VOLUME III

ORIGINAL REPORTS
ON GLUTAMATE
(Part 2)

IGTC May 1974

39

p13

Volume 3-**Original Reports on Glutamate** (Part 2)-IGTC-5/74

REPORT ON AN INVESTIGATION OF THE TOXICITY OF L-MONOSODIUM GLUTAMATE, DL-MONOSODIUM GLUTAMATE AND L-GLUTAMIC ACID

TO

INTERNATIONAL MINERALS AND CHEMICAL CORPORATION

Part I

ARTHUR D. LITTLE, INC.
CAMBRIDGE 42, MASSACHUSETTS
JULY 30, 1952
ISSUED MARCH 15, 1953
C-58047

TABLE OF CONTENTS

	Page
LETTER OF TRANSMITTAL	
INTRODUCTION	1
ACUTE TOXICITY	4
CHRONIC TOXICITY	7
NUTRITIONAL DATA	10
GROWTH AND DEVELOPMENT	23
FOOD INTAKE	29
BLOOD EXAMINATIONS	34
GENERAL BEHAVIOR	43
FERTILITY OF TEST ANIMALS	45
GENERAL PHYSICAL CONDITION	53
SURVIVAL DATA	55
PATHOLOGICAL EXAMINATIONS	61
OCCURRENCE OF SPONTANEOUS TUMORS	69
MICROSCOPIC EXAMINATIONS	73
GLUTAMIC ACID IN BLOOD AND RELATIONSHIP TO TUMORS	85
DISCUSSION	89
CONCLUSION	92
SUMMARY	93
APPENDIX	96
PHOTOGRAPHS	

LIST OF FIGURES

		Page
1.	Total Per Cent Weight Gain of Rats on Control Diet and on Control Diet Supplemented by L-Monosodium Glutamate	25
2.	Total Per Cent Weight Gain of Rats on Control Diet and on Control Diet Supplemented by DL-Monosodium Glutamate	26
3.	Total Per Cent Weight Gain of Rats on Control Diet and on Control Diet Supplemented by L-Glutamic Acid	27
4.	Average Food Consumption in Grams per Day--Female Weanling Rats	28
5.	Average Weekly Per Cent Weight Gain --Female Weanling Rats	32

LIST OF TABLES

		Page
I.	Intake of Supplement - Daily and Over Two Year Period	12
II.	Assay Data for Supplemented Rat Food (In Per Cent)	13
III.	Vitamin A and Fat Content of Supplemented Purina Laboratory Chow	17
IV.	Niacin Content of Supplemented Purina Laboratory Chow	18
V.	Riboflavin Content of Supplemented Purina Laboratory Chow	19
VI.	Thiamine Content of Supplemented Purina Laboratory Chow	20
VII.	Vitamin B_{12} Content of Supplemented Purina Laboratory Chow	21
VIII.	Summary of Vitamin Assay Data	22
IX.	Average Blood Counts of Female Rats (355 Days of Age) after 241 Test Days	36
X.	Average Blood Counts of Male Rats (339-353 Days Age) after 241 Test Days	37
XI.	Average Blood Counts of Male Rats (373-380 Days Age) after 241 Test Days	38
XII.	Average Blood Counts of Rats (Both Sexes) after 567 Test Days	39
XIII.	Average Blood Counts of Female Rats after 681 Test Days	40
XIV.	Average Blood Counts of Male Rats after 681 Test Days	41

LIST OF TABLES, CONTINUED

		Page
XV.	Normal Range of Blood Values in the Laboratory Rat	42
XVI.	Number and Size of Litters by Generation	49 and 50
XVII.	Average Number and Size of Litters Produced by Rats (F_0-F_4) on Supplemented Diets	51
XVIII.	Clinical Condition and Survival Rates of Rats Fed Supplemented Diets for 182 Test Days	56
XIX.	Clinical Condition and Survival Rates of Rats Fed Supplemented Diets for 368 Test Days	57
XX.	Clinical Condition and Survival Rates of Rats Fed on Supplemented Diets for 547 Days	58
XXI.	Clinical Condition and Survival Rates of Rats Fed Supplemented Diets for 730 + Days	59
XXII.	Post-mortem Examinations	65
XXIII.	Gross Post-mortem Findings in Rats Sacrificed at 548 Test Days	66
XXIV.	Distribution of Gross Abnormalities	67
XXV.	Location Gross Abnormalities	68
XXVI.	Presumptive Percentage Incidence of Tumors	70
XXVII.	Summary Comparison of All Test Rats with Control Rats at 730 + Test Days	72
XXVIII.	Distribution of Microscopic Abnormalities Exclusive of Tumors	82
XXIX.	Statistical Distribution of Abnormalities	83
XXX.	Glutamic Acid Content of Blood Filtrates	87

Arthur D. Little, Inc.
RESEARCH · ENGINEERING · INVESTIGATION
CAMBRIDGE 42, MASSACHUSETTS

March 15, 1953

Dr. Paul D. V. Manning
International Minerals and Chemical Corporation
20 North Wacker Drive
Chicago 6, Illinois

Dear Dr. Manning:

We are sending you herewith a report of an investigation of the toxicity of L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid. The work was carried out over a two-year period during which these compounds were fed to albino rats as supplements to nutritionally adequate diets. A control group of rats was fed on the same diet without supplement and maintained under identical conditions. The acute toxicity of these compounds was determined by administering a single large dose orally to rats. The doses found to be lethal to fifty per cent of the rats to which they were administered (LD_{50}) were as follows:

L-monosodium glutamate	19.9 grams per kilogram body weight
DL-monosodium glutamate	10.3 grams per kilogram body weight
L-glutamic acid	More than 23 grams per kilogram body weight

The concentrations of the test materials fed to rats were 0.1 per cent and 0.4 per cent of the total diet. Observations were made on growth, nutritional and general physical status, and fertility. The mortality rates for each group were carefully recorded as well as the occurrence of abnormalities such as dental anomalies, abnormal eye conditions and occurrence of tumors. Post-mortem examinations were made of all rats, and evaluation of tissues and organs made by microscopic examination as well as by gross inspection.

No evidence was seen of chronic toxicity resulting from the administration of the materials under test. The incidence of abnormalities was approximately the same in control animals, and the survival rates showed no significant differences. The lesions seen after death were consistent with the advanced age of the animals, and were chiefly low-grade inflammatory lesions in the lungs and kidneys.

A number of tumors (in every case benign) were seen. These were chiefly fibroplastic in type. They were more numerous in female than in male animals, but were not more numerous in test animals than in the control group. No adverse effect was manifested by the test materials on the fertility of the rats to which they were fed.

The report is presented in two parts. The first describes the organization of the experimental work, the results of post-mortem examinations and the general conclusions. The second part contains a detailed description of the tissue examinations for record purposes.

It is our conclusion that we have observed no chronic toxic effect to be exerted by L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid, and that such abnormalities as we observed in the living animals and at autopsy existed in approximately the same incidence in the control as in the test animals. The anatomical tissue changes which we observed and which are described in detail are more properly relatable to the age of the animals than to the test materials.

Respectfully submitted,

Arthur D. Little, Inc.

INTRODUCTION

The controversial position of "intentional" food additives in recent years has made objective investigations of their so-called "chronic" toxicity a matter of necessity. Investigation of the toxicity of the substances which are added to food differs materially from the toxicity of drugs, for the use of the former may extend throughout the life span of the consumer, whether human or animal. For many chemical substances, there may exist a large body of data covering pharmacodynamic action, biochemical fate and therapeutic value, but definitive and descriptive information regarding the effect of repeated ingestion in small amounts may be lacking. Into this category fell glutamic acid and its sodium salt, although there existed extensive information acquired on an empirical basis.

The purpose of the investigation reported here was to acquire under controlled conditions, objective information regarding the effect of feeding L-glutamic acid, L-monosodium glutamate and DL-monosodium glutamate to laboratory animals. The feeding program was carried out over more than two years during which time observations were made on the general physical condition and behavior, growth, fertility, mortality rates, and gross and microscopic changes in body organs and tissues by post-mortem examination. Determinations were made of the lethal dose (LD_{50}) when administered via the gastrointestinal tract. The species

used was the albino rat, and observations were made on control groups as well as on rats fed a standard diet supplemented by one of the compounds under test.

The chronic toxic effects of a chemical substance may be manifested in a variety of forms, either as alterations in normal physiological and biochemical processes observable during life, or they may be expressed as anatomical alterations which are perceptible only after death or sacrifice of the subject animal. Nutritional changes may result from the inadequacy of the diet due to the addition of large amounts of supplementary material. Changes may be seen in cardiorenal, neuromuscular, or gastrointestinal function. It is only by comprehensive observation and recording of all such changes plus any random observations that a total picture of the presence or absence of chronic toxicity can be established.

If chronic toxicity is high and manifests itself early in the course of controlled observations, it is probably adequate to limit the duration of experimental work. When no evidence of toxicity is seen or the evidence is so limited as to make early generalizations of non-toxicity hazardous, the feeding of the test material should be extended over two years, or the approximate life span of the animal, whichever is shorter. The director of the Division of Pharmacology of the U. S. Food and Drug Administration has stated that any toxicological data on a food additive based on work of less than two years duration can be given extremely small weight.

Almost any chemical substance can be said to be toxic if the dosage be sufficiently high. In general, toxicity is considered to be the capacity of a substance to produce injury, while hazard is the probability that injury will result from the use of the substance in the quantity and manner proposed. From our findings with respect to both acute and chronic toxicity of L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid, it is apparent that a wide and adequate margin exists between the amount which will produce damage and that which will be ingested by humans under reasonably normal conditions. No evidence was derived that normally adequate diets were reduced in nutritional value by adding any of the three substances examined.

Glutamic acid is a normal constituent of mammalian tissue, and is found in free form in brain, cerebral cortex, heart, spleen and thymus. It is a non-essential amino acid, although a major constituent of body proteins and enters into many normal metabolic processes. It is an intermediate in the synthesis of folic acid and a growth accelerator. Glutamic acid was first isolated in 1866 by Ritthausen and is said to be the only chemical substance other than certain of the carbohydrates which can support the metabolism of isolated brain tissue. The Asiatic peoples, notably the Japanese and Chinese have used monosodium glutamate as a food additive for several centuries without suspension of associated toxicity.

ACUTE TOXICITY

The data covering the determination of the acute toxicity of the three compounds examined in this investigation was described in detail in a previous report dated August 4, 1950. It is summarized here for reference purposes. The single lethal doses for 50 per cent of rats (LD_{50}) to which the compounds were administered by a stomach tube were found to be

L-monosodium glutamate	=	19.9 grams per kilogram weight.
DL-monosodium glutamate	=	10.3 grams per kilogram weight.
L-glutamic acid	=	a) more than 30 grams per kilogram body weight in rats. b) more than 23 grams per kilogram body weight in rabbits.

No exact lethal dosage level was found for glutamic acid. According to the classification of Hodge[1], these compounds can be considered "Practically non-toxic" in the case of DL-monosodium glutamate and "Relatively harmless" in the case of the other two compounds.

Rats and rabbits were used to determine the LD_{50} for L-glutamic acid and rats only in determining this level for DL-monosodium glutamate and L-monosodium glutamate.

---

[1] Hodge, H. C. and Sterner, J. H.; Tabulation of Toxicity Classes. Am. Ind. Hyg. Assoc. Quart. 10.93, 1949.

L-MONOSODIUM GLUTAMATE

To each of 28 albino rats was administered a solution of the compound in distilled water (90-95 percent). The dosage level tolerated by 50 percent of the rats to which it was administered was 19.9 grams per kilogram of body weight.

DL-MONOSODIUM GLUTAMATE

To each of 57 albino rats was administered a solution of the compound in distilled water (50-60 percent) by stomach tube.

This compound was less soluble in distilled water than was L-monosodium glutamate and when the calculated dose exceeded a volume of 12 ml, it was given in divided doses with an hour interval between. The LD_{50} was found to be 10.3 grams per kilogram body weight.

L-GLUTAMIC ACID

To each of 36 albino rats was administered L-glutamic acid as a suspension in 1.0 percent carboxymethyl cellulose (which is known to be non-toxic). For levels requiring a volume greater than 12 ml. it was necessary to give an additional dose after an interval of one hour. Administration of the calculated amount of L-glutamic acid to rats was never completely accomplished for, even with uniform suspension, the compound settled out in part and was difficult to extrude from the delivery syringe through a catheter.

L-glutamic acid was tolerated by rats at all levels ranging from 8.1 to 30.3 grams per kilogram body weight. The low solubility of L-glutamic acid and the difficulty of administration in concentrations sufficient to produce toxic effect necessitated the repetition of the procedure in another species. Rabbits were used and the dosages administered ranged from 3.8 to 23.0 grams per kilogram. One animal died during administration and at autopsy, fluid was found in the lungs. Presumably death resulted from asphyxia rather than from the toxic effects of L-glutamic acid. Administration of the suspension of L-glutamic acid in 1.0 percent carboxymethylcellulose was more successful than in rats for a larger stomach tube could be used, which minimized the "settling" of the suspended material before being deposited in the animal's stomach.

CHRONIC TOXICITY

Young, adult, albino rats obtained from Sprague-Dawley, Inc., Madison, Wisconsin, were used as test animals. Six hundred rats weighing approximately 250 to 350 grams and aged about three months when the experimental work started, were divided according to age and sex and caged in sub-groups of 7-8 rats. Female rats were born between August 21 and 31, 1949 and the male rats were born between August 3 and September 12, 1949. Six test groups of 75 individual rats each and one control group of 150 rats were established. The rats were divided as evenly as possible according to sex among the six test groups; the control group contained a slightly larger number of females than males. For each supplement to be tested two groups of rats were set aside; one group was fed on a standard diet adequate for their nutritional needs with added supplement to the extent of 0.1 percent of the total weight of the ration. The second group received 0.4 percent supplement added to the standard diet. The distribution of rats according to sex is indicated below:

Supplement	Number of Animals		
	Males	Females	Total
0.1% L-MSG*	40	35	75
0.4% L-MSG	35	40	75
0.1% DL-MSG	40	35	75
0.4% DL-MSG	35	40	75
0.1% L-GA	40	35	75
0.4% L-GA	35	40	75
Control Diet	61	89	150

The rats were housed in wire mesh cages in groups of seven or eight. Food in pellet form was offered in mesh feeders which were accessible ad libitum to the rats. Water was supplied from non-dripping bottles (providing a "demand" supply) so attached that only the outlet projected into the cage. A sliding metal tray containing sawdust was in position under a mesh floor to catch droppings and waste. The sawdust was renewed each day. Cages, feeders, and water bottles were thoroughly cleaned periodically.

The cage room was maintained at constant temperature of 74° F. and approximately 50 per cent relative humidity. The lighting

---

*Throughout this report, the following abbreviations, when used, are intended to indicate the following:

L-MSG	=	L-monosodium glutamate monohydrate.
DL-MSG	=	racemic DL monosodium glutamate monohydrate.
L-GA	=	L-glutamic acid.

conditions were made constant by rendering the windows opaque and regulating the daily period during which artificial illumination was in use. The feeders were refilled as required.

It should be noted here that the Sprague-Dawley strain of rats used by us is highly inbred. This has the advantage of producing uniformity of response, but at the same time exaggerating any tendency to which the strain is particularly susceptible.

NUTRITIONAL DATA

The test supplements were mixed with the food and incorporated into pellets at the Experimental Ration Laboratory of the Ralston Purina Company, St. Louis. The supplements were obtained from Dr. M. J. Blish, Research Division, International Minerals and Chemical Corporation, Rossford, Ohio, who also arranged for assays of the actual pellets consumed. The Ralston Purina Company was not able to prepare a pellet the same size as that of the basic ration. It was shorter (1/2 - 1 inch) and slightly smaller in diameter (3/8 - 1/2 inch) but was acceptable to the rat. The only differences between the pellets fed to animals in the test and control groups were the presence of the supplement and slightly different size. The Ralston Purina Company reported some difficulty in getting a uniform concentration of the supplements into pellet form in lots weighing less than a quarter of a ton. Preparation of such a quantity was undesirable because of probable vitamin loss before such a large lot of food was entirely consumed.

Representative samples of the unsupplemented food were sent at intervals to the Rossford plant where they were analyzed for the appropriate supplement under the direction of Dr. M. J. Blish. For the reasons stated above, the actual content of dietary supplement varied from the intended concentration because of the relatively small lot which was prepared at one time. In all cases, the actual concentration

was greater than the intended concentration so that all animals received more supplement during the course of the experimental work than either 0.1 or 0.4 per cent would have furnished. The theoretical and actual intake daily and for the two-year feeding program is shown in TABLE I. These data are based on the average amount of food consumed during a controlled measurement survey upon which comment is made elsewhere in the report. The results of the assays for supplements are shown in TABLE II. The maximum variation from the intended concentration is not outside the limits of tolerable error in this type of experiment.

The glutamate supplement concentrations of 0.1 and 0.4 per cent were selected as those approximating the probable average daily range of intake for a human subject using monosodium glutamate in the concentration recommended, in all suitable foods; these levels were established on the recommendation of Mr. L. B. Sjostrom and Dr. S. E. Cairncross.

TABLE I

INTAKE OF SUPPLEMENT - DAILY AND OVER TWO-YEAR PERIOD

Supplement	Amount of Supplement (in grams) Received Daily				Amount of Supplement (in grams) Received Over the Two Year Feeding Program			
	Theoretical		Actual (average of assay values)		Theoretical		Actual (average of assay values)	
	Male	Female	Male	Female	Male	Female	Male	Female
0.1 percent L-MSG	.04	.02	.08	.04	30.29	16.79	59.1	32.7
0.4 percent L-MSG	.17	.09	.18	.10	121.18	67.16	132.9	73.2
0.1 percent DL-MSG	.04	.02	.11	.06	30.29	16.79	80.3	44.5
0.4 percent DL-MSG	.17	.09	.23	.13	121.18	67.16	166	92
0.1 percent L-GA	.04	.02	.06	.04	30.29	16.79	47.5	26.4
0.4 percent L-GA	.17	.09	.18	.10	121.18	67.16	132.1	73.3

In all instances the actual amount received in grams was greater than the theoretical amount.

Arthur D. Little, Inc.

TABLE II

ASSAY DATA FOR SUPPLEMENTED RAT FOOD (IN PERCENT)

Date of Report and Percent of Supplement Found

Supplement Represented	January 30, 1950	February 21, 1950	June 7, 1950	July 13, 1950	October 16, 1950	February 28, 1951	June 6, 1951	September 26, 1951	Average
0.1 percent L-MSG	0.20	0.39	0.16	0.18	0.17	0.12	0.17	0.17	.195
0.4 percent L-MSG	0.43	0.43	0.42	0.48	0.47	0.43	0.40	0.45	.439
0.1 percent DL-MSG	0.29	0.29	0.30	0.24	0.24	0.28	0.21	0.27	.265
0.4 percent DL-MSG	0.51	0.57	0.50	0.59	0.59	0.61	0.47	0.55	.549
0.1 percent L-GA	0.13	0.16	0.17	0.14	0.19	0.16	0.14	0.17	.158
0.4 percent L-GA	0.42	0.49	0.41	0.43	0.45	0.44	0.40	0.45	.436

VITAMIN ASSAYS

Vitamin assays for vitamin A, B_{12}, niacin, riboflavin and thiamine were made on samples of supplemented rat food used in this study at the Food Technology Laboratories, Massachusetts Institute of Technology under the direction of Dr. Samuel A. Goldblith. A summary of the results is presented in tabular form. The samples assayed had been stored for various periods of time. (Tables III - VIII)

An effort was made to exhaust a lot of food before starting to feed a newly prepared lot to minimize vitamin loss and general deterioration. The assays were made between October 8 - 28, 1950, of two lots of food supplemented with 0.4 percent L-monosodium glutamate and on three samples with 0.4 percent L-glutamic acid prepared at different times. Each vitamin assay was started on the same day, in order to favor consistent results. This is especially important in microbiological assays, so that the same standard curve can be used for all data.

VITAMIN A

There was loss of vitamin A in some samples, but in no case did the loss reduce the amount present to less than the minimum nutritional requirement of rats, i.e. 40 U.S.P. units/100 gms. of food.

NIACIN

There appeared to be a definite loss of niacin on storage, except in the case of the sample containing 0.4 percent L-monosodium glutamate. Any variation less than ± 10 percent is not significant due inherent errors in microbiological assays.

RIBOFLAVIN

The extent of riboflavin loss is of the same order of magnitude as that for niacin. As with the niacin assays, there was more loss in the sample stored for eight months than in the sample stored for three months (0.4 percent L-glutamic acid).

THIAMINE

With respect to thiamine, the losses, if any, were not great. In some samples, a slight gain was shown, probably due to errors in the experimental method or loss of moisture.

VITAMIN B_{12}

The B_{12} losses may be actual in the case of the sample containing 0.4 percent L-monosodium glutamate as well as in that containing 0.4 percent L-glutamic acid. In both cases, however, because of the inherent inaccuracies of B_{12} assays, the actual extent of the losses cannot be stated with exactness. However, it was apparent that no animal showed the effect of B_{12}-avitaminosis.

An appreciable loss of riboflavin and niacin occurred in less than eight months' storage of the rat food containing 0.4 percent

L-glutamic acid. A small loss occurred in three months storage of that containing 0.4 percent L-monosodium glutamate. This did not affect the nutritional status of the rats appreciably since a new lot of food was ordered every three months. Thiamine appeared to be stable on storage of the food, although there seemed to be a slight loss on storage of the 0.4 percent L-monosodium glutamate sample. When losses occurred on storage, the extent of loss appeared to bear no relation to the glutamate derivative present.

Variations in the initial vitamin content of the basic ration were not significant for the losses which occurred of niacin and riboflavin were of far greater magnitude than the variation in different samples. The analyses showed that more of the vitamins assayed were present than were required for proper nutrition of rats, even in samples stored for a period greater than six months. No lot of food was sufficiently large to last more than this period.

TABLE III

VITAMIN A AND FAT CONTENT OF SUPPLEMENTED PURINA LABORATORY CHOW

Sample Identification	Date of Preparation of Pellets	Moisture (in percent)	(Dry Basis) Average in percent	Vitamin A (USP units/gm) (Dry Basis) Average
0.4 percent L-MSG	6/14/50	4.92	7.50	351
0.4 percent L-MSG	9/29/50	5.78	6.85	321
0.4 percent L-GA	1/23/50	4.95	6.66	355
0.4 percent L-GA	6/14/50	4.94	7.50	217
0.4 percent L-GA	9/29/50	6.62	6.79	279

TABLE IV

NIACIN CONTENT OF SUPPLEMENTED PURINA LABORATORY CHOW

Sample Identification	Date of Preparation of Pellets	Moisture (in percent)	Niacin Content (Wet Basis) Average	(γ /gm) (Dry Basis) Average
0.4 percent L-MSG	6/14/50	4.92	30.6	32.2
0.4 percent L-MSG	9/29/50	5.78	32.9	35.0
0.4 percent L-GA	1/23/50	4.95	26.2	27.5
0.4 percent L-GA	6/14/50	4.94	31.5	33.1
0.4 percent L-GA	9/29/50	6.62	41.5	44.4

Arthur D. Little, Inc.

TABLE V

RIBOFLAVIN CONTENT OF SUPPLEMENTED PURINA LABORATORY CHOW

Sample Identification	Date of Preparation of Pellets	Moisture (in percent)	Riboflavin Content (Wet Basis) Average	(γ /gm) (Dry Basis) Average
0.4 percent L-MSG	6/14/50	4.92	13.8	14.5
0.4 percent L-MSG	9/29/50	5.78	15.6	16.5
0.4 percent L-GA	1/23/50	4.95	12.0	12.6
0.4 percent L-GA	6/14/50	4.94	14.1	14.8
0.4 percent L-GA	9/29/50	6.62	16.5	17.6

Arthur D. Little, Inc.

TABLE VI

THIAMINE CONTENT OF SUPPLEMENTED PURINA LABORATORY CHOW

Sample Identification	Date of Preparation of Pellets	Moisture (in percent)	Thiamine Content (Wet Basis) Average	(γ /gm) (Dry Basis) Average
0.4 percent L-MSG	6/14/50	4.92	2.98	3.14
0.4 percent L-MSG	9/29/50	5.78	3.38	3.59
0.4 percent L-GA	1/23/50	4.95	3.28	3.45
0.4 percent L-GA	6/14/50	4.94	3.09	3.25
0.4 percent L-GA	9/29/50	6.62	3.36	3.59

TABLE VII

VITAMIN B_{12} CONTENT OF SUPPLEMENTED PURINA LABORATORY CHOW

Sample Identification	Date of Preparation of Pellets	Moisture (in percent)	Vitamin B_{12} (Wet Basis) Average	(γ /gm) (Dry Basis) Average
0.4 percent L-MSG	6/14/50	4.92	0.038	0.040
0.4 percent L-MSG	9/29/50	5.78	0.054	0.057
0.4 percent L-GA	1/23/50	4.95	0.038	0.040
0.4 percent L-GA	6/14/50	4.94	0.031	0.033
0.4 percent L-GA	9/29/50	6.62	0.042	0.045

TABLE VIII

SUMMARY OF VITAMIN ASSAY DATA*

Description	Date of Preparation	Vitamin A (γ/gm) (Dry Basis)	Vitamin A Percent Loss or Gain in Storage	Niacin (γ/gm) (Dry Basis)	Niacin Percent Loss or Gain in Storage	Riboflavin (γ/gm) (Dry Basis)	Riboflavin Percent Loss or Gain in Storage	Thiamine (γ/gm) (Dry Basis)	Thiamine Percent Loss or Gain in Storage	Vitamin B12 (γ/gm) (Dry Basis)	Vitamin B12 Percent Loss or Gain in Storage
0.4 percent L-MSG	6/14/50	351	+ 9.3	32.2	- 8.0	14.5	-12.1	3.14	-12.5	.040	-20.8
0.4 percent L-MSG	9/29/50	321	-	35.0	-	16.5	-	3.59	-	.057	-
0.4 percent L-GA	1/23/50	355	27.1	27.5	-38.0	12.6	-28.4	3.45	3.9	.040	-11.2
0.4 percent L-GA	6/14/50	217	-22.2	33.1	-25.5	14.8	-15.9	3.25	9.5	.033	-26.6
0.4 percent L-GA	9/29/50	279	-	44.4	-	17.6	-	3.59	-	.045	-

*Comparison is between theoretical amount and amount actually found.

Arthur D. Little, Inc.

GROWTH AND DEVELOPMENT

The growth and development of all rats proceeded at a normal rate. At regular intervals all rats were weighed and the amount of weight gain or loss recorded. At first, the times of weighing all rats consecutively was scheduled as nearly as possible to be five or six weeks apart until the rats had reached their maximum growth. Data on weights from 0-106 test days was reported on March 27 and August 4, 1950. This will not be repeated here, but is summarized in Figures 1-3, which show that for both male and female rats on all supplemented diets, the absolute and per cent weight gain from 0-206 test days was greater than for control rats of both sexes.

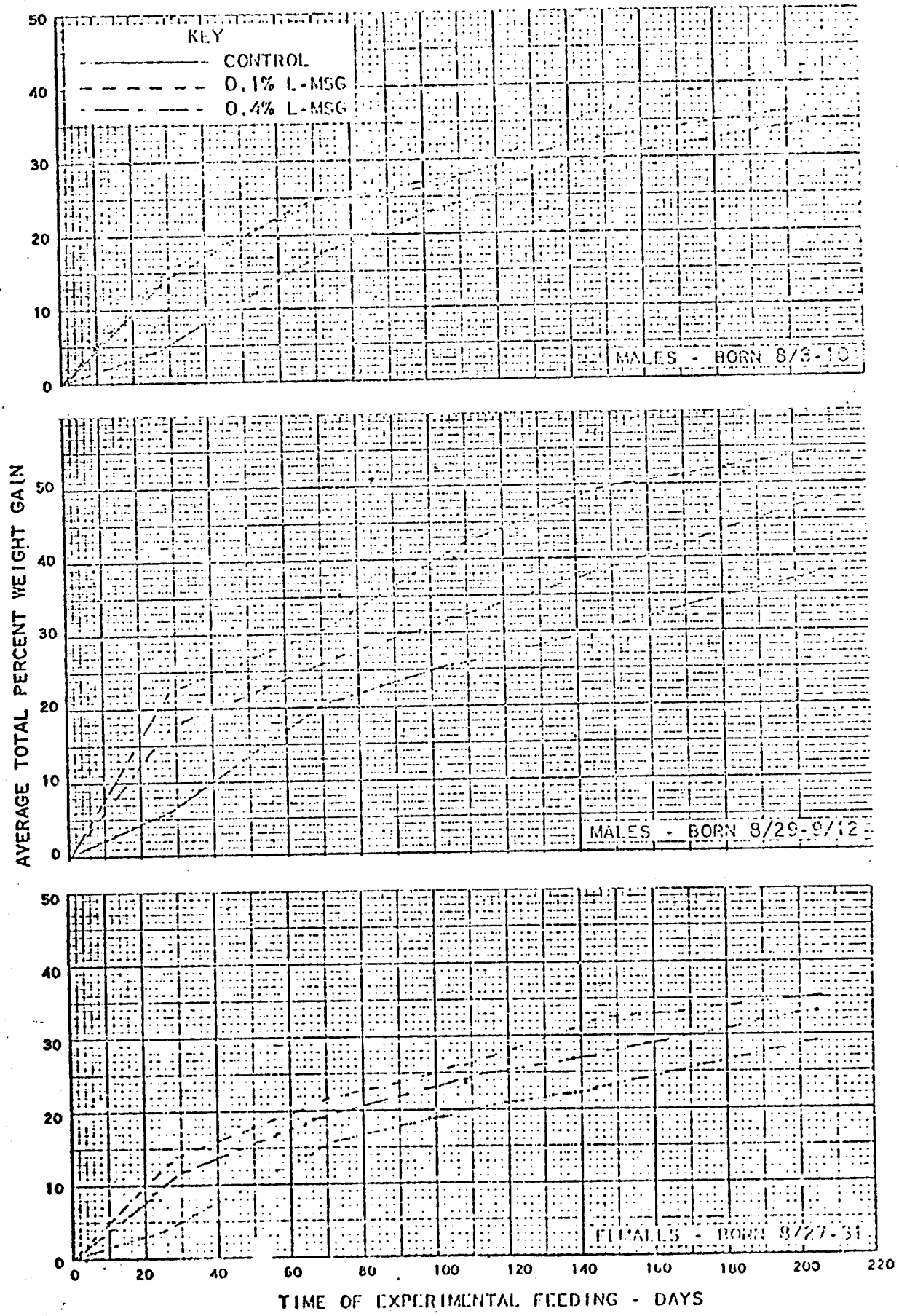
Ten series of complete weighings were made including the weight at zero day and the final weight before autopsy. The ninth weighing was made when the feeding program had been in progress twenty months. The tenth was the weight taken at time of autopsy. The following table lists the intervals between weighing of all rats:

Weighing Series	Number Test Days
1	0
2	29
3	71
4	106
5	144
6	206
7	254
8	309
9	606
10	Terminal

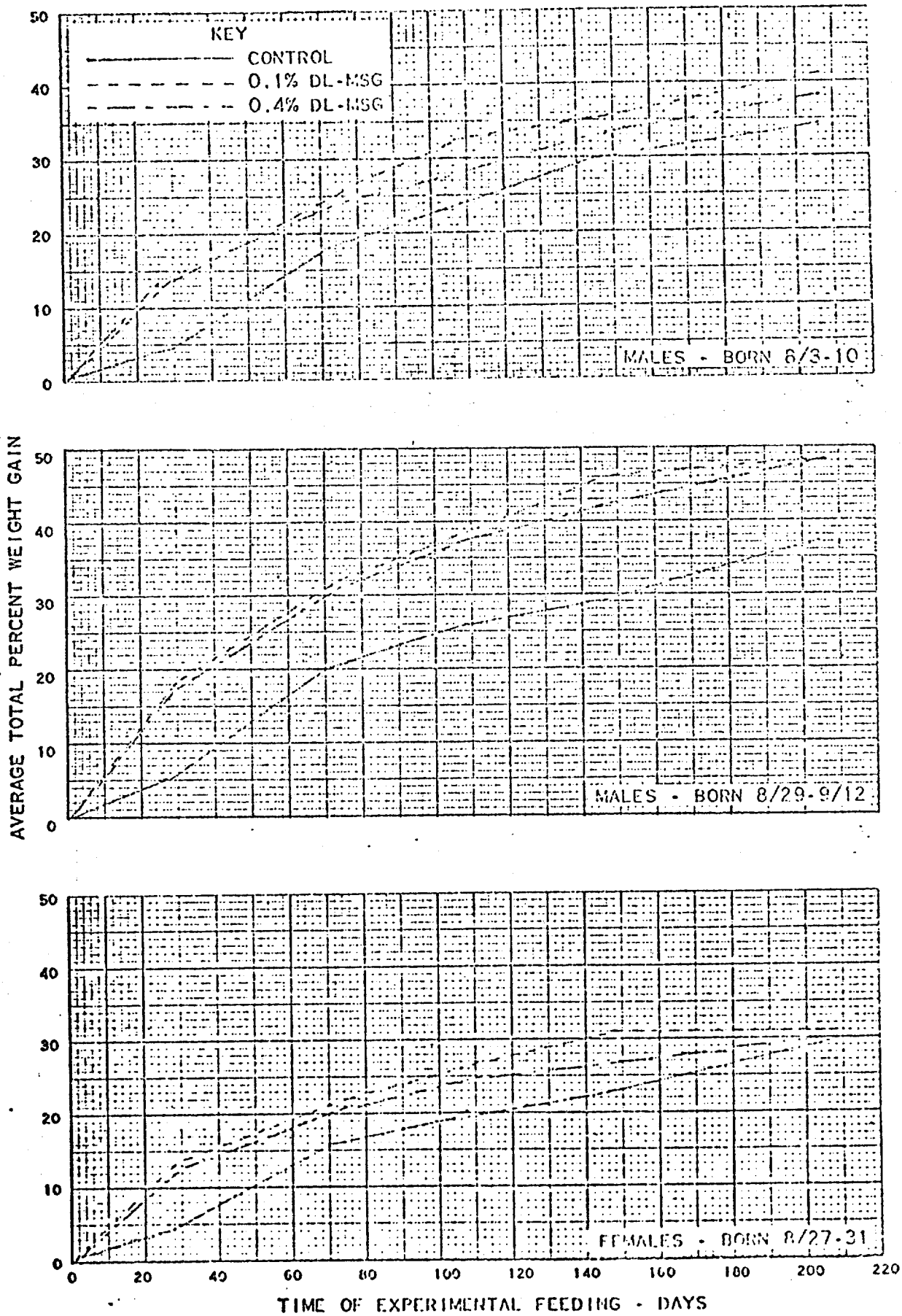
The per cent of weight gain and weight loss for a random selection of animals was calculated after each weighing series over a ten-month period. At that time, maximum growth had been obtained and the incidental factors affecting weight were not related to growth. Several animals failed to ingest their usual weight of food because of dental abnormalities, and this was soon reflected in their body weight. After 300 test days, the losses and gains in weight were irregularly distributed throughout all rat groups and does not seem to indicate any particular trend. We believe the most significant data regarding body weight is that covering 206 test days at which time the rats were aged approximately ten months. After that time, the weight curve leveled and was not significantly altered for any group as a whole. Certainly there was no marked adverse effect exerted on the early growth and development by the test supplements. The effect, if any, was a slightly favorable one in terms of weight gain especially in male rats on supplemented diets.

FIGURE 1

TOTAL PERCENT WEIGHT GAIN OF RATS ON CONTROL DIET AND ON CONTROL DIET SUPPLEMENTED BY L-MONOSODIUM GLUTAMATE



TOTAL PERCENT WEIGHT GAIN OF RATS ON CONTROL DIET
AND ON CONTROL DIET SUPPLEMENTED BY
DL-MONOSODIUM GLUTAMATE



Arthur D. Little, Inc.

FIGURE 3

TOTAL PERCENT WEIGHT GAIN OF RATS ON CONTROL DIET AND ON CONTROL DIET SUPPLEMENTED BY L-GLUTAMIC ACID

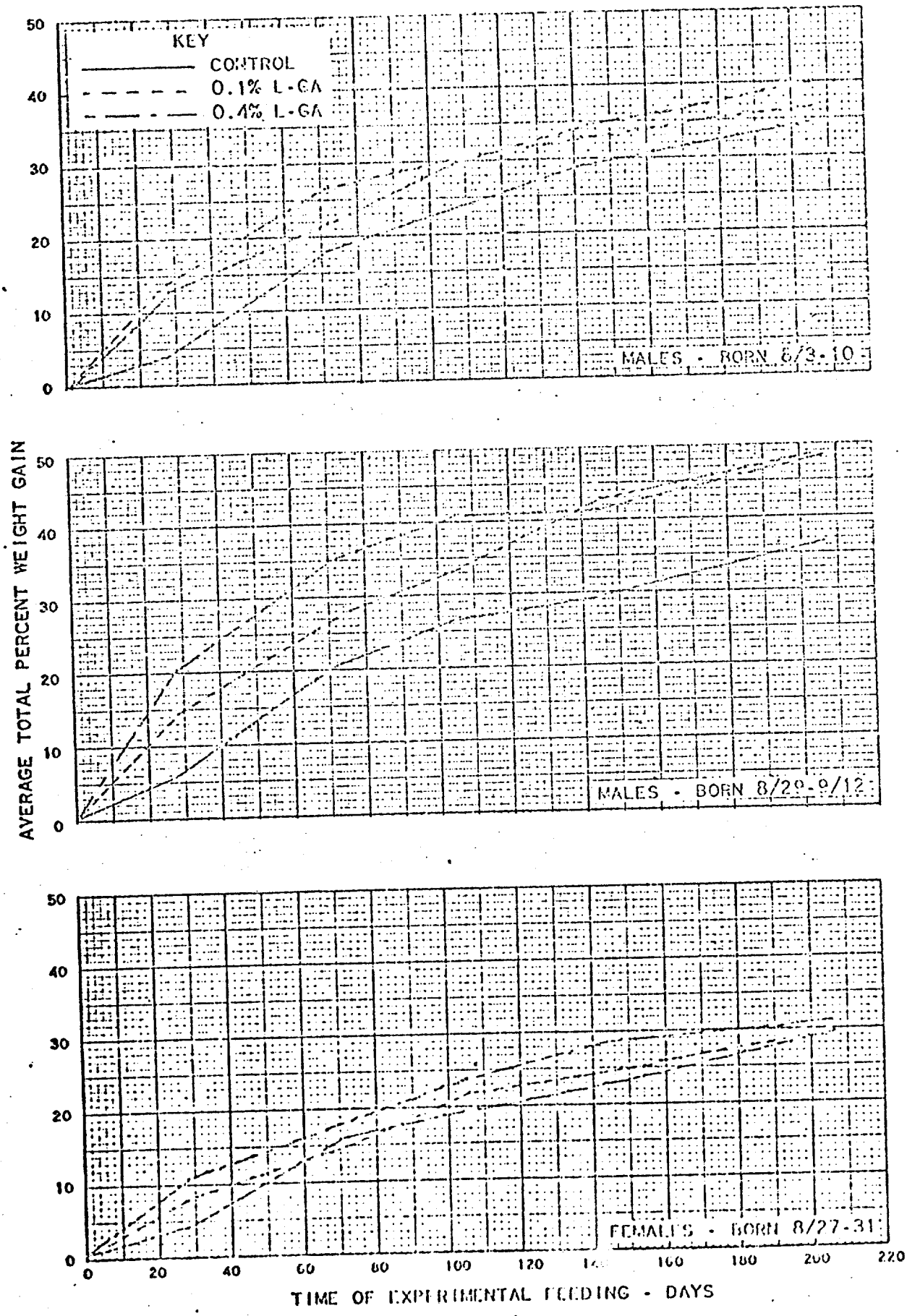
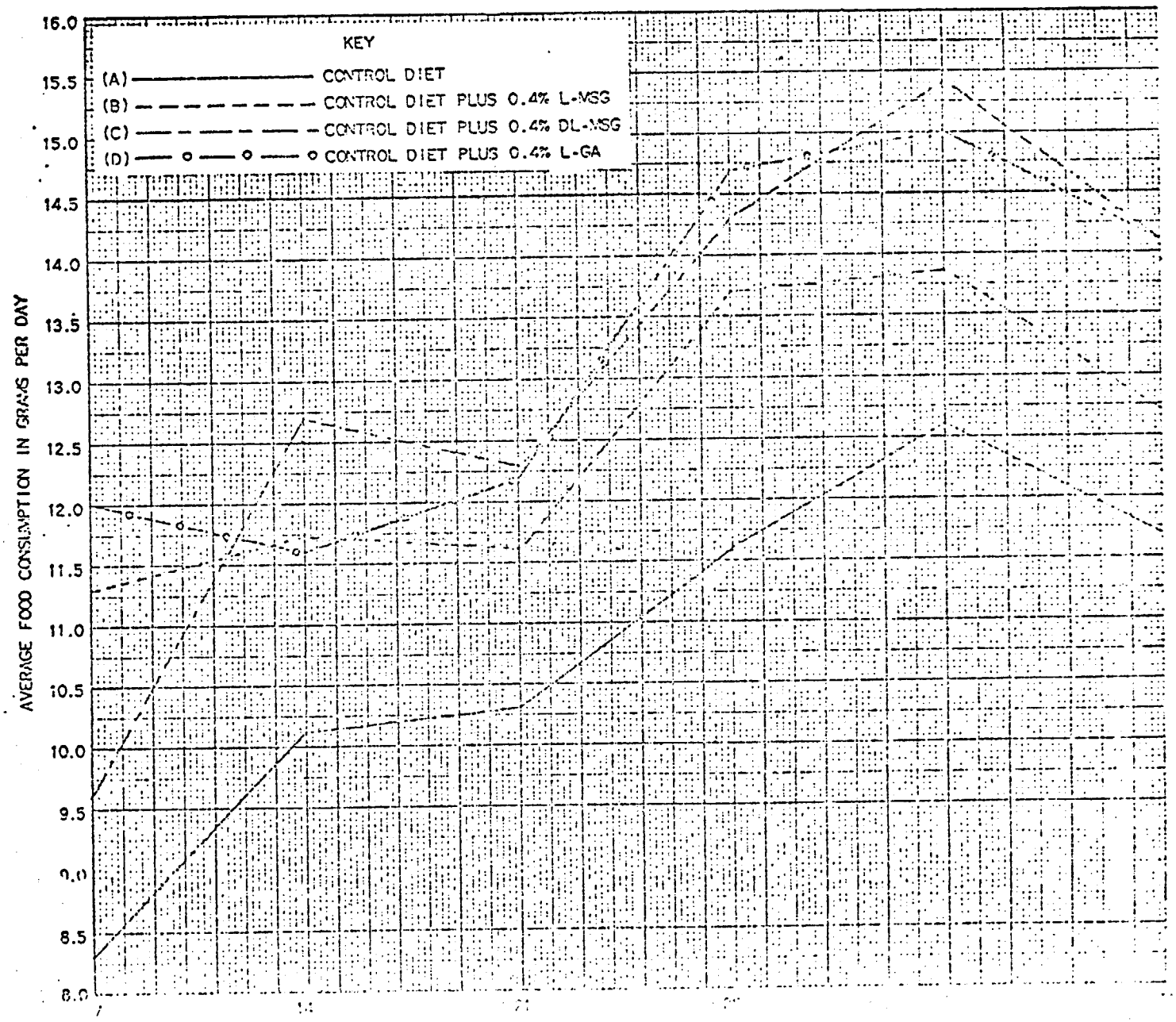


FIGURE 4

AVERAGE FOOD CONSUMPTION IN GRAMS PER DAY - FEMALE WEANLING RATS



Arthur D. Little, Inc.

FOOD INTAKE

The method of feeding from wire holders permitted the rats to eat ad libitum. While the net intake per rat varied from day to day, it was found to be remarkably constant throughout the investigation on the basis of average daily intake in grams per rat, measured over a period longer than two months. A controlled study was undertaken at 180 test days in which the amount of food offered and the amount consumed was weighed daily for selected rats from each feeding group. An effort was made to determine the water intake at the same time but this proved to be impractical since pressure from the animal's body against the glass nipple caused water to be released into the bottom of the cage introducing a large element of error. The information to be obtained did not warrant the time which would have been required to obtain accurate volume measurements; we estimate the average daily water intake for all rats to have been approximately 32 ml. per day.

When the work had been in progress for six months, food consumption measurements were made for all groups, test as well as control. The several age groups represented in the original stock, the different dietary groups and the two sexes were represented. Each rat used in the substudy was caged separately and was fed and watered independently. Animals were selected whose body weight and development at that time were representative of the entire group. Due measurement of spillage was made (averaging 10 grams per day). From the accumulated data, it was determined that for all animals, test and control, the average daily intake of food was 21.4 grams. Some considerable variation was noted in

a given rat from day to day, but the trends in all groups were consistent. Thus, if the intake was high on one day, it tended to be less on the subsequent day and vice versa. The intake per day increased as the rats reach maturity, at which time the intake tended to become stabilized and so continued until late in the work when the rats had become senile. At that latter time, the daily intake tapered off to a certain extent. In rats which developed tumors, often of large size, there appeared to be no significant increase in food intake. The rats were returned to their original cages after the measurements of food intake were completed and continued in their original groups to the end of the investigation.

At the request of Dr. Paul D. V. Manning a second investigation of individual food intake was made, but a new group of rats was used. These were weanling rats (Sprague-Dawley strain). It had been noted from data presented in a previous progress report submitted by us on August 4, 1950 that rats fed on supplemented diets showed a greater weight gain than did those on the control diet. Some relationship between amount of food intake, stimulation of appetite by the supplements and net weight gain appeared to be a possibility. Weanling rats were selected so that measurements of intake and weight gain might be made during the period of maximum normal weight gain.

Twenty-four female weanling rats (Sprague-Dawley) weighing between 35 and 50 grams were housed together as a group on control food for a week of general observation. As a period of further acclimatization each rat was individually caged for a second week and fed from a wire feeder containing the control diet of Ralston Purina Laboratory Chow.

At the beginning of the study (0 day) each rat was weighed and the feeders filled with appropriate food, i.e., the control food plus 0.4 per cent of supplement. Six animals were used in each of the four groups.

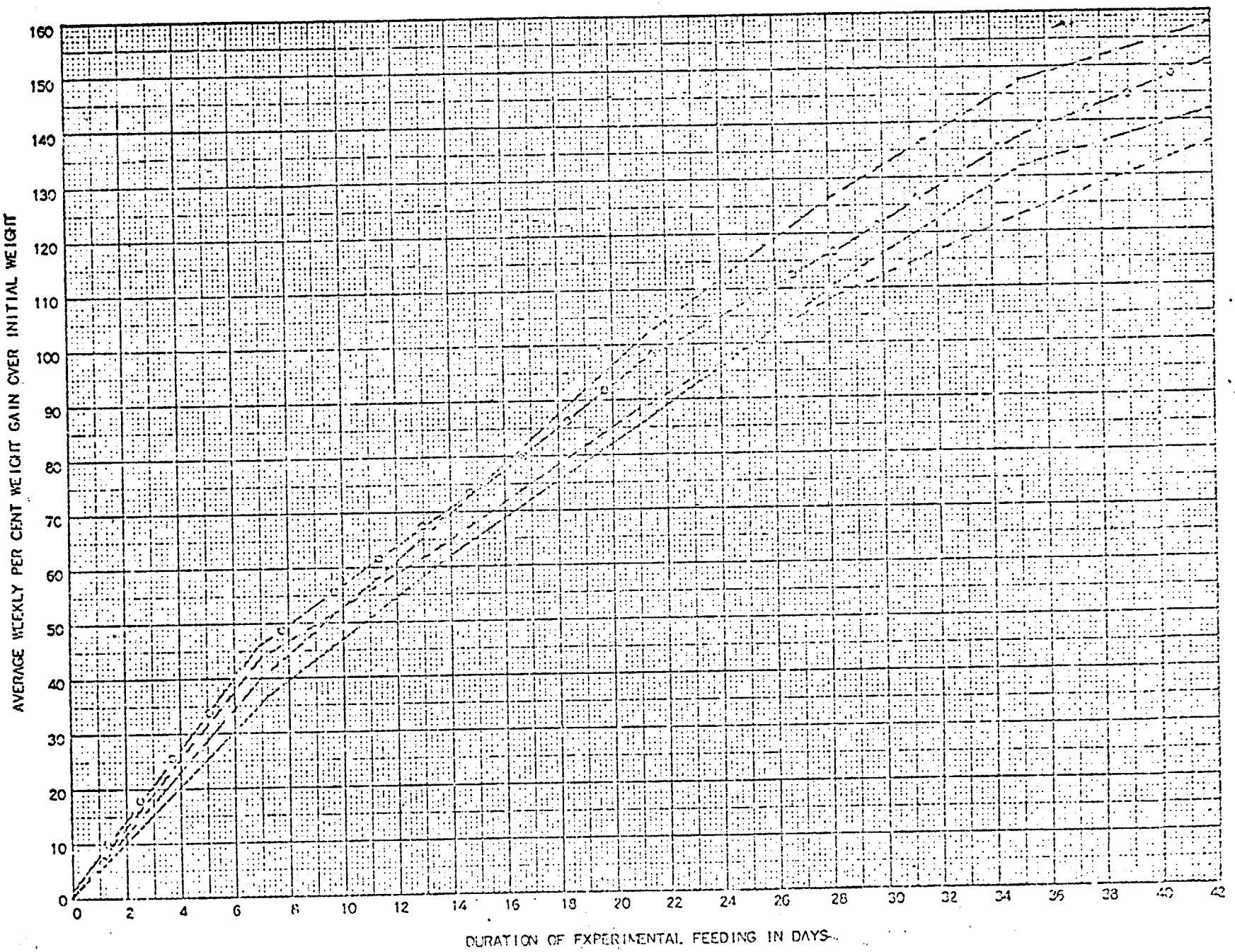
The food consumption was recorded daily by weighing each feeder and subtracting the spillage which was carefully collected on pieces of paper towel of known weight placed in the tray below the feeder for this purpose. Four successive daily weighings were made and the fifth included the weekend consumption so that whenever possible, a seven-day week was accounted for. The average per diem consumption on a week's basis was calculated for each animal as well as the average per diem consumption per group on a weekly basis. The graphic presentation (Figure 4) gives the average food consumption per day for a six-week period for each group.

The rats weighed weekly and the per cent weight gain over the initial weight calculated. An attempt was made to have the initial weight calculated. An attempt was made to have the initial animal weights similar in each group, but in a few instances compromises had to be made. It was interesting to note that the largest animals at zero day were not necessarily the ones to show the most rapid or greatest weight gain. The accompanying chart (Figure 5) shows the average per cent weight gain per week over the initial weight for each group.

The per diem intake of food in grams increases with the growth of the rats. Although the difference between the intake of control food and that which had been supplemented was never great, it was always lower for control food. Similarly, the growth increase was faster for

FIGURE 5

AVERAGE WEEKLY PER CENT WEIGHT GAIN - FEMALE WEANLING RATS



Arthur D. Little, Inc.

rats fed supplemented diets until the 26th day when the weight gain for rats fed a supplement of 0.4 per cent L-monosodium glutamate fell below those rats fed on the control diet or on diets supplemented by 0.4 per cent DL-monosodium glutamate or 0.4 per cent L-glutamic acid, remaining lower until the end of the experiment. The greatest increase in weight was seen in rats fed on control diet plus 0.4 per cent DL-monosodium glutamate which appears noteworthy because of the reported physiological inactivity of racemic isomers. In general, the differences in weight gain are not significant but demonstrate a small but favorable and consistent effect of the three supplements on food intake and weight gain.

Throughout the two-year period, male rats consumed more food than did females. This was uniformly true for control and supplemented diets at both concentrations. In the last few months of breeding when the age was about 2-1/2 years or more, the daily intake dropped for all groups, an occurrence which is customary in all normal aged mammals for the metabolic requirements are decreased markedly in senility. Activity decreased in parallel manner. It appears, further, from a comparison of weight gain and food intake that rats fed on supplemented food gained more in terms of percentage increase over their initial weight in a shorter period than did the control animals. This may have been due to a specific stimulation of appetite by the supplement, to a higher specific dynamic action imparted to the ration by the supplement or to a different level of motor activity than was seen in control rats. It is our belief that a combination of factors operated to increase the daily weight of food consumed, and to stimulate weight gain.

BLOOD EXAMINATIONS

At intervals, hematological examinations were made of random animals from each test group. Both male and female rats were represented. Representative findings are presented in a series of summary tables which are typical of the blood picture after 241, 567 and 681 test days. (a table showing normal values for the laboratory rat is also presented.) (TABLES IX-XV).

Complete blood counts included the erythrocyte and leukocyte count, the hemoglobin concentration expressed in grams per 100 ml. blood, and a differential count of the leukocytes present. The method of counting erythrocytes and leukocytes followed standard procedures; Hayem's diluting fluid was used for red blood cells, and a three per cent aqueous solution of glacial acetic acid was used as the diluting fluid for white blood cells. The hemoglobin determinations were based on the Sahli scale of 17 grams of hemoglobin per 100 ml. of blood. These values were then converted to a scale of 14.6 grams of hemoglobin per 100 ml. of blood which is the normal hemoglobin concentration in rat blood. The differential smear was stained with Wright's stain and the distribution in type of leukocytes expressed in per cent.

The blood examinations showed the deviations from so-called normal blood values to be minimal. There was a slight tendency in all groups to monocytosis and in female rats when examined at 681 test

days a slight eosinophilia was found. An average hemoglobin figure of 12.4 grams per 100 ml. blood was found which is slightly below the normal value cited in the pertinent literature, but is within the range of normal hemoglobin values. A similarly low value for hemoglobin was found in control rats which minimizes the significance of the finding. The increase in monocytic cells, especially when it occurs later in the course of the study, may be related in some manner to the evidences of a low-grade infectious process seen in post-mortem microscopic examination of the viscera. (These cells are importantly concerned with phagocytosis and immunity.)

In general, the blood picture of these rats was not seriously altered at any time from the normal range of values found elsewhere. The deviation from the values for the control group found by us was slight. There appeared to be no evidence of pathological changes in physical condition reflected in the blood.

TABLE IX

AVERAGE BLOOD COUNTS OF FEMALE RATS (355 DAYS OF AGE) AFTER 241 TEST DAYS

Supplement	Erythrocytes (millions/mm^3)	Hemoglobin (grams/100 ml)	Leukocytes (count/mm^3)	Differential Count				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
0.1% L-MSG	9,240,000	11.9	13,050	6	2	1	88	3
0.4% L-MSG	8,590,000	12.2	11,750	16	2	0	76	6
0.1% DL-MSG	8,255,000	12.1	14,030	8	1	0	84	7
0.4% DL-MSG	8,325,000	12.3	15,850	12	0	0	79	9
0.1% L-GA	8,680,000	12.4	14,750	4	0	0	89	7
0.4% L-GA	8,350,000	12.6	12,300	17	0	0	75	8
Control	7,245,000	12.1	12,350	8	0	0	80	12

TABLE X

AVERAGE BLOOD COUNTS OF MALE RATS (339-353 DAYS AGE) AFTER 241 TEST DAYS

Supplement	Erythrocytes (millions/mm^3)	Hemoglobin (grams/100 ml)	Leukocytes (count/mm^3)	Differential Count				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
0.1% L-MSG	9,355,000	12.9	13,150	12	0	0	83	5
0.4% L-MSG	10,955,000	13.3	8,050	20	0	0	79	1
0.1% DL-MSG	8,745,000	12.9	16,075	28	0	0	71	1
0.4% DL-MSG	8,750,000	12.6	14,450	13	1	0	72	9
0.1% L-GA	8,705,000	12.9	13,600	21	0	0	75	4
0.4% L-GA	8,015,000	12.3	13,925	18	0	0	76	6
Control	9,055,000	12.8	12,550	16	0	0	75	9

Arthur D. Little, Inc.

TABLE XI

AVERAGE BLOOD COUNTS OF MALE RATS (373-380 DAYS OF AGE) AFTER 241 TEST DAYS

Supplement	Erythrocytes (millions/mm^3)	Hemoglobin (grams/100 ml)	Leukocytes (count/mm^3)	Differential Count				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
0.1% L-MSG	9,325,000	12.3	11,550	21	1	0	75	3
0.4% L-MSG	8,700,000	11.8	11,575	21	1	1	74	3
0.1% DL-MSG	8,840,000	12.0	10,225	22	1	1	71	5
0.4% DL-MSG	8,905,000	12.2	12,750	9	1	0	84	6
0.1% L-GA	9,400,000	12.8	13,250	11	1	0	84	4
0.4% L-GA	8,360,000	13.1	17,700	15	1	0	77	7
Control	8,845,000	13.4	11,300	8	0	0	84	8

Arthur D. Little, Inc.

TABLE XII

AVERAGE BLOOD COUNTS OF RATS (BOTH SEXES) AFTER 567 TEST DAYS

Supplement	Erythrocytes (millions/mm^3)	Hemoglobin (grams/100 ml)	Leukocytes (count/mm^3)	Differential Count				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
0.1% L-MSG	8,030,000	12.6	7,800	28	1	0	68	3
0.4% L-MSG	10,340,000	12.9	8,940	20	1	0	72	7
0.1% DL-MSG	7,740,000	13.8	11,030	28	1	0	64	7
0.4% DL-MSG	8,800,000	13.1	13,275	20	1	1	72	6
0.1% L-GA	8,490,000	12.8	11,850	24	2	0	69	5
0.4% L-GA	8,405,000	13.0	13,175	19	2	0	75	4
Control	No control values done at this time.							

Arthur D. Little, Inc.

TABLE XIII

AVERAGE BLOOD COUNTS OF FEMALE RATS AFTER 681 TEST DAYS

Supplement	Erythrocytes (millions/mm^3)	Hemoglobin (grams/100 ml)	Leukocytes (count/mm^3)	Differential Count				
				Neutrophils	Eosinophils	Basocytes	Lymphocytes	Monocytes
0.1% L-MSG	6,990,000	11.6	13,225	27	1	1	57	14
0.4% L-MSG	7,610,000	11.7	10,675	32	1	0	63	4
0.1% DL-MSG	9,485,000	12.4	11,800	25	2	1	65	7
0.4% DL-MSG	7,675,000	9.7	22,225	38	1	0	49	12
0.1% L-GA	7,530,000	11.2	24,580	23	4	1	65	7
0.4% L-GA	8,155,000	10.9	13,125	37	1	1	54	7
Control	8,190,000	11.6	11,625	33	1	0	61	5

TABLE XIV

AVERAGE BLOOD COUNTS OF MALE RATS AFTER 681 TEST DAYS

Supplement	Erythrocytes (millions/mm^3)	Hemoglobin (grams/100 ml)	Leukocytes (count/mm^3)	Differential Count				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
0.1% L-MSG	6,865,000	11.6	17,525	36	3	1	55	5
0.4% L-MSG	7,210,000	9.5	25,250	27	1	0	61	11
0.1% DL-MSG	8,945,000	12.2	14,200	19	3	0	70	8
0.4% DL-MSG	9,111,000	12.7	15,550	24	1	0	67	8
0.1% L-GA	9,280,000	12.4	14,725	21	0	1	72	6
0.4% L-GA	8,970,000	11.9	20,175	36	1	0	56	7
Control	9,090,000	11.7	21,750	27	3	1	54	15

TABLE XV

NORMAL RANGE OF BLOOD VALUES IN THE LABORATORY RAT[1, 2]

	Average	Range
Erythrocytes (millions/mm^3 blood)	8,900,000	7,200,000 - 9,600,000
Blood Hemoglobin Concentration (grams per 100 ml.)	14.8	12-17.5
Leukocytes (count/mm^3)	14,000	5,000 - 25,000
Differential Count (distribution per 100 cells)		
Neutrophils	22	9-34
Eosinophils	2.2	0-6
Basophils	0.5	0-1.5
Lymphocytes	7.3	65-84
Monocytes	2.3	0-5

(1) AIR FORCE TECHNICAL REPORT No. 6039, Standard Values in Blood, July 1951.

(2) Gardner, Mary V.; Blood Picture of Normal Laboratory Animals, J. Franklin Inst. 243, 77, 1947.

GENERAL BEHAVIOR

Throughout the course of the investigation, the behavior of all rats was normal and typical for their age and sex. During the day, activity was limited as it tends to be for most laboratory rodents. In the early evening and at night, motor activity increased to a great extent. This was noted for all groups, test and control. As the rats aged, motor activity decreased. No differences in general behavior were seen in rats fed on a diet supplemented by L-glutamic acid although there may have been a tendency for their offspring to mature earlier than did the offspring of rats fed on other supplemented diets.

The rats, when first received, were normally aggressive when handled, but soon became oriented to their surroundings and much gentler when picked up. Except in rats who developed tumors which prevented ambulation because of the location, all rats surviving over 730 days were normal in activity up to the time when sacrificed.

As the experimental work progressed, it appeared that rats fed on glutamate supplemented diets seemed to be in better condition than were the control rats. Their coats seemed sleeker, their general alertness seemed notably greater. As both groups aged and approached senility, the differences grew less. All rats surviving

730 + days appeared to be aged and to have suffered considerable loss of vigor. It would have been difficult at 730 days to have distinguished test and control rats by their external appearance alone.

FERTILITY OF TEST ANIMALS

One of the phenomena which is importantly stressed in long term toxicity studies is the effect of the material under test on fertility. To determine the anatomical changes which may have occurred in the sex organs after prolonged feeding is insufficient; in those animals of our series which survived over two years, there were observed in some rats both gross and microscopic anatomical changes. These were for the most part, changes which may be categorized as "senile", but gave no indication of the integrity of function of the reproductive organs during the phase of the study when the rats were vigorous and mature. An effort was made to learn the effect of the test supplements on reproduction in all groups, including controls, during the first year. Lehman[1] suggested the breeding of subject animals for two generations. In some groups, we were able to continue to the fifth generation. Our original stock is referred to as F_0 and subsequent generations as F_1, F_2, etc. The diet of the young was the same as that fed the parents as soon as they were weaned.

The breeding study was initiated with the original group of animals (F_0) when they had been on the feeding program approximately

---

(1) Lehman, A. J.; Proof of Safety, J. Am. Pharm. Assoc., 40, 305, 1951.

seven months. Four females and one male were selected from each of the six test groups and the control group and caged together. The females were examined periodically until pregnancy was apparent, at which time they were removed to individual whelping cages. For each litter, the following data was recorded: identity of mother, date of weaning, number in litter and distribution by sex. The litter identity could be retraced at any time by a system of notching the ears like that used on the F_0 rats.

The females quickly became pregnant, when opportunity was presented, but many neglected their litters immediately after they were born. In the group fed on a diet supplemented with 0.1 per cent L-monosodium glutamate, one female was replaced because a tumor became apparent during pregnancy. It was estimated that 15 to 20 per cent of all the pups survived. It was difficult to determine the survival rate of rat pups accurately, since some rats after delivery destroyed and ate part or all of their litters, in some cases before they were observed by attendants.

A second and third study was initiated at nine and eleven months, using four different males and females from each group. The same difficulty was encountered in determining the survival rate. It was not surprising that more litters did not survive since the rats were nine months of age when first bred. Although this is not old for breeding, it is an advanced age for delivering a first litter, since fertility is at its maximum when rats are from 100 - 300 days of age.

Two females of the F_1 generation in the 0.1 per cent L-glutamic acid group, and one of the three control females became pregnant before they were separated from their male litter mates. One delivered a litter of eight males and two females at approximately 61 days of age. The second female delivered a litter of 4 males and 6 females at approximately 69 days. One of the three females from the control group delivered a healthy litter of six males at approximately seventy-nine days of age.

The successful delivery of an F_2 litter, albeit unplanned, by females so young in age is not common but has been reported. The age at which fecundation and pregnancy is possible is controversial. In a personal communication by Shay to Griffith and Farris[1] observations on 250 male and 262 female Wistar rats demonstrated the vagina to be patent in about 60 per cent of the females between 35 - 50 days of age (range 15-67 days) and that the testes had descended in 85 per cent between 18-31 days of age, (range 15 - 51 days). Regarding an especially well-fed and tended group of rats, Donaldson[2] cites observations made by Greenman and Duhring[3]

---

[1] Griffith, J. Q. and Farris, E. J.; The Rat in Laboratory Investigation. J. B. Lippincott, 1942, p. 3.

[2] Donaldson, H. H.; The Rat: Data and Reference Tables. Memoirs of the Wistar Institute of Anatomy and Biology, No. 6, Philadelphia, 1924.

[3] Greenman, M. J. and Duhring, F. L.; Breedings and Care of the Albino Rat for Research Purposes. Published by the Wistar Institute of Anatomy and Biology, Philadelphia, 1931. Second edition.

that exceptional females in such a group case their litters at 55 and 57 days of age. These rats therefore became pregnant at 33 and 35 days.

When the F_1 generation of our rats had reached ten months of age, the breeding study was resumed. Since the surviving F_2 generation of controls at that time was all male, a second attempt was made to produce an F_2 control generation. The F_1 and F_2 generations of the 0.1 per cent L-glutamic acid group were also successfully bred. One female of the F_1 generation in the 0.1 per cent DL-monosodium glutamate group became pregnant and was isolated. Before delivery, she died from an unknown cause, leaving no female with which to reproduce this generation. Several females of the F_0 generation (approximately 21 months of age) were caged with males with the hope that one of them might still be fertile, but no pregnancies ensued.

Since the breeding program at its inception was planned solely to observe fertility, its course was interrupted. In the later stages of the breeding program emphasis was concentrated on the dietary groups and generations of particular interest, namely, the 0.1 per cent L-glutamic acid group, the 0.4 per cent L glutamic acid group, and the control group.

Fairly large populations of F_3 and F_4 rats from these three groups were bred. Partial data on the number of rats of each generation in each group are presented on the following pages. (TABLES XVI and XVII).

TABLE XVI

NUMBER AND SIZE OF LITTERS BY GENERATION

Group	Generation	Number Litters	Total Number of Individuals	Average per Litter	Males	Average per Litter	Females	Average per Litter
0.1% L-MSG	F_1	3	13	4.3	10	3.3	3	1.0
	F_2	5	20	4.0	9	1.8	11	2.2
	F_3	23	65	2.8	32	1.4	33	1.4
0.4% L-MSG	F_1	3	13	4.3	10	3.3	3	1.0
	F_2	1	3	3.0	1	1.0	2	2.0
	F_3	3	14	3.6	4	1.3	10	3.3
	F_4	2	12	6.0	4	2.0	8	4.0
0.1% DL-MSG	F_1	2	6	3.0	5	2.5	1	0.5
0.4% DL-MSG	F_1	1	6	6.0	4	4.0	2	2.0
	F_2	4	17	4.2	6	1.5	11	2.9
	F_3	8	50	6.3	28	3.5	22	2.7
	F_4	2	9	4.5	5	2.5	4	2.0

(Continued)

Arthur D. Little, Inc.

TABLE XVI

NUMBER AND SIZE OF LITTERS BY GENERATION (CONTINUED)

Group	Generation	Number Litters	Total Number of Individuals	Average per Litter	Males	Average per Litter	Females	Average per Litter
0.1% L-GA	F_1	2	7	3.5	5	2.5	2	1.0
	F_2	3	34	1.3	19	6.3	15	5.0
	F_3	18	133	7.4	61	3.3	72	4.1
	F_4	11	51	4.6	27	2.6	24	2.0
	F_5	4	24	6.0	11	2.8	13	3.2
0.4% L-GA	F_1	1	8	8.0	5	5.0	3	3.0
	F_2	2	11	5.5	6	3.0	5	2.5
	F_3	6	49	8.1	23	3.9	26	4.3
	F_4	2	10	5.0	5	2.5	5	2.5
Control	F_1	1	7	7.0	3	3.0	4	4.0
	F_2	3	16	5.3	7	2.3	9	3.0
	F_3	10	56	5.6	24	2.4	32	3.2
	F_4	12	48	4.0	25	2.0	23	1.9

TABLE XVII

AVERAGE NUMBER AND SIZE OF LITTERS PRODUCED BY RATS (F_0 - F_4) ON SUPPLEMENTED DIETS

Group	Total Number of Successful Matings Represented	Average Number Litters/Generation	Average Number per Litter	Average per Litter (Males)	Average per Litter (Females)
0.1% L-MSG	31	10.3	2.9	1.6	1.3
0.4% L-MSG	9	10.5	4.7	2.1	2.6
0.1% DL-MSG	2	2	3.0	2.5	0.5
0.4% DL-MSG	15	3.7	5.5	2.9	2.6
0.1% L-GA	38	7.2	6.6	3.5	3.0
0.4% L-GA	11	2.8	7.0	3.4	3.6
Controls	26	6.5	4.7	2.1	2.6

The data is probably of low statistical significance since maximum opportunity for breeding was not presented to all groups. The distribution between male and female offspring is approximately equal, discounting statistical differences resulting from the small number of rat matings represented. There appears to be a trend toward smaller litters in the F_4 generation, but before this point could be established, breeding under more exactly controlled conditions would be necessary.

It is a reasonable conclusion that there was no impairment to fertility in the F_0 generation rats which had been on a supplemented diet for seven to nine months before breeding. The successive generations (F_1 - F_5 bred from the initial group (F_0) and which were also fed the corresponding supplemented diet as the parent stock were capable of producing healthy, normal offspring.

GENERAL PHYSICAL CONDITION

Throughout the first year, the general physical condition of all rats was good. Their growth and development was satisfactory and only a few died from natural causes or were sacrificed because of some specific abnormal condition. At approximately 368 test days, a number of animals had developed tumors and a few had begun to show eye changes involving the cornea. This abnormality was distributed throughout the test and control groups but at 368 days was not seen in the 0.1 per cent L-monosodium glutamate or the 0.1 per cent DL-monosodium glutamate groups. The eyes showed a white film covering the cornea with some enlargement. Both eyes were never involved in one animal. Soon after its first appearance the whiteness disappeared, and in the more seriously affected rats, the eye developed a condition of keratoconus with areas of vascularization. The less seriously involved cases developed corneal opacities. In a few rats, congenital anomalies interfered with normal eye development. Paralyses were seen in a few rats late in the feeding period and could usually be related to tumors involving the legs or adjacent mammae.

Dental anomalies were seen of various types and were often so marked or advanced as to interfere with eating. A common type was that in which one or more teeth in the upper or lower jaw were missing. In some rats the teeth required regular clipping because of

spiral elongation of the incisors due to the failure of attrition. This condition was most common in a group of male control rats. According to Griffith and Farris[1] "when by accident one of the incisors is broken or when malocclusion occurs, the non-attrition of the opposing incisor and its continuous growth results in an elongation or so-called overgrowth. The term 'overgrowth' is misleading. In reality this elongation is not related to the rate of growth of the tooth, but is the result of the lack of wear, just as long hair can be the result not of overgrowth, but of lack of trimming."

As the rats aged, their fur tended to become yellow, but no group was affected in this respect more than another.

In the early days of this investigation, it appeared that animals fed on glutamate-supplemented diets grew more rapidly, were more alert and generally were superior to the control animals. This impression continued until approximately one year after the beginning of our observations. At that time, and subsequently up to the termination of the experimental observations, there appeared to be a lesser difference between test and control animals. At 730 experimental days, all surviving animals, whether test or control were approximately equal in general condition but there remains some reason to believe that glutamate-fortified diets may cause earlier physical and sexual maturation in rats.

---

(1) Griffith, J. Q. and Farris, E. J.; The Rat in Laboratory Investigation. J. B. Lippincott, 1952, p. 114.

SURVIVAL DATA

The rats were counted daily at the time of feeding and cleaning cages. At least twice a week, each rat was carefully examined and his general condition described and recorded in individual histories. Deaths were recorded in terms of elapsed test days and whenever the post-mortem condition permitted, an examination was made. The cannibalistic tendencies of rats limited the observations unless the body was discovered immediately after death. For this reason, rats who were in severe distress or obviously moribund were often sacrificed to permit an autopsy to be made. Thus, the figures on mortality (or survival) are not exact. Such errors as exist are conservative for the survival rate may have been higher and the number of rats sacrificed might have been reduced but the survival rate would not have been lower. An additional reduction in the colony occurred as a result of two series of autopsies in which 71 animals were intentionally sacrificed at 63 or 548 test days. Due allowance is made for the deliverately sacrificed animals in computing percentage of surviving rats.

A series of tables is presented showing survival data and general clinical condition at 182, 368, 548 and 730 test days, (Tables XVIII - XXI).

TABLE XVIII

CLINICAL CONDITION AND SURVIVAL RATES OF RATS FED SUPPLEMENTED DIETS FOR 182 TEST DAYS

Supplement	Number in Original Group	Number Sacrificed	Percentage Natural Deaths	Percentage Survival	Percentage Abnormal Conditions*			
					Tumors	Dental Anomalies	Eye Condition	Miscellane
0.1% L-MSG	75	7	0	100	1.5	0	0	0
0.4% L-MSG	75	7	1.5	98.5	1.5	0	0	1.5
0.4% L-MSG	75	6	0	100	0	0	0	0
0.4% DL-MSG	75	6	1.5	98.5	0	1.5	0	0
0.1% L-GA	75	6	0	100	0	0	1.5	0
0.4% L-GA	75	6	1.5	98.5	0	1.5	0	1.5
Control	150	15	0.7	99.3	0.7	6.7	0	0.7

*Computed with allowance for
a) Animals who died as a result of existing condition.
b) Animals sacrificed because of existing condition.
c) Animals surviving with existing condition.

Arthur D. Little, Inc.

TABLE XIX

CLINICAL CONDITION AND SURVIVAL RATES OF ANIMALS FED SUPPLEMENTED DIETS FOR 368 DAYS

Supplement	Number in Original Group	Number Sacrificed	Percentage Natural Deaths	Percentage Survival	Percentage Abnormal Conditions*				
					Tumors	Dental Anomalies	Eye Conditions	General Debility	Miscel-laneous
0.1% L-MSG	75	9	3.0	97.0	6.0	1.5	0	0	1.5
0.4% L-MSG	75	8	1.5	98.5	2.9	0	2.9	0	0
0.1% DL-MSG	75	6	1.4	98.5	1.4	0	0	0	1.5
0.4% DL-MSG	75	6	4.3	95.7	0	2.9	1.5	0	2.9
0.1% L-GA	75	7	2.9	97.0	1.5	1.5	2.9	0	3.0
0.4% L-GA	75	7	2.9	97.0	2.9	0	2.9	0	2.9
Control	150	16	6.8	93.3	4.5	7.4	2.8	0.7	4.5

*Computed with allowance for
a) Animals who died as a result of existing condition.
b) Animals sacrificed because of existing condition.
c) Animals surviving with existing condition.

Arthur D. Little, Inc.

TABLE XX

CLINICAL CONDITION AND SURVIVAL RATES OF RATS FED ON SUPPLEMENTED DIETS FOR 547 DAYS

Supplement	Number in Original Group	Number Sacrificed	Percentage Natural Deaths	Percentage Survival	Percentage Abnormal Conditions*				
					Tumors	Dental Anomalies	Eye Condition	General Debility	Miscellaneous
0.1% L-MSG	75	13	8.0	92.0	14.5	3.2	1.6	3.2	1.5
0.4% L-MSG	75	10	7.7	92.4	6.1	0	3.8	0	9.2
0.1% DL-MSG	75	6	14.5	85.5	4.3	0	0	0	13.0
0.4% DL-MSG	75	7	11.8	88.4	5.9	4.4	1.4	1.4	8.8
0.1% L-GA	75	9	10.7	89.4	10.6	3.0	4.5	3.0	12.1
0.4% L-GA	75	7	8.8	91.2	5.9	0	1.5	0	4.4
Control	150	18	12.1	87.9	9.0	7.0	3.8	0.8	9.8

*Computed with allowance for a) Animals who died as a result of existing condition.
b) Animals sacrificed because of existing condition.
c) Animals surviving with existing condition.

TABLE XXI

CLINICAL CONDITION AND SURVIVAL RATES OF RATS FED SUPPLEMENTED DIETS FOR 730+ DAYS

Supplement	Number in Original Group	Number Sacrificed	Percentage Natural Deaths	Percentage Survival	Percentage Abnormal Conditions*				
					Tumors	Dental Anomalies	Abnormal Eye Conditions	Paralyses	Miscel-laneous
0.1% L-MSG	75	22	45.3	54.7	54.7	5.6	5.6		28.3
0.4% L-MSG	75	21	37.6	62.4	39.6	1.8	7.5	0	33.9
0.1% DL-MSG	75	13	41.9	58.1	29.0	0	1.6	0	27.4
0.4% DL-MSG	75	17	41.5	58.5	34.4	6.9	6.9		32.7
0.1% L-GA	75	18	42.2	57.8	40.3	3.5	3.5		29.8
0.4% L-GA	75	14	47.5	52.5	42.6	3.2	4.9		36.0
Control	150	32	40.7	59.3	42.4	16.9	7.6	0	21.2

*Computed with allowance for
a) Animals who died as a result of existing condition.
b) Animals sacrificed because of existing condition.
c) Animals surviving with existing condition.

Arthur D. Little, Inc.

At 730 + test days, the animals were aged approximately 2-1/3 years. Donaldson[1] has stated that survival of the albino laboratory rat to three years represents an age equivalent to 90 years in man. On that basis, each of these rats at 730 days was at an age equivalent in human terms to 70 years of age and could be expected to show senile changes. It was a matter of some surprise to us that only a few showed general debility at this time. The survival rate in no group, either test or control was less than 52.5 per cent ranging up to 62.4 per cent (0.4 per cent L-monosodium glutamate). The percentage survival for all test rats was 58.8 per cent. (Sherman has stated that 33-1/3 per cent survival after two years is a very acceptable rate for nutritional studies.)

---

[1] Donaldson, H. H.; The Rat, Memoirs of Wistar Inst. of Anat. and Biol., No. 6, Philadelphia, 1924.

PATHOLOGICAL EXAMINATIONS

It had originally been planned that the colony would be progressively reduced to watch the development of pathological processes, if any. A group of 48 rats, representing 12 from the control group and 6 from each of the test groups was sacrificed after 63 test days by intraperitoneal injection of nembutal. Since neither gross nor microscopic findings showed abnormal changes, the initial plan was modified. Another factor influencing this decision was a conference held with Dr. Arnold J. Lehman and associates of the Division of Pharmacology, U. S. Food and Drug Administration. It was emphasized in discussion that if the colony was progressively reduced, adequate data on survival could not be accumulated. The risk of missing reversible pathological lesions which do not apparently affect the clinical condition of the animals was present but the best evidence of the non-toxic nature of the materials under test are living animals in good clinical condition.*

As the project progressed, each animal was examined regularly, and those with any indication of abnormalities such as tumors, dental anomalies, paralyses, pneumonia, atypical eye conditions, or general debility were sacrificed whenever the condition had progressed to the point where it was obvious that the

---

*Lehman, A. J.; Proof of Safety. J. Amer. Pharm. Assoc. 40:305, 1951.

rat would not survive. As the colony aged, spontaneous deaths due to unknown causes increased. Whenever possible, post-mortem examinations were made, but in animals whose deaths occurred at night or during a weekend period, decomposition had progressed too far before the body could be examined.

After 548 test days, (1 1/2 years), a group of 23 animals was sacrificed and autopsy performed. Gross examination revealed no evidence of toxic effects from the supplements under test. When a two year test period had elapsed the remaining animals were autopsied.

The method of sacrifice was by an intraperitoneal injection of veterinary nembutal. Pieces of tissue were reserved from the lungs, heart, thyroid, trachea, esophagus, stomach, intestine, kidneys, liver, spleen, gonads, and any other tissue warranting special attention. If a tumor presented a heterogeneous structure, several pieces of tissue were taken. The tissues were fixed in a 10 per cent formalin solution in preparation for histological treatment. The body weight, tumor weight, (if present), and principal organ weights were recorded. The organs from over 300 rats were examined.

The gross post-mortem findings are described in Part II in tabular form divided according to supplement and giving a description of each organ showing gross pathology, indicating number

of test days, method of sacrifice, and other pertinent data. Those organs which were normal are not described. The animals whose tissues were completely normal and not remarkable in any respect are also omitted from these tables.

The 48 rats autopsied on the 63rd test day showed no apparent pathology on gross inspection. No evidence of gastrointestinal irritation was seen, and the heart, kidneys (with one exception), lungs, spleen, pancreas and reproductive organs were entirely normal. One animal fed on the 0.4 per cent DL-monosodium glutamate diet showed an abnormality of both kidneys resembling, if not identical with hydronephrosis. The kidneys seemed shrunken and on longitudinal section, the pelves were seen to be dilated, with wasting of the renal tissue. The thinning of the renal substance was more marked in the left kidney than in the right. No gross evidence of obstruction of the ureters was seen. The bladder was normal in appearance, not distended, and there was no apparent blocking of the urethra. Adjacent organs were normal and did not appear to be pressing on the kidneys.

The liver weights were recorded of the first group of animals autopsied and the ratios of liver weight to total body weight were presented in a progress report dated March 27, 1950.

Twenty-three rats were sacrificed in a second autopsy group with three rats from each test group and from the control

group (with one extra rat from the 0.4 per cent monosodium glutamate and 0.4 per cent L-glutamic acid groups). The organs of the extra animals at these two levels were photographed in situ when the abdominal cavity was exposed. The pictures demonstrate the generally good physical and nutritional status of these animals after an 18 month period on supplemented diets. The animals were sacrificed as before with an intraperitoneal injection of nembutal. (Occasionally, other anaesthetics were used in this study.) When the body cavity was opened, all the viscera were of good color and consistency. The final body weight and the principal organ weights were recorded, but on analysis of the data no specific trends could be observed. The gross post-mortem observations were not remarkable except in a few rats which are described in a table which follows. The findings do not suggest toxic effects of the supplements because they are not confined to a single group and are varied in type.

The incidence of gross abnormalities in 284 animals surviving two years or longer was higher in the control group (82.1)* than in all test animals (58.3) or in any test group. The highest incidence in the test group was in the 0.4 per cent L-monosodium glutamate group (75.6) and the lowest in the 0.4 per cent DL-monosodium glutamate group (29.6). The mean incidence was 70.2.

---

*This figure refers to average number of gross lesions per animal x 100.

TABLE XXII

POST-MORTEM EXAMINATIONS

Test Group	Final Examinations*		Earlier Examinations	Total Examinations
	Complete	Partial		
0.1% L-MSG	31	10	22	63
0.4% L-MSG	37	19	19	75
0.1% DL-MSG	41	11	13	65
0.4% DL-MSG	44	8	17	69
0.1% L-GA	36	19	18	73
0.4% L-GA	39	20	14	73
Control	56	14	32	102
TOTAL	284	101	135	520

* At 730 test days or later.

TABLE XXIII

GROSS POST-MORTEM FINDINGS IN RATS SACRIFICED AT 518 TEST DAYS

Identification and Sex	Supplement	Description of Abnormal Organ
8 30 F	0.1 per cent L-monosodium glutamate	Lung: Small cystic area on surface.
23 11 M	0.1 per cent DL-monosodium glutamate	Skin: Small hardened area 1/2" below right axillary margin
28 01 F	0.1 per cent DL-monosodium glutamate	Lung: Very small cystic area on surface.
36 03 M	0.4 per cent DL-monosodium glutamate	Kidney: Small, collagenous. tumor adjacent to left kidney.
50 01 F	0.1 per cent L-glutamic acid	Lung: Two small cystic areas on surface.
61 10 F	Control	Fibrous mass present in right axillary region, firmly adherent to skin but loosely attached to ribs. On section, appeared to be fibrous connective tissue, convoluted but not vascularized.

TABLE XXIV

DISTRIBUTION OF GROSS ABNORMALITIES*

Test Group	Number Animals Represented	Number Showing Pathology	ORGANS INVOLVED															Total
			Lungs	Liver	Kidneys	Spleen	G. I. Tract	Seminal Vesicles	Uterus	Testes	Ovary	Trachea	Preputial Gland	Heart	Adrenal	Bladder		
0.1% L-GA	31	13	8	2	5	2	2	2	-	-	-	-	-	-	-	-		21
0.4% L-GA	37	20	15	2	8	-	-	-	1	1	-	1	-	-	-	-		28
0.1% DL-MSG	41	16	9	5	5	4	-	-	2	-	1	-	1	-	-	-		27
0.4% DL-MSG	44	8	3	-	6	2	-	-	1	-	-	-	-	1	-	-		13
0.1% L-GA	36	14	12	4	3	4	-	-	-	-	-	-	-	-	1	-		24
0.4% L-GA	39	13	9	2	3	1	-	-	2	1	-	-	-	1	-	1		20
Control	56	33	21	8	6	3	-	1	4	1	2	-	-	-	-	-		46
Total	284	117	77	23	36	16	2	3	10	3	3	1	1	2	1	1		179

*Exclusive of tumors and based on post-mortem observation of animals surviving 730+ test days.

Arthur D. Little, Inc.

LOCATION GROSS ABNORMALITIES

Supplement	Number Animals Represented	External Abnormalities		Tumors			Tissues Showing Gross Changes
0.1% L-MSG	18	Anemia	1	16	Lungs	8	Liver
		Paralysis	1		Kidneys	5	G. I. Tract
		Corneal Opacity	1		Spleen	2	Seminal Vesicles
		Dental Anomaly	1				
0.4% L-MSG	25	Corneal Opacity	1	17	Lungs	15	Trachea
		Debility	2		Kidneys	8	Uterus
					Liver	2	Testes
0.1% DL-MSG	20	Corneal Opacity	2	10	Lungs	9	Uterus
		Keratoconus	2		Kidneys	5	Preputial Gland
		Paralysis	2		Liver	5	Ovary
					Spleen	4	
0.4% DL-MSG	16	Keratoconus	1	10	Kidneys	6	Heart
		Paralysis	1		Lungs	3	Uterus
		Corneal Opacity	1		Spleen	2	
		Corneal Vascularity	1				
0.1% L-GA	18	Paralysis	2	16	Lungs	12	Kidneys
		Debility	2		Spleen	4	Adrenal
		Keratoconus	2		Liver	4	
0.4% L-GA	18	Paralysis	1	12	Lungs	9	Testes
					Kidneys	3	Spleen
					Liver	2	Heart
					Uterus	2	Bladder
Controls	40	Anemia	2	26	Lungs	21	Spleen
		Dental Anomaly	1		Liver	8	Ovary
		Paralysis	1		Kidneys	6	Testes
		Corneal Vascularity	1		Uterus	4	Seminal Vesicles

Arthur D. Little, Inc.

OCCURRENCE OF SPONTANEOUS TUMORS

At the end of one year of observation, a number of rats showed swellings which were presumed to be spontaneous tumors. These findings are described in detail in Part II of this report. The incidence of tumors in the various groups at 182, 368, 547 and 730 days is shown in TABLE XXVI. Microscopic sections were made of a representative number of tumor specimens and they were found to be chiefly adenomatous in type and with no exception, benign in type.

The comment of Dr. Lloyd C. Fogg, our consultant in pathology, regarding these tumors (based on microscopic examination) follows:

"None of the external outgrowths suggest any positive etiological relation to the dietary supplements.

"The tumors, both in the test animals and the controls, show a general pattern sharply divided between sexes. The tumors of the females resemble adenomas of sweat glands or of the breast, some of which are actively secreting and some that show extensive fibrosis.

"The outgrowths in the male are associated with skin lesions, some of which are metaplastic, or cystic, or fibrotic.

"A striking observation is the frequency and the uniformity in the type of outgrowth in the female. With no exception, all the outgrowths were probably associated with mammary gland proliferation. It is suggestive that this represents a predilection in the females of the Sprague-Dawley strain for this type of growth."

TABLE XXVI

PRESUMPTIVE PERCENTAGE INCIDENCE OF TUMORS*

Test Days	182	368	547	730
0.1% L-MSG	1.5	6.0	14.5	54.7
0.4% L MSG	1.5	2.9	6.1	39.6
0.1% DL-MSG	0	1.4	4.3	29.0
0.4% DL-MSG	0	0	5.9	34.4
0.1% L-GA	0	1.5	10.6	40.3
0.4% L-GA	0	2.9	5.9	42.6
Control	0.7	4.5	9.0	42.4

*Based on observations during life.

A summary comparison of all groups of rats, i.e., test with control rats, at 730+ days is shown in TABLE XXVII. It can be seen that there is no significant difference in the incidence, severity, extent or character of tumors in all test rats, all control rats and all rats. The incidence of tumors in control rats is 1.7 per cent higher than in all rats surviving for two years.

The abnormalities perceptible on gross inspection were chiefly seen in lungs, liver and kidney, but were not in every case verified as serious pathological changes by microscopic examination (TABLE XXV). In many cases, microscopic examination revealed lesions which were not apparent to gross inspection. However, gross and microscopic examinations confirmed each other on two points:

1) The most numerous abnormalities occurred in lungs, kidneys and spleen in both test and control groups.
2) The abnormalities were those associated with age.

TABLE XXVII

SUMMARY

COMPARISON OF ALL TEST RATS WITH CONTROL RATS AT 730+ TEST DAYS

			Percentage Abnormal Conditions	
	Percentage Survival	Tumors	Dental Anomalies	Miscellaneous*
Average All Test Rats	57.3	40.1	3.5	31.3
Average All Rats	57.6	40.7	5.4	29.8
Control Rats	59.3	42.4	16.9	21.2

* This category includes conditions of such diversity that further classification would require extensive subgrouping. See Part II for tabulation of miscellaneous abnormalities.

MICROSCOPIC EXAMINATIONS

The tissue specimens taken at autopsy were prepared for microscopic examination by standard techniques and stained with hematoxylin and eosin. They were examined and screened by Dr. M. G. Gray and reviewed by Dr. Lloyd C. Fogg, our consultant in pathology. Approximately 1400 sections were prepared from tissue material resulting from 520 post-mortem examinations. Of these 960 sections were reviewed by Dr. Fogg. A description of abnormal sections will be found in Part II. Sections not described in that place can be assumed to have shown no abnormalities or to have been entirely consistent with the age of the animal represented.

In summary, it can be said that the chief microscopic changes were an increase in connective tissue elements and the general shrinkage of organic structures which characterize the elderly animal. No evidence was found that could be directly or indirectly related to toxic activity on the part of the supplements fed. The low-grade inflammatory process seen in lungs and kidneys of control animals and in the kidneys of test animals is very similar to that seen in a group of senile C-57 mice maintained to a comparable age and used in a correlative study. It is probably the result of a low-grade infection but the causative organism is not known to us.

The incidence of benign tumors appears to be related to the highly inbred strain of rats used (Sprague-Dawley) and is discussed in Part II. There was no essential difference in the type of pathological changes seen in control and test rats. The characteristics which were sought for in particular were those of chronic toxic character. Occasionally an acute lesion was found but these were not confined to any group. It is to be expected in a group of the size with which we were concerned that occasional lesions would be found. The variety and random distribution leads us to conclude that they should not be ascribed to the effect of the supplementary compounds.

CONTROL GROUP

There was no obvious pathology in the liver, genital glands, spleen, or heart. The lungs show foci of alveolar fibrosis and pinpoint accumulations of lymphocytes around the bronchi or bronchioles which can be related to a low-grade infection.

The kidneys showed evidence of low-grade, chronic infection expressed by foci of inflammatory reaction such as dilated tubules, concretions and an occasional fibrotic glomerulus.

L-MONOSODIUM GLUTAMATE GROUP

No clear-cut difference from the control group was seen nor did any significant difference appear on later examination of the DL-monosodium glutamate or L-glutamic acid groups. The kidneys

of animals fed at both levels showed a more extensive chronic nephritis but this appearance did not approach acute intensity in any section.

DL-MONOSODIUM GLUTAMATE GROUP

There was no distinctive difference between this group and the controls. There was a suggestion that the low-grade infection noted in the controls showed a marginal intensification in these animals.

L-GLUTAMIC ACID GROUP

There was seen in this group a suggestion of irritation in the gastro-intestinal mucosa. However, the observation was not peculiar to this group but appeared slightly intensified. No correlative changes in accessory organs was seen.

For a detailed description of the microscopic abnormalities, the reader's attention is directed to Part II. For convenience, a summary and comparison of the findings for all groups is presented here. Animals are represented who were sacrificed at 63, 548, 745, 752, 759 and 830 test days; animals showing no lesions are not included. When the lesions were seen, they were of the type described below.

Control Group

Liver: Essentially normal. Only one case of focal hepatitis was noted in an aged animal.

Kidney: Foci of cortical and medullary inflammation which are highly variable in intensity from small infrequent foci of congestion to numerous dilated tubules with concretions and fibrotic glomeruli. The inflammation was universal in the older animals.

Lungs: In the 14 animals studied, all sections showed accumulations of lymphocytes around bronchioles with some fibrosis.

Spleen: In the younger animals, the tissue was normal. Characteristics of age such as mild congestion and disorganized red and white pump appeared in the older groups.

Heart: Normal. No pathology.

Intestine: Of four animals, 745 and 752 days old, two had an abscess, one showed worms in the lumen, and one, corroded epithelium. Other sections normal.

Genital Glands: Normal.

	L-Monosodium Glutamate Group	
	0.1 per cent	0.4 per cent
Liver:	Of 18 rats, 15 were normal. Three had traces of inflammation as shown by pinpoint foci of lymphocytic infiltration around blood vessels.	Three rats showed mild lymphocytic infiltration as in 0.1 per cent group.
Lungs:	Foci of fibrosis and areas of inflammation around bronchi or bronchioles, varying from a trace to extensive involvement. There were two lung abscesses.	Mild chronic irritation. Two with marked congestion and edema. One with possibility of lobar pneumonia.
Kidney:	In general, there were foci of inflammation around blood vessels, sometimes only in cortex but occasionally extending to renal pelvis. In extreme cases, expressed as dilated tubules with concretions. Diagnosed as mild, chronic interstitial nephritis.	Similar to appearance seen in 0.1 per cent group but not more severe.
Intestines:	Chronic irritation as evidenced by presence of sterile abscesses, eroded epithelium or polyposis.	No abscesses seen but otherwise as seen in 0.1 per cent group.
Ovary:	Several corpora lutea seen but no obvious pathology. No primary follicles. Senile ovaries.	No pathology seen.
Trachea and Esophagus:	No pathology.	One section showed disorganized epithelium.
Spleen:	In older animals, there was some congestion and disorganization, typical for senility. No obvious pathology.	As seen in 0.1 per cent group.
Heart:	No pathology.	No pathology.

Genital Glands:	No pathology.	No pathology.
Aorta:	One section sclerotic.	
Salivary Glands:	No pathology.	No pathology.
Testis:	No pathology.	Practically depopulated of germinal cells; senile. No obvious pathology.

DL-Monosodium Glutamate Group

	0.1 per cent	0.4 per cent
Liver:	In 2 rats (777 and 779 test days old) there was evidence of focal lobular necrosis and suggestion of portal cirrhosis.	One section showed hypertrophied epithelium in gall bladder. Not diagnosed as malignant, but probably indicates irritation. All other sections normal.
Kidney:	Changes varying from normal to evidence of mold chronic interstitial nephritis.	Appearance as in 0.1 per cent group but somewhat intensified.
Lung:	Changes varied from normal to foci of fibrosis with pinpoints of inflammatory areas around bronchi and bronchioles. 2 cases showed edema and minute hemorrhagic areas in aged animals.	Same appearance as in 0.1 per cent group.
Spleen:	In young animals, no change from normal. In older animals, senile appearance as shown by disorganization and mild congestion.	Same appearance as in 0.1 per cent group.
Heart:	No pathology.	No pathology.
Trachea and Esophagus:	2 sections show mild, chronic irritation in glandular epithelium, non-malignant.	No section showed disorganization of tracheal epithelium but could be an artefact.
Adrenal:	No obvious pathology.	
Ovary:	Senile appearance but no obvious pathology.	
Intestine:	In 1 case (779 test days), chronic enteritis. No evidence of malignancy.	One section shows extensive papillary-type folds in epithelial layer extending into lumen suggesting partial obstruction. No evidence of malignancy.

L-Glutamic Acid Group

	0.1 per cent	0.4 per cent
Liver:	Essentially normal in all animals represented.	Two animals over 700 days old showed foci of necrotic tubules.
Kidney:	Mild chronic, interstitial nephritis evidenced by foci of dilated tubules with concretions, thickened vascular walls and areas of lymphocytic infiltration.	Same as 0.1 per cent animals.
Lungs:	Varying degrees of inflammation around bronchioles and fibrosis. No obvious edema or congestion.	Same as 0.1 per cent animals.
Spleen:	All sacrificed at 63 and 548 days were normal. Those at 688 days or later showed disorganization characteristic of senility. No obvious pathology.	Same as 0.1 per cent animals.
Intestine:	Alterations in epithelium in animals sacrificed at 740 test days or later ranging from sterile abscesses to fibrotic villi with eroded mucosae.	Same as 0.1 per cent animals.
Esophagus and Trachea:	No pathology.	Same as 0.1 per cent animals.
Testis:	Typical of senility; sparsely populated with germ cells. No obvious pathology.	
Heart:	No pathology.	No pathology.
Ovary:	Normal except for involutional changes.	No pathology.
Stomach:	No pathology.	No pathology.
Adrenal:	One section somewhat disorganized but no obvious pathology seen.	No pathology.

	0.1 per cent	0.4 per cent
Genital Glands:	No pathology.	No pathology.
Skin:	No sections.	Keratinous cyst, n malignant, in one r

TABLE XXVIII

DISTRIBUTION OF MICROSCOPIC ABNORMALITIES EXCLUSIVE OF TUMORS

Test Group	Number Animals Represented	Number Sections Examined	Lungs	Kidneys	Spleen	G. I. Tract	Liver	Trachea	Testes	Ovary	Aorta	Esophagus	Bronchus	Adrenal	Total Number Abnormalities
0.1% L-MSG	63	154	18	13	12	3	4	-	-	-	1	-	-	-	52
0.4% L-MSG	75	162	9	11	8	6	2	1	1	1	-	-	-	-	38
0.1% DL-MSG	65	190	7	7	7	1	3	1	-	1	-	1	-	-	28
0.4% DL-MSG	69	173	10	13	9	1	4	1	-	-	-	-	1	-	39
0.1% L-GA	73	236	10	7	8	8	0	-	1	-	-	-	-	1	35
0.4% L-GA	73	163	14	9	12	6	3	-	1	-	-	-	-	-	45
Average for Test Animals	- -	- -	11.3	13.3	9.7	4.1	2.6	*	*	*	*	*	*	*	39.5
Controls	102	305	14	10	5	4	1	1	-	-	-	-	-	-	35
Total	520	1383	82	70	63	29	17	4	3	1	1	1	1	1	272

*Less than 1.0

Arthur D. Little, Inc.

TABLE XXIX

STATISTICAL DISTRIBUTION OF ABNORMALITIES

	Gross*	Microscopic**
Incidence (Average number lesions per animal x 100)		
Controls	82.1	34.3
All Animals	63.0	52.3
All Test Animals	58.3	56.6
0.1% L-MSG	67.7	82.5
0.4% L-MSG	75.6	50.7
0.1% DL-MSG	65.8	43.0
0.4% DL-MSG	29.6	56.5
0.1% L-GA	66.6	47.9
0.4% L-GA	51.4	61.8
Mean Incidence	70.2	45.4
Deviation from Mean Incidence		
All Animals	-7.2	+6.9
All Test Animals	-11.9	+11.2
All Controls	+11.9	-11.2

*284 animals surviving 730+ test days

**1383 sections from 520 rats coming to autopsy

An examination of Table XXIX shows a statistical comparison of the incidence of gross and microscopic abnormalities. We do not believe that the volume of data available is adequate for further statistical analysis but the trends are worthy of comment. It is noteworthy that the data for gross and microscopic abnormalities are derived from groups of animals of different sizes. The criteria for indicating severity of abnormality was the same (so far as possible) for both observations, and was based on a scale ranging from 0-4. Thus

0 = Essentially negative, no pathology
1 = Trace
2 = Apparent
3 = Obvious
4 = Severe

Only changes rated two or higher are included in the statistical analysis. However, many gross abnormalities consisted solely of departures from normal color and consistency, while the microscopic examinations represent only verifiable anatomical alterations.

Although the incidence of microscopic abnormalities is higher in test animals than in controls, the data presented above regarding percentage survival in control and test animals suggests that for all animals represented, the microscopic lesions seen among test animals were not of such severity that they contributed significantly to mortality. In all categories represented in TABLE XXVIII, the abnormalities represented are, with few exceptions, consistent with senility. The figures for incidence of microscopic abnormalities do include tissues from rats dying earlier in the experiment, while the figures for gross abnormalities include only those surviving 730 days or longer.

GLUTAMIC ACID CONTENT OF BLOOD AND ITS RELATIONSHIP TO TUMORS

The claims[1] of relationship between the glutamic acid level of blood and the presence of malignant tumors led us to have analyses made of rat blood from animals fed on diets containing added 0.4 per cent L-monosodium glutamate, 0.1 and 0.4 per cent L-glutamic acid and from others fed on the control diet. Rats with obvious tumors and others without tumors were selected, the latter to be used as a secondary control. Blood samples were taken between 754 and 759 days by decapitation.

The blood was collected in beakers which had been coated with 0.1 ml. of a dilute solution of Heparin. The beakers were rotated while drying over an electric plate to spread the Heparin uniformly. Since the female rats had the largest number of tumors, male rats with tumors were selected whenever possible to make as even a division by sex as possible. An autopsy was then performed on those animals sacrificed for blood and observations made of the gross appearance of organs and tumors. Pieces of tissue were saved from each organ and tumor for histological preparation.

The quantity of blood plasma required was 10 ml. per test, but this provided two 5 ml. samples for duplicate determinations. Approximately 6-8 ml. could be obtained from the female rat and 10-15 ml. from the male rat.

---

(1) Beaton, J. R., McGanity, W. J., and McHenry, E. W.; Plasma Glutamic Acid Levels in Malignancy. Canad. M. Ass. J. 65, 219 1951.

Arrangements were made for Dr. Samuel P. Bessman of the Children's Hospital Research Foundation of Washington, D. C., to make determinations of glutamic acid content on plasma filtrates sent him by us. Dr. Bernard Gould, of the Massachusetts Institute of Technology prepared the filtrates. Each filtrate represented a pooling of the blood of four animals from each group according to Dr. Bessman's directions.

The general procedure for preparing filtrates required the centrifugation of heparinized blood to separate the red cells from the plasma. A protein-free filtrate was then prepared by treating 2 ml. plasma with 1 ml. of fresh, cold 15 per cent trichloracetic acid. The precipitated protein was then separated by centrifugation as rapidly as possible. The supernatant fluid was poured off and frozen as quickly as possible and stored in a "deep-freeze" cabinet. The prepared filtrates were sealed in glass vessels and shipped to Dr. Bessman by Air Express.

The glutamic acid content of the blood filtrates as determined by Dr. Bessman is presented on the following page, (TABLE XXX).

TABLE XXX

GLUTAMIC ACID CONTENT OF BLOOD FILTRATES*

	Animals without Tumors		Animals with Tumors	
	mg % Glutamic Acid	mg % Glutamine	mg % Glutamic Acid	mg % Glutamine
Control	2.75	10.38	2.62	9.75
	2.42	9.06	2.42	9.06
	3.25	10.25	2.91	10.69
0.1% L-GA	2.32	10.39	2.12	9.02
	3.32	10.35	2.66	11.01
0.4% L-GA	2.24	11.20	2.18	8.62
	2.40	9.55	1.52	11.98
0.4% L-MSG	2.12	10.35	3.11	9.68
	2.58	10.92	2.59	10.58

*Each value represents a filtrate prepared by pooling the blood samples of four rats.

From the values obtained there is no difference in glutamic acid content of the blood between animals in this series with tumor development and those without. There are no significant differences among the three dietary groups or in comparison with the control group. Later microscopic examination showed all the tumor specimens to have been benign.

Because of the non-malignant character of the tumors represented the preceding data neither substantiates nor disproves the contention of McHenry and his associates that the plasma glutamic acid content is elevated in animals which are hosts to malignant tumors.

DISCUSSION

For adequate evaluation, any toxicological investigation must be considered in light of the use which will be made of the test materials or the conditions under which human subjects will be exposed. To say categorically that a substance is or is not toxic would savor of the dogmatic. All chemical substances have a toxic level, although for one reason or another, (such as low solubility and other physico-chemical characteristics) it may be impossible to expose a human to that concentration. We might expect that a large proportion of the subject animals would develop pathological lesions or show metabolic, cardiac, renal or hepatic insufficiency if under the experimental conditions obtaining, the test material exhibited chronic toxic activity. We would further expect that under the stress of special situations such as pregnancy, lactation, fatigue or tumor formation that the signs of chronic toxicity would become increasingly manifest, if potentially present. Ultimately, we might expect that an effect would be exerted on survival rates in comparison to those of control animals.

The development of abnormal changes resulting from the chronic toxic effects of chemical substances under investigation would vary in severity and in the time required for their maximum development. Regression in a pathological process might occur and if so, a lesion might not come to observation. If this were

true, the lesion would in all probability be so minor in significance that its total effect on the living subject animal would be negligible. We might also expect to see some effect on the fertility of test animals and their ability to beget, conceive, deliver and rear another generation. Nutritional deficiencies might be observed due to primary toxic interference with absorption or metabolism or to a secondary effect on an organ concerned with metabolism, storage or excretion.

None of these changes were consistently seen in test animals of any group or in control animals. Those pathological changes observed in tissues and organs were of the type commonly associated with the natural operation of the aging process. In general, all our test animals coming to autopsy were found to have muscles of good consistency, size and firmness and in all but a few animals the distribution of fat was normal for their age. Hemorrhage from body orifices was not seen. Fever was observed only in a few animals moribund from traumatic wounds inflicted by their cage mates. Commonly, animals (and humans) bearing tumors show febrile reactions to proteins liberated from tumor tissue and absorbed into the blood stream. Such proteins may also affect the red blood cells and blood forming organs. Changes of this type were entirely lacking in both control and test animals. The mild inflammatory changes observed at autopsy were distributed through test and control groups and are considered to be a senile phenomenon. Senility can be

expressed in several other forms in laboratory animals. The microscopic changes which are commonly seen in the elderly rat are chiefly in the sex organs. The senile ovary fails to show any primary follicles and the sterile testis becomes depopulated of germ cells. The lung of the aged animal shows fibrosis and focal edema with thickening of the connective tissue walls. The kidneys show mild but chronic inflammation which may proceed to a frank glomerulonephritis with varying degrees of fibrosis while the senile spleen shows diffuse disorganization of the red and white pulp. The gastro-intestinal tract is not regularly affected in the aging process although there is a loss of muscle tone without necessarily accompanying micro-anatomical changes.

The dental anomalies and corneal changes upon which comment has been made are not apparently related to diet. Overgrowth of teeth in rats has been reported by other investigators and a number of publications have appeared dealing with eye changes in the inbred albino rat. The incidence of tumors and survival rate is approximately the same for control rats, all test rats and all rats which suggests that no lethal or toxic factors acted upon one group which did not act to the same extent on the others.

CONCLUSION

In consideration of the observations made during life and the post-mortem findings both gross and microscopic, and with due evaluation of the type of abnormalities and their distribution between test and control groups, it is our conclusion that no evidence of chronic toxicity was seen in this investigation to be exerted by L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid.

SUMMARY

An investigation of the acute and chronic toxicity of L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid is reported. The study was carried out over two years during which albino rats (Sprague-Dawley strain) were fed on a standard diet appropriate to their nutritional needs to which had been added the test materials to the extent of 0.1 or 0.4 per cent. A control group of animals was maintained on the standard diet without supplement under identical conditions. The age of rats at the beginning of the feeding period was approximately three months, and the division by sex was approximately equal. Each test group for each supplemented diet was comprised of 75 rats and the control group numbered 150, a total of 600 rats.

The single lethal dose (LD_{50}) was determined for these compounds and found in rats to be: for L-monosodium glutamate, 19.9 grams per kilogram; for DL-monosodium glutamate, 10.3 grams per kilogram; and for L-glutamic acid, more than 30 grams per kilogram. The LD_{50} was determined also in rabbits for L-glutamic acid and found to be more than 23 grams per kilogram.

Assays were made of the standard ration for vitamin content and found to be adequate. Although the content of the supplements was not exact, it was found on assay to be slightly higher than intended so that all animals received more than the

minimal amount. Observations were made of general physical condition, behavior, activity and fertility of all test animals during the feeding period of two years and little difference was seen between the control and test groups. The per cent of survivors among test rats, control rats and all rats was found to vary not more than ± 1.0 per cent from a median value of 58.3 per cent at 730 test days. For animals of this age (approximately 2-1/3 years) this survival rate is about 25 per cent higher than the expected survival rate, according to Sherman.

A number of spontaneous tumors, all of them benign, developed in both test and control rats. The incidence was approximately the same for all test rats, all control rats and all rats; the variation from the median incidence of 41.1 per cent was not greater than ± 1.3 per cent for test rats, control rats or all rats. We believe that the high incidence of tumors in the strain of rats used is due to close inbreeding.

Pathological examinations, either complete or partial by gross and microscopic inspection were made of 520 rats. The abnormalities seen varied from test group to test group and from control to test group. In general, the type of lesions seen were consistent with the age of the rat at time of death and in most rats were associated with the aging process. The most common type of lesion in rats surviving to 730 days or longer was a low-grade inflammatory

process involving lungs, kidneys, spleen and occasionally other organs. Other abnormalities varied so greatly in type or were so random in their distribution in both test and control groups that they do not appear to be associated with chronic toxic characteristics of any of the supplements fed.

On the basis of our observations made in living animals and at post-mortem examination, it is our conclusion that we have seen no evidence of chronic toxicity to be exerted by L-monosodium glutamate, DL-monosodium glutamate or L-glutamic acid.

REPORT ON AN INVESTIGATION OF THE TOXICITY OF L-MONOSODIUM GLUTAMATE, DL-MONOSODIUM GLUTAMATE AND L-GLUTAMIC ACID

TO

INTERNATIONAL MINERALS AND CHEMICAL CORPORATION

Part II

ARTHUR D. LITTLE, INC.
CAMBRIDGE 42, MASSACHUSETTS
JULY 30, 1952
ISSUED MARCH 15, 1953
C-58047

TABLE OF CONTENTS

	Page
OCCURRENCE OF SPONTANEOUS TUMORS	2
SURGICAL REMOVAL OF TUMORS	16
APPENDIX	19
SECTION 1 - GROSS PATHOLOGICAL FINDINGS IN RATS FED ON SUPPLEMENTED AND A CONTROL DIET	20
SECTION 2 - DESCRIPTION OF MICROSCOPIC PATHOLOGY	64
SECTION 3 - DESCRIPTION OF TUMORS	80
SECTION 4 - CLASSIFICATION OF TUMORS BY MICROSCOPIC EXAMINATION	103
SECTION 5 - PHOTOGRAPHS	110

LIST OF TABLES

TABLE NO.		PAGE
I	INCIDENCE OF TUMORS	5
II	PRESUMPTIVE PERCENTAGE INCIDENCE OF TUMORS	6
III	DATA ON TUMORS (SUMMARY)	7
IV	CLASSIFICATION OF TUMORS	9
V	RATIO OF TOTAL BODY WEIGHT TO TUMOR WEIGHTS OF MALE AND FEMALE RATS	10
VI	INCIDENCE TUMORS BY SEX	11
VII	TUMOR CHARACTERISTICS	12
VIII	EFFECT OF SURGICAL REMOVAL OR TRANSPLANTATION OF TUMORS	18

For purposes of reference, detailed data concerned with gross and microscopic appearance of rat tissues are presented in Part II, together with data on spontaneous tumors. Although of interest to a limited group of readers, the findings are chiefly important as matters of record for it is largely upon post-mortem findings in laboratory animals that the essential conclusions are based. In effect, the material in Part II of this report is an appendix.

OCCURRENCE OF SPONTANEOUS TUMORS

At the end of one year of observation, a number of rats showed swellings which were presumed to be spontaneous tumors. Previous to 368 test days, tumors had been seen in one control rat and in one rat from each of the groups fed on L-monosodium glutamate. At 368 test days, the number had increased so that rats from all groups except that fed on 0.4 per cent DL-monosodium glutamate showed tumor growth. The percentage incidence at that time in the 0.1 per cent L-monosodium glutamate group was 6.0 per cent and in the control group, was 4.5 per cent. The incidence for all rats at this time was 2.7 per cent, and for all test rats was 2.5 per cent which is significantly lower than for the control group.

At one and a half years (547 test days) the percentage incidence ranged from 4.3 per cent in the group fed a supplement of 0.1 per cent DL-monosodium glutamate to 14.5 per cent for the group fed a supplement of 0.1 per cent L-monosodium glutamate. Making a similar comparison to that above, the percentage incidence for all test rats was 7.9 per cent, for all rats was 8.0 per cent and for controls was 9.0 per cent.

Between one and a half and two years, the incidence of presumptive tumors increased very greatly. At 730+ days, the incidence varied from 29.0 per cent in the group fed a supplement of 0.1 per cent DL-monosodium glutamate to 54.7 per cent in the 0.1

per cent L-monosodium glutamate group. However, rats fed on a supplement of 0.4 per cent L-monosodium glutamate showed an incidence of 39.6 per cent which was next to the lowest for all groups. At two years, (730 + days) the average percentage incidence for all rats was 40.7 per cent, for all test rats 40.1 per cent and for control rats was 42.4 per cent. From these data it appears that although the rate of development of tumors varies between test and control rats, the presumptive* incidence at two years is approximately the same. The male rats were more resistant to spontaneous tumor formation than females, but this is not peculiar to the Sprague-Dawley strain.

The incidence among female rats was approximately three and a half times that seen in male rats. One of four female rats with tumor showed multiple tumor sites. Since these were chiefly mammary adenomas, it is probable that they did not represent true metastases but were continuous along the course of the interconnecting mammary tissue. Ten times as many tumors in female rats were found on the ventral surface as on the dorsal surface or on mixed body surfaces. In male rats, only one showed multiple

---

*The term "presumptive" is used in discussing tumor growth when the data is based on observations made during life. At autopsy, a number of suspected tumors were found to be swellings, cysts, etc., but not verified tumors. Thus, the verified incidence of tumors is slightly lower than the presumptive incidence.

tumors. The tumor sites among male rats were equally divided between the dorsal and ventral surfaces. The comparative absence of internal tumors in both sexes is noteworthy.

The typical tumor in male rats appeared on either dorsal or lateral body surfaces and was horny or wart-like. It was invariably external to the body wall and on section often contained a granular material or milky secretion which may have represented cystic regression.

Of 90 tumors carefully examined microscopically, more than one-half were purely adenomatous in type. The next highest category was that of fibrosarcoma represented by 11 tumors. Mixed types of tumors were seen in 10 specimens. The origin was not conclusively established for 46 tumors but because of the sites involved, they probably originated in mammary tissue or in connective tissue adjacent to mammary tissue. It was possible to verify mammary origin in 33 tumors.

The appearance of tumors is described in tabular form below. In referring to mammary tumors, the mammae are designated as pectoral, abdominal or inguinal according to the nomenclature used by Greene.[1]

---

(1) Greene, E. C.; Anatomy of the Rat. Trans. Am. Phil. Soc., 27, Philadelphia, 1935.

TABLE I

INCIDENCE OF TUMORS*

Test Group	Original Number of Rats	Number with Tumors	Percentage Incidence
0.1% L-MSG	75	41	54.7
0.4% L-MSG	75	30	39.6
0.1% DL-MSG	75	22	29.0
0.4% DL-MSG	75	26	34.4
0.1% L-GA	75	31	40.3
0.4% L-GA	75	32	42.6
Control	150	64	42.4
Total	600	246	41.0

Average percentage incidence:

All test rats	40.1
All rats	40.7
Control rats	42.4

* Based on observations during life up to 730 test days.

TABLE II

PRESUMPTIVE PERCENTAGE INCIDENCE OF TUMORS*

Test Days	182	368	547	730
0.1% L-MSG	1.5	6.0	14.5	54.7
0.4% L-MSG	1.5	2.9	6.1	39.6
0.1% DL-MSG	0	1.4	4.3	29.0
0.4% DL-MSG	0	0	5.9	34.4
0.1% L-GA	0	1.5	10.6	40.3
0.4% L-GA	0	2.9	5.9	42.6
Control	0.7	4.5	9.0	42.4

* Based on observations during life.

TABLE III

DATA ON TUMORS* (SUMMARY)

Test Group	Number of Rats with Tumors			Number of Rats with Multiple Tumors			Location on Body Surface					
							Dorsal		Ventral		Mixed	
	M	F	Total	M	F	Total	M	F	M	F	M	F
0.1% L-MSG	3	12	15	0	4	4	1	1	1	11	1	0
0.4% L-MSG	3	12	15	0	3	3	1	0	2	11	0	1
0.1% DL-MSG	4	7	11	0	3	3	0	0	3	7	1	0
0.4% DL-MSG	2	6	8	0	3	3	0	0	0	6	2	0
0.1% L-GA	4	11	15	0	1	1	4	2	0	9	0	0
0.4% L-GA	1	12	13	0	1	1	0	0	0	11	1	1
Control	5	17	22	1	4	5	2	1	2	15	1	1
Total	22	77	99	1	19	20	8	4	8	70	6	3

* Based on gross autopsy findings in animals surviving 730 + test days.

Arthur D. Little, Inc.

All tumors were benign (by microscopic examination), most were encapsulated and external to the body wall. The consistency was usually fibrous and firm. A few had ulcerated and become cystic and some showed regression. A table is presented showing the relation of tumor weight to total body weight:

$$\text{i.e.} \quad \frac{\text{body weight}}{\text{tumor weight}}.$$

Thus, the smaller the ratio, the larger was the tumor. This data shows little more than trends and is not considered to be significant or to warrant firm conclusions since the size of the tumor was a function of its age which in turn is related to whether the animal was sacrificed soon after the tumor became apparent or whether it was allowed to live. In only a few cases did the size or condition of the tumor require the sacrifice of the animal. Determination of the functional body weight was rendered difficult in rats bearing large tumors. No marked losses of body tissue were seen except in animals with tumors which had grown to large size. This may have been due to the utilization of food by the tumor substance resulting in deprivation of normal tissues and subsequent shrinkage. (Emaciation is a concomitant of tumor growth, when uninterrupted and in general, the effect is greater at first on muscular than on predominantly fatty structures.)

TABLE IV

CLASSIFICATION OF TUMORS

Test Group	TYPE										ORIGIN						
	Adenoma	Fibrosarcoma	Mixed	Fibroadenoma	Fibroma	Carcinoma	Adenocarcinoma	Sarcoma	Unclassifiable	TOTAL	Unknown	Mammary	Epithelial	Sweat Gland	Uterine	Epidermoid	TOTAL
0.1% L-MSG	5	3	3	-	-	-	-	-	-	11	8	3	-	-	-	-	11
0.4% L-MSG	10	2	1	2	1	-	-	-	-	16	6	6	2	1	1	-	16
0.1% DL-MSG	3	-	1	1	2	-	2	1	-	10	6	4	-	-	-	-	10
0.4% DL-MSG	5	-	1	-	1	1	-	-	-	8	5	3	-	-	-	-	8
0.1% L-GA	5	3	3	-	-	-	-	-	-	11	8	3	-	-	-	-	11
0.4% L-GA	10	2	1	2	1	-	-	-	2	18	7	6	2	2	1	-	18
Control	11	1	-	1	-	2	-	1	-	16	6	8	-	-	-	-	16
Total	49	11	10	6	5	3	2	2	2	90	46	33	4	3	2	2	90

Arthur D. Little, Inc.

TABLE V

RATIO OF TOTAL BODY WEIGHT TO TUMOR WEIGHTS OF MALE AND FEMALE RATS

0.1% L-MSG		0.4% L-MSG		0.1% DL-MSG		0.4% DL-MSG		0.1% L-GA		0.4% L-GA		Control	
M	F	M	F	M	F	M	F	M	F	M	F	M	F
4.6	4.4	3.5	2.2	2.1	2.2	8.2	1.8	2.3	2.2	118.7	2.3	68.9	1.6
19.6	1.6	75.7	2.8	20.0	4.3		2.0	2.3	3.1		21.0	172.6	1.3
2.6	3.0		3.4	3.2	5.4		2.7	118,7	9.6		3.2	8.7	2.7
	2.5		6.5		40.9		4.0		11.8		5.3	483.0	4.6
	5.9		4.9		3.2		3.7		11.0		9.8		47.4
	30.3		3.4		3.3		1.9		170.1		9.4		12.8
	4.9		4.2		2.2		139.3		4.0		2.4		9.4
	13.0		15.3				1.9		3.2				2.2
	9.2		3.9										6.8
	4.4		1.9										8.9
	2.9												3.2
verage: 8.9	7.5	39.6	4.85	8.4	8.8	8.2	19.7	41.1	26.8	118.7	7.6	183.3	9.2

Arthur D. Little, Inc.

TABLE VI

INCIDENCE TUMORS BY SEX*

Supplement	Number Animals Represented	Number with Tumors			Percentage Incidence		
		M	F	Total	M	F	Total
0.1% L-MSG	3.1	3	12	15	9.7	38.7	48.4
0.4% L-MSG	37	3	12	15	8.1	32.4	40.5
0.1% DL-MSG	41	4	7	11	9.8	17.1	26.9
0.4% DL-MSG	44	2	6	8	4.5	13.5	18.0
0.1% L-GA	36	4	11	15	11.1	29.2	40.3
0.4% L-GA	39	1	12	13	2.6	30.7	33.3
Control	56	5	17	22	8.8	30.4	39.2
Total	284	22	77	99	7.7	27.7	35.4

* Based on gross autopsy findings in animals surviving 730 + test days.

TABLE VII

TUMOR CHARACTERISTICS*
(Gross Observations)

Location According to Body Quadrant

Test Group	Right Anterior		Right Posterior		Left Anterior		Left Posterior		More Than One Quadrant	
	M	F	M	F	M	F	M	F	M	F
0.1% L-MSG	0	4	1	2	2	4	0	1	0	1
0.4% L-MSG	1	3	0	2		2	0	3	2	2
0.1% DL-MSG	3	0	1	1	0	0	0	1	0	5
0.4% DL-MSG	0	0	0	1	0	0	0	1	2	4
0.1% L-GA	0	1	0	3	2	2	1	4	1	1
0.4% L-GA	1	1	0	1	0	4	0	2	0	4
Controls	4	3	0	2	2	2	0	4	0	5
Total	9	12	2	12	6	14	1	16	5	22

* Based on gross autopsy findings in animals surviving 730 + test days.

Test Group	External to Body Wall		Encapsulated		Consistency: Firm		Consistency: Soft		Consistency: Mixed or Unspecified	
	M	F	M	F	M	F	M	F	M	F
0.1% L-MSG	2	10	2	10	2	9	1	2	0	2
0.4% L-MSG	3	11	1	10	1	9	3	2	0	0
0.1% DL-MSG	3	8	2	8	2	6	1	0	1	1
0.4% DL-MSG	0	6	0	6	0	5	2	0	0	1
0.1% L-GA	3	10	2	10	3	6	0	5	1	0
0.4% L-GA	1	12	1	11	1	9	0	3	0	0
Controls	5	16	1	16	0	13	5	3	0	1

The mechanism leading to death in tumor-bearing animals is commonly a progression from ulceration to infection, then resulting in general sepsis, exhaustion and death. Death, when it occurred naturally in our rats, whether tumor-bearing or tumor-free did not follow such a sequence.

When the high incidence of tumors in all groups became apparent, we wrote to the Sprague-Dawley, Inc., requesting any available data regarding the incidence of tumors in this strain of rats. Our letter in part follows:

November 6, 1951

"We have found an excessively high incidence of tumors in this group of animals. They vary in size and rate of growth within wide limits. That the tumors result from factors other than those intrinsic to the experiment is fairly obvious, for control rats show a slightly higher incidence than do the test rats. We have removed tumors surgically with complete success and there has been no evidence of recurrence at the primary site or elsewhere in operated rats. Tumor transplants in rats previously free from tumors show no evidence of viability in the new sites. There are a few animals which show multiple tumors, but since they are breast tumors, we can not fairly draw any inferences as to metastasis.

"Our question is, therefore, have you any data derived under control conditions as to the incidence of tumors in Sprague-Dawley rats? Has it been obtained in your own laboratory or elsewhere? What types have predominated and have you any evidence of malignancy?"

A reply dated November 8, 1951 and signed by Mr. E. R. Dawley suggests that no exact information as to the incidence of spontaneous tumors exists for this strain. It must be considered, however, that the maintenance of rats to the age represented by our group is unusual and that no previous investigation may have been carried on sufficiently long to permit this data to be accumulated. Mr. Dawley's letter (in part) follows:

> "We have no quantitative data on the incidence of tumors in our rats. We usually can find a few old discarded female breeders which have tumors. Last May we sent five of these animals to our consultants at the University of Wisconsin. They reported as follows:
>
> 'The diagnosis is based on the histological and gross lesions. Histologically, the tissue sections revealed a well lined glandular arrangement with epithelial cells of normal appearance. Though several mitotic figures were seen in the epithelial cells, such figures were hardly characteristic. There was a certain degree of sclerosis. Since the tumors were well localized subcutaneously without any effect on general condition, the diagnosis is adenoma with sclerosing features.'
>
> "All of our present production is derived from an original 15 rats taken by Caesarian in 1940. Since that time we have never found a malignant tumor in any animal."

In light of the similarity of figures for incidence of tumors at 730+ days, i.e.

All rats	40.7 per cent
All test rats	40.1 per cent
Control rats	42.4 per cent

we are unable to relate the development of tumors conclusively to the supplementary materials fed over the two-year period.*

---

* In the tables referring to tumors some variation in the totals may be observed. This results from differences in the criteria used for establishing tumor growth. For example, in living rats, all persistent swellings were assumed to be tumors. Post-mortem examination of several suspected tumors showed them to be non-neoplastic. Not all tumors could be examined microscopically because of their grossly disorganized condition when autopsy was done.

REPORT ON

A STUDY OF L-MONOSODIUM GLUTAMATE, DL-MONOSODIUM GLUTAMATE AND L-GLUTAMIC ACID WITH RESPECT TO POTENTIAL CARCINOGENICITY

TO

INTERNATIONAL MINERALS AND CHEMICAL CORPORATION

C-58049

ARTHUR D. LITTLE, INC.
Cambridge 42, Massachusetts
July 21, 1952
Issued January 13, 1953

Table of Contents

	Page No.
Letter of Transmittal	1
Introduction	2
General Discussion of Carcinogenesis	5
Criteria for Establishing Presence of Tumors	9
Experimental Animals Description - Bases for Selection	10
Blood Examinations	20
General Physical Condition and Examination for Tumors	25
Mortality Data	36
Gross Findings at Autopsy	40
Microscopic Examinations	52
Discussion	68
Summary	70
APPENDIX	
Tables Covering Detailed Physical Examinations	72
Cumulative Population Records	111
Notes on Gross Pathological Examinations	120
Notes on Atypical Microscopic Findings	139
Photographs	147

Arthur D. Little, Inc.
RESEARCH · ENGINEERING · INVESTIGATION
CAMBRIDGE 42, MASSACHUSETTS

July 21, 1952

Dr. Paul D. V. Manning
Vice President, Research Division
International Minerals and Chemical Corporation
20 North Wacker Drive
Chicago 6, Illinois

Dear Dr. Manning:

We are sending you herewith our report on a study of the potential carcinogenicity of L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid. This work has been carried out by the use of C-57 black mice who were maintained under controlled experimental conditions for two years during which time they were fed standard laboratory diets supplemented by the materials under test. At the end of the two-year period, the surviving mice, including a control group, were sacrificed and a careful post-mortem examination of their tissues made.

The tissue changes were those consistent with advanced age and were chiefly senile alterations of the kidney, liver and lungs. A few mice showed areas of lymphocytic infiltration in these tissues, but all were of low-grade, subacute type. There were no appreciable differences between the control animals and those fed on diets supplemented as indicated above. At autopsy, a single tumor was found. It was an adenoma of the lung (benign) and its type was established by microscopic examination. The survival rate was approximately the same for control and test animals at the end of the experiment. The C-57 mouse is notoriously combative; many deaths resulted from fighting and the bodies of survivors were much scarred from this cause.

It is our conclusion based on the detailed observations reported herein that L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid do not possess carcinogenic properties.

Respectfully submitted,

Arthur D. Little, Inc.

INTRODUCTION

From time to time, the attention of persons engaged in cancer research has been attracted to the role of the amino acids in the etiology and metabolism of neoplastic tissues. Kogl and Erxleben [1] announced in 1939 that the protein of cancerous tissue is distinguished from normal proteins by the occurrence of amino acids of unnatural stereochemical configuration, in particular D-glutamic acid,-(the natural form, now referred to as L-glutamic acid). Five years later, after more experimental work, the existence of stereochemical abnormality as a characteristic of tumor protein remained unsettled.

Kogl and Erxleben did not indicate the function of D-glutamic acid, nor did they suggest whether its presence is due to altered metabolic processes in neoplastic cells, or to its elaboration as an organic defense against carcinogenic factors. The synthesis and testing of glutamic acid conjugates in the treatment of cancer (Diopterin and Triopterin), which in man has caused regression of tumors and analgesia, has not elucidated the observation of Kogl and Erxleben to any extent. Pfeiffer commented upon Kogl's work at a symposium on monosodium glutamate sponsored by the Quartermaster Food and Container Institute for the Armed Forces at Chicago in 1948, and questioned whether cancerous growths or embryonic cell rests may not be matured by glutamic acid. In 1951, a paper was published by Beaton, McGanity and McHenry [2] of Toronto in which they claimed that the concentration of

---

(1) Kogl, F. and Erxleben, H.; Zur Ätiologie der malignen Tumoren I. Mitteilung über die Chemie der Tumoren. Z. physiol. Chem. 258, 57, 1939.

(2) Beaton, J.R., McGanity, W.J. and McHenry, E.W; Plasma Glutamic Acid Levels in Malignancy. Canad. M. Ass. J. 65, 219, 1951.

blood glutamic acid was greatly increased in animals and human subjects suffering from malignant tumors. Such increase was said to be peculiar to malignant tumors and could be utilized for diagnostic purposes; the order of elevated concentration was several times that encountered in the blood of animals and human subjects who were hosts for benign growths. Whether the glutamic acid reportedly involved in tumor metabolism is of etiological significance, or whether it is a metabolite of tumor tissue has never been clearly established so far as we can learn.

The uncertain significance of glutamic acid in tumor tissue, its increasing therapeutic use, and the lack of information on its side actions have made it appropriate for study. The manufacturers of glutamic acid and its salts were properly interested in learning whether the compounds possessed any carcinogenic action, and to that end authorized a study of this phenomenon; it is the results of that investigation which are reported herewith. The closely related monosodium glutamate was included because it is the principal derivative of glutamic acid used in food.

In planning a study of this general type, in which the route of administration was to be gastro-intestinal, several factors warranted reflective consideration. The choice of species and variety of experimental animal was probably of greatest importance. The duration of the experimental work had to be sufficient to insure that the carcinogenic action, if any, would be allowed to operate throughout the entire life span of the selected species. An animal of uniform genetic constitution was required, and one for which an impressive body of data was available with respect to its response to carcinogenic agents. The incidence of

spontaneous tumors in the selected species should be low, but it should have been established that the selected species would be able to support tumor growth without succumbing immediately to the effect of a neoplasm. Should death occur early, it is possible that many tumors might have failed of observation before the size of the tumor mass became appreciable.

Our objective was not comparable to that of the investigator who transplants known tumor tissue to a susceptible host, nor could it be compared with the problem confronting an investigator who wishes to observe spontaneously-occurring tumors. Essentially, we wished to expose the subject animals to factors of unknown weight with respect to their tumor-producing properties. Our objective differed, too, from that in which the significance of a suspected carcinogen with respect to its industrial hazard is of interest. In that latter situation, the exposure is most commonly of the epithelial tissues and their appendages. Throughout our investigation, it has been important to remember that the projected use of the test materials might be repeated (in human subjects) through a large part of the life span whether used therapeutically (L-glutamic acid) or as an ingredient of food (L-monosodium glutamate). The racemic isomer (DL-monosodium glutamate) although not commonly ingested by humans, warranted study in view of its possible future use.

GENERAL DISCUSSION OF CARCINOGENESIS

The awareness of tumors in plants and animals is almost as ancient as man. The knowledge of tumor behavior has advanced materially in the twentieth century, but the basic facts regarding causation are almost as obscure as they were several centuries ago. Carcinogenic potency, i.e. the ability of a chemical substance to cause tumors, is a property widely distributed among chemical compounds, and there is little or no correlation between carcinogenicity and chemical structure.(1) The difference in tissue response to a suspected carcinogen is a matter of general concern, but it is of lesser importance when the carcinogen is administered by mouth than when applied to the body surface, since it will be more uniformly distributed by the blood stream. The establishment of the appropriate dosage is important, and if variation from the optimum is necessary, it had better be on the side of more than less. The latent period of carcinogens is frequently given too little consideration in studies of this type, but if the material is administered throughout the life span of the animal, it will be of minimal significance.

All neoplastic growths may be referred to as "tumors"; further classification may depend on such characteristics as site, invasiveness, cell type, embryonic origin of host tissue, or rapidity of growth. Tumors, by very derivation of the name, are swellings, yet they do not cause an increase in the size of normal cellular elements. Their intrinsic danger lies in their tendency to encroach on normal vital tissues. Malignant tumors are invasive, and have no capsules, but the benign

---

(1) Hartwell, J.L.; Survey of Compounds....Tested for Carcinogenic Activity Second edition, 1951. United States Public Health Service, Publication No. 149.

type push normal structures out of the way or press upon them. The benign type do not metastasize and are usually enclosed in a fibrous capsule or sac. Their growth is confined to localized areas and the growth rate is moderate. Malignant tissues, on the contrary, encroach on and encompass any tissues in their way. They penetrate the lymphatic and blood vessels and tend to metastasize or spread to distant areas. Malignant tumors originate from primitive or embryonic types of cells which never reach mature function. They reproduce by cell division; they are never encapsulated but infiltrate rapidly into surrounding tissues. Metastases occur when a few malignant cells are carried by the lymph and blood vessels to new sites. The rapid growth, the tendency to recur after surgical removal, and the dissemination of toxic products of tumor metabolism quickly endanger the life of the host.

A tumor, whether benign or malignant, is essentially an autonomous new growth of tissue, whose cells possess atypical structure and manifest atypical behavior. Tumor tissue possesses no useful function, and there is no limit to its possible extent of growth. If growth is uninterrupted, it can destroy the host by utilizing nutritive material, thus depriving normal tissue of sustenance, giving rise to a "wasting" in all tumor-ridden tissues. Tumors may arise from embryonic cell rests* so called, or from normal body cells. Growth is by cell division or mitosis, and may take place by expansion in which case there occurs the formation of a connective tissue capsule due to the pressure of the growth on the surrounding, supporting cells. Growth may also take

---

* Embryonic cell rests represent a persistance of immature cells, ordinarily found only in fetal life.

place by infiltration in which case the spread of the tumor occurs along tissue spaces and via the lymphatic vessels. A secondary growth of the same type may occur at some distance from the primary site. This latter type of growth is more characteristic of malignant growths than of the benign type. Incomplete removal of tumor tissue may result in recurrence with an increase in the growth rate.

The connective tissue type forms its own stroma or supporting structure and vascular supply. Epithelial tumors, on the other hand, are formed around stroma from the surrounding tissue, and their blood vessels arise from the same source. No new lymphatic vessel or nerve elements are formed in tumors. As the tumor increases in size, greater nutritional demands are made upon the host, and metabolic changes result from pressure upon or infiltration of vital organs. Degenerative changes in tumors may result from deficient blood supply; the tumor may become necrotic in whole or in part, pouring waste products into the blood stream. Sometimes only a cyst is found to indicate the earlier presence of a fully developed tumor which has been resorbed except for the cystic relic.

Benign tumors are usually slow-growing. They increase in size by expansion and are usually encapsulated, but do not infiltrate the surrounding tissues, nor do they metastasize. They are non-recurrent if removed surgically. Some benign tumors are believed capable of metamorphosis to a malignant form such as that which occurs in mammary tumors in mice.

Tumors are commonly classified on the basis of type and origin involved, but in either case, the histological characteristics are important in diagnosis. The tumor is named according to the tissue it

resembles and in which it occurs. However, certain malignant tumors do not resemble any normal tissue, and their most characteristic appearance when examined microscopically, is the total lack of cellular organization. Tumors which develop in tissues having their origin in the ectodermal layers of the embryo become carcinomas, while those coming from connective tissue of mesodermal origin (cartilage, bone, muscle) become sarcomas. The latter are usually far more malignant than carcinomas, and develop in individuals of younger age than do the former type, whose onset occurs in middle or late life. The typical sarcoma cell is spindle-shaped, but may be round or giant in type. The most malignant forms have a great number of round cells. Ulceration is a common event in the life of a tumor. In this condition, a cavity develops and fills with tissue fluid. The walls of the tumor may regress and leave only scar tissue behind.

CRITERIA

In a study of carcinogenesis, the animals represent biological tools. Their nutritional and general physical status must be followed, and the criteria for verifying the existence of a tumor must be established in general terms before the experimental work begins. They may be altered in some details as the work proceeds, but the general criteria will remain unchanged. Sufficient time must elapse for the latent period of a carcinogen to be completed.

Obviously, the principal criterion will be formation of a lump, either exterior to the body wall or palpable in deep tissues and organs. When a tumor develops in a laboratory animal, its clinical condition eventually degenerates and is marked by loss of weight, malnutrition, ruffling of the hair, generalized weakness, and possibly lowered body temperature. The animal may appear shrunken in size or it may show respiratory distress and generalized accumulation of fluid in the tissues (edema). In a sick animal, any local hardness or undue firmness of the tissues, any asymmetry of the abdomen, failure to eat, appearance of emaciation or disturbance of respiration warrants close daily observation until the cause becomes apparent. If comparative survival rates are to be determined, the animals can not be sacrificed indiscriminately, but if they appear to be moribund, sacrifice and immediate post-mortem examination is indicated. In the C-57 black strain of mice, overnight delay may mean that should an animal die, its cage mates will have cannibalized the entire body by morning.

Once tumor formation is suspected, the body must be searched for possible metastases to new sites, and any tendency toward regression in size must be individually evaluated. The ultimate and conclusive proof of the existence of a tumor will depend upon post-mortem examination of the tumor-bearing host and histological examination of the suspect tissues.

Arthur D. Hills Jr.

EXPERIMENTAL ANIMALS

DESCRIPTION - BASES FOR SELECTION

The selection of suitable experimental animals received careful consideration. The advice of a number of persons familiar with tumor growth was sought; without exception, they recommended mice, and in particular, one of the strains bred so effectively at the Roscoe B. Jackson Memorial Laboratory at Bar Harbor, Maine. At this institution, established in 1929 "for the study of cancer, genetics and behavior", over a million mice a year are bred and the permanent mouse colony numbers 150,000. Although many strains were destroyed at the time of a disastrous forest fire in 1947, all had been reconstituted by gifts from institutions to whom the Jackson Laboratory had previously supplied mouse stock. Great emphasis is placed there on the maintenance of inbred stocks of mice and systematic breeding has produced mice strains which are 98 percent (or more) homozygous so that when raised under uniform experimental conditions, these animals are for practical purposes, a single mouse, "blessed for the experimenter's purpose with many times nine lives".(1) The greater uniformity

---

(1) Lancet. Editorial. 262, 6701, 1952.

of inbred* stock permits detection of quantitative differences in the reaction of animals than would be possible with hybrid stock.

When the general nature of our problem was explained to one of the resident investigators at the Jackson Laboratory, Dr. George Snell, he recommended without hesitation that we use the Jackson strain denoted "C-57 black". These result from brother-sister matings. Occasionally, an internal sarcoma has been seen in this species, but for the most part they are free of spontaneous tumors. Dr. Snell suggested that we use only male** mice of the C-57 black strain since they are notoriously pugnacious and cannibalistic when caged near females of the same variety, unless caged individually. Later experience proved that other factors than the presence of females stimulated pugnacious behavior! We wished to use 800 individuals so that an individual caging scheme would have been formidable.

---

*By inbreeding, (brother x sister) or by linebreeding (mother x son or daughter x father) it is possible to bring about an increase in the proportion of homozygous gene pairs. A given line will become as pure as it can be in 30-40 generations. Further matings after that time will result in spontaneous mutations sufficient in number to outweigh the value of continued inbreeding. Thus, inbreeding results in reduction of the number of variations for a given characteristic and produces a greater uniformity of response to drugs and carcinogens. Inbred stocks may show some reduction in so-called "hybrid vigor" but this can be minimized by decreasing the size of the newly born litter by culling the runts.

**Little and his associates had established in 1939 that when spontaneous tumors occur in the C-57 black strain, the incidence is the same in both sexes (Little, C.C., Murray, W.F., and Cloudman, A.M., Genetics of Non-Epithelial Tumor Formation in Mice. Am. J. Cancer. 36, 431, 1939)

Another investigator who had used the C-57 black mouse advised us that we would not see a spontaneous tumor in fewer than 10,000 individuals. In previous reports, we have referred to this type mouse as "highly susceptible" to tumors. In light of increased information from various sources and our own observations, we would wish to modify this to state that the strain is not highly susceptible to spontaneous tumors. It is susceptible to carcinogenic chemicals and the tissues of this strain are well able to support tumor growth of this origin.

In November, 1949, we obtained 800 male mice of the C-57 black strain, approximately 28 days of age.

In the C-57 black mouse, no mammary tumors are expected to occur, except in breeding females (one percent) and lung tumors are almost non-existent. Approximately 90 percent or more C-57 black mice can be expected to die free from spontaneously developing tumors.

The description furnished with the mice by the Jackson Laboratory indicated that they were recorded as "C-57 black, subline 6, inbred 27 generations with considerable inbreeding previous to that. The origin of this stock was by mutation from the C-57 brown strain. They show high fertility and have a genetic constitution described as aa".

EXPERIMENTAL CONDITIONS

The C-57 black mice were approximately four weeks old when received from the Jackson Laboratory; the ages within the total group did not vary more than two weeks. For a two week period, the mice were quarantined to eliminate sick or sub-standard individuals. Ultimately, they were divided into six test groups of 100 mice each. Two hundred mice were reserved for a control group.

The basic ration was PURINA LAB CHOW and the test compounds were incorporated in it in pellet form. This ration has been found to be appropriate to the nutritional requirements of the mouse and has been used successfully in our laboratory for ten years. The individual ration fed to the test groups contained one of the supplements at 1.0 percent or 4.0 percent concentration. Thus, the seven groups were fed throughout the experiment as follows:

Group	1	200	mice	basic ration				control
"	2	100	"	basic ration plus				1% L-Glutamic Acid
"	3	100	"	"	"	"		4% L-Glutamic Acid
"	4	100	"	"	"	"		1% L-Monosodium Glutamate
"	5	100	"	"	"	"		4% L-Monosodium Glutamate
"	6	100	"	"	"	"		1% DL-Monosodium Glutamate
"	7	100	"	"	"	"		4% DL-Monosodium Glutamate

The selection of levels of 1.0 and 4.0 percent was to bring the supplement to concentrations ten times those of supplements fed in a parallel study of the chronic toxicity of the same compounds in rats. The higher concentrations fed to the mice bears some approximate relation (with respect to glutamic acid) to the therapeutic dosage level in human subjects.

The mice were housed in wire mesh cages of adequate size in groups

of 20. Five cages were required for each test group, and ten for the control group. The floors of the cages were of wire mesh with draw pans beneath containing sawdust, which was replaced each day to insure a clean floor at all times. The food was supplied in pellet form and a constant supply provided by placing the pellets in a wire mesh feeder. Water was supplied from glass bottles which were wired to the outside of the cage and closed with a rubber stopper through which a glass tube led into the cage, providing a "demand" source of water. Cages and water bottles were washed frequently. The temperature of the cage room was maintained automatically at 75° F. and the relative humidity at 45-50 percent.

The supplemented ration was prepared at the Experimental Food Division of the Ralston Purina Company, Checkerboard Square, St. Louis. The standard ration,* Ralston Purina Laboratory Chow, ordinarily in

---

* Copy of analysis attached to each bag

GUARANTEED ANALYSIS

Crude Protein (Min.) .	23.0%
Crude Fat (Min.) . . .	5.0%
Crude Fibre (Max.) . .	6.0%
N.F.E. (Min.)	44.0%

INGREDIENTS

Meat meal, dried skimmed milk, wheat germ, fish meal, liver meal, dried beet pulp, corn grits, oat middlings, soybean oil meal, dehydrated alfalfa meal, molasses, vitamin B_{12} feed supplement, riboflavin supplement, brewers' dried yeast, thiamin, niacin, vitamin A and D feeding oils, D activated plant sterol, 1% steamed bone meal, .5% iodized salt, .02 % manganese sulphate.

pellet form, was reground and mixed with the proper concentration of the supplement and repelleted before shipment directly to us. The food for the control animals was purchased from a local supplier with assurance from Mr. Emmett Johnson of the Ralston Purina Company of the uniformity of the basic ration from either source.

Whenever a new shipment of the supplemented food was received by us, representative samples were taken and sent to Dr. M.J. Blish, Supervisor of Organic Chemical Research of the Amino Acid Division of International Minerals and Chemical Corporation, Rossford, Ohio, for analysis of the amount of supplement actually contained. To avoid vitamin loss, large supplies were not stored, and each lot was completely used before starting another.

The results of the assays made at Rossford are summarized in the table which follows:

ASSAYS OF MOUSE RATION WITH SUPPLEMENTS

Date of Report and Amount of Supplement Found (in Percent)

Presumptive Supplement	1/30/50	2/21/50	10/16/50	2/28/51	9/26/51	Average
1.0% L-MSG.H20	1.37	1.01	1.70	0.86	0.82	1.15
4.0% L-MSG.H20	3.38	3.68	3.30	3.10	3.50	3.37
1.0% DL-MSG.2H20	1.17	1.06	1.10	0.89	1.10	1.06
4.0% DL-MSG.2H20	3.64	3.61	3.80	3.40	3.90	3.67
1.0% L-GA	1.56	0.95	1.00	1.10	1.00	1.12
4.0% L-GA	3.74	3.59	3.10	3.60	3.70	3.55
Control	0.05% L-GA					

The reported values show some discrepancy from the intended amount of supplement incorporated into pellets. The Ralston Purina Company reported some difficulty in obtaining a uniform dispersion of supplement in lots less than one half ton. The probability of major vitamin loss during storage before use made it inadvisable to have half-ton lots prepared at one time.

The average discrepancy of actual supplement content from intended content was ± 13 percent, tending to be higher than intended for the 1.0 percent supplement and lower for the 4.0 percent supplement. However, the average discrepancy for the food intended for mice was not greater than limits of error inherent in work of this type.

Previously reported figures (Report of March 22, 1950) of actual food consumption for a group of mice selected at random indicated an average daily food intake of 6.8 - 7.5 grams per day per mouse. From observations throughout the feeding program the food consumption did not vary significantly. As the mortality rate increased, the feeders did not require refilling as often, but the food consumption remained relatively constant per animal. On the basis of the probable food consumption per individual mouse, and the average concentration of supplement, by assay, the actual intake was probably that shown in the following table:

THEORETICAL AND PROBABLE INTAKE OF SUPPLEMENTS

Supplement	Theoretical Intake per Mouse Day	Probable Intake	Theoretical Intake Two Mouse Years	Probable Intake Two Mouse Years
1.0% L-MSG	0.068 - 0.075 gm.	0.0781- 0.0863 gm.	49.64 - 54.75 gm.	57.013 - 62.999 gm.
4.0% L-MSG	0.272 - 0.300 gm.	0.230 - 0.253 gm.	198.56 - 219.0 gm.	167.900 - 184.690 gm.
1.0% DL-MSG	0.068 - 0.075 gm.	0.072 - 0.0795 gm.	49.64 - 54.75 gm.	52.560 - 58.035 gm.
4.0% DL-MSG	0.272 - 0.300 gm.	0.250 - 0.275 gm.	198.56 - 219.0 gm.	182.500 - 200.750 gm.
1.0% L-CA	0.068 - 0.075 gm.	0.076 - 0.084 gm.	49.64 - 54.75 gm.	55.480 - 61.320 gm.
4.0% L-CA	0.272 - 0.300 gm.	0.241 - 0.266 gm.	198.56 - 219.0 gm.	175.930 - 194.180 gm.
Control	0.0034-0.0037 gm. (Per mouse day)			

Arthur D. Little, Inc.

There was no conclusive evidence that one supplement caused a greater total food intake than any other. In these mice, the nutritional status was good and did not contribute significantly to the mortality rate throughout the experiment.

The mice fed 4.0 percent DL-MSG received careful attention in view of the slightly higher toxicity of the racemic form demonstrated when determination of the acute toxicity (LD_{50}) of these compounds was made in rats. It was found to possess no greater toxic effects than either of the other supplements.

BLOOD EXAMINATIONS

The blood of representative mice was examined at intervals for correlation with their general condition. Blood was obtained by incising the tail vein. The total erythrocyte and leukocyte count, a differential count, and a hemoglobin determination, whenever possible, were made. The Thoma standard type blood pipettes were used. Hayem's diluting fluid was used in making the erythrocyte count and 3 percent acetic acid was used as a diluent for the leukocyte count. The differential count was made by staining the blood smear with Wright's stain. One hundred leukocytes were counted and their distribution among the five main classifications of neutrophils, eosinophils, basophils, lymphocytes, and monocytes was expressed as percentages of total leukocytes. Hemoglobin determination by the Sahli method was not entirely successful since the total blood volume in the mouse is very small.

The following tables present the blood values of mice at 241-251 test days and 275-280 test days which are representative of all determinations made. A table of normal blood values is given.

In a later series of blood counts (275-280 Test Days) hemoglobin determinations were made by matching the tail blood of the mouse with the Tallquist Hemoglobin scale. The determinations are expressed in such terms that 15.0 grams of hemoglobin (average value for mouse) per 100 ml. of blood is equivalent to 100 percent. The range for the normal mouse is 12.0 - 17.1 grams hemoglobin per 100 ml. blood.

The average blood values found are within the established normal range for the laboratory mouse. The relatively high white counts for the groups fed 1.0 percent L-MSG and 1.0 percent L-GA are noteworthy. In view of other normal blood values, especially the differential count in both groups, no significance is attached to the elevated white count in these animals.

The tumor-bearing host, both human and animal, is often found to suffer from blood dyscrasias. From the representative data presented and other blood examinations made at random intervals, no evidence of significant abnormalities in the blood cytology was seen at any time.

AVERAGE BLOOD VALUES IN C-57 MICE AFTER 241-251 TEST DAYS

Supplement	Erythrocytes Millions/mm^3 Blood	Hemoglobin (grams/100 ml. Blood)	Leukocytes Count/mm^3	DIFFERENTIAL COUNT				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
1% L-MSG	9,410,000	13.5	16,900	17	1	0	74	9
4% L-MSG	10,005,000	13.5	10,925	25	1	0	63	11
1% DL-MSG	7,750,000	11.0	13,150	16	3	0	74	6.5
4% DL-MSG	9,780,000	----	8,650	23	0	0	66	11
1% L-GA	10,942,000	12.5	16,100	20	1	0	70	10.5
4% L-GA	10,765,000	----	13,200	31	1	0	53	15
Control	9,000,000	6.5	10,100	28	0.5	0	65	7.5

AVERAGE BLOOD VALUES IN C-57 MICE AFTER 275-280 TEST DAYS

Supplement	Erythrocytes Millions/mm^3 Blood	Hemoglobin (grams/100 ml. Blood)	Leukocytes Count/mm^3	DIFFERENTIAL COUNT				
				Neutrophils	Eosinophils	Basophils	Lymphocytes	Monocytes
1% L-MSG	8,765,000	11.7	16,700	22	0	0	68	10
4% L-MSG	9,675,000	11.7	12,700	28	0	0	60	12
1% DL-MSG	9,905,000	11.7	16,200	12	1	0	83	5
4% DL-MSG	10,145,000	10.9	18,500	17	0	0	73	10
1% L-GA	9,580,000	11.8	21,600	27	1	0	62	10
4% L-GA	12,975,000	10.5	16,770	21	0	0	69	10
Control	9,510,000	12.5	11,180	15	0	0	77	8

Arthur D. Little, Inc.

NORMAL RANGE OF BLOOD VALUES IN THE LABORATORY MOUSE*

	Average	Range
Erythrocytes (Millions/mm^3 Blood)	9,229,000	5,500,000 - 13,900,000
Blood Hemoglobin Concentration (grams per 100 ml.)	15.05	12.0 - 17.1
Leukocytes (count/mm^3)	14,080	7,800 - 22,500
Differential Count (Distribution per 100 cells)		
Neutrophils	20.62	8.0 - 57.9
Eosinophils	2.58	0.0 - 15.1
Basophils	rare	-----
Lymphocytes	66.58	36.2 - 89.8
Monocytes	5.73	0.7 - 14.0

*1) Standard Values in Blood. Air Force Technical Report No. 6039, July 1951.

2) Gardner, M.V.; The Blood Picture of Normal Laboratory Animals. A Review of the Literature 1935-1946. J. of Franklin Institute, 243, 172, 1947.

GENERAL PHYSICAL CONDITION AND EXAMINATION FOR TUMORS

At intervals, each mouse was examined for swellings, asymmetry of body, lesions of skin and appendages, bleeding from body orifices and other findings indicative of poor clinical condition or suggestive of tumor formation (see section above on Criteria). A series of eight complete physical examinations, plus a final examination at autopsy of each individual C-57 mouse was made during two years' time. The data below shows the interval and age of mice at the time of examination.

Examination	Date	No. Test Days	Approximate Days of Age
1	January 5, 1950	30	58
2	May 1-16, 1950	146-152	174-180
3	July 11-14, 1950	215-220	243-248
4	September 18, - November 13, 1950	286-342	314-370
5	December 12, 1950 - January 14, 1951	370-403	398-431
6	May 18-23, 1951	529-534	557-562
7	October 2-3, 1951	664	692
8	November 21, 1951	715	743

In addition, random examinations were made at more frequent intervals. Complete notes on the condition of animals at each complete physical examination are given in the appendix.

There was no evidence of any tumor formation or other neoplastic activity at the time of the first complete physical examination. The only lesions seen were injuries which were obviously sustained in

fighting. There appeared to be a definite pattern of attack on the part of a single aggressor in a cage for the mice which had been attacked showed similar anatomical distribution of injuries. Usually, one mouse showed no injuries at all!

The condition of the mice at the second physical examination followed the same general pattern as described for the first. Although injuries from fighting were still present, they were not increased in number or severity. In some groups the previous injury had healed entirely, especially in the group fed a supplement of L-monosodium glutamate. Those being fed DL-monosodium glutamate were at this time suffering more numerous injuries from fighting.

In one mouse in the group fed 4.0 percent L-glutamic acid a small, internal mass was palpated in the genital region. The mouse was isolated for observation. Forty-four days later, the mass was no longer palpable and the mouse was returned to the original group. It is believed that the mass in question may have been an unretracted testis.

The third physical examination was made July 11-14, 1950, after 215-220 test days, when the age of mice was 243-248 days. Each individual mouse was handled, the body carefully palpated and examined for lumps, ulcerations, loss of hair or other grossly perceptible changes.

The injuries from fighting were greatly reduced in number from the two previous examinations. One animal in the 1.0 percent L-monosodium glutamate group had lost a leg but moved about as agilely as the others. The mice who showed signs of possible

neoplastic growth were identified for continued observation.

One month later, or at 245 test days, they were re-examined. The observations are presented below:

Cage 1	The lump was still present, not enlarged, freely movable.
Cage 10	No evidence of lump
Cage 24	" " " ".
Cage 27	A very small lump on dorsal surface was still present under skin, not enlarged.
Cage 30	No change from previous examination. The lump appeared to be just under the skin, possibly an area of infection from fighting injuries.
Cage 37	Lump in genital region under skin was very prominent, similar in appearance to that seen in Cages 27 and 30.

The palpable lumps described in mice from Cage 1 and Cage 10 (of which there was no evidence in the latter one month later) may well have been the unretracted testes. This was suggested as the probable explanation in the one case described in a previous examination. The disappearance of the small lumps on the dorsal surface of two mice can not be explained unless the mass was an area of ulceration which was absorbed on healing. The lumps which were still present were external to the body wall in the region of the genitals. The incidence of suspicious swelling at time of this examination was confined to one mouse on the 4.0 percent L-glutamic acid supplemented diet and two on the control diet.

On September 18, 1950 (286 test days) and on several days up to November 13, 1950 (342 test days) the fourth physical examination was made for presence of tumors or other suspicious lesions.

The mice which showed suspicious swellings and other surface changes were identified for continued observation. One mouse on the 4.0 percent DL-monosodium glutamate supplemented diet had a wart on his chin. One mouse at the 1.0 percent DL-monosodium glutamate level and two on the 4.0 percent L-glutamic acid supplemented diet each had a small lump (approximately 1/16" in diameter) on the ventral surface above the genital region but it was difficult to determine whether it was the beginning of a tumor or a point of healing of a previously injured area. Two mice fed on the control diet showed a similar lump visible to the eye in the same region which was palpable. When re-examined later at different times, there was no change in appearance or size.

The incidence of lesions suspected of being tumors at the fourth examination is presented below in tabular form:

Supplement	Number of Mice With Suspect Lesions	Description
1% L-MSG	0	None
4% L-MSG	0	None
1% DL-MSG	1	One mouse with a hard lump under skin near penis. (Possibly an area of infection)
4% DL-MSG	1	One mouse with a wart on chin
1% L-GA	0	None
4% L-GA	2	Two mice with a lump under the skin on ventral surface near genitals. (Possible areas of infection)
Control	2	Two mice with a lump on ventral surface above genitals

The descriptions of suspected neoplasms present the same pattern previously discussed and are not verified tumors. From their location involving the endocrine system and reproductive organs, it is believed that they may be the result of the aging process.

At the time of the fifth examination the study had been in progress approximately one year. An interesting observation at this time was the noticeable graying of the fur of some animals. A colony count was made as well as a description of the physical condition of each animal. A great many injuries resulted from fighting. Several had badly bitten faces, some had swollen, infected legs, others had crookedly healed broken legs or had completely lost one limb; some shortened tails or not any tail at all. The genital and anal region of many was bitten and infected. Many had scabs over the back and the ears had been chewed.

At one year, the persisting suspect lesions were equally distributed between control and test groups of mice.

Supplement	Mice with Suspicion of Developing Tumors	Description of Suspected Tumor
1% L-MSG	0	None.
4% L-MSG	1	Small wart on chin.
1% DL-MSG	0	None.
4% DL-MSG	1	Projecting wart on chin.
1% L-GA	0	None.
4% L-GA	1	Questionable lump above genitals (Condition from previous report unchanged).
Control	3	Scabbed areas (like lumps) external to body wall above the genitals.

It was concluded at the end of one year that no bona fide tumors had been observed in any living mouse. Such suggestive lesions as were seen failed to increase in size; many had disappeared on subsequent examination.

The sixth physical examination was made May 18-23, 1951 and a detailed description is recorded in the appendix of the condition and appearance at this time. The pugnacious habits persisted with the same general results described previously. In addition, some mice showed corneal opacities with scabbed and crusted lids from injuries sustained in fighting; some also had an eye exudate of questionable origin. These conditions occurred in all groups including the control animals. There were numerous chewed ears, scabbed backs and faces, swollen legs, infected anal and genital areas, and amputations. Some were denuded of fur in large areas over the back or genitals. The possibility of tumor formation having its origin in traumatic irritation was not ignored.

Observations of lesions believed to be incipient tumors are tabulated from the data recorded at the sixth complete physical examination.

Supplement	Original Number in Group	Number of Mice Showing Suspect Lesions	Description
1% L-MSG	100	2	Small lumps above genitals.
4% L-MSG	100	0	None.
1% DL-MSG	100	1	Small lump on ventral surface above genitals.
4% DL-MSG	100	2	Small lump on ventral surface above genitals.
1% L-GA	100	4	Each mouse had evidence of a small lump above genitals.
4% L-GA	100	2	Each mouse had evidence of a small lump above genitals.
Control	222	5	Each mouse had a small lump above the genitals.

One month later when these animals with suspect swellings were re-examined there was no indication in any one of the previously described hard lumps above the genitals. The lumps may have been liquefied and resorbed or they may have been due to injuries which were healing. Certainly none had increased in size nor were other lesions present in these mice. It was concluded that the swellings were not related to neoplastic activity and were not due to carcinogenic activity of the food supplements.

The seventh general physical examination was made October 2-3, 1951 after 664 test days at an average age of 692 days. At this time the mice were at the age when spontaneous tumors might have been expected, if any were to occur.

The incidence of swellings suggestive of incipient neoplasms are summarized below: (Does not include one animal on 4.0 percent L-glutamic acid supplemented diet with a small blood blister under right hind leg.)

Supplement	Number of Mice Involved	Description of Suspected Incipient Neoplasms
1% L-MSG	1	Small lump on lower genital region.
4% L-MSG	0	None.
1% DL-MSG	0	None.
4% DL-MSG	2	One mouse with lump near left testis. One mouse with lump on chest near right side.
1% L-GA	0	None.
4% L-GA	4	One mouse with lump on upper part of chest. One mouse with lump near region of genitals. One mouse with lump near testis. One mouse with small lump on back (may be injury from fighting).
Control	10	Eight with lumps in genital region (possibly the prostate gland). One mouse with lump on right side of genitals. One with possible internal tumor.

The suspected incipient neoplasms were of two general types. One type observed is described as a lump under the skin but external to the body wall in the region of the genitals, which had usually disappeared at subsequent examination, and if still present was not enlarged. The lump in some cases suggested trauma. Another possibility considered was that it may have been an involvement of the prostate gland, but when these animals came to autopsy, no abnormality was seen. The second type observed was described as a lump on the chest.

In order to explore the exact nature of these findings, four mice were autopsied, two from the 4.0 percent L-glutamic acid supplemented diet and two from the control group. Post-mortem examination showed no gross indication of tumor formation. The lump near the genitals on the mouse on the 4.0 percent L-glutamic acid supplemented diet was seen to be a small cyst containing a thick, yellow material. The lump was under the skin but external to the abdominal cavity.

The swelling on the mouse with an enlargement in the chest area on the 4.0 percent L-glutamic acid supplemented diet was an enlarged thyroid gland but was non-tumorous. The mouse on the control diet with a questionable visceral tumor presented no gross evidence of neoplasm at autopsy. The second mouse on the control diet sacrificed for exploration of the small lump under the skin near the genitals presented a similar picture to the one described on the 4.0 percent L-glutamic acid supplemented diet. The lump under the skin near the genitals was yellow and hard. Adjacent to it was a yellow leaf-like structure later identified as the bulbo-urethral gland. A more detailed account of autopsy findings is presented in the section on pathological findings.

On November 21, 1951, the eighth complete physical examination was made. This represented observations after 715 test days and an average age of 743 days. They were extremely senile mice and the survivors had outlived the average life span by approximately 25 percent. In the detailed description (see appendix) the occupants of several cages had been combined into a single cage because of their decreased numbers.

The incidence of lesions suspected of being tumors at the eighth examination is presented below in tabular form:

Supplement	Number of Mice With Suspect Lesions	Description
1% L-MSG	0	None
4% L-MSG	3	Two had lumps near genitals; one had internal lump (Palpable in region of liver).
1% DL-MSG	0	None
4% DL-MSG	2	One mouse had lump on chest near throat; one had lump near genitals
1% L-GA	0	None
4% L-GA	0	None
Control	3	Three instances of lumps near the genital region

Summarizing, then, the mice showed the results of injuries sustained in fighting, many of which resulted in death. There were, in some mice, swellings which invariably subsided and did not continue to increase in size. No authenticated tumors were seen during life in any animal.

The general physical condition of the mice at the pre-sacrifice inspection followed the same pattern as at previous examinations. Fighting had persisted and many had bald patches or scabbed areas over the body surface. A favorite site for attack had been the eyes. On some mice both eyes were completely closed because of scabbed or crusted lids; others had opacities of either or both eyes. The usual cases of chewed ears, broken but crookedly healed legs, and graying of fur were present but to the same extent in both test and control groups.

Parasites in the laboratory mouse are not uncommon and if the infestation in a colony is unchecked may result in many deaths. The most common parasites in the C-57 mouse are Endamoeba muris, Giardia muris and Coccidia muris. In our colony, no evidence of infestation by the former two were seen but a few animals showed signs of old coccidiosis when they came to autopsy. Because of our caging scheme, cross infestation was probably held to a minimum.

Hemorrhagic septicemia is sometimes seen in laboratory mice and is caused by an organism named Erysipelothrix. Two other common infectious diseases are pseudotuberculosis of rodents (Corynebacterium pseudotuberculosus). and arthritis of the mouse

(Streptobacillus moniliformis) in which there is marked edema of the legs and tails. A few animals in our group showed the latter condition but in all but two cases it cleared spontaneously without specific treatment.

We must consider the possibility that the low-grade infectious process found at autopsy may have been mouse typhoid caused by Salmonella organisms (Salmonella typhimurium and Salmonella enteriditis). This disease is characterized by loss of normal activity. The affected mouse sits with its head hunched on its shoulders; its hair is ruffled and the coat gloss disappears. There is loss of appetite, conjunctivitis and increased respiration. Death usually occurs within one week. The post-mortem appearance shows enlargement of the liver and spleen with serosanguineous fluid in the peritoneal cavity. The lungs may be free from involvement or they may show pin-point hemorrhages with congestion and hyperemia. In our mice, the diagnosis of mouse typhoid is questionable in view of the long survival.

The low incidence of disease and of parasitic infestation is believed due to the quarantine period at the beginning of the study during which any animal found to be in less than perfect condition was eliminated and a substitution made in the actual test group.

MORTALITY DATA

From the numerical counts of each group which were made at the time of complete physical examinations, tables showing the population at approximately three months intervals were made to show survival ratios.

Because of heavy losses in the control group early in the experiment, replacements were added. However, due allowance for these was made in computing losses and in evaluating other data depending on the total number of animals represented.

Deaths were due to three main causes, namely: a) sacrifice, b) known trauma from fighting or accidental causes and c) unexplained causes.

Many unexplained deaths occurred as a result of injuries received from combat. Whenever possible, the aggressive mouse in a cage was isolated, but this was not usually possible until identification had been made by the loss of several cage mates. Another difficulty in determining accurate mortality rates was the trait of cannibalism among C-57 black mice. Frequently, the only evidence remaining of a deceased mouse was a small amount of skin and fur in the bottom tray or a few bone fragments. Occasionally, a mouse would disappear without trace. Deaths from fighting or of unexplained cause were recorded daily but the body, if found, was often so much eaten that a post-mortem examination was futile. A daily record was maintained of known casualties and injuries and their effect on the population record. (A few mice died of known accidental causes such as being crushed in feeder or cage door.)

Most varieties of laboratory mouse can be expected to live to the age of eighteen months; they have been known to live until three years of age but this is exceptional. After they reach the age of average life expectancy, they are physiologically senile and the clinical appearance and post-mortem condition of organs and tissues will be consistent with their age. This was found to be true in our colony in all animals surviving more than fifteen months. No increase in alertness or better physical condition was seen in mice whose diets were supplemented with L-glutamic acid over the condition of other test mice or controls.

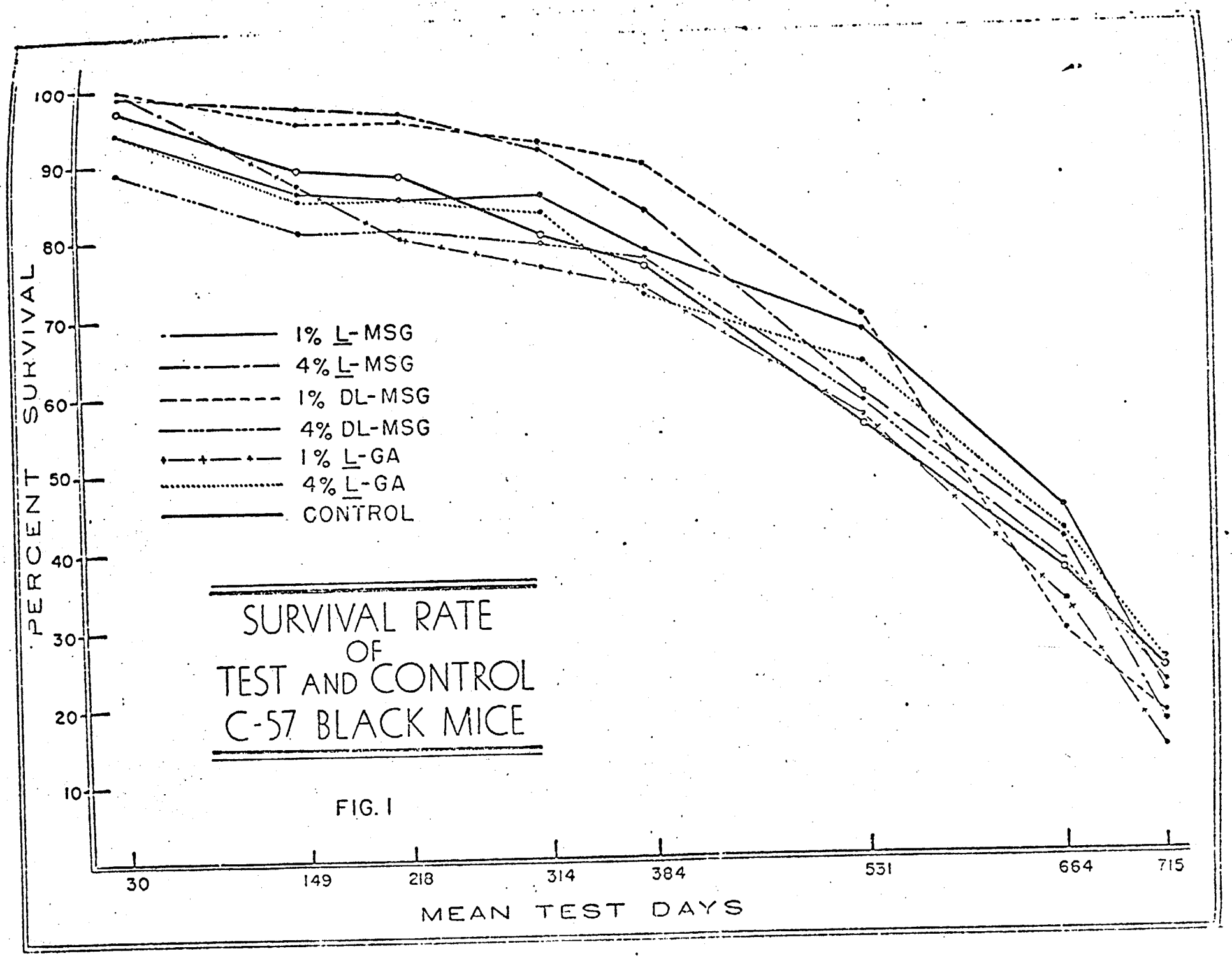
The survival rate during the first year of observation was approximately the same for all test groups and for the control group. The maximum loss during this period occurred in the 1.0 percent L-glutamic acid group (24 percent). The smallest loss during this period was seen in the group receiving 1.0 percent DL-monosodium glutamate (8 percent). From that point to the end of the test period, the cumulative losses varied so that at one point, the greatest may have been in the control group and at another in any one of the test groups. The cumulative survival rates are shown in an accompanying table, and in complete tables entitled "Cumulative Population Record" in the Appendix. The final survival rates show variations between test groups and controls (from 13 percent in the 1.0 percent L-glutamic acid groups to 24 percent in the 4.0 percent L-glutamic acid groups and 24 percent in the control group). The average final survival figure for all test mice was 19 percent which should be compared with 24 percent

for the control group. The total number of survivors (both test and control) after 733 days for all mice was 19.8 percent. In view of the large number of deaths from trauma, the difference between the survival rate for test mice and controls is apparently of minimal significance.

After one year, the number of deaths in all groups increased very rapidly (see Figure 1). At 664 test days, approximately one third of the original mice were living; 51 days later (715 test days) the number of survivors was reduced to less than one quarter of the original number. At no time did a large number of mice in a single group die within a short period either in test or control groups, which corroborates the findings in the collateral study of chronic toxicity in rats. In the latter six months of the test period many more deaths were due to natural causes than in the first eighteen months. The correlation between physical appearance, senile deaths and autopsy findings in the mice surviving to 733 test days* is very close. Certainly no deaths characterized by cachexia and emaciation, fever and any other signs associated with neoplastic growth were seen at any time. We feel confident that carcinogenicity of any of the supplements was not the major lethal factor.

---

*To obtain age at death, 28 days should be added. This was the age at the beginning of the experiment.


PERCENT SURVIVAL
100
90
80
70
60
50
40
30
20
10
1% L-MSG
4% L-MSG
1% DL-MSG
4% DL-MSG
1% L-GA
4% L-GA
CONTROL
SURVIVAL RATE
OF
TEST AND CONTROL
C-57 BLACK MICE
FIG. I
30
149
218
314
384
531
664
715
MEAN TEST DAYS

SURVIVAL RATES (IN PERCENT)

Mean Test Days	30	149	218	314	384	531	664	715
1% L-MSG	94	86	85	85	78	67	44	20
4% L-MSG	99	97	96	91	83	59	40	16
1% DL-MSG	100	95	95	92	89	69	28	17
4% DL-MSG	89	81	81	79	77	58	37	21
1% L-GA	100	87	80	76	73	56	32	13
4% L-GA	94	85	85	83	72	63	41	24
Average - all test mice	96	89	87	84	78	62	37	19
Control	97.0	89	88	80	76	55	36	24

Average interval between complete examinations 90 days

Total survivors at 733 test days (all groups) 19.8 percent

Arthur D. Little, Inc.

GROSS FINDINGS AT AUTOPSY

At the end of the two years, the surviving animals were sacrificed with an overdose of veterinary nembutal and pathological examinations made. A description of the grossly abnormal findings will be found in the Appendix. Pieces of tissue were taken from the principal organs such as the lungs, heart, liver, kidney, spleen, intestine, testes, seminal vesicles, and preputial (genital) glands. Any other tissues showing lesions or suggestive of neoplastic changes were also reserved for microscopic examination. They were prepared by standard histological procedures and ultimately stained with hematoxylin and eosin. A representative number of tissues, normal to visual inspection, were examined microscopically and are described, if remarkable, elsewhere in this report. Whenever possible, during the course of the work, mice which were found dead or dying were examined pathologically. We have mentioned above the fragmentary remains of the bodies of mice unless found soon after death and before their cage mates had eaten them.

A total of 202 post-mortem examinations were made, of which 159 were considered to be complete. They were done after 730-762 test days. Since the average age of mice at the beginning of the experimental work was 28 days (± 3 days), the age at autopsy of this group was 758-790 days.

The distribution of complete post-mortem observations among groups was reasonably uniform and although representing only one fifth of the initial number of animals, it is believed by us that the findings were consistent and representative.

Test Group	Final Autopsies	Earlier Autopsies	Total
1 % L-monosodium glutamate	20	5	25
4 % L-monosodium glutamate	16	7	23
1 % DL-monosodium glutamate	17	6	23
4 % DL-monosodium glutamate	21	6	27
1 % L-glutamic acid	13	7	20
4 % L-glutamic acid	24	5	29
Control group	48	7	55
Total	159	43	202

As noted above, the exterior appearance was that of old mice, much scarred by fighting and showing the result of numerous injuries. Lacerations, abrasions and healed leg fractures were commonly seen. The coat color was often altered and the uniform leaden grey-black was rusty and often much lighter than is found in young specimens. On section, numerous gross abnormalities were seen, varying in severity from simple changes in the color of viscera to pneumonic consolidation in two control animals. Only a single verified tumor was seen, a lung adenoma occurring in an animal who had been fed on a diet supplemented with 1.0 percent DL-monosodium glutamate. The actual diagnosis was made by microscopic examination but an alteration in the consistency of the lung was apparent by visual inspection. The detailed observations are presented in the Appendix but a summary of the incidence of abnormalities other than minor changes in color is presented here to show the location with respect to organs involved and the number observed in each group of mice.

LOCATION OF GROSS POST-MORTEM ABNORMALITIES

Supplement	Tissue Involved	Total	External Appearance	Total	Lesions Suggestive of Abnormal Growth	Total
1% L-MSG	Liver 4 Kidney 3 Lung 1 Testes 1	9	Corneal Opacity 3	3	None	0
4% L-MSG	Liver 1 (abscess) Testes 2	3	Corneal Opacity 2	2	Liver abscess 1 Genital swelling 2	3
1% DL-MSG	Lung 1 Spleen 1 Seminal vesicles 2 Preputial gland 1	5		0	Genital swelling 1 Growth on seminal vesicles 1	2
4% DL-MSG	Lung 3 Liver 1 (abscess) Seminal vesicles 3 Kidney 3 Spleen 2 Testes 3	15		0	None	0
1% L-GA	Thyroid 2 Lung 1 Spleen 2 Seminal vesicles 2 Testes 1	8		0	Enlarged thyroid 2 Genital swelling 1	3

Arthur D. Little, Inc.

LOCATION OF GROSS POST-MORTEM ABNORMALITIES (CONTINUED)

Supplement	Tissue Involved	Total	External Appearance	Total	Lesions Suggestive of Abnormal Growth	Total
4% L-GA	Kidney 1 Spleen 2 Seminal vesicles 2 (hypertrophy) Testes 1 Thyroid 1 Preputial gland 1 (atrophy)	8	Corneal Opacity 1	1	Thyroid cyst 1 Peritoneal cyst 1 Genital swelling 2	4
Control	Thyroid 4 Preputial gland 7 Seminal vesicles 6 Heart 1 Lung 5 (consolidation in 2) Kidney 1 Liver 3 Spleen 7	34	Corneal Opacity 4	4	Thyroid cyst 1 Genital swelling 3 Subcutaneous lump 1	5

STATISTICAL DISTRIBUTION OF GROSS ABNORMALITIES

Incidence of Gross Abnormalities
(Average number lesions per animal x 100)

Controls	70.8
All Animals	51.5
All Test Animals	43.4
Mean incidence	57.1

Deviation from mean incidence

All animals	-5.6
All Test animals	-13.7
Controls	+13.7

Arthur D. Little, Inc.

In the following summaries, tissues and organs not specifically described were considered entirely normal and not remarkable.

COMMENT ON FINDINGS BY TEST GROUPS

1.0 percent L-monosodium glutamate group

The changes in the livers in this group worthy of note were chiefly paleness. The livers of two mice showed tiny yellow spots on the surface. The kidneys of two animals were small and shrunken. One spleen had a small, circumscribed, whitened area near the edge. One testis showed gross atrophy, apparently associated with senile changes and loss of function. Three animals showed corneal opacity, obviously traumatic in origin.

4.0 percent L-monosodium glutamate group

A liver abscess, partially healed and having much fibrous tissue at its margin was the chief gross abnormality seen in this group. One testis had herniated into the peritoneal cavity but did not appear otherwise damaged. The left testis in another animal was hemorrhagic but the right testis was unchanged. Corneal opacity was seen in two animals, but only one eye was involved.

1.0 percent DL-monosodium glutamate group

The general condition of this group was good. There were no corneal opacities. The lung of one animal showed calcification of one lobe; apparently the process followed an old pulmonary infection. The spleen in one individual was shrunken and appeared atrophic. Both seminal vesicles in one animal were definitely hypertrophic and markedly enlarged. A palpable abdominal swelling (before the body cavity was opened) was seen to be a swelling of the preputial gland. Comment on this finding is made elsewhere in the report; this may

have been the remains of an old abscess. Microscopic examination proved that it was not carcinogenic in nature but resembled an acute process which had subsided rapidly.

4.0 percent DL-monosodium glutamate

The animals in this group showed more gross pathological changes than did those in any other test group. Although the external appearance was good, on section it was found that the viscera of nine animals showed pathological lesions. Especially notable were the kidneys of three animals; one showed evidence of an old and widespread hemorrhage. Another showed gross enlargement and hypertrophy, being almost twice the normal size but also showing microscopic evidence of cystic changes and loss of function. A third kidney was hydronephrotic and showed cystic changes in the pelvis. The kidneys of several other animals were altered to a minor degree, chiefly in color, tending to be pale. These were not considered to be more than suggestive of a renal toxic effect manifested by the supplement at the 4.0 percent level. In three animals, the lungs were dark red, possibly hemorrhagic. One mouse showed the results of a coccidial infection in the form of a liver abscess. The spleen of a single animal was hemorrhagic and in another animal, the spleen was enlarged. The seminal vesicles of two animals were enlarged and had been palpable during life as nodules. One seminal vesicle had herniated and another showed considerable atrophy. The testes of one mouse were enlarged and cystic; of another, definitely enlarged while a third mouse showed a scrotal hernia. The incidence of gross abnormalities in this group was greater than for

any other test group (71.4 percent) and was slightly greater than that for the control group (70.8 percent).

1.0 percent L-glutamic acid group

The thyroid gland in two animals showed enlargement. One mouse showed a curious mottling of the lungs. Two animals showed abnormal spleens, one being enlarged and the other showing a mottled surface. In two animals, the seminal vesicles were altered in size, one being enlarged and the other reduced in size. The testes in one mouse were so shrunken as to be almost invisible. This was also true of the preputial gland in another mouse.

4.0 percent L-glutamic acid group

In one animal, both kidneys were pale and enlarged; two spleens showed the same type of mottling remarked upon in the 1.0 percent L-glutamic acid group. Two animals had enlargement of the seminal vesicles and one animal had an atrophic, shrunken testis. The preputial gland in another mouse in this group was atrophic but otherwise normal in appearance.

Control group

In the large group of grossly atypical organs seen in the control group, no changes were seen that had not been observed in other groups with the exception of a calcified mass lying freely in the abdominal cavity. In one animal, the thyroid was shrunken in size but in five others, it was enlarged to a noteworthy degree. In five animals, the preputial gland was enlarged and in two others it was shrunken. The subcutaneous lump noted in the summary table was found to be an apparently normal preputial gland which was surrounded by

fibrous tissue and losely attached to the skin of the lower abdomen. In five animals, the seminal vesicles were enlarged and in one they were small in size, presumably atrophic. A thyroid cyst was found in the neck of a single animal and in the same animal the myocardium appeared dark red in color but was otherwise normal. The lungs of five animals showed a generalized mottling. Two other animals showed moderately advanced consolidation of the lungs; pulmonary complications had not been evidenced by their clinical condition before death. Three livers were unusual in appearance, one being cystic to visual inspection, one granular in texture and one enlarged to a moderate degree. The kidneys of the group were essentially normal although in one animal, they appeared small in size. In seven animals, the spleen appeared changed in appearance. In three animals, the spleen was mottled; in three other animals, it was enlarged and in one animal, it was decreased in size. Corneal opacity was present in four animals in this group.

The chief and most significant observations were that the genital swellings noted in the living mice appeared in all cases noted to be enlargement of one of the genital glands or organs. None were found on microscopic examination to show neoplastic changes. Throughout the course of the project, these swellings had been carefully observed and considered suggestive of abnormal growth. However, both gross and microscopic criteria proved that such was not true. A second observation which appears significant was the kidney pathology in animals fed on 4.0 percent DL-monosodium glutamate. It has been

suggested that this isomer is toxic* to the kidney. In light of these findings, it may warrant independent investigation. The high incidence of pathological changes in this group (71.4 percent) must be compared with an incidence of 51.5 percent in all animals, both test and control and again with an incidence of 43.2 percent in animals on supplemented diets. The incidence of lesions in 71.4 percent of the animals maintained on standard ration plus 4.0 percent DL-monosodium glutamate is very slightly higher than seen in the control group (70.8 percent). No other test group approached this level except that fed on 1.0 percent L-glutamic acid (61.5 percent). However, in view of the few animals in this latter group coming to complete autopsy (13) the number of gross abnormalities may weight the incidence unduly, particularly in light of the lower incidence of gross abnormalities in the 4.0 percent L-glutamic acid group which showed an incidence of gross abnormalities of only 33.3 percent.

Perhaps the most significant comparison is between all test animals and all control animals. Here, the incidence of abnormalities for control animals is 27.6 percent higher than for test animals. For all animals, the incidence of abnormalities is 8.3 percent higher than for the test animals but this may be a strongly weighted figure.

It would be possible to argue that maintaining mice (and presumably other animals) on diets supplemented by L-glutamic acid

---

*A higher LD_{50} in rats was found for DL-monosodium glutamate than for L-monosodium glutamate or L-glutamic acid.

or its derivatives had a favorable effect in reducing pathological lesions. We are satisfied to conclude that no marked pathological changes are produced by feeding these diets over the life span of the animal.

The incidence of suggestive abnormal growths have little meaning since the suspect lesions were found to be non-carcinogenic, usually non-tumorous and probably non-neoplastic. It is of passing interest to note that the variation from the mean incidence of suspect lesions of three summary groups, (i.e. all animals, all test animals, control animals) is $\pm$ 0.65 percent.

MICROSCOPIC EXAMINATIONS

The tissue specimens reserved at autopsy for microscopic examination were prepared by standard techniques and stained with hematoxylin and eosin. They were examined and screened by Dr. M.G. Gray of our staff and reviewed by Dr. Lloyd Fogg, our consultant in pathology. Approximately 800 sections were prepared from tissue material resulting from 159 + post-mortem examinations. Of these, 340 were included in the review by Dr. Fogg. A comment on atypical sections will be found in the Appendix. The appearance of all other sections was either not remarkable or consistent with the age of the animal.

The total number of sections examined was 798 of which 141 showed abnormalities chiefly associated with age. One section permitted the diagnosis of "benign adenoma of the lung" in an animal fed on 1.0 percent DL-monosodium glutamate. Several showed abscess formation but were free from neoplastic changes.

In selecting tissues for microscopic examination a representative number were taken from each group. In addition, all tissues which appeared grossly abnormal were prepared for microscopic examination. The organs represented were: trachea, bronchi, lungs, heart, kidneys, spleen, pancreas, submaxillary gland, thyroid, stomach, liver, intestine, skin.

1.0 percent L-Monosodium Glutamate Group

Number sections examined	76
Number showing abnormalities	30

Liver	4
Kidney	5
Lungs	9
Intestine	2
Stomach	1
Spleen	8
Testes	1

Liver: Foci of inflammation around central veins; foci of necrotizing parenchyma cells. Inflammation varies in degree from mild cellular infiltration to necrosis.

Kidney: Interstitial inflammation; fibrosis of glomeruli and tubular atrophy. Focal necrosis in renal pelvis. Cortical tubules involved in one section. In another section, chronic interstitial nephritis involving glomeruli and tubules.

Lung: Inflammatory foci around bronchioles; lymphocytic infiltration. Focal fibrosis of parenchyma. Occasional mild congestion. Massive abscess in one section.

Intestine: Polyp formation in colon, focal corrosion of epithelium.

Stomach: Hyperkeratosis with hyperplasia of underlying stratum germinativum.

Spleen: Congestion, with disorganization of red and white pulp. Senile appearance.

Testes: Reduced germinal population; consistent with non-functional, senile organ.

4.0 percent L-Monosodium Glutamate Group

Number sections examined	84
Number showing abnormalities	22

Liver	3
Kidney	5
Spleen	5
Lung	4
Testes	4
Genital gland	1

Liver: Accumulation of blood cells around central vein with foci of cellular necrosis. General distribution of hyperchromatic cells with trace of hepatitis (3 sections).

Kidney: Some interstitial inflammation with focal glomerular fibrosis. Trace of focal glomerulo-nephritis (4 sections). Exudative infiltration of intertubular connective tissue with focal atrophy of tubular cells. Inflammatory infiltration by lymphocytes.

Spleen: Mild congestion; disorganized pulp; typical of senile spleen. Thickened trabeculae (5 sections).

Lung: Focal fibrosis with reduction in number of expanded alveoli. Occasional focal edema. Collection of lymphocytes around bronchioles.

Testes: Germinal cells reduced in number (4 sections).

GENERAL COMPARISON OF MICROSCOPIC FINDINGS L-MONOSODIUM GLUTAMATE GROUP (751 TEST DAYS)

	1.0 percent	4.0 percent
Liver:	Sections about equally divided between those showing no pathology and those showing traces of hepatitis as evidenced by foci of inflammatory cells around veins which also affect neighboring parenchymal cells.	Same as 1.0 percent group.
Kidney:	Sections vary from those showing no pathology to extensive focal areas indicating interstitial inflammation, the effect extending to glomerular fibrosis and atrophy of tubules. Some focal necrosis in renal pelvis.	All sections show some focal interstitial inflammatory indications involving nuclear toxicity in glomeruli, or fibrosis. In one case, there were foci of atrophied tubular cells, some dilated tubular areas which in a few foci showed casts. General mild chronic nephritis.
Lungs:	Most sections showed pinpoint areas of inflammation around bronchi and bronchioles as evidenced by infiltration of lymphocytes. Focal fibrosis of parenchyma. One lung has an abscess.	Only one animal showed pinpoint areas of inflammation. Others showed focal fibrosis, traces of edema or reduction in number of expanded alveoli as might be expected in lungs of aged animals.
Spleen:	All spleens show a mild congestion with a general lack of organization of red and white pulp. No actual pathology.	Two normal animals. Remainder as in 1.0 percent group.
Heart:	Normal.	Normal.
Genital Glands:	No pathology.	One showed focal abscess.
Testes:	Reduced population of germinal cells as might be expected in senile organ. No pathology.	Same as in 1.0 percent.
Intestinal Tract:	One case of focal corrosion of epithelium, otherwise no pathology. One case of polyp formation in colon. One case of hyperkeratosis with concomitant hyperplasia of underlying stratum germinativum.	No pathology.

1.0 percent DL-Monosodium Glutamate Group

Number sections examined 105

Number showing abnormalities 22

Liver	4
Kidney	6
Lungs	5
Spleen	4
Testes	3

Liver: Pinpoint foci of inflammation around some central veins, involving necrosis of parenchyma.

Kidney: Pinpoint areas of interstitial inflammation in cortex evidenced by accumulations of lymphocytes and degenerating tubule cells. Many fibrotic glomeruli.

Lung: Reduction in number of alveoli with slight focal fibrosis. Peribronchiolar inflammation.

Spleen: Mild congestion. Red and white pulp not clearly demarcated. Trabeculae enlarged.

Testes: Reduction in number of germinal cells in tubules.

4.0 percent DL-Monosodium Glutamate Group

Number sections examined	103
Number showing abnormalities	25

Liver	4
Kidney	7
Lung	5
Spleen	5
Testes	4

Liver: Pinpoint areas of inflammation around central vein, hepatic vein and arteries.

Kidney: Foci of interstitial inflammatory reactions, mostly collections of lymphocytes. Some necrosis in renal pelvis.

Lung: Focal fibrosis; reduced number of alveolar sacs. Peribronchiolar inflammation. Some fibrosis.

Spleen: Mild congestion with disorganization of red and white pulp.

Testes: Reduced population of germinal cells and tubules.

GENERAL COMPARISON OF MICROSCOPIC FINDINGS DL-MONOSODIUM GLUTAMATE GROUP (720-751 TEST DAYS)

	1.0 percent	4.0 percent
Liver:	Pinpoint foci of inflammation around central veins; in these areas there is a trace of necrosis of parenchyma. Nuclei stain intensely.	Same as in 1.0 percent.
Lung:	Some focal fibrosis with reduced number of alveoli, present in all animals. In one section there are areas of inflammation around bronchioles as evidenced by accumulation of lymphocytes. In another case, a lung tumor, benign.	All slides show inflammatory changes around br chioles, in some cases, extensive. Focal fibr sis, reduced number of alveolar sacs. This is suggestive of aged lung.
Kidney:	In all sections there are pinpoint areas of interstitial inflammation in cortex, involving glomeruli and tubules. Also present in renal pelvis in some sections.	Pinpoint foci of interstitial inflammatory reactions, mostly collection of lymphocytes. Cases vary from above to involvement of extens areas in cortex involving tubules and connecti tissue but not glomeruli. One case of a concr tion in pelvis.
Spleen:	Mild congestion. Red and white pulp not clearly demarcated. Trabeculae thickened. Suggests aged spleen in every case.	Same as 1.0 percent.
Skin:	Normal	Normal.
Submaxillary Gland:	Normal	Normal.
Genital Glands:	Normal	Normal.
Testes:	Reduction in number of germinal cells in tubules, as in aged or senile testis; no pathology.	Same as in 1.0 percent.

Arthur D. Little, Inc.

GENERAL COMPARISON OF MICROSCOPIC FINDINGS DL-MONOSODIUM GLUTAMATE GROUP (720-751) TEST DAYS (CONTINUED)

	1.0 percent	4.0 percent
Heart:	Normal	Normal
Pancreas:	Normal	Normal
Digestive Tract:	Normal	Normal
Thyroid:	Normal	Normal
Trachea:	Normal	Normal

Arthur D. Little, Inc.

1.0 percent L-Glutamic Acid Group

Number sections examined 105

Number showing abnormalities 32

Lung	6
Spleen	9
Liver	5
Kidney	4
Seminal Vesicles	3
Intestine	2
Testes	3

Lung: Inflammation of bronchi; thickening of alveolar sacs. Fibrosis.

Spleen: Mild congestion.

Liver: Perivascular inflammation; hyperchromatism and binucleated cells.

Kidney: Mild pelvic inflammation. Fibrosis of glomeruli.

Seminal Vesicles: Epithelial disorganization.

Intestines: Focal destruction of epithelium.

Testes: Senile.

4.0 percent L-Glutamic Acid Group

Number sections examined 118

Number showing abnormalities 16

Lung	7
Spleen	4
Liver	2
Kidney	1
Testes	1
Seminal vesicles	1

Lung: Bronchiolar inflammation; fibrosis, focal edema.

Spleen: Focal abscess.

Liver: Hyperchromatic nuclei.

Kidney: Necrosis in pelvic tubule cells.

Testes: Sparse germinal cells.

Seminal vesicles: Areas of corroded epithelium.

GENERAL COMPARISON OF MICROSCOPIC FINDINGS L-GLUTAMIC ACID GROUP (752 TEST DAYS)

	1.0 percent	4.0 percent
Lung:	Nearly every slide showed traces of inflammation around bronchi or bronchioles. Some fibrosis of walls of parenchyma.	Every section showed some indication of inflammation around bronchi or bronchioles, varying from slight to extensive.
Submaxillary gland:	Normal	Normal
Spleen:	All sections show a mild congestion. General disorganization of red and white pulp. No obvious pathology. Interpreted as indication of senility.	Varied from normal, through mild congestion to one case with an abscess.
Liver:	Frequent binucleate cells. General slight hyperchromatism of nuclei. No obvious pathology.	Slight hyperchromatism to normal.
Kidney:	Only traces of inflammation such as collection of lymphocytes around connective tissue in pelvis.	Normal. One slide showed slight necrosis in tubule of pelvis.
Seminal Vesicles:	Foci of corroded epithelium.	Normal to one case of corroded epithelium.
Intestines:	Foci of corroded epithelium.	Normal
Testes:	Germinal tubules only sparsely populated indicating senility.	Terminal tubules sparsely populated; senile
Heart:	Normal	Normal

Control Group

Number sections examined 207

Number showing abnormalities 45

Liver	9
Lung	11
Kidney	7
Skin	1
Bladder	1
Testes	6
Spleen	8
Seminal Vesicle	2

Liver: Perivascular lymphocytic infiltration; some hyperchromatism. One section shows general necrosis.

Lung: Peribronchiolar lymphocytic infiltration. Reduced number of alveoli. Inflammation of lymph nodes around bronchus.

Kidney: Interstitial inflammation; scattered glomerular fibrosis.

Skin: Cyst in sub-cutis.

Bladder: Lymphocytic infiltration in epithelium.

Seminal Vesicles: Some semi-solid particles in lumen. Foci of corrosion in epithelium.

Spleen: Mild congestion. Red and white pulp not clear demarcated. Some thickening of trabeculae.

SUMMARY OF MICROSCOPIC FINDINGS - CONTROL GROUP

Liver:	In general, sections show pinpoints of accumulation of lymphocytes around veins. One section shows necrosis of one lobe.
Lung:	Generally mild inflammation as indicated by lymphocyte collections around bronchioles. Focal fibrosis and reduced number of alveolar sacs, typical of aged lung.
Kidney:	Some interstitial areas of inflammation as indicated by presence of lymphocytes. Extent of effect varies from: (a) Rarely affecting glomeruli. (b) Rare fibrotic glomeruli. (c) Some necrosis in distal medullary tubules, otherwise negative. (d) Rare dilated tubules. Suggests a trace of chronic nephritis.
Spleen:	Mild congestion. Red and white pulp not clearly demarcated. Some thickening of trabeculae. Generally suggests aged spleens.
Seminal Vesicles:	Essentially normal. One case of casts and another of a focus of corroded epithelium.
Submaxillary Gland:	Normal
Heart:	Normal
Testis:	Germinal cells reduced in number. Otherwise no pathology. Typical of senile testis.
Skin:	Normal
Bladder:	Normal. Focus of infection in epithelial lining in one section.
Thyroid:	Normal.

DISTRIBUTION OF ABNORMALITIES

Supplement	No. sections examined	No. showing Abnormalities	Liver	Kidney	Lungs	Spleen	Testes	Intestine	Stomach	Genital Gland	Seminal Vesicles	Skin	Bladder
1% L-MSG	76	30	4	5	9	8	1	2	1	-	1	1	1
4% L-MSG	84	22	3	5	4	5	4	-	-	1	-	-	-
1% DL-MSG	105	22	4	6	5	4	3	-	-	-	-	-	-
4% DL-MSG	103	25	4	7	5	5	4	-	-	-	-	-	-
1% L-GA	105	32	5	4	6	9	3	2	-	-	3	-	-
4% L-GA	118	16	2	1	7	4	1	-	-	-	1	-	-
Average for Test Animals		25	3.7	4.7	6	6	2.6	*	*	*	*	*	*
Control	207	45	9	7	11	8	6	-	-	-	2	1	1
Total	798												

*Less than 1.0

The absence of microscopic evidence of tumor formation is obviously the most significant observation which can be made. With absence of gross evidence of tumor growth, we can safely conclude that under the conditions of this experimental study that the carcinogenicity of any one of the supplements is zero.

In the control animals there was microscopic evidence of low-grade infection in the liver, lungs and kidney which is not unusual in aged animals. In isolated cases, corroded epithelium of genital glands or a focus of infection in the bladder was noted. As isolated cases, it can be concluded that they represent nonspecific etiology, having minimal significance.

In general, no marked pathology was found in any group of mice. The animals fed with DL-monosodium glutamate showed no clear-cut differences from the controls. There was a suggestion that the low-grade infection noted in the controls showed a marginal intensification in these animals which can not be ascribed even indirectly to specific supplements. There is no evidence that potential carcinogenesis can be related to it.

The mice fed with L-monosodium glutamate, likewise, did not show clear-cut differences from the controls. The kidneys reveal more extensive, mild chronic nephritis than did those of the control group with no distinction between the 1.0 percent or 4.0 percent concentrations fed. No evidence was found suggesting carcinogenesis related to this supplement.

The livers and kidneys of mice fed on a supplement of L-glutamic acid showed slightly less evidence of inflammation. Foci of corroded

epithelium were noted in the seminal vesicles and intestines, but it is not clear that this could be traced to the specific diet. In general, there existed no significant microscopic difference between this group and any other.

On the basis of the microscopic data, we conclude:

1) There is no evidence of carcinogenicity possessed by the supplements tested.

2) There is evidence of a low-grade inflammatory process in all animals, probably related to their age.

3) The supplements did not produce any characteristic pathology. Such microscopic alterations as were found are consistent with the age of the animals.

DISCUSSION

With one exception, a benign lung adenoma in a mouse fed on DL-monosodium glutamate, no tumors were observed in any mouse included in the colony, either test or control. To have concluded that L-monosodium glutamate, DL-monosodium glutamate or L-glutamic acid possessed carcinogenic properties, it would have been necessary to have observed abnormal neoplastic tissue activity in a significant percentage of the total group.

The absence of specific pathological lesions other than those consistent with the advanced age of the subject mice,(which at the termination of the experimental work represented from 90-120 percent of the average life expectancy for the C-57 black mouse) makes it possible to conclude that under the conditions of this investigation the supplements fed do not possess toxic properties, and that they do not cause cumulative effects resulting in organic tissue damage when fed for almost the entire life span. The failure to find tumors, with the single exception noted, leads to a conclusion that the supplements are not carcinogenic when fed as a part of the normal dietary intake throughout life.

The majority of pathological examinations were made on a definitely aged group of animals. Because the major objective was the identification of abnormal growths of carcinogenic origin, the changes which occurred as a part of the aging process did not confuse the total picture. A secondary and somewhat adventitious objective was to observe general toxicological phenomena which might have been related to the supplements fed during the two-year period.

In an investigation of this type, certain limitations are inherent. Of these we are aware, and have given due consideration to them in evaluating our findings. The outstanding limitation is the high mortality rate,

resulting chiefly from the combative behavior of the strain of mice used. The reasonably uniform distribution of deaths at various intervals among the test groups would seem to demonstrate that deaths are less particularly related to the supplements fed than to the behavior of the C-57 black mouse. Fortunately, the number of animals used was sufficiently large so that a representative number survived to the end of the experimental work.

SUMMARY

An investigation of the potential carcinogenicity of L-monosodium glutamate, DL-monosodium glutamate and L-glutamic acid has been carried out for a two-year period using the C-57 black mouse. No tumors were authenticated with the exception of a single benign adenoma of the lung occurring in a mouse fed DL-monosodium glutamate. This diagnosis was not made during life, but was based solely on the microscopic appearance of the lung tissue. Such organic tissue changes as were observed were consistent with the advanced age of the subject mice at the termination of the experimental work. There were no significant differences between the appearance and behavior during life of test and control groups, and the appearance of the tissues after sacrifice was essentially the same for both test and control animals. It is our opinion that no evidence has been derived in the investigation reported above that any of the test materials fed for a period representing approximately 90-120 percent of the average life span of the mouse strain used, possess carcinogenic properties.

THE FREE L-GLUTAMIC ACID CONTENT OF FOODS (Report No. 2)

by

Seiichi Maeda, Sadanari Egushi and Hiroshi Sasaki*
Kasei-Gaku Zasshi (Journal of Home Economics)
12 (2) 1969? p. 105-06

*The Aji-no-moto Company, Ltd., Foodstuffs Research Laboratory

INTRODUCTION

For the purpose of making a determination of the correlation between the tast of ordinary foods in the state in which they are usually offered and free L-glutamic acid content, a microbiological quantitative analysis was made to measure the free L-glutamic acid content of 53 types of foods comprising seafood, vegetables, meats and mushrooms. A report was rendered on the fact that there is a high free L-glutamic acid content in such foods as tomatoes, arkshell, short neck clam, oysters, squid, mushrooms, dried cuttlefish and soup stock kelp.[1]

For the purpose of making additional studies of the correlation between the taste of food and the free L-glutamic acid content, 65 types of seafood, fruits and vegetables, meats, eggs, mushrooms, soup stock, etc., which had not been used for the analysis covered in the previous report[1], were analyzed. The results obtained are given in Report No. 2.

EXPERIMENTAL METHOD

1. Samples

A total of 65 types of foodstuffs, comprising 16 types of seafood, 29 types of vegetables, three types of meat, two types of eggs, 11 types of mushrooms and four types of soup stock, as shown in Tables 1 - 6 were used.

2. Preparation of the Samples

The samples were prepared as summarized in the previous report,[1] and used as samples for measuring water content, pH and free L-glutamic acid.

3. Microbiological Determination

The measurements of free L-glutamic acid in the samples prepared as described above were made by the method covered in the previous report.[1]

EXPERIMENTAL RESULTS AND DISCUSSION

Tables 1 - 6 show the numerical values for the water content, pH and free L-glumatic acid in the samples. As reported in the previous report,[1] the free L-glutamic acid content was high in those foods which have a relatively strong taste. It was also reconfirmed that the free L-glutamic acid content in mushrooms and the soup stock foods was particularly high.

SUMMARY

1. Measurements were made by microbiological quantitative analysis to determine the free L-glutamic acid content of 65 types of seafood, vegetables, meats, eggs, mushrooms and soup stock (materials that were not analyzed for the previous report[1]).

2. A particularly high free L-glutamic acid content was detected in the mushrooms and the soup stock.

In conclusion, sincere thanks are expressed to the Aji-no-moto Company, Ltd. for granting permission to publish this report and to Dr. Fumikazu Toi, the Chief of the Foodstuffs Research Laboratory for his valuable advice during the course of this study.

(Received September 13, 1960).

BIBLIOGRAPHY

1. Maeda, Eguchi, Sasaki: Kaseigaku Zassahi (Domestic Science Magazine) Vol. 9, page 163, 1958.

Table 1. The Free L-Glutamic Acid Content of Foods (Seafoods)

Sample	Water Content	pH	Free L-Glutamic Acid Content: Within the sample mg %	Free L-Glutamic Acid Content: In dry state mg %
Flounder	78.2	6.29	11	51
Yellow tail	61.5	5.79	9	23
Thread herring (*Chatoessus punctatus*)	73.2	6.50	21	79
Saury	79.6	6.40	58	284
Cod	80.6	6.48	9	46
Akaji	87.1	7.11	1	8
White Croaker	81.6	6.97	13	71
Lancelet	80.2	6.52	32	162
Conger	76.0	6.28	16	67
Mudfish	77.1	7.03	22	96
Angler	85.2	6.21	51	344
Angler liver	44.9	6.30	63	114
Octopus ocellatus	90.1	6.47	126	634
Sea urchin	68.4	6.05	192	610
Sea Cucumber	94.6	7.23	17	315
Quahog	82.7	6.37	13	87

Table 2. The Free L-Glutamic Acid Content of Foods (Vegetables)

Sample	Water Content	pH	Free L-Glutamic Acid Content: Within the sample mg %	Free L-Glutamic Acid Content: In dry state mg %
Chinese cabbage(hakusai)	95.9	5.90	100	2440
Chinese cabbage (komatsuna)	87.7	6.40	64	520
Starwort	88.7	6.20	27	239
Brassica japonica	82.9	6.12	48	271
Rape	91.8	6.55	54	660
Katsuona	90.9	6.24	28	308
Trefoil	95.7	6.18	41	950
Tetragonia expansa	92.7	6.70	33	450
Parsley	94.5	6.11	36	650
Miura radish	94.7	6.22	59	1110
Turnip	92.3	6.50	63	820
Taro (yatsugashira)	77.9	5.54	11	50
Yamato potato	73.1	5.70	81	301
Chinese yam	97.1	5.75	36	1240
Taro (Colocasia antiquorum)	80.8	6.32	10	52
Yam (Dioscorea)	84.9	5.69	26	172
Irish potato	81.0	6.20	102	538
Arrowhead	70.7	6.55	32	109
Taro stock	97.0	5.72	5	167
Horse bean	78.0	6.63	28	128
Burdock	81.7	5.90	18	99
Lotus root	87.3	5.90	56	440
Aralia cordata	95.6	5.81	21	478
Malt	93.3	5.95	20	300
Bamboo shoots (Kyoto prod)	92.2	6.01	41	526
Bamboo shoot (Tokyo prod)	81.1	6.44	39	206
Celery	93.4	5.90	32	485
Cauliflower	88.0	7.32	74	617
Broccoli	83.7	6.32	171	1050

Table 3. The Free L-Glutamic Acid Content of Foods (Meats)

Sample	Water Content	pH	Free L-Glutamic Acid Content	
			Within the sample mg %	In dry state mg %
Quail	70.1	6.35	173	580
Duck (leg)	78.0	6.05	69	314
Duck (breast)	78.0	5.86	50	227

Table 4. The Free L-Glutamic Acid Content of Foods (Eggs)

Sample	Water Content	pH	Free L-Glutamic Acid Content	
			Within the sample mg %	In dry state mg %
Chicken	73.4	7.30	23	87
Quail	71.5	7.62	35	132

Table 5. The Free L-Glutamic Acid Content of Foods (Mushrooms)

Sample	Water Content	pH	Free L-Glutamic Acid Content: Within the sample mg %	Free L-Glutamic Acid Content: In dry state mg %
Hokuro	94.5	5.70	20	360
Chichitake	99.1	5.82	24	2660
Kumatake	15.8		1400	1660
Mushroom (tea)	90.7	5.35	180	1980
Shoro	91.2	6.20	130	1480
Amashiro Tonko*	20.0	5.95	290	360
Kami Tonko	18.3	6.12	250	310
Nami Tonko	18.6	6.38	140	170
Kamikoshin	18.4	5.88	180	220
Erishita	18.2	5.93	220	270
Furuishita	17.9	6.12	160	190

*These are mushrooms that are used for seasoning in Chinese cooking.

Table 6. The Free L-Glutamic Acid Content of Foods (Soup Stocks)

Sample	Water Content	pH	Free L-Glutamic Acid Content: Within the sample mg %	Free L-Glutamic Acid Content: In dry state mg %
Scallop	11.7		340	380
Prime kelp	11.0		1100	1240
Common kelp	16.6		1600	1920
Riijiri kelp	11.1		2240	2520

Glutamate-Induced Hyperglycemia.* (31899)

ROBERT MARCUS AND GERALD REAVEN (Introduced by David Rytand)
Department of Medicine, Stanford University School of Medicine, Palo Alto, Calif.

During the course of other investigations glutamic acid was noted to produce significant hyperglycemia in normal rats. Further studies have been conducted in an effort to define the mechanism of this unexpected response, and the results are presented here.

Materials and methods. Male Sprague-Dawley rats, weighing between 200 and 300 g, were used in all experiments, and assigned to groups with the aid of a table of random numbers. Experiments were generally performed after an overnight fast (14 hours), but some studies were carried out following 48 hours of fasting. Test substances were prepared as 0.15 molar aqueous solutions, and injected intraperitoneally. Blood was obtained from non-anesthetized animals by cardiac puncture either 30 or 60 minutes after administration of the test substances. Glucose concentration was determined by the glucose-oxidase method (Glucostat, Worthington Biochemical Corp.).

Adrenalectomized animals were maintained on standard laboratory rat chow, but their drinking water was replaced with 0.9% sodium chloride. They were used on the fourth post-operative day. Spontaneous ACTH activity was suppressed by administration of dexamethasone, 25 μg per 100 g body weight, 2 hours prior to injection of test substances. This dose has been found to inhibit the corticotrophin-releasing response to non-specific stress(1).

Pyruvate-2-C^{14}, alanine-U-C^{14}, and glutamate-3,4-C^{14}, obtained from New England Nuclear Corp., were added to non-labeled solutions of pyruvate, alanine and glutamate. The test solutions were injected intraperitoneally into rats fasted 14 hours. Each rat received 0.4 moles of "cold" and 2 μc of tracer amounts of C^{14}-labeled substrate/100 g body weight. Blood was obtained 30 minutes after injection. Plasma was separated into 2 aliquots: one used to measure plasma glucose concentration (glucose oxidase) and the other to isolate glucose by thin layer chromatography(2). The glucose spot was scraped off the plate, and eluted; the eluate was divided into 2 aliquots: one aliquot was used for determination of radioactivity (Packard Liquid Scintillation Counter), and the other for glucose measurement (glucose oxidase).

Results and discussion. The degree of hyperglycemia produced by glutamate is seen in Table I. Elevation of plasma glucose concentration induced by glutamate was significantly greater (t-test) than that following equimolar amounts of alanine at 30 and 60 minutes or glucose 60 minutes after injection (P in all cases was <0.01).

Effects of increasing amounts of glutamate on plasma glucose concentration at 60 minutes are seen in Fig. 1. Although 0.05 mmoles/100 g body weight produced a small elevation of plasma glucose, significant hyperglycemia was not achieved until the dose reached 0.1 mmoles/100 g body weight. Maximum hyperglycemia was reached with 0.2 mmoles/100 g body weight, and doubling this dose did not lead to any significant further elevation. Detailed analysis of the glutamate dose response curve made it seem unlikely that the rise in plasma glucose concentration was solely due to conversion of glutamate to glucose. Plasma glucose concentration increased from 88 to 173 mg%, an increment of 85 mg%, when the dose of glutamate was raised from 0.01 to 0.20 mmoles/100 g body weight. The additional amount of glutamate responsible for this rise in plasma glucose would be equal to 0.456 mmoles in a 240 g rat (0.19 mmoles/100 g body weight $\times$ 2.4). Since 1 mole of glutamate is converted to 1 mole of phosphoenol-pyruvate, and 2 moles of the latter are required for 1 mole of glucose, the additional glutamate could produce, at most, 0.228 mmoles of glucose. If glutamate was entirely converted to glucose, and if none of this newly formed glucose was utilized, the net increment in total body glucose would

*This work was supported by Research Grant HE. 08506 from Nat. Inst. Health.

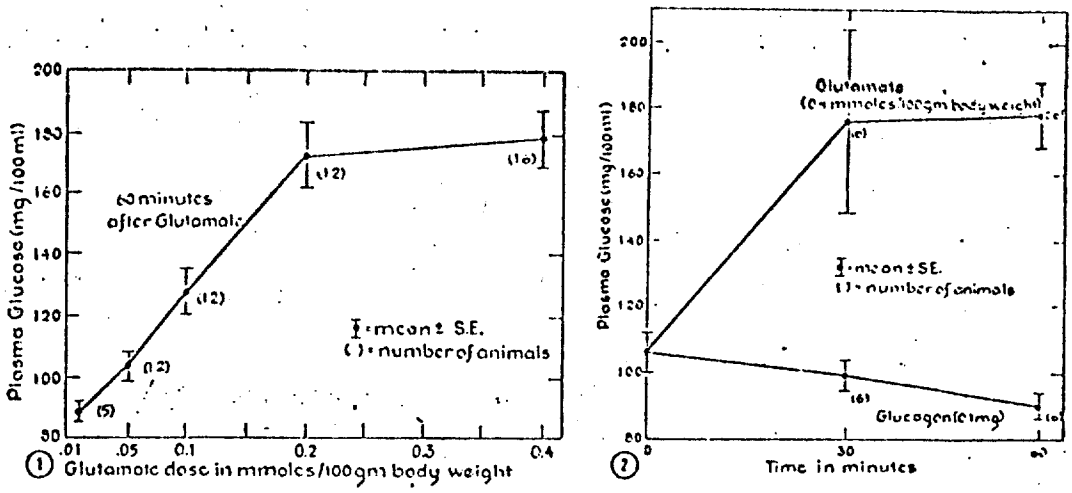


Fig. 1. Response of plasma glucose concentration to administration of increasing amounts of glutamate.

Fig. 2. Comparison of glucagon and glutamate adminstration on plasma glucose concentration of rats fasted for 14 hr.

equal 41 mg (0.228 mmoles × 180 mg/mmole). As stated above, the effect of the additional 0.19 mmoles of glutamate was to increase plasma glucose concentration 85 mg%. If one assumes that glucose is confined to the extracellular space, and that its volume of distribution approximates 25% of body weight, *i.e.*, 60 ml in a 240 g rat, the net increase in total body glucose produced by the additional 0.19 mmoles per 100 g body weight would be 51 mg (0.85 mg/ml × 60 ml), or 10 mg more than could be accounted for by the quantitative conversion of glutamate to glucose and complete inhibition of glucose removal. Consequently, the possibility that glutamate produced hyperglycemia solely by its conversion to glucose seemed unlikely.

The possibility that glutamate produces hyperglycemia by conversion to glucose was further studied by measuring the relative incorporation of several C^{14}-labeled substrates into glucose. Rats fasted 14 hours received i.p. injections of either alanine, pyruvate or glutamate solutions, to which C^{14}-labeled tracer amounts of the same substrate had been added. The percent of administered isotope present as glucose 30 minutes later was determined, and results are seen in Table II. The amount of radioactivity in glucose in animals receiving C^{14} glutamate was not greater than that following administration of C^{14} alanine or pyruvate.

TABLE I. Response of Plasma Glucose Concentration to Intraperitoneal Administration of Glucose, Glutamate and Alanine (.4 mmole/100 g Body Weight). Fasting plasma glucose concentration = 102 ± 3.2 (8).

Substrate	Plasma glucose (mg % ± SE) 30 minutes	60 minutes
Glucose	164 ± 7.2 (9)	133 ± 4.1 (16)
L-glutamate	176 ± 28.0 (6)	178 ± 9.2 (16)
L-alanine	121 ± 7.8 (6)	130 ± 4.2 (9)

( ) = No. of animals.

These results suggested that glutamate-induced hyperglycemia most likely resulted from glycogenolysis or gluconeogenesis. The possibility that glutamate produced hyperglycemia by stimulating glycogenolysis was investigated next by comparing the effects of glutamate with those of a large dose of glucagon (0.1 mg) in rats fasted 14 hours. The results are seen in Fig. 2. A maximal dose of glucagon did not increase plasma glucose in these rats, suggesting that glycogen stores are severely depleted in a 14-hour fasted rat. Since the effect of glutamate was still evident, it seems unlikely that glutamate acts by increasing conversion of glycogen to glucose.

The possibility that glutamate-induced hyperglycemia results from stimulation of the pituitary-adrenal axis was then examined. Results are seen in Table III, and indicate that adrenalectomy abolished the hyperglycemic

TABLE II. Incorporation of Labeled Precursors into Glucose.

Isotope injected	Non-labeled compound injected (.4 mmole/100 g)	No. of animals	% Isotope in glucose 30 min after injection (mean ± SE)*
Alanine-U-C^{14}	Alanine	4	8.7 ± .54
Pyruvate-2-C^{14}	Pyruvate	4	6.8 ± 1.07
Glutamate-3,4-C^{14}	Glutamate	4	6.1 ± 1.02

* Calculated on basis of plasma glucose concentration, plasma glucose specific activity, and assumption that glucose space is 25% of body wt.

TABLE III. Effect of Adrenalectomy and ACTH Suppression on Plasma Glucose Response to Glutamate (.4 mmole/100 g Body Weight) in Rats Fasted 14 Hours.

Experimental condition	No. of animals	Elevation of plasma glucose (mg %),* mean ± SE
Control	12	64 ± 9
Adrenalectomy	6	6 ± 4
Dexamethasone (25 μg/100 g body wt)	6	58 ± 11

* Rise in plasma glucose 60 min after receiving glutamate.

by increasing new glucose production, and suggest that this may result from stimulation of gluconeogenesis. The possibility that this action may be related to the recent suggestion (3) that gluconeogenesis is dependent upon transport of 4-carbon fragments across the mitochondrial membrane is currently being investigated.

Summary. Plasma glucose rose linearly with increasing amounts of glutamate up to a maximal dose of 0.2 mmoles/100 g body weight. The glutamate effect could not be explained by stoichiometric conversion of glutamate to glucose. Glutamate produced hyperglycemia after 14-hour fast, at which time glucagon had no effect on glucose concentration. Adrenalectomy abolished the glutamate effect; ACTH suppression with dexamethasone did not. Glutamate supplementation increased the plasma glucose response to pyruvate in excess of the additive effects of either substrate alone. It is suggested that glutamate produces hyperglycemia by stimulating new glucose production, and that this most likely results from increased gluconeogenesis.

TABLE IV. Effect of Glutamate Supplementation on Plasma Glucose Response to Pyruvate Administration.

Group	No. of animals	Elevation of plasma glucose (mg %),* mean ± SE
Pyruvate (.2 mmole/100 g body wt)	14	9 ± 3
Glutamate (.2 mmole/100 g body wt)	21	32 ± 6
Pyruvate (.2 mmole) + glutamate (.2 mmole/100 g body wt)	28	72 ± 8

* Rise in plasma glucose 60 min after injection.

effect of glutamate. Suppression of ACTH activity did not alter the glutamate effect. These results indicate that although intact adrenals are necessary for glutamate to produce hyperglycemia, acute stimulation of the pituitary-adrenal axis is not the cause of this phenomenon.

Finally, an attempt was made to demonstrate directly that glutamate stimulates gluconeogenesis. These results are seen in Table IV. Pyruvate administration to rats fasted 48 hours produced a slight increase in plasma glucose concentration. The concomitant administration of pyruvate and glutamate markedly increased plasma glucose concentration, and this rise was greater than could be accounted for by the cumulative effects of pyruvate plus glutamate (t-test, $P<.02$). These results further support the thesis that glutamate produces hyperglycemia

1. Kendall, J. W., Jr., Endocrinology, 1962, v71, 452.

2. Nikkilä, E. A., Ojala, K., Life Sci., 1964, v3, 243.

3. Lardy, H. A., Paetkau, V., Walker, P., Proc. Nat. Acad. Sci., 1965, v53, 1410.

Received November 18, 1966. P.S.E.B.M., 1967, v124.

OBESITY INDUCED BY MONOSODIUM GLUTAMATE IN MICE

SHIGERU MATSUYAMA,[1] YOSHIO OKI[2] and YUITSU YOKOKI[1]

(Received for publication November 22, 1972)

In newborn mice subcutaneous injections of monosodium glutamate (MSG) induced acute pyknosis of nuclei in the regions around the basal parts of the third ventricle of the hypothalamus. The effective doses used were more than 1.0 mg of MSG per gram of body weight. Newborn mice which had received a single dose or a series of injections of MSG showed a syndrome of obesity after maturation. This syndrome was most remarkable in a group treated with a single injection of 4.0 mg/g of MSG. A single treatment with 2.0 mg/g of MSG had almost the same effect as a serial treatment with 5 doses of 2.0 mg/g each given every other day by the subcutaneous route. No obese animals were observed in a group injected with 0.5 mg/g five time every other day. Animals grown up to be obese showed no remarkable changes in the reproductive system or in the sexual cycles of the female.

In newborn rats, MSG was injected subcutaneously, but no morphological lesions were noticeable in the brain after this treatment. In adult rats, the spontaneous multi-unit activities of arcuate nuclei were observed before and after MSG treatment. No remarkable changes were seen throughout the observation period.

Preliminary observations made by Camus & Roussy[11] in 1920 and by Bailey & Bremer[5] in 1921 suggested that extreme obesity might be due to hypothalamic lesions. There have been many reports dealing with both obesity and the central nervous system.

Olney[29] reported that in newborn mice a single or serial subcutaneous injection of monosodium glutamate (MSG) induce acute neuronal necrosis in several areas of the brain and finally the development of obesity. He pointed out that the preoptic and arcuate nuclei of the hypothalamus were selectively destroyed, together with scattered neurons within the median eminence, but that no acute changes occurred to any other hypothalamic area or the pituitary.

Hetherington & Ranson[21] observed more than 30 years ago that electrolytic destruction of the ventromedial portion of the hypothalamus led to obesity in rats. Since that time, the role of this portion of the brain (satiety center) in the control of food intake has been the subject of numerous investigations. It is well-known that destruction of the ventromedial area of the hypothalamus by electrolytic[2,4,20,21,28] or mechanic[22,24,28] means or by chemical administration[8,9,14,18,25,27,31,33] leads to obesity in mice and rats. Olney,[29] however, showed complete destruction of the arcuate nuclei of the hypothalamus and the unaffected state of the ventromedial nuclei following obesity.

It is also well-known that the hypothalamus is the most important region that controls the secretion of gonadotropins. It seems that the destruction of the preoptic and arcuate nuclei and median eminence

マウスにおけるグルタミン酸モノナトリウムによる肥満症
1 松山 茂，横木勇逸： Third Research Division, National Institute of Animal Health, Kodaira, Tokyo, 187 Japan
2 大木与志雄： Fourth Research Division of the same institute.

may be most effective to disturb the reproductive function in female and male mammals.[6,7,13,16,23,26]

This report deals with obesity induced by MSG in mice and changes in the reproductive function of mice and rats after a single or serial injections of MSG in the neonatal stage.

MATERIALS AND METHODS

Experiment 1: A total of 136 mice of 13 litters of the ICR strain were used in this experiment. Within 24 hours after birth, the young of the same litter were divided into two groups, male and female. Half the animals of each sex group were treated with MSG in the following manner.

(1) A single subcutaneous injection of 4 mg per gram of body weight of MSG within 24 hours after birth.

(2) A single subcutaneous injection of 2.0 mg/g of MSG within 24 hours after birth.

(3) Serial subcutaneous injections of 2.0 mg/g of MSG every other day from 1 to 9 days of age.

(4) Serial subcutaneous injections of 0.5 mg/g of MSG every other day from 1 to 9 days of age.

The other half of the animals of each sex group were not treated to serve as controls. Uniform litter mates were kept with their mother in each cage placed in a room under controlled environment until weaning. After weaning, males and females were separated from each other. The treated and control mice of the same sex and of the same litter were kept in the same cage, fed Laboratory Foods, and given tap water freely. All the animals were bred in the same conditions and weighed individually every week over a period from the second to the 40th week or later.

At about 14 weeks of age, 13 female mice were used for checking the sexual cycle. Of them, seven had been chosen from among those treated with 4.0 mg/g or 5 doses of 2.0 mg/g each of MSG and presenting remarkable obesity, and six from among those treated with 5 doses of 0.5 mg/g each of MSG and showing no obesity and from among the controls. Vaginal smears were prepared from all the mice and observed every day for 16 days.

At 330 days of age, some of these mice were sacrificed for macroscopic and microscopic examinations.

Experiment 2: For the observation of acute neuronal changes in several areas of the brain, more than 40 newborn mice of the ICR strain were used. All the animals, 1 or 2 days old, were killed some time between 1 and 48 hours after a single subcutaneous injection of MSG (dosage varying from 0.1 to 4.0 mg/g). The brains were fixed in 10 per cent buffered formalin and embedded in paraffin. They were sectioned transversely, stained with hematoxylin and eosin, and examined by light microscopy.

Experiment 3: For the study of acute brain damage, male and female rats of the Wistar-Imamichi strain, 1 to 2 days old (supplied by the Dobutsu Hanshoku Kenkyusho, Inc.), received a single subcutaneous injection of MSG. The dose of MSG injected was 0.1 ml. The total dose was 4.0 mg per gram of body weight. Control rats were injected with almost the same volume of saline. All the animals were killed 1 to 3 hours post-treatment. Brains were fixed in 10 per cent buffered formalin, sectioned transversely, and stained with hematoxylin and eosin for light microscopy.

Experiment 4: To study a possibility for glutamate treatment in the neonatal stage to destroy the arcuate nuclei and disturb the reproductive function in the female rat, almost the same procedures were applied to newborn rats. Two litters of rats of the Wistar-Imamichi strain were used. Three female rats of the same litter received subcutaneous injection of MSG (1.0 mg/g) daily over a period from 1 to 5 days after birth. Four female rats from another litter received 2.0 mg/g of MSG by the same method. On and after the 51st day of age, vaginal smears were observed in all these animals every day for more than 40 days.

Experiment 5: To study the acute effect of large doses of MSG on the brain activity of adult rats, an experiment was carried out with four male rats of the Wistar-Imamichi strain, weighing about 250 g. The animals were anesthetized with urethane before surgical operation, in which parts of the cranical bone and dura mater were removed to allow the insertion of recording micro-electrodes. The microelectrodes consisted of stainless steel insect pins (size No. 0) coated with Epico 1000 (Nippon Yushi Co., Ltd.), except at the tip which was less than 5 μ in diameter.

Spontaneous multi-unit discharges of the arcuate nuclei were observed by a dual beam oscilloscope (Nihon Kohden, Type VC 7). Electrodes were oriented in such manner as indicated in the stereo-taxic atlas of De Groot.[15]

After the recording of unit discharges for more than 1 hour as control period, all the animals were injected intraperitoneally with 4.0 mg/g of MSG, except one which was slowly given MSG through the cannul inserted into the femoral vein. Successive injection with more than 4.0 mg/g of MSG was performed on the former three animals 30 minutes after the first treatment.

In this experiment, all the procedures and observations were carried out in an air conditioned room. The t-test was used for statistical analysis of all the data obtained.

RESULTS

Experiment 1: Growth records for the treated and untreated control animals are shown in Figs. 1 and 2, respectively. There was no difference in body weight between the treated and control animals until seven weeks of age. The treated animals which had received a single subcutaneous injection with 4.0 or 2.0 mg/g of MSG and five serial injections with 2.0 mg/g each of MSG surpassed the control animals and those treated with five doses of 0.5 mg/g each of

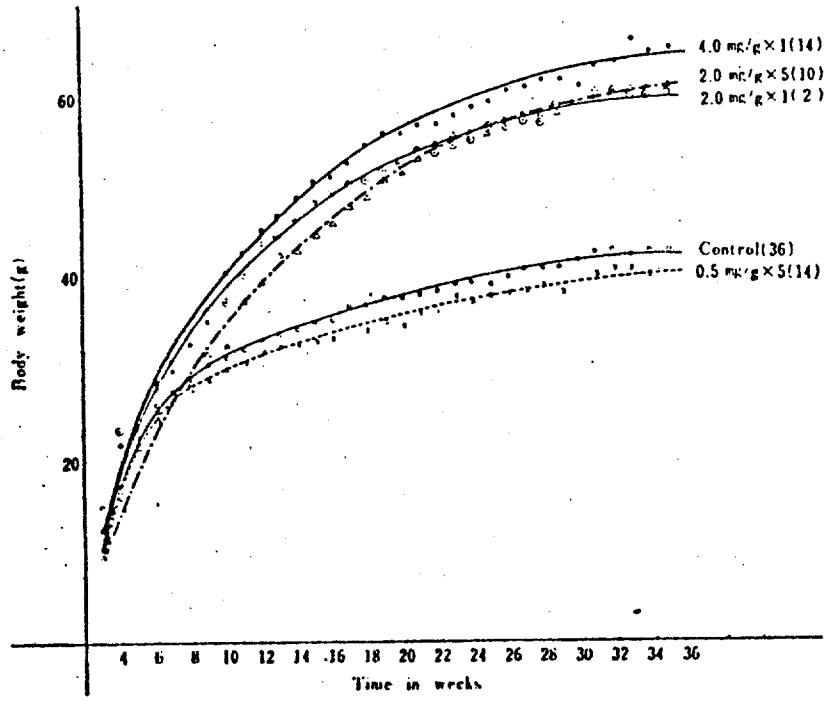


Fig. 1. Growth records covering the third to 36th week of age for experimental and control female mice after a subcutaneous injection of monosodium glutamate. Each symbol represents a mean. The number of animals used is shown in parentheses.

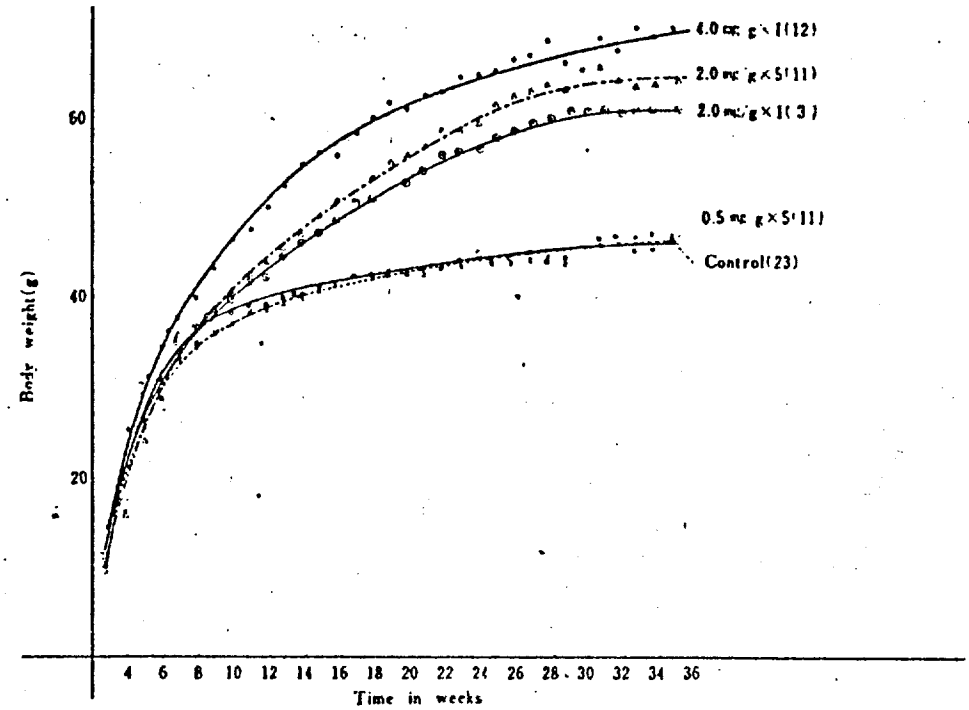


Fig. 2. Growth records covering the third to 36th week of age for experimental and control male mice after a subcutaneous injection of monosodium glutamate. Each symbol represents a mean. The number of animals used is shown in parentheses.

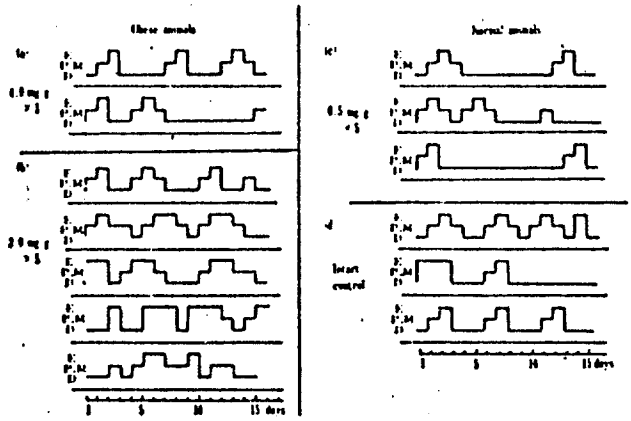


Fig. 3. Estrous cycle in obese and non-obese mice. E: estrous stage P.M.: proestrous or metestrous stage D: diestrous stage
(a) Animals treated with a single subcutaneous injection of 4.0 mg/g of monosodium glutamate (MSG) within 24 hours after birth.
(b) Animals treated with serial subcutaneous injections of 2.0 mg/g of MSG every other day from 1 to 9 days of age.
(c) Animals treated with serial subcutaneous injections of 0.5 mg/g of MSG every other day from 1 to 9 days of age.

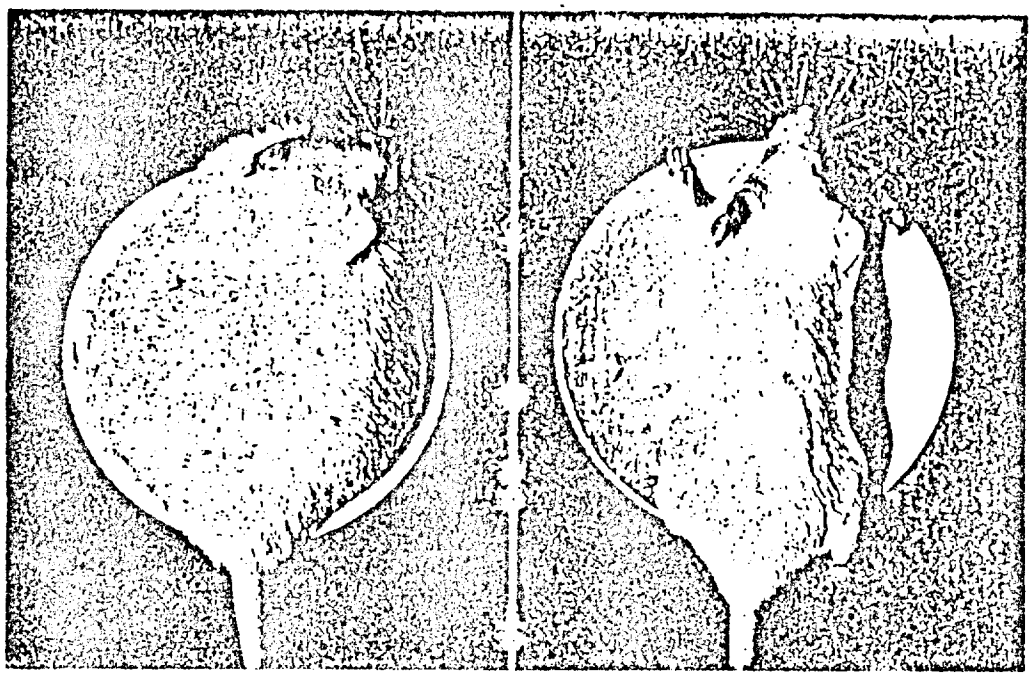


Fig. 4. A 12-month-old male mouse of ICR strain which was treated with a single subcutaneous injection of 4.0 mg/g of monosodium glutamate, in the neonatal stage is shown (left) beside the control (right). It weighs 100 g, while the control does 44 g.

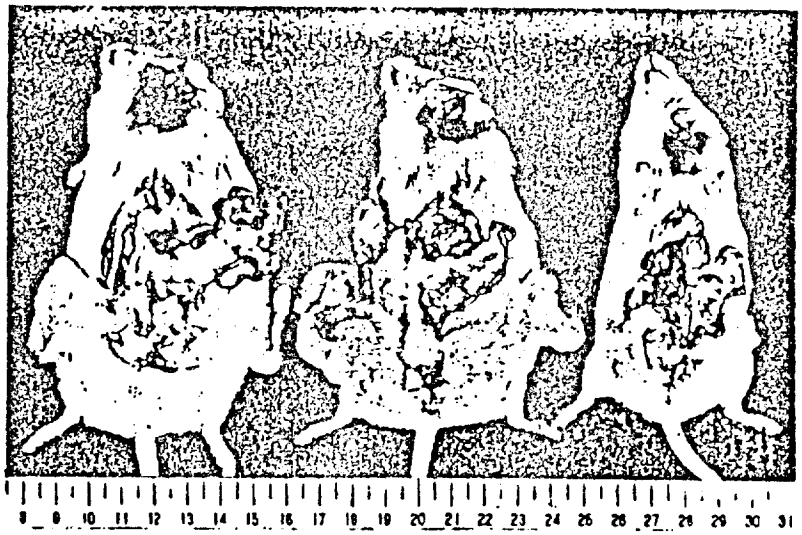


Fig. 5. A view of the mesenteries of mice of the same litter autopsied at 11 months of age. Right: A female not treated (control). - Middle: A female treated with monosodium glutamate (obesity). Left: A male treated with monosodium glutamate (obesity).

MSG in body weight at about 8 or 9 weeks of age. The treated animals, except those treated with 5 doses of 0.5 mg/g each of MSG, continued to gain weight after this period. Then the difference in body weight became greater and greater. Although these

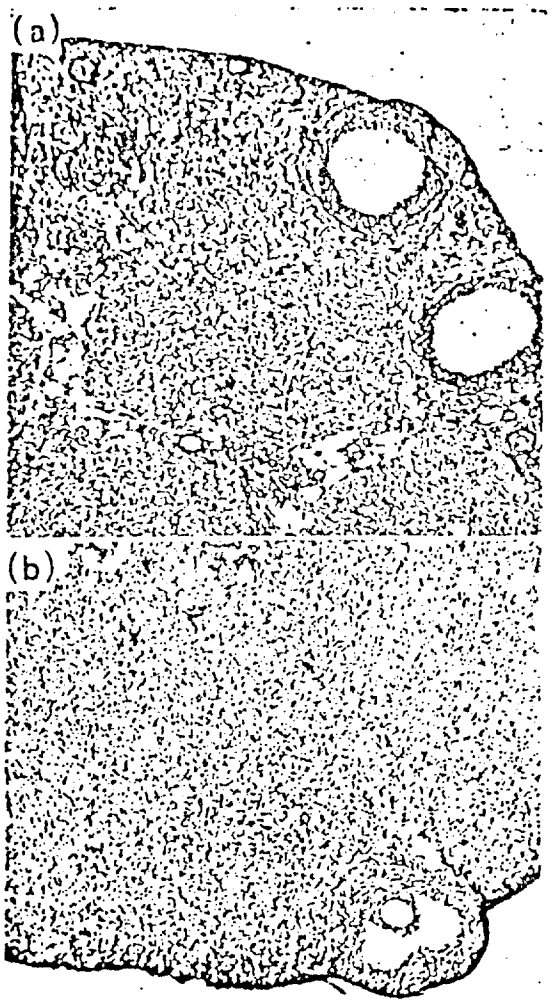


Fig. 6. Ovaries of an obese experimental mouse (a) and a non-obese control mouse (b). They contain follicles and corpora lutea. Hematoxylin and eosin (HE) staining, ×40.

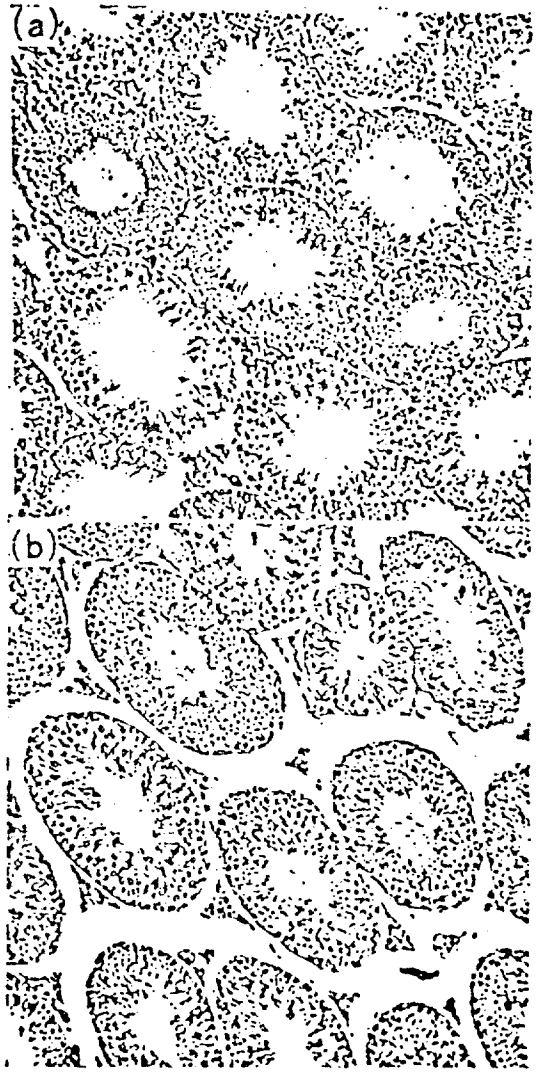


Fig. 7. Testes of an obese experimental mouse (a) and a non-obese control mouse (b). They show normal spermatogenesis. HE staining, ×80.

obese animals were heavier than normal control ones, they were almost the same in body length as the control ones. The fur coat of the animals which showed obesity seemed to bristle up and lost its sleekness.

Fig. 3 shows the sexual cycle of female mice which were obese or not obese. So far as the observation of vaginal smears was concerned, all the animals, including intact controls, showed no completely regular cycle. The two groups consisting of obese and normal animals, respectively, showed cyclic changes in their vaginal smears.

The heaviest body weight in this experiment was recorded in the group having received a single injection with 4.0 mg/g of MSG. A male mouse of this group weighed over 100 g at 385 days of age (Fig. 4). He was plump and a ball in shape. His fur coat was not so sleek as that of the control.

Autopsy was performed at 11 months of age. Invariably, the fat animals had enormous accumulations of adipose tissue in the

Table 1. *Effect of monosodium glutamate (MSG) injection in newborn mice on adult organ weights*

Group	Number of mice	Body weight (g)	Liver (g)	Kidneys (mg)	Spleen (mg)	Heart (mg)	Pancreas (mg)	Adipose tissue in abdomen (g)	Fur coat (except head) (g)	Adrenals (mg)
					Females					
Control	5	47.8± 6.5	2.3±0.6	526±31	131±19	166± 9	270±28	4.1±1.4	7.0±1.0	11.4±1.1
Obese animals (MSG treated)	5	68.2±11.3*1	2.4±0.2	496±45	121± 7	158± 6	207±13*1	11.7±1.6*3	10.3±1.3*1	11.2±0.8
					Males					
Control	6	42.2± 2.3	2.4±0.2	755±29	124±19	219±11	285±27	0.9±0.4	5.5±0.2	5.5±0.6
Obese animals (MSG treated)	6	64.9± 5.5*1	3.6±0.4	752±50	148± 9	203±19	347±23*1	3.9±0.8*3	12.1±0.5*3	6.6±0.5

The value given is the mean ± standard error of the mean.

*1 Difference from the value for the control group is significant ($P<0.05$).

*2 Difference from the value for the control group is significant ($P<0.01$).

*3 Difference from the value for the control group is significant ($P<0.001$).

Table 2. *Effect of monosodium glutamate (MSG) injection in newborn mice on adult reproductive organ weights*

Group	Females			Males				
	Number of mice	Ovaries (mg)	Uterus (mg)	Number of mice	Testes (mg)	Epidi-dymides (mg)	Seminal vesicles and coagulating glands (mg)	Prostates (mg)
Control	5	31.2±4.5	266±57	6	247±28	121± 7	371±37	24.3±4.5
Obese animals (MSG treated)	5	26.8±6.8	253±51	6	251±26	122±13	422±70	28.0±2.3

The value given is the mean ± standard error of the mean.

abdomen. In the mesenteries around the kidneys and along the aorta, the deposition of fat was so marked that it was sometimes difficult to find and excise the adrenals. This change was more remarkable in female than in male animals (Fig. 5).

In the obese animals, the liver underwent fatty degeneration, which was not so serious, and it was a little heavier than that of the control (Table 1). No significant differences were observed in the weight of liver, kidney, spleen, heart, adrenal, uterus, testis, epididymis, seminal vesicle (including the coagulating glands), or prostate between the obese animals and the controls (Tables 1 and 2). In both groups, the ovaries had follicles and corpora lutea (Fig. 6). In the obese animals, normal spermatogenesis was seen in the testes (Fig. 7) and highly motile sperms could be recognized in the epididymides. Most of the organs of the obese animals were indistinguishable from the counterparts of the controls in the histological examination. The pancreas of the female obese animals was significantly lighter than that of the controls at $p<0.05$. On the contrary, the pancreas of the male obese animals was heavier than that of the controls. The obese animals had significantly heavier

Table 3. *Appearance of pyknotic nuclei in the hypothalamus of newborn mice after a subcutaneous injection of monosodium glutamate (MSG)*

Dose of MSG	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr
4.0 mg/g	±	+	+	+	+	+
2.0 mg/g	±	+	+	+		+
1.0 mg/g	—			±		
0.5 mg/g	—			—		
0.25 mg/g	—			—		
0.1 mg/g	—			—		
0.0 mg/g	—			—		

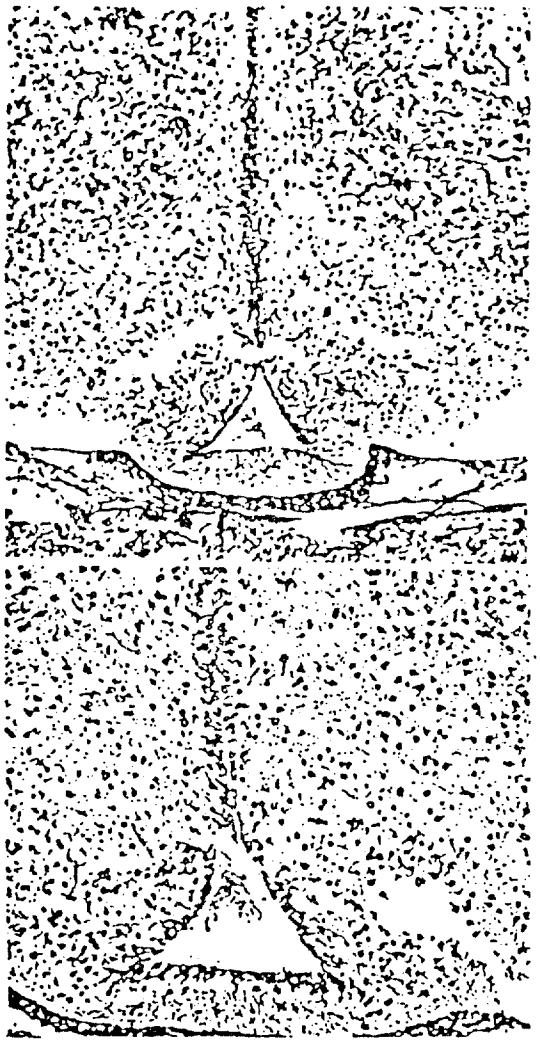


Fig. 8. Sections through the hypothalamus of a 1-day-old mouse showing a lesion 1 hour after (upper, ×80), and 2 hours after (lower, ×120), a subcutaneous injection of 2 mg/g of monosodium glutamate. Pyknotic nuclei can be seen around the third ventricle. HE staining.

adipose tissue in the abdomen and the body coat than the controls.

Experiment 2: Pyknotic nuclei could be seen in the hypothalamus of newborn mice which had received a single injection with 2.0 or 4.0 mg/g of MSG (Fig. 8 and Table 3). This change appeared 1 hour after the treatment. In animals which had received 1.0 mg/g of MSG, a pyknotic tendency could be recognized 8 hours after the administra-

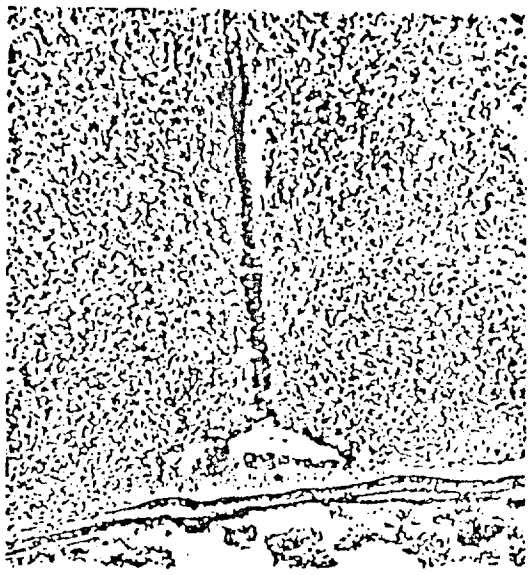


Fig. 9. Section through the hypothalamus of a 1-day-old rat 3 hours after a single injection of 4.0 mg/g of monosodium glutamate. No morphological lesions are seen in the brain. HE staining, ×80.

tion. Pyknotic nuclei were scattered mainly in the arcuate nuclei and median eminence. Some of them were seen in a part of the ventromedial nuclei of the hypothalamus. No animals which had been injected with less than 0.5 mg/g of MSG were affected to such extent as to show pyknotic nuclei in the hypothalamus.

Experiment 3: In newborn rats killed 1 to 3 hours after a single injection with MSG, light microscopic examination of the brain failed to reveal any remarkable change in the preoptic nuclei, arcuate nuclei, ventromedial nuclei, or median eminence (Fig. 9).

Experiment 4: In all the experimental rats, except two, vaginal smears were examined on and after the 51st day of age. These two rats had been treated with 5 doses of 2.0 mg/g each of MSG, but their closed vagina made it impossible to collect vaginal smears. One of those two animals opened her vagina on the 58th day of age and the other did not allow to check her sexual cycle until the 81st day of age. None

of the treated animals, including those which showed the late start of the sexual cycle, exhibited a long suspension of the cycle.

Experiment 5: Spontaneous multi-unit discharges of the arcuate nuclei showed quite irregular activities in adult rats during the control period before MSG-treatment. In all the animals, about 10~500 pulses per 10 secondes were recorded during the control period. Within 2 hours after the treatment, the multi-unit activities of the arcuate nuclei of the hypothalamus did not exhibit any remarkable change. Only one animal showed a decrease in discharge 3 hours after the treatment. In it the mean firing rate during this period was almost one-third of that during the control period. This animal died within 4 hours after the treatment.

DISCUSSION

At the beginning of the 1940's, Hetherington & Ranson[21] demonstrated that obesity was regularly followed by bilateral electrolytic destruction of the ventromedial nuclei of the hypothalamus in the rat. Brobeck and his colleagues[10] soon replicated these observations and concluded that the obesity could be attributed almost entirely to an increased food intake. It is easy to understand that when energy intake consistently exceeds energy expenditure, the inevitable result is obesity.

On the contrary, Olney[29] pointed out that the obesity caused by MSG treatment had a characteristic of less food consumption, and that the areas of neuronal necrosis by this method were completely different from the so-called satiety center. According to him, acute brain damage was found in a newborn mouse, rat, and monkey[28,30] after a single administration of MSG, and the lesion affected areas around the preoptic and arcuate nuclei and median eminence. Generally, the destruction of these areas of the hypothalamus seriously affects the reproductive function.[13,1[illegible],26] In this investigation, the authors were interested in the relationship between the brain damage caused by MSG treatment and the reproductive function.

The authors could duplicate almost the same damage on the brain in newborn mice. The majority of neurons in the arcuate nuclei of the hypothalamus, and neurons scattered around the third ventricle were necrotic. Their nuclei were pyknotic. The dose of MSG proved to be effective for treatment was a little different from that reported by Olney,[29] who demonstrated that neuronal necrosis had developed within a few hours after treatment with a dose of 0.5 mg/g. In the present experiment, pyknotic nuclei could be seen after treatment with a dose of over 1.0 mg/g. No brain lesions were induced by treatment with doses of less than 0.5 mg/g each. It could not be concluded that a complete destruction might have occurred to these region. It was recognized that the majority of neurons of the ventromedial nuclei were free from changes, but it was very difficult to determine whether the neurons in the outer zone of the ventromedial nuclei were affected or not. These results coincided with those of observation on growth record in mice for a long period after the glutamate treatment. There was no significant difference in the growth curve between the controls and mice which had been given 0.5 mg/g of MSG five times. Mice which had received a single injection with 2.0 mg/g of MSG showed the remarkable features of obesity in spite of the dose of administration, which was less than a total of 5 doses of 0.5 mg/g each in the preceding mice. A group of mice which had received a single injection with 4.0 mg/g of MSG surpassed a group of mice which had been given a serial treatment with 5 doses of 2.0 mg/g each in body weight. These facts indicate that the brain lesions which may induce obesity depend not only upon the dose of administration but also upon the time of treatment.

Obese animals showed a tremendous accumulation of adipose tissue, although there was a marked sex difference. Even when body weight was the same, female mice had significantly much more fat pads in the

abdomen than male mice (Fig. 5, Tables 1 and 3). This result suggests that the deposition of adipose tissue in this experiment may also be under the control of sexual hormone. The pancreas was lighter in obese female mice than in the controls. On the other hand, it was heavier in obese male mice than in the controls. This discrepancy is hard to explain.

No data on food consumption were recorded consistently in the present experiment, but it seemed that obese animals might have consumed a little more food than the controls after 20 weeks of age in this experiment.

Like Adamo & Patner,[1] the present authors failed to duplicate acute brain damage in rats. Since the experimental approach used in rats was almost the same as that used in mice, it is very difficult to determine the cause of this difference. One of the factors for determination may be the time when the blood brain barriers were accomplished.

The female mice and rats which had been treated with MSG in the neonatal stage showed cyclic changes in their vaginal smears. Especially, obese mice in which the brain might have been damaged in the neonatal stage showed almost the same estrous cycle as the normal controls. There were no macro- or microscopical differences in the female or male reproductive organs between obese mice and the normal controls. These facts indicate that the regions of the arcuate nuclei and median eminence were not completely destroyed, because the destruction of these regions causes serious disturbances in the reproductive function of mammals. Since no observation on the reproductive capacity of obese animals was attempted in the present experiment, it could not be determined whether these animals were sterile or not. It seemed, however, that it might hardly be possible for them to take a mating behavior because of their plump bodies. Then, not only a mating test but also a method of artificial insemination should be employed for discussing their breeding ability.

Olney[29] reported that when an adult mouse was treated with a large dose (6 mg/g) of MSG, the arcuate nuclei of its hypothalamus were completely destroyed along with the neuronal constituents of the median eminence. In adult rats, however, no remarkable change was observed in hypothalamic activities after treatment with a large dose (>4 mg/g) of MSG by the intraperitoneal or intravenous route. Spontaneous multi-unit activities of the arcuate nuclei were quite different from one individual to another, but the number of discharges before the treatment was little different from that after the treatment in each case. This suggests that the large dose of MSG may have had no acute influence on the activities of the hypothalamus in adult rats. It is difficult to clarify the cause of the discrepancy between the results obtained from adult rats in this experiment and those obtained from adult mice by Olney.[29] The same question can be raised about the effect of MSG on neonatal mice and rats. A possibility exists, however, that these differences may be due to the variation in species susceptibility. Anyway, it is true that a single treatment with MSG induces marked obesity in newborn mice. It seems that the cause of this obesity may be different from two other chemical compounds, gold thioglucose and bipiperidyle mustard, which destroy the ventromedial nucleus ("satiety center") of the hypothalamus. The obesity caused by the MSG-treatment is much milder than that by these two chemical compounds, which induce obesity after hovering between life and death.

ACKNOWLEDGMENTS

The authors thank Miss Toshiko MIYAZAKI for her excellent technical assistance.

LITERATURE CITED

1) Adamo,N.J. & Patner,A.: Monosodium glutamate: Lack of effects on brain and reproductive function in rats. *Science* 169, 673-674 (1970).
2) Anand,B.K. & Brobeck,J.R.: Hypothalamic

control of food intake in rats and cats. *Yale J. Biol. Med.* 24, 123-140 (1951).

3) Anand, B.K.: The nervous regulation of food intake. *Physiol. Rev.* 41, 677-708 (1961).

4) Averill,R.L.W. & Purves,H.D.: Differential effects of permanent hypothalamic lesions on reproduction and lactation in rats. *J. Endocrinol.* 26, 463-477 (1963).

5) Bailey, P. & Bremer, F.: Experimental diabetes insipidus. *Arch. Intern. Med.* 28, 773-803 (1921).

6) Bogdanove, E.M. & Halmi, N.S.: Effects of hypothalamic lesion and subsequent propylthiouracil treatment on pituitary structure and function in the rat. *Endocrinology* 53, 274-292 (1953).

7) Bogdanove,E.M.: Selectivity of the effects of hypothalamic lesions on pituitary-tropic hormone secretion in the rat. *Endocrinology* 60, 689-697 (1957).

8) Brecher,G. & Waxler,S.H.: Obesity in albino mice due to single injection of gold thioglucose. *Proc. Soc. Exp. Biol. Med.* 70, 498-501 (1949).

9) Brecher,G. et al.: The brain lesion of gold thioglucose obesity. *J. Exp. Med.* 121, 395-401 (1965).

10) Brobeck,J.R., Tepperman,J. & Long,C.N.H.: Experimental hypothalamic hyperphagia in the albino rat. *Yale J.Biol.Med.* 15, 831-853 (1943).

11) Camus,J. & Roussy,G.: Experimental researches on the pituitary body. Diabetes insipidus, glycosuria and those dystrophies considered as hypophyseal in origin. *Endocrinology* 4, 507-522 (1920).

12) Corbit,J.D. & Atellar,E.: Palatability, food intake, and obesity in normal and hyperphagic rats. *J. Comp. Physiol. Psycol.* 58, 63-67 (1967).

13) Davidson,J.M. & Ganong,M.F.: The effect of hypothalamic lesions on the testes and prostate of male dogs. *Endocrinology* 57, 355-362 (1960).

14) Debons, A.F. et al.: Localization of gold in mouse brain in relation to gold thioglucose obesity. *Amer. J. Physiol.* 202, 743-750 (1962).

15) De Groot,J.: The rat forebrain in stereotaxic coordinates. 1-40, N.V. Noord-Hollusch Utigevers Maatschappij, Amsterdam (1967).

16) Dey, F.L. et al.: Disturbance in reproductive functions caused by hypothalamic lesions in female guinea pigs. *Amer.J.Physiol.* 129, 39-46 (1940).

17) Dey,F.L.: Changes in ovaries and uteri in guinea pigs with hypothalamic lesions. *Amer. J.Anat.* 69, 61-87 (1941).

18) Epstein,A.: Reciprocal changes in feeding behavior produced by intrahypothalamic chemical injections. *Amer. J. Physiol.* 199, 969-974 (1960).

19) Ganong,W.F., Fredrickson,D.S. & Hume,D.M.: The effect of hypothalamic lesions on thyroid function in the dog. *Endocrinology* 57, 355-362 (1955).

20) Herrero, S.: Radio-frequency-current and direct-current lesions in the ventromedial hypothalamus. *Amer. J. Physiol.* 217, 403-410 (1969).

21) Hetherington, A.W. & Ranson, S.W.: Hypothalamic lesions and adiposity in rats. *Anat. Rec.* 78, 149-172 (1940).

22) Hoebel, B.G. & Teitelbaum, P.: Hypothalamic control of feeding and self-stimulation. *Science* 135, 373-377 (1962).

23) Hume, D.M.: Hypothalamic localization for the control of various endocrine secretions. *In*: Jasper, J.J., ed., Reticular Formation on the Brain. 231-248, Little Brown, Boston, Massachusetts (1966).

24) Jansen, G.R. & Hutchinson, C.F.: Production of hypothalamic obesity by microsurgery. *Amer. J. Physiol.* 217, 487-493 (1969).

25) Marshall, N.B., Barnett, R.J. & Mayer, J.: Hypothalamic lesions in gold thioglucose injected mice. *Proc. Soc. Exp. Biol. Med.* 90, 240-244 (1955).

26) Matsuyama, S. et al.: Testosterone sensitive centers in the brain of male rat. *Jap.J.Vet.Sci.* 30, Suppl. 200-201 (1968). [In Japanese. Summary of oral presentation.]

27) Mayer, J. & Marshall, N.B.: Specificity of gold thioglucose for ventromedial hypothalamic lesions and hyperphagia. *Nature* 178, 1399-1400 (1956).

28) Mayer, J. & Thomas, D.W.: Regulation of food intake and obesity. *Science* 156, 328-337 (1967).

29) Olney, J.W.: Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science* 164, 719-721 (1969).

30) Olney, J.W. & Sharpe, L.G.: Brain lesions in an infant rhesus monkey treated with monosodium glutamate. *Science* 166, 386-388 (1969).

31) Perry, J.H. & Liebelt, R.A.: Extra-hypothalamic lesions associated with gold thioglucose induced obesity. *Proc. Soc. Exp. Biol. Med.* 106, 55-57 (1961).

32) Reynolds,R.W.: Ventromedial hypothalamic lesions without hyperphagia. *Amer. J. Physiol.* 204, 60-62 (1963).

33) Rutman,R.J., Lewis,F.S. & Bloomer,W.D.: Bipiperidyle mustard, a new obesifying agent in the mouse. *Science* 153, 1000-1002 (1966).

The Effect of L-Glutamic Acid and Other Amino-acids in Hypoglycaemia

By W. MAYER-GROSS and J. W. WALKER
Department of Clinical Research, Crichton Royal, Dumfries

(*Received* 26 *April* 1948)

While it is generally agreed that glucose is the only substrate able to maintain the function of the brain in intact animals under normal conditions, opinions are still divided on the part played by certain amino-acids and related substances in cerebral metabolism, particularly in the absence of glucose.

In vitro experiments by Quastel & Wheatley (1932) have shown that L-glutamic acid is oxidized by brain tissue in the absence of glucose. This work has been developed by Krebs (1935) with special reference to glutamine and glutaminase. Weil-Malherbe (1936) suggested that L-glutamic acid fulfils a specific function in relation to carbohydrate metabolism in cerebral tissue.

Using eviscerated animals Bollmann & Mann (1931) and Maddock, Hawkins & Holmes (1939) found that the intravenous injection of the substances known to be oxidized by the brain tissue *in vitro* does not maintain the electrical activity of the brain nor relieve the symptoms of hypoglycaemia. Klein & Olsen (1947) have shown that in cats anaesthetized with sodium amytal substances, such as lactate, pyruvate, L-glutamate and succinate, do not pass from the blood to the brain in significant quantity. Conversely, in the normal human subject a ready exchange of lactate and pyruvate takes place between the blood and the brain as demonstrated by Himwich & Himwich (1946).

The work of Nord (1926) should be mentioned here. We are indebted to Dr R. B. Fisher for drawing our attention to this after a preliminary publication of our results (Mayer-Gross & Walker, 1947). Working with rabbits, Nord produced a rise in the blood glucose by intravenous injection of glutamic and aminoacetic acids, an effect which was abolished after adrenalectomy. He concluded that this effect was due to hypersecretion of adrenalin and subsequent mobilization of glucose.

Further evidence of a direct effect of glutamic acid on cerebral function was provided by clinical observations. Price, Waelsch & Putnam (1943) observed that the administration of DL-glutamic acid hydrochloride reduced the number of *petit mal* attacks in epileptics but was without effect upon major seizures. Waelsch & Price (1944) subsequently showed that this effect was due to the L-glutamic acid fraction and not to any shift in the acid-base balance of the blood. Zimmermann & Ross (1944) and Zimmermann, Burgemeister & Putnam (1946), confirming these observations, have claimed a favourable influence of orally administered L-glutamic acid upon the ability of rats to learn a simple maze and upon the intellectual development of defective children. At the same time Unna & Howe (1945) have shown that the vomiting induced by the injection of L-glutamic acid is probably central in origin since it can be prevented by narcosis.

While the results of *in vitro* experiments could not be expected to be in complete agreement with those in the intact animal, the wide differences in the experiments quoted above suggested the desirability of a study of the immediate effect upon the human subject of relatively large quantities of L-glutamic acid. The original observation of Quastel & Wheatley (1932) that L-glutamic acid was only oxidized by brain tissue in the absence of glucose suggested that patients in a state of hypoglycaemic coma would prove to be suitable subjects for these experiments.

EXPERIMENTAL AND RESULTS

The subjects of the experiments were otherwise healthy patients undergoing Sakel's insulin coma treatment for schizophrenia. They had, except where otherwise mentioned, received the optimum dose of insulin to produce hypoglycaemic coma. Coma was defined as a state of unconsciousness from which the patient could not be roused by sensory stimulation; if stimulation provoked any movement, it was of a general nature and in no way local or purposeful. The blood-glucose levels, estimated by the method of King, Haslewood & Delory (1937), varied in coma between 7 and 12 mg./100 ml., the mean being 10 mg./100 ml. The results of this method closely approximate to true glucose values and we found it reproducible to ± 2 mg./100 ml. At the time of the onset of coma 1 mg. of atropine sulphate was injected subcutaneously to minimize the excessive gastric, bronchial and salivary secretion produced by insulin.

Glutamic acid

The results of the oral administration of L-glutamic acid hydrochloride (Waelsch & Price, 1944; Zimmerman & Ross, 1944; Zimmerman *et al.* 1946) suggested that it might be absorbed in sufficient amount from the intestinal canal to serve as a substrate for cerebral metabolism. The substance was first given in hypoglycaemic coma by stomach tube.

No effect upon consciousness could be observed in a series of 10 cases in which quantities of up to 100 g. of L-glutamic acid hydrochloride were administered.

In a further series of 14 experiments 20 g. of L-glutamic acid hydrochloride were added to the 200 g. of sucrose normally administered by stomach tube for the purpose of terminating hypoglycaemic coma; in 11 cases no effect upon the recovery of consciousness could be observed. The patients did not wake up more quickly than after glucose alone. The remaining three experiments, however, carried out on the same subject, did suggest that the L-glutamic acid hydrochloride might have some effect. This subject had never been known to recover from coma after the oral administration of sucrose. He recovered only after a subsequent intravenous injection of glucose. In these three experiments the addition of 20 g. of L-glutamic acid hydrochloride to the 200 g. of sucrose had the effect of rendering unnecessary the intravenous injection of glucose, consciousness being restored within 20 min. of the oral administration of the mixture. Without the 20 g. of L-glutamic acid hydrochloride intravenous injection of glucose was invariably necessary.

An injectable preparation of L-glutamic acid was prepared by cautious addition of 50% NaOH (w/v) to a saturated solution of L-glutamic acid hydrochloride. The two solutions were cooled with ice and mixed with constant stirring in a large mortar packed round with crushed ice. The L-glutamic acid at first precipitated was redissolved, and the pH was taken to 7·2 (pH meter). A solution which contained the equivalent of 25 g. of L-glutamic acid and 10–11 g. of NaCl/100 ml. was obtained, and was sterilized by filtration through a Ford 'Sterimat' grade SB.

This preparation (80 ml.≡20 g. L-glutamic acid) was injected intravenously in a series of 45 experiments carried out on 31 patients in hypoglycaemic coma. The injection was made over a period of 2 min. Blood glucose was determined before the injection and at intervals of 3 min. from its termination. The subjects were closely watched for returning consciousness over a period of 15 min., and, if at the end of this period consciousness was not restored, an intravenous injection of glucose was administered. The criteria adopted as signs of consciousness were ability to obey spoken requests and to answer simple questions rationally. In some cases, where speech was delayed, the fact that the patient sat up and swallowed sips of water was considered a sign of consciousness.

In 26 of these 45 experiments the subject became sufficiently conscious to satisfy these criteria while in the remaining 19 cases consciousness was not restored. In many of the latter a considerable decrease in the depth of coma was noticeable, e.g. cessation of hyperventilation and return of normal respiration, disappearance of muscular spasm and twitchings, opening of the eyes, fixation and following the observers' movements, sighing, yawning, stretching, etc.

In all 45 experiments an increase in the blood glucose level was observed. In the 26 subjects recovering consciousness the blood-glucose level at the time of the recovery of consciousness, and in the remaining 19 cases the highest level reached, was noted. These values are set out in columns (1) and (6) of Table 1. The two sets of values are not greatly different from each other, the means being 25 and 22 mg./100 ml. respectively. The significance of the rise of blood glucose will be discussed later. There was the possibility that the large quantity of sodium chloride contained in the injected preparation of L-glutamic acid could have some effect upon the return of consciousness. For this reason a series of nine identical experiments was carried out in which the comatose subjects were given an intravenous injection of 80 ml. of a solution of sodium chloride containing 11 g./100 ml. In no case was there any effect upon the state of consciousness nor any significant effect upon the blood-glucose level, the highest level reached being 16 mg./100 ml. while the mean was 13 mg./100 ml. The values are set out in column (10) of Table 1.

Aminoacetic acid

The result of the experiments with L-glutamic acid suggested their extension to other amino-acids. Because of its ready availability aminoacetic acid was selected; and an injectable preparation containing 25 g./100 ml. was made, the pH being adjusted to 7·2 by means of a few drops of sodium hydroxide solution. The solution was sterilized by intermittent heating to 100° and stored at 37° as the amino-acid had a tendency to crystallize at lower temperatures.

A series of 26 experiments was carried out with this preparation under similar conditions to those in the experiments with L-glutamic acid, each subject receiving 20 g. of aminoacetic acid. In 13 experiments the patient became sufficiently conscious to satisfy the standard condition while in the remaining experiments this degree of consciousness was not reached although in many cases the coma became considerably less deep. The blood-glucose level at the time of the return of consciousness or, alternatively, the highest level reached in the time of the experiment was noted; these figures are set out in columns (2) and (7) of Table 1. The mean values were 24 and 20 respectively.

p-*Aminobenzoic acid*

A further series of experiments was carried out using a solution of the sodium salt of *p*-aminobenzoic acid, each subject receiving 20 g. of the acid. In four of these six experiments consciousness was restored while the remaining two did not reach this stage. The blood glucose values of this short series are set out in columns (3) and (8) of Table 1, the mean values being 22 and 24 mg./100 ml. respectively.

The significance of the rise of blood glucose

The result of these four series of experiments suggested that the injection of 20 g. of the amino-acid had been responsible for the return to consciousness of the subjects. The mechanism of this result was, however, in some doubt since the administration of

Table 1. *Relationship of blood glucose values and consciousness after the administration of amino-acids, glucose and sodium chloride*

Consciousness restored: blood glucose values (mg./100 ml.)					Remaining unconscious: blood glucose values (mg./100 ml.)				
Glutamic acid	Glycine	*p*-Amino-benzoic acid	Glucose only, oral	Glucose only, intravenous	Glutamic acid	Glycine	*p*-Amino-benzoic acid	Glucose oral and intravenous	NaCl
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
21	23	28	55	53	21	21	23	36	9
16	32	19	54	55	19	14	25	25	12
28	25	23	51	37	26	20	—	35	16
26	23	19	30	32	21	23	—	42	12
23	25	—	37	35	30	26	—	46	14
28	28	—	40	53	25	17	—	23	12
28	19	—	39	48	35	19	—	19	12
32	28	—	35	42	30	19	—	24	12
35	28	—	37	35	17	23	—	22	16
25	21	—	37	37	23	19	—	30	—
34	16	—	34	40	25	27	—	19	—
37	21	—	—	37	32	21	—	34	—
28	19	—	—	—	30	16	—	25	—
23	—	—	—	—	16	—	—	35	—
25	—	—	—	—	16	—	—	19	—
21	—	—	—	—	14	—	—	27	—
35	—	—	—	—	16	—	—	28	—
25	—	—	—	—	16	—	—	27	—
21	—	—	—	—	14	—	—	35	—
27	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—
Mean: 25	24	22	42	42	22	20	24	29	13

the amino-acid had always been accompanied by a rise in the blood-glucose level above the average level of 10 mg./100 ml. found in hypoglycaemic coma. The work of Nord (1926) would suggest that the return to consciousness was entirely due to this increase in blood glucose which he proved to be a sympathomimetic effect. On the other hand, Nord had failed to obtain this effect when the quantity of amino-acid injected was less than 2 g./kg.; while in none of our experiments did the quantity of amino-acid exceed 0·4 g./kg. Furthermore, no clinical signs of hyperadrenalism such as a rise in pulse rate or in pulse pressure were seen. Previous investigations by ourselves (Mayer-Gross & Walker, 1945) had shown that consciousness was not restored until a blood-glucose level had been reached that was about double the average figures of 22–25 mg./100 ml. obtained in these experiments. For this reason it seemed probable that the elevation of blood glucose was not solely responsible for the return of consciousness. It seemed desirable, however, to arrange control experiments on an identical pattern in order to determine the critical level of blood glucose at which consciousness was restored.

In the first of two series of experiments 200 g. of sucrose were administered to subjects in hypoglycaemic coma by a stomach tube. Blood-glucose level was determined at 3 min. intervals, and the subject closely watched for the appearance of the signs of consciousness satisfying the previously mentioned criteria. The blood-glucose level at the time of the recovery of consciousness was noted.

In a second series 3 g. of glucose were injected intravenously into patients in coma and similar observations of blood glucose and consciousness were made. Out of a total of 42 experiments consciousness was restored in 23 while in 19 cases the patient remained in coma. The blood-glucose levels at which consciousness was restored are set out in columns (4) and (5) of Table 1, the mean values being 42 in each case. In the 19 cases where there was no restoration of consciousness the highest values determined within 15 min. after the administration of glucose were noted. These values are set out in column (9) of Table 1 the mean value being 29 mg./100 ml.

Statistical analysis of Table 1 shows that there are clearly no significant differences between the means of columns (1), (2) and (3), nor any significant differences

between those of columns (4) and (5). These columns may, therefore, be taken together. The grouped means are, for the amino-acids 24·53 and for the glucose, oral and intravenous, 41·34; the difference is 16·81, the standard error of difference is 1·89 and the ratio of the difference to its standard error 8·9. The chance probability of this is less than 0·001 and the difference between the means is highly significant.

It may, therefore, be concluded that the rise of blood glucose following injection of amino-acids was insufficient, in itself, to produce the return of consciousness. Taking column (9), which gives the blood-glucose values in experiments where consciousness was not restored, the group mean is 21·00, and the difference between this and the grouped mean of columns (1)–(3) is 4·46. The standard error of this difference is 1·96 and the ratio of difference to its error 2·28. The chance probability is less than 0·05 which attains a level of significance commonly accepted for clinical experiments. In other words, even in cases in which consciousness was not restored by glucose, the blood-glucose level was significantly higher than that of the cases in which it had been restored by amino-acids.

Effect of glutamic acid on the blood urea

An attempt was next made to determine whether the glucose responsible for the rise in blood-glucose level, after the injection of L-glutamic acid, was formed by the synthesis of glucose from the amino-acid. If this were so it would be necessary for deamination to occur, and, as a result of this, a rise in the blood-urea level. In 12 experiments in which 20 g. of L-glutamic acid were injected into patients in hypoglycaemic coma, parallel observations were made upon the blood-glucose level, the blood-urea level and upon the state of consciousness. The blood-urea levels were determined by the method of King *et al.* (1937) which we found reproducible to ±1 mg./100 ml. The results are summarized in Table 2. In eight cases consciousness was restored at an average blood-glucose level of 28 mg./100 ml. with a slight rise in blood urea in four and a slight depression in the other four. Four cases did not recover consciousness, while their mean blood-glucose level rose to 25, in two with a slight rise in the blood-urea level and a slight fall in the remaining two.

Table 2. *Parallel observations on consciousness, blood glucose and blood urea after intravenous injection of 20 g. of L-glutamic acid in hypoglycaemic coma*

Conscious		Unconscious	
Blood glucose (mg./100 ml.)	Rise or fall in blood urea (mg./100 ml.)	Blood glucose (mg./100 ml.)	Rise or fall in blood urea (mg./100 ml.)
35	+2	23	−3
35	+2	25	−3
28	±2	25	+5
23	−3	28	+3
21	+6	—	—
32	−1	—	—
25	−5	—	—
25	−2	—	—
Mean 28		25	

Succinic acid

Succinic acid is one of the substances related to glutamic acid which may be converted in the body into glucose (Soskin & Levine, 1946). The problem was whether this could take place within the 15 min. period of our experiments and affect the hypoglycaemic condition. A solution of succinic acid suitable for intravenous injection was prepared by adding small quantities of succinic acid to a solution of sodium succinate until the pH was reduced to 7·2. The quantities were so adjusted that the final product contained the equivalent of 1 g. of succinic acid/10 ml.

In view of the expectation of very rapid oxidation of the succinic acid by the tissues of the body it was considered necessary to inject the preparation as rapidly as possible. An immediate difficulty was encountered, however, in the violent erythema that accompanied the intravenous injection of the substance, and, for this reason, it was deemed inadvisable to carry out the injection too quickly and to administer more than the equivalent of 10 g. succinic acid.

In a series of 14 experiments no change in the state of consciousness of the hypoglycaemic subject could be seen, nor was there any marked change in the concentration of the blood glucose, the greatest change in the latter being a rise of 5 mg./100 ml.

A number of determinations of the blood-succinic acid concentration were made by the method devised by Forssman (1941). The average concentration obtained was 11·6 mg./100 ml. the highest being 17·2 mg./100 ml. and the lowest 7·3 mg./100 ml. The results are summarized in Table 3.

Table 3. *The effect of intravenous injection of succinic acid upon blood-glucose and blood-succinic acid concentrations of subjects in hypoglycaemic coma*

(Subjects remained in coma.)

No.	Succinic acid injected (g.)	Rise in blood glucose (mg./100 ml.)	Blood succinic acid concentration immediately after injection (mg./100 ml.)
1	2	3	—
2	4	5	—
3	4	3	—
4	8	2	—
5	8	2	—
6	10	2	7·3
7	7	3	10·9
8	12	4	17·2
9	10	4	16·3
10	10	2	11·0
11	10	3	12·2
12	10	5	9·7
13	10	2	8·2
Mean		3	11·6

Individual reactions

Although the amino-acid experiments and glucose controls could not always be performed in the same individuals, parallel experiments and controls were possible in 19 subjects. While in some subjects the impression was gained that easy recovery from coma, or its persistence after injection of amino-acids was peculiar to the individual, critical examination of the experiments as a whole, and of the parallel tests in particular, showed that individual reaction patterns were rare. Success and failure in restoring consciousness seemed to depend mainly on the duration of coma before the administration of the amino-acid.

An interesting phenomenon occurred in one female patient who, in the normal process of recovery from hypoglycaemic coma after the oral administration of sucrose, invariably passed through a transient but violently emotional phase characterized by much weeping. When she was awakened by L-glutamic or aminoacetic acid the emotional phase did not occur. The patient became completely conscious and able to converse intelligently without emotional upset. Nevertheless, upon her subsequently drinking sucrose solution, the emotional reaction developed. This observation was made in four experiments on successive days.

Glutamic acid in the absence of hypoglycaemia

A series of nine experiments was made in which 20 g. of L-glutamic acid were injected intravenously to non-hypoglycaemic (fasting) subjects. The clinical condition and the blood-glucose level of the patient were closely watched for 20 min. after the injection. In spite of adminstration of atropine, as

Table 4. *Blood glucose (mg./100 ml.) before and after intravenous injection of 20 g. of L-glutamic acid to non-hypoglycaemic (fasting) subjects*

Before glutamic acid	After glutamic acid				Maximum rise or fall
	5 min.	10 min.	15 min.	20 min.	
83	83	83	93	83	+10
65	72	70	65	70	+7
79	67	79	77	79	−12
70	79	77	79	77	+9
101	95	98	88	88	−13
90	97	90	95	99	+9
100	89	72	98	95	−28
97	97	99	90	93	−7
82	80	84	82	82	+2

previously described, the vomitive effect of the L-glutamic acid (Unna & Howe, 1945) was invariably present and was much more marked than in the hypoglycaemic subjects. While there was no detectable effect upon the patient's mental state, there was a generalized sensation of tingling associated with the injection, which had always passed off before vomiting occurred. The effect on blood glucose was very variable; it was raised in four cases, depressed in four cases and not significantly changed in one case. The results which are summarized in Table 4 suggest that the observations of Quastel & Wheatley (1932) *in vitro* held true *in vivo* and that L-glutamic acid was not utilized in the presence of glucose.

DISCUSSION

The work quoted in the introductory remarks suggests two explanations of the restoration of consciousness after the injection of amino-acids. Neither accounts for all our observations.

In the first place, the reaction of hypoglycaemic subjects could be due to oxidation of L-glutamic acid by the brain in the absence of glucose (Quastel & Wheatley, 1932). Identical reactions obtained with aminoacetic and *p*-aminobenzoic acids make this interpretation doubtful since Krebs (1935) found L-glutamic acid to be the only amino-acid oxidized by brain tissue.

The second explanation traces the effect of amino-acids to the invariable rise in blood glucose. This may be due to (1) general stimulation during injection, (2) conversion of amino-acid to glucose, or (3) sympathomimetic action.

(1) While a small rise of blood glucose may be produced in hypoglycaemic coma by any external stimulation the parallel experiments with 11% sodium chloride solution have shown that this rise is not equal to that produced by amino-acids.

(2) From the absence of change in blood urea, after the injection of glutamic acid and from the absence of a rise in blood glucose after injection of succinic acid, it seems improbable that glucose is derived from the amino-acid injected. Although it is feasible that glutamic and aminoacetic acids are converted into glucose within the body, this is unlikely in the case of *p*-aminobenzoic acid.

(3) Sympathomimetic action (Nord, 1926), although not observed clinically, cannot be ruled out in our experiments and may cause the rise of blood glucose. Whatever the cause of this rise, however, it cannot account for the recovery of consciousness in a large proportion of cases. Control experiments and statistical analysis of Table 1 have shown that it was too small for this purpose. Furthermore, the injection of glutamic acid into non-hypoglycaemic subjects has completely failed to produce the significant rise of blood glucose demonstrated in Nord's experimental animals. Similarly, the vomiting, much more marked without than with hypoglycaemia, points to a different action of glutamic acid in the two conditions. An explanation based only on the glucose rise therefore fails to account for our observations. Presumably, therefore, the amino-acids exert some additional influence on the metabolism of the nerve cell.

The nature of this influence is at present obscure. An excess of simple amino-acids may replace fractions of the protein molecule for some of their

functions thus releasing them for a more specific purpose in relation to carbohydrate metabolism. This might result in more efficient utilization of glucose and so in the recovery of consciousness at a lower level of blood glucose.

SUMMARY

1. Oral administration of L-glutamic acid to patients in hypoglycaemic coma was without effect except in one subject.

2. Intravenous injection of 20 g. of L-glutamic acid restored consciousness to subjects in hypoglycaemic coma in 26 of 45 experiments, and modified the depth of coma in the remaining 19 experiments.

3. Similar effects were produced by the injection of 20 g. of aminoacetic and *p*-aminobenzoic acids.

4. In all cases where amino-acids were injected intravenously into hypoglycaemic subjects there was a rise in blood glucose, which was, however, in itself inadequate for the restoration of consciousness.

5. Injection of L-glutamic acid into subjects in hypoglycaemic coma was without significant effect on blood urea.

6. Injection of succinic acid into hypoglycaemic subjects was without significant effect on blood glucose or on state of consciousness.

7. Intravenous injection of L-glutamic acid into non-hypoglycaemic subjects failed to produce any significant effect on blood glucose, but produced vomiting far more strongly than in hypoglycaemic subjects.

We wish to express our gratitude to Dr P. K. McCowan, Physician Superintendent of Crichton Royal, for his interest in our work and for his permission to publish the results. Our thanks are also due to Miss A. Beattie, sister-in-charge of the Insulin Department, for her collaboration.

REFERENCES

Bollmann, J. L. & Mann, F. C. (1931). *Amer. J. Physiol.* 94, 683.
Forssman, S. (1941). *Acta Physiol. Scand.* 2, Suppl. 5.
Himwich, H. E. & Hahum, L. H. (1932). *Amer. J. Physiol.* 101, 446.
Himwich, H. E. & Himwich, W. A. (1946). *J. Neurophysiol.* 9, 133.
King, E. J., Haslewood, G. A. D. & Delory, G. E. (1937). *Lancet*, i, 886.
Klein, J. A. & Olsen, N. S. (1947). *J. biol. Chem.* 167, 1.
Krebs, H. A. (1935). *Biochem. J.* 29, 1951.
Maddock, S., Hawkins, J. E. & Holmes, F. (1939). *Amer. J. Physiol.* 125, 551.
Mayer-Gross, W. & Walker, J. W. (1945). *Brit. J. exp. Path.* 26, 81.
Mayer-Gross, W. & Walker, J. W. (1947). *Nature, Lond.*, 160, 334.
Nord, F. (1926). *Acta Med. Scand.* 65, 1.
Price, J. C., Waelsch, H. & Putnam, T. J. (1943). *J. Amer. med. Ass.* 122, 1153.
Quastel, J. H. & Wheatley, A. H. M. (1932). *Biochem. J.* 26, 725.
Soskin, S. & Levine, R. (1946). *Carbohydrate Metabolism.* Chicago: University Press.
Unna, D. & Howe, E. E. (1945). *Fed. Proc.* 4, 138.
Waelsch, H. & Price, J. C. (1944). *Arch. Neurol. Psychiat., Lond.*, 51, 393.
Weil-Malherbe, H. (1936). *Biochem. J.* 30, 665.
Zimmermann, F. R., Burgemeister, B. B. & Putnam, T. J. (1946). *Arch. Neurol. Psychiat., Lond.*, 56, 489.
Zimmermann, F. T. & Ross, S. (1944). *Arch. Neurol. Psychiat., Lond.*, 51, 446.

Studies on the Metabolism of Semen

5. CITRIC ACID IN SEMEN

By G. F. HUMPHREY and T. MANN, *Molteno Institute, University of Cambridge*

(*Received* 23 *April* 1948)

The semen of man and certain other higher mammals is distinguished by a very high content of citric acid (Scherstén, 1929, 1936; Dickens, 1941; Huggins & Neal, 1942; Lardy & Phillips, 1945; Barron & Huggins, 1946*a*, *b*; Humphrey & Mann, 1948). The acid originates in the accessory glands of reproduction, chiefly the seminal vesicles, and in this respect it resembles another more recently discovered component of semen, namely fructose, which has similarly been shown to be secreted mainly in the seminal vesicles (Mann, 1946). The present study was undertaken primarily with the object of investigating the possibility that there may exist a link between the two substances with regard to their formation, distribution or function in the reproductive organs and semen. In the course of this study it was established that the process of generation and maintenance in semen of both fructose and

To the Editor. Until I read the correspondence of Dr. Ho Man Kwok in the April 4 *Journal*, I was convinced that my own Chinese-restaurant syndrome could easily have been remedied with a placebo.

The first experience came at lunchtime in a New York City establishment in 1956. Subsequent four or five occurrences took place during evening meals at a Chinese restaurant in Chicago, during 1961 and 1962. From 1962 to 1964 I lived on a fairly consistent Chinese diet in the Hawaiian Islands — uneventfully. Upon return to the mainland at the end of 1964 and during the period of 1965 and 1966, I dined fairly often at Chinese restaurants in southern California and in New Hampshire, with a renewal of the experience each time. It was only at the New York restaurant and once at the New Hampshire restaurant that the attack was most severe. My most recent visits during the past two years to a Chinese restaurant in Harrisburg, Pennsylvania, were uneventful.

Like Dr. Ho Man Kwok's, my trouble began about 20 minutes after I started the meal. However, the symptoms differed: they began with severe aching in the muscles down both sides of the back of the neck and spread into the shoulders, shoulder blades and upper spine. This was accompanied on each occasion with a profuse, cold sweat about the face and under the armpits. The climax to each experience was a most uncomfortable tightness on both sides of the head just forward of the ears in the temple area. It felt as though, at any second, the sides of my head would burst; this symptom lasted no more than a minute, and the entire syndrome disappeared quite rapidly.

It should be pointed out that there were occasions, but few, when I dined in the New York, Chicago, southern California and New Hampshire restaurants when the syndrome did not appear at all.

THOMAS J. MCCAGHREN
Managing Editor
Pennsylvania Medicine

AN ATTEMPTED REVERSAL OF THALIDOMIDE EMBRYOPATHY IN THE RAT BY GLUTAMINE

J. D. McColl, M. Globus,[1] and S. Robinson
Research Laboratories, Frank W. Horner Ltd., Montreal, Que.

Received July 31, 1964

Abstract

Skeletal, cardiovascular, and urethral defects were observed in the 21-day-old offspring of Sprague–Dawley rats following administration of thalidomide (2% of diet) during the entire gestation period. The fetuses of animals which received either *l*-glutamic acid or *l*-glutamine (2% of diet each), in addition to thalidomide, showed essentially the same incidence and type of gross defects as those from the thalidomide-treated mothers.

The ineffectiveness of these essential growth factors to reduce the embryopathy significantly is interpreted to indicate that they are not predominately involved in the teratogenic action of thalidomide in the rat.

Introduction

The biochemical lesion responsible for thalidomide-induced embryopathy has not been determined. Various investigators (1–6) have employed protozoan, microbiological, or some in vitro system in which to test the possible anti-metabolite action of the drug.

Because cleavage of the thalidomide molecule yields simple compounds, such as glutamic and phthalic acid, the interaction with glutamic acid – glutamine metabolism (7) has been suggested as the cause of the biochemical lesion. The general resemblance of thalidomide-induced malformations to those produced by other glutamine antagonists (8) was additional stimulus for investigation of this system. Williams (6) observed that one of the metabolites of thalidomide inhibited glutamine synthetase of rat brain. Frank (1) reported that nicotinamide reversal of thalidomide-induced inhibition of growth of a protozoa was enhanced in the presence of glutamine. Recently Boylen *et al.* (9) observed that *l*-glutamine (but not *l*-glutamic acid) reduced the incidence of malformations produced by 3- and 4-hydroxyphthaloylglutamine in the developing chick.

It has been reported (10, 11) that certain skeletal and cardiovascular defects could be produced in the fetus of rats following chronic oral administration of thalidomide. The present study was designed to test, in a mammalian species, the hypothesis that thalidomide embryopathy results from interference with glutamine synthesis by attempting to reduce teratogenicity by the concurrent administration of glutamine or glutamic acid.

Methods

Sprague–Dawley (Cesarean-derived) rats from Charles River Breeding Laboratories were used and maintained on Purina Laboratory Chow.

The breeding procedures have been described in detail elsewhere (10, 11).

[1]Present address: Department of Zoology, Western Reserve University, Cleveland, Ohio.

Briefly, the drugs were given to all animals for 3 days before mating and to pregnant females for the subsequent gestation period. The following treatments were administered by admixing the compound in powdered chow: (*a*) thalidomide 2%, (*b*) thalidomide 2% + *l*-glutamic acid* 2%, (*c*) thalidomide 2% + *l*-glutamine* 2%, (*d*) glutamic acid 2%, and (*e*) controls (normal diet).

Pregnancy was determined by observation of vaginal smears and body weight gain, and the pregnancy rate recorded. Body weight and food consumption were regularly recorded. On the 21st day the animals were delivered by hysterotomy and the viability, number, and body weight of the fetuses determined. The uterus was examined for resorption sites. Offspring were killed and either dissected under low power magnification (20 ×) for visceral anomalies or deep frozen for subsequent study. Skeletons were stained with alizarin red by a modification of the method of Dawson (12) and examined microscopically for defects.

Statistical analyses were performed by the use of Binomial Probabilities (13).

Results

Results, expressed as a percentage occurrence of the fetuses examined, are summarized in Table I. Maternal administration of thalidomide (group A) resulted in a significant reduction of litter size, increased occurrence of uterine resorption sites, and reduction of pregnancy rate. Body weight of the fetus was not different from controls.

Thalidomide increased the incidence of malformations that occur spontaneously to a small degree in the strain of rat employed (11). In the interpretation of such studies it is important that the "normal" development of the experimental animal be known, and that adequate controls are included in the design to determine the incidence of incipient anomalies. In thalidomide-treated animals, in comparison to controls, there was a significant increase in the occurrence of skeletal malformations. These were usually confined to defects indicative of inhibition of ossification of the vertebral centra, the ribs, and skull. The vertebral centra were also fragmented. A high incidence of missing fifth sternebrae and fragmented sternebrae was noted. The ribs were branched and fused. These findings are similar to those previously reported (10, 11, 14–17).

Cardiovascular deformities consisting of intraventricular septal defects were significantly increased both in incidence and severity. In control animals these defects consisted of small foramen in the membranous portion of the septum which "normally" closed by the 17th day. In the treated animals the openings ranged from small to large, extending considerably into the muscular part (11). An enlarged right auricle was also a persistent finding.

Among the viscera hydroureter was the only gross defect seen. In some animals there was an urethral atresia; however, it was impossible by gross methods to determine if it caused the hydroureter in all instances.

Fetuses from the rats which received *l*-glutamic acid (group B) or *l*-glutamine

*Nutritional Biochemical Corporation.

TABLE I

Thalidomide-induced malformations in the Sprague–Dawley rat

	Control	Thalidomide	Thalidomide + *l*-glutamic acid	Thalidomide + *l*-glutamine
Number of litters	31	31	6	8
Number of offspring	351	209	38	48
Mortality at birth	1.4%	2.8%	0	6.2%
Mean litter size	11.0±0.6[b]	6.0±0.9	6.3±0.7	6.0±0.8
Mean fetus weight, g	5.4±0.3	5.2±0.2	5.3±0.3	4.8±0.2
Resorption sites/litter	0.1±0.05	4.8±0.9	5.6±0.6	3.5±0.5
Pregnancy rate[a]	98%	73%	56%	80%
	%	%	%	%
I. Vertebral anomalies				
Thoracic				
Fragmented	0.6	27**	0	4.2
Inhibition of ossification	35	62**	68**	73**
Lumbar				
Fragmented	0	2.8*	4**	0
Inhibition of ossification	0	9.3**	4**	15**
II. Sternal anomalies				
Malformed	3.0	14**	32**	21**
Inhibition of ossification	3.5	3.8	0	10
Missing 5th sternebra	0.6	72**	72**	69**
III. Rib anomalies				
Malformed	0	3.2**	0	
Extra centers	33	0.6**	12**	2**
IV. Skull anomalies	0	10**	0	19**
V. Limb anomalies	0	0	0	4**
VI. Cardiovascular				
Septal defects	12.7	71**	64**	50**
Aortic arch defects	0	0	4**	6.2**
Enlarged rt. auricle	0	13**	8**	0
VII. Hydronephrosis	0.8	52**	28**	27**

[a]Includes resorption sites.
[b]S.E.M. *Significant $P = <0.05$ from control.
**Significant $P = <0.01$ from control.

(group C) in addition to thalidomide showed essentially the same defects as seen in the thalidomide-treated group A. There were, however, a few significant differences; for example, the incidence of fragmented thoracic centra was less and, with *l*-glutamine, the incidence of fragmented lumbar vertebrae. On the other hand, the percentage of malformed sternebrae was higher.

In group C in a lower incidence of septal defects and enlarged right auricle was seen, but the severity of septal defects was greater than in group A.

Fetuses of animals which received *l*-glutamic acid (group D) were similar to controls and are not included in Table I. Another control group with *l*-glutamine alone was not included because of the cost involved.

Discussion

Under the specific conditions of the experiment, except for the minor differences noted, the concurrent oral administration of *l*-glutamine failed to modify the gross teratogenic effect induced by thalidomide.

As the critical level of thalidomide in the embryo necessary to induce malformation is not known, it is impossible to be certain of the tissue levels of glutamine that might be required to overcome a postulated block in its synthesis. While tissue levels were not determined, it is noteworthy that Tigerman and MacVicar (18) demonstrated a 20% increase in the content of rat liver, kidney, lung, spleen, heart, and skeletal muscle following the feeding of 3.8 g of *l*-glutamine per kilogram of food for 10 days. As 20 g/kg of food was fed in our study it is likely that this increased level was obtained if not exceeded.

Despite these reservations our negative results are in agreement with those of Evered and Randall (4), who failed to demonstrate any significant inhibition by thalidomide of the uptake of *l*-glutamine in intestinal mucosa in the rat. Narrod and King (19) were unable to obtain evidence that thalidomide acted in vivo as a glutamine antagonist by measuring its effect on mouse liver nicotinamide enzymes. Hirschberg *et al.* (20) found no alteration produced by thalidomide in the activity of glutamic dehydrogenase or glutamine oxaloacetic transaminase in the brain or liver in vivo or in vitro, in normal and pregnant mice.

Fabro *et al.* (21) observed no effect of thalidomide, or nine of its metabolites, on glutamine synthetase, decarboxylase, or dehydrogenase in rat brain. They concluded also that thalidomide had no effect on energy-yielding processes in the rabbit fetus. Felisati (22) also reported failure to reverse thalidomide-induced embryopathy in the rabbit with doses of 350 mg/kg of *l*-glutamine.

While Boylen *et al.* (9) were able to reverse some of the defects produced by hydroxylated analogues of thalidomide in the chick with *l*-glutamine, the current evidence would seem to indicate that inhibition of glutamine biosynthesis is not the primary lesion produced by thalidomide in the mammalian species as represented by rats, mice, and rabbits.

Acknowledgment

Our thanks are due for the skillful technical assistance of Mr. Salvatore Galloro.

References

1. O. Frank, H. Baker, S. H. Hunter, and H. Sobotka. Proc. Soc. Exptl. Biol. Med. **114**, 326 (1963).
2. C. Nystrom. Scand. J. Clin. Lab. Invest. **15**, 102 (1963).
3. W. Lammers and A. Manten. Pharm. Weekblad, **98**, 458 (1963).
4. D. F. Evered and H. G. Randall. Brit. Med. J. **2**, 610 (1963).
5. S. Roath, M. W. Elves, and M. C. G. Israels. Lancet, **1**, 249 (1963).
6. R. T. Williams. Lancet, **2**, 724 (1963).
7. J. W. Faigle, H. Keberle, W. Riess, and K. Schmid. Experientia, **18**, 389 (1962).
8. M. L. Murphy. Ciba Found. Symp. Congenital Malformations, London, 78 (1960).
9. J. B. Boylen, H. H. Horne, and W. J. Johnson. Can. J. Biochem. **42**, 35 (1964).
10. J. D. McColl, M. Globus, and S. Robinson. Experientia, **19**, 183 (1963).
11. J. D. McColl, M. Globus, and S. Robinson. Toxicol. Appl. Pharmacol. In press.
12. A. B. Dawson. Stain Technol. **1**, 123 (1926).
13. D. Mainland. Elementary medical statistics. Saunders, Philadelphia. 1952. p. 260.
14. H. J. K. Obbink and L. M. Dalderup. Experientia, **12**, 645 (1963).
15. D. Felisati. Lancet, **2**, 725 (1962).
16. C. T. G. King and F. J. Kendrick. Lancet, **2**, 1116 (1962).
17. G. F. Som
18. H. Tiger
19. S. A. Nap
20. E. Hirsch
21. S. Fabro, 90, 5P
22. D. Felisat

17. G. F. Somers. Biochem. Pharmacol. Suppl. 12, 47 (1963).
18. H. Tigerman and R. MacVicar. J. Biol. Chem. 189, 793 (1951).
19. S. A. Narrod and C. T. G. King. Lancet, 1, 1374 (1963).
20. E. Hirschberg, M. Osnos, S. Bryant, and J. E. Ultmann. Science, 143, 1343 (1964).
21. S. Fabro, H. Schumacher, R. L. Smith, R. B. L. Stagg, and R. T. Williams. Biochem. J. 90, 5P (1964).
22. D. Felisati. Lancet, 1, 724 (1964).

S 4-2

EBERT, A.G.

Vol. 1 No. 2
FEBRUARY 1970

NUTRITION REPORTS INTERNATIONAL

Anthony A. Albanese, Ph.D.
Editor-in-Chief

Published Geron-X, Inc., Los Altos, Calif.

BLOOD AND BRAIN LEVELS OF GLUTAMIC ACID IN YOUNG RATS GIVEN MONOSODIUM GLUTAMATE

J.M. McLaughlan, F.J. Noel, H.G. Botting and J.E. Knipfel
Food and Drug Research Laboratories, Department of National Health and Welfare, Ottawa Canada

Abstract

There has been concern about the safety of monosodium glutamate (MSG). Questions such as size of dose, ingestion with food, route of administration and brain levels of glutamic acid required answers. Blood levels of glutamic acid in young rats were very high after ingestion of large doses of MSG but brain levels were little affected. Ingestion of MSG with meat delayed, but did not prevent, the increase in blood glutamic acid. Similar high blood levels of glutamic acid were obtained after oral or subcutaneous administration of a large dose of MSG. After moderate oral doses, comparable to human intakes, blood levels remained normal.

Introduction

Monosodium glutamate (MSG) has been widely used for several years by the food industry as a flavor enhancer. Recent reports that MSG is the cause of the Chinese restaurant syndrome and that it may produce brain damage in young mice is obviously of concern to many. This compound is the sodium salt of glutamic acid and experiments with humans have shown that synthetic L-glutamic acid also produces the Chinese restaurant syndrome in adult man (1). As this amino acid constitutes 10 to 25% of all food proteins, the question arises why as little as 4 g of MSG produces the syndrome in many subjects (1). MSG taken along with the meal usually has no disturbing effects. The Chinese restaurant syndrome typically results from taking wonton soup, containing MSG, on an empty stomach and it has been suggested that prior ingestion of food delays the absorption of the compound.

Neame and Wiseman (2) reported that glutamic acid is very actively transaminated in the intestinal wall and that there is only a slight increase in plasma glutamic acid after ingestion of moderate amounts of glutamic acid. Increased plasma levels which occur with intravenous infusion of glutamic acid in humans have been associated with nausea and vomiting (3).

More information on the effect of size of dose, variability and presence of food with MSG was obviously needed. In view of Olney's report (4) that MSG injected subcutaneously produces brain damage in young mice, another pertinent question concerned the route of administration of the compound.

Methods

The general procedure is outlined and exceptions in a specific experiment are pointed out when the experiment is described under results.

Male weanling Wistar rats were fed a commercial rat chow until they weighed 90-110 g. After an overnight fast a solution of MSG was administered by stomach tube; 30 minutes later the animal was anaesthetized with ether, decapitated and blood collected in heparinized tubes. Brains were quickly excised and frozen. Proteins in plasma and brain were precipitated with tungstate. Treatment groups consisted of 8 rats and deproteinized samples were pooled to give a single sample. Free amino acids in the protein-free filtrates were determined using a Beckman autoanalyzer with a lithium buffer.

Finely ground freeze-dried veal was suspended in 0.9% sodium chloride or in solutions of MSG; the meat was administered at the rate of 2 mg/g body weight.

Results

In the first experiment, plasma amino acid levels were determined initially and at 10 minute intervals after administration of MSG (0.2 mg per g body weight) alone or with meat, blood being taken by heart puncture. Plasma glutamic acid rose rapidly (Figure 1), reaching a level of 70.5 g μ moles/100 ml ten minutes after the oral dose of MSG and reaching a peak value of 85 μ moles/100 ml twenty minutes after the treatment. After an initial fall, plasma glutamine remained almost steady. Plasma threonine levels (and levels of most other neutral amino acids for which data are not shown) remained relatively constant during the sampling period. Aspartic acid, however, increased from 5.8 μ moles/100 ml to a peak value of 13.8 μ moles/100 ml twenty minutes after administration of the compound. When MSG was given with meat, plasma glutamic acid rose slowly but after thirty minutes reached a level which was almost as high as the peak value for MSG given alone.

In the second experiment the effects of size of dose and of meat taken with MSG were investigated. Ingestion of 0.1 mg of MSG per gram body weight produced no increase in the level of plasma glutamic acid but increases which were proportional to the amount of MSG were obtained with two higher dosage levels of the compound (Table I). Plasma glutamic acid levels with the largest dose of MSG tended to be higher when MSG was given alone than when it was ingested with meat. Neither glutamic acid in brain nor glutamine in plasma and brain appeared to be altered as a result of MSG ingestion.

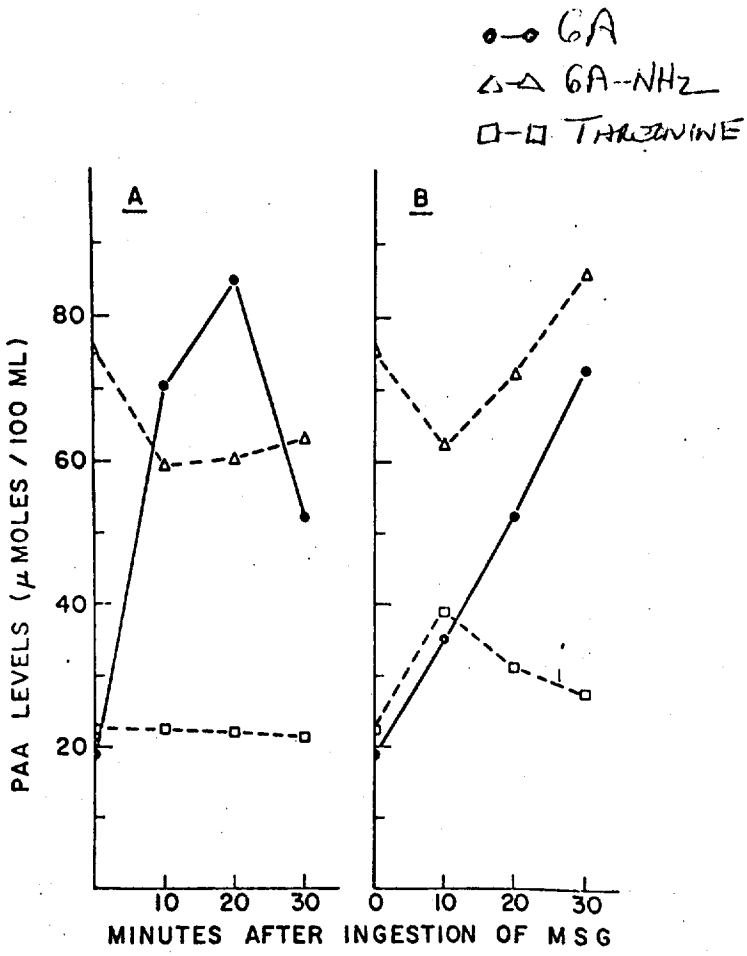


Figure 1. Relationship between plasma amino acid (PAA) levels and time after ingestion of MSG alone (A) or with meat (B). The solid line represents glutamic acid; the dotted line and triangles - glutamine; dotted line and squares - threonine.

Table I. Plasma and brain levels of glutamic acid and glutamine 30 minutes after ingestion of MSG or MSG and meat[1].

Oral dose MSG (mg/g body wt)	Meat[2]	Plasma		Brain	
		Glutamic acid	Glutamine	Glutamic acid	Glutamine
0	-	10.5	47.6	1025	206
0.10	-	10.2	51.5	1192	256
0.50	-	44.1	63.5	1266	272
2.50	-	251.0	71.1	1370	262
0	+	18.7	72.3	1317	264
0.10	+	15.2	65.5	1426	290
0.50	+	39.2	70.3	1337	258
2.50	+	155.6	72.6	1452	302

[1] All values expressed in μ moles per 100 ml or 100 g tissue.

[2] Freeze-dried veal administered at rate of 2 mg/g body weight.

Table II. Plasma and brain levels of glutamic acid and glutamine after an oral dose or subcutaneous injection of MSG (0.5 mg/g body wt)[1].

Treatment	Number of daily treatments	Plasma		Brain	
		Glutamic acid	Glutamine	Glutamic acid	Glutamine
Saline (PO)[2]	1	21.6	81.7	1152	261
Saline (SC)[3]	1	15.7	47.1	1316	335
MSG (PO)	1	95.3	67.9	1135	270
MSG (SC)	1	111.7	55.5	1203	287
Saline (PO)	7	12.1	51.9	1199	268
Saline (SC)	7	19.4	47.0	1188	274
MSG (PO)	7	102.2	64.8	1503	346
MSG (SC)	7	129.8	55.5	1172	236

[1] Results are expressed in μ moles per 100 ml or 100 g tissue.

[2] PO = by mouth.

[3] SC = by subcutaneous injection.

In the third experiment rats were permitted to feed from 08.30 to 16.30 hours and MSG (0.5 mg/g body wt) was administered daily at 08.20 hours by subcutaneous injection or by mouth for 7 days. Other rats on the same feeding schedule received MSG only on the seventh day. Glutamic acid and glutamine levels in plasma and brain were determined (Table II). Glutamic acid levels of rats given saline varied from 12.1 to 21.6 μ moles/100 ml of plasma with a mean value of approximately 17 μ moles. Plasma glutamic acid levels were similar (111.7 and 129.8 μ moles) when MSG was given by injection or by mouth (95.3 and 102.2 μ moles). There was no real evidence that several daily doses of the compound produced higher plasma levels of glutamic acid than a single dose or that there was adaptation to MSG. Amounts of brain glutamic acid and of plasma and brain glutamine did not appear to be affected by MSG.

The fourth experiment was done to confirm the finding in experiment 2 that plasma glutamic acid was not increased with low oral doses of MSG. The results are given in Table III. Plasma glutamic acid remained low until the dose exceeded 0.1 mg/g body weight.

The fifth experiment, carried out with younger rats (40-50 g), was done to correlate possible brain damage due to very large oral doses of MSG, with plasma glutamic acid levels; results are also given in Table III. Initial blood samples were taken from behind the eye (venous blood from the orbital sinus) 30 minutes after dosing. Variation among replicate pooled samples (plasma

of 4 rats) was very large but a plot of these data revealed a straight line relationship between the amount of plasma glutamic acid and magnitude of the dose of MSG. Plasma aspartic acid also showed a marked increase reaching 38.6 μ moles/100 ml plasma at the highest dosage level. A second blood sample was taken from each rat 4 hours after administration of the compound; plasma levels of rats given the two lower doses were 15.2 and 12.2 μ moles/100 ml respectively, whereas rats given the 8 mg dose had a mean plasma glutamic acid level of 292 μ moles/100 ml. (Brains have not yet been examined histologically.)

Table III. Relationship between oral dose of MSG and plasma glutamic acid levels in young rats[1].

Dose (mg.g body wt)	Experiment 1	2	3	4	5			
0	19	10	22	10	13	11	-	-
0.05				6				
0.1		10		7				
0.2	52			17				
0.4				52				
0.5		44	95					
2.0					218	115	229	
4.0					360	384	789	444
8.0					1338	829	1076	684

[1] Values expressed in μ moles per 100 ml of plasma. Plasma pooled from 8 rats except in experiment 5 in which plasma from 4 rats was pooled.

Discussion

An arithmetic plot of the data for all experiments summarized in Table III revealed a linear relationship between the plasma level of glutamic acid and MSG for doses above 0.2 mg/g body weight. Apparently the lowest doses of MSG produced no change in the amount of plasma glutamic acid but it is possible that a slight increase occurred and subsided before blood samples were taken 30 minutes after administration of the compound. Nevertheless, this finding is in agreement with the report of Neame and Wiseman (2). It is of interest that the lowest dose (0.05 mg/g body weight) corresponds to the amount which a 70 kg man might consume in a bowl of wonton soup, i.e. 3.5 g of MSG. Schaumberg (1) reported that susceptible humans experienced the Chinese restaurant syndrome with amounts of MSG ranging from 1-12 g suggesting great individual variation. The present studies with rats also indicate great variability in response to this compound. This variability was overcome by the use of large

numbers of rats.

The Chinese restaurant syndrome does not occur when MSG is taken with food (1). These studies showed that food taken with MSG delayed but did not prevent the increase of glutamic acid in plasma. However, the blood picture was quite different in the two situations. When MSG was taken alone, only glutamic and aspartic acid were elevated but when meat was taken with MSG the concentration of most amino acids was increased, thereby maintaining a balanced amino acid pattern in the plasma.

In view of the large amount of glutamic acid present in brain tissue, it seemed possible that the brain might take up glutamic acid very avidly from the blood. (Normally blood glutamic acid is relatively constant.) However, glutamic acid in blood would be expected to rise markedly after subcutaneous injection of MSG. If brain tissue concentrated blood glutamic acid then brain damage might result as has been reported by Olney (4). However, these studies showed that no marked increase in brain glutamic acid occurred even when blood glutamic acid was 25 times the normal fasting level. This may indicate the existence of a very efficient blood-brain barrier or rapid metabolism of the amino acid by brain tissue.

It is of interest that blood glutamic acid levels were almost as high when MSG was given orally as when administered by subcutaneous injection. However, the dose (0.5 mg/g body wt) which was high in physiological terms was the lowest level given by Olney in his study with mice. Daily injections of MSG did not lead to accumulation of glutamic acid in plasma; this was not surprising in view of the finding that the very high blood level obtained with a 4.0 mg/g dose (equivalent to 280 g for a 70 kg human) was down to normal fasting level within 4 hours.

These studies are in accord with the findings of Meame and Wiseman (2) concerning the threshold level of glutamic acid for absorption from the intestine. However, the existence of this threshold means that there is no relationship between low (physiological amounts) doses of MSG and high doses; this conclusion casts serious doubts on the validity of animal studies with high doses of MSG and the extrapolation results to human situations.

Acknowledgements

The authors thank Dr. T.K. Murray for his helpful suggestions and Mr. R.L. Brien for technical assistance.

References

1. Schaumberg, H.H., Byck, R., Gerstl, R., and Mashman, J.H. Monosodium L-glutamate: Its pharmacology and role in the Chinese restaurant syndrome. Science 163, 826 (1969).

2. Neame, K.D., and Wiseman, G. The transamination of glutamic and aspartic acids during absorption by the small intestine of the dog in vivo. J. Physiol. 135, 442 (1957).

3. Levey, S., Marroun, J.E., and Smyth, C.J. Serum glutamic acid levels and the occurrence of nausea and vomiting after the intravenous administration of amino acid mixtures. J. Lab. Clin. Med. 34, 1238 (1949).

4. Olney, J.W. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. Science 164, 719 (1969).

J. Neurol. Neurosurg. Psychiat., 1951, 14, 47.

AN INVESTIGATION INTO THE EFFECTS OF GLUTAMIC ACID ON HUMAN INTELLIGENCE

BY

J. R. MILLIKEN and J. L. STANDEN

From the Institute of Psychiatry, the Maudsley Hospital, London

Since 1943 there have been many reports of investigations conducted to determine whether the oral administration of supranormal quantities of glutamic acid is followed by an improvement in cognitive and other functions. Both rats and human beings have been used as subjects, and the literature shows that both positive and negative results have been obtained. Working with rats, Albert and Warden (1944) and Zimmerman and Ross (1944) have reported improvements in performance in problems of the maze type, but point out that no significant changes in weight or growth were observed. Hamilton and Maher (1947) reported that the experimental animals "showed a significantly higher activity level than the controls" on the three-table test of "reasoning", but that they observed no significant differences in actual test scores on this test or on tests of timidity which were administered after the three-table test. Entirely negative results were obtained by Stellar and McElroy (1948) and Marx (1948) from measures of test performance and of weight, and the latter also observed no increases in the activity level. With human beings as subjects, Price, Waelsch, and Putnam (1943) and Waelsch and Price (1944) have reported that patients suffering from petit mal improved in behaviour and in the frequency and severity of their seizures. With mentally retarded subjects, improvement in performance on cognitive tests has been reported by Gasorek (1948), Zimmerman, Burgemeister, and Putnam (1948), and Albert, Hoch, and Waelsch (1946). The last named reported improvement on personality tests also. Zimmerman, Burgemeister, and Putnam (1949a, and b) reported improvements in mongoloid patients on verbal tests, and in behaviour and general physical condition. The same investigators (1946, 1947) found in mentally retarded patients suffering from convulsive disorders that they improved in performance on psychological tests of personality and of cognitive functions, as well as in activity level and behaviour. Schizophrenic subjects were used by Ewalt and Bruce (1948) and Kitzinger, DeVere, Cartwright, and Shapiro (1949), who reported improvements in personality (as estimated by tests), activity level, and behaviour. Harney (1950) found that glutamic acid had a beneficial effect upon the mental age, personality, and school achievement of retarded girls.

The present experiment was designed to test the hypothesis that the oral administration of glutamic acid to human subjects would be followed by improvements in their scores on psychological tests of intelligence, both of the verbal and the performance type. In view of the reports of improvement in personality and behaviour, the body sway test was also included, on account of its brevity, simplicity, and its correlation with neuroticism (Eysenck, 1947). To this was subsequently added the static ataxia test, which has the same advantages (Eysenck, 1947). The additional hypothesis was that the administration of glutamic acid would be followed by a decrease in the scores on these tests of neuroticism.

The administration of the experiment, including the selection and matching of the subjects, was the responsibility of the psychiatrist (J. R. Milliken). The actual testing, and the analysis of the results after the completion of the testing, were carried out by the psychologist (J. L. Standen), who was unaware which subjects were experimental and which control until the testing was finished.

Plan of the Experiment

Three main groups of subjects were chosen: (1) adult patients in a mental defectives' institution; (2) child patients in the same institution; (3) boys of a normal range of intelligence, living in a home.

The last-named group was subdivided into "seniors" and "juniors", the seniors being all those over the age of 10, and those under 10 who were able to complete the Thurstone primary mental abilities test which was taken by all the boys over 10. The remainder of the boys constituted the junior group.

It was found possible at a later stage of the experiment

to add another group of adult defectives, so that the experiment was actually conducted with five distinct groups of subjects. For convenience of reference, they were described as "Adult Defectives Group I", "Adult Defectives Group II", "Defective Children", "Senior Normals", and "Junior Normals".

Each of these groups was divided, after the initial testing, into an experimental section ("E") and a control section ("C"). In every case, the members of the E section received glutamic acid, and the members of the C section received a control mixture during the same period. At the end of the period (four months in the case of the three defective groups, and three in the case of the normals) every subject was again tested, with the same or equivalent tests as on the first occasion. The roles of the two sections within each group were then reversed, so that subjects who were formerly experimental became controls, and vice versa, for a second period of treatment of the same duration as the first, at the end of which the test battery was administered for the third time.

At the beginning of the experiment each subject in the E section was matched as closely as possible, on the basis of age, sex, and intelligence, with a subject in the corresponding C section.

Selection of the Subjects

Adult Mental Defectives, Group I.—A preliminary selection was made of 88 adult patients in the Manor Hospital, Epsom, who would be in the 21–29 age group when the experiment began. From this number there were then rejected (*a*) those with a history of epilepsy or organic nervous disease; (*b*) those who showed any signs of organic nervous disease on physical examination; (*c*) those who were in isolated wards of the hospital in numbers insufficient to justify the special supervision necessary; (*d*) those whose I.Q.s were below 45 on the new Stanford-Binet scale.

There were 36 remaining, of whom only 26 continued to receive glutamic acid or the placebo regularly for four months, and 23 then completed the second period of four months. The I.Q. range of the 26 was 48–72. Those who failed to complete the experiment did so for reasons beyond the experimenters' control.

Defective Children.—All children in the hospital whose ages were between 10 and 14 years were first selected, and from these were rejected those who fell into categories (*a*), (*b*), and (*d*) as defined above. There remained 20, of whom 16 completed both the four month periods. Their I.Q. range was 41–66.

Normal Boys.—All the boys in Dr. Barnardo's Home, New Lodge, Windsor, whose ages were between six and 15 were first chosen, and from these were rejected those who fell into categories (*a*) and (*b*), and also those who were likely to leave the home before the end of the experiment. Of the number remaining, all those over 10 were tested with the Thurstone primary mental abilities group test. Those between eight and 10 were given the same test, and the ten boys who were most successful, as measured by the sum of the scores on the word-meaning and the reasoning tests, were included in the senior group. The remainder constituted the junior group. Sixty-one members of the senior group completed the first period of three months, and 56 completed the second period. In the junior group, 24 completed the first period, and 21 the second period. The members of the senior group were tested with the Wechsler-Bellevue intelligence scale I, and their I.Q.s ranged from 65 to 112, with the exception of two boys, whose I.Q.s were 58. The I.Q. range of the juniors, who were tested with the new Stanford-Binet scale, was 67–112.

Adult Mental Defectives, Group II.—This group was added when the second stage of treatment had been reached by Group I. It consisted entirely of women, who were selected according to the same criteria as Group I, except that the lower I.Q. limit was set at 40. Twelve patients became available, with I.Q.s ranging from 43 to 74. They received only one period of treatment (four months).

The subjects in each group were paired on the basis of age, sex, and intelligence (i.e. the Binet I.Q. in all cases except the senior boys, whose intelligence was measured, for this purpose, by the sum of the scores made on the word-meaning and the reasoning sections of the P.M.A.), and the result of a coin-toss determined which member of each pair was allocated to the E section of the group.

Materials and Administration

The l-glutamic acid solution (sodium glutamate) was made up by mixing l-glutamic acid in distilled water and neutralizing with caustic soda solution to *p*H 7, the strength of the final solution being such that 1 g. glutamic acid was contained in 4 ml. Sacch. ust. was added to approximate the colour to that of the control solution. The control solution was made up of yeast extract 2, sodium chloride 3, aq. chlor. 25, water to 100.

Both these solutions had a similar appearance and taste. They were either made up freshly each week or stored in a refrigerator up to a maximum of three weeks. As a check on the l-glutamic acid content of the solution used, two specimens were determined by l-glutamic acid decarboxylase. Both were initially described as containing 25 g. glutamic acid per 100 ml. The first, tested after five months, during nearly the whole of which time it had been kept in a refrigerator, contained 20·1 g./100 ml. The second, tested after three weeks kept mostly at room temperature, contained 19·9 g./100 ml.

The solutions were administered by mouth in water twice or three times a day, with or before a meal. In all cases the beginning dose of glutamic acid was 12 g. per

day, and this was increased to a maximum of 20–36 g. per day in different subjects. Those in the control groups received corresponding amounts of the control solution.

In all cases the period of administration of each solution was not less than 12 weeks in the two normal groups, and 15–16 weeks in the three defective groups. After stopping the administration of either solution to any subject, the intelligence and other tests were administered after an interval of not less than six hours nor more than two days; within a week of the discontinuance, the administration of the other solution was begun.

The l-glutamic acid and control solutions were labelled "Mixture Z" and "Mixture A", and neither the staff nor the subjects were aware which mixture contained glutamic acid.

Clinical Progress

Before the treatment began the subjects were interviewed, examined physically, and told that they were to be given a special kind of tonic, which, it was hoped, would make them quicker, and that they would be tested before and after to find out if they had benefited. With a few exceptions they seemed anxious to cooperate, and in only five cases was it necessary to discontinue the treatment because of the subject's refusal to take the tonic.

The noticeable effects of the administration were few. In two adults vomiting occurred on several occasions after glutamic acid, but not after the control mixture. Many complaints of a feeling of nausea, particularly in the morning, were reported. Among the children, 28 complained of feeling sick after the glutamic acid, and four after both solutions. Six children vomited on several occasions after glutamic acid.

No other effect was noted throughout the whole period of the experiment, except that the Superintendent of the boys' home noted that two of the boys, generally difficult to manage and subject to violent outbursts of temper, had ceased to have any outbursts of temper while they were receiving Mixture Z (glutamic acid), but that within one week of starting to have Mixture A, their former behaviour had reasserted itself. One of the boys was then put on Mixture A and the other on Mixture Z. During the following week the boy on the control mixture had several temper outbursts, while the other had none. At the beginning of the next week the mixtures were exchanged, and during this week neither of the boys was reported to be difficult. Eight months after the cessation of the treatment, the Superintendent reported that both boys had reverted to their former behaviour; arrangements are being made for them to resume the treatment. The I.Q. (Wechsler) of one of the boys, aged 14 years 3 months at the beginning of the experiment, was 58. It had increased to 68 after three months on the control mixture, and to 73 after three months on glutamic acid. The corresponding I.Q.s of the other boy, aged 10 years 5 months were 78, 83, and 86. Increases of this order were frequent in both the E and C sections of the group (senior normal).

In view of the few side-effects noted, it was decided to give rapidly increasing doses of glutamic acid to a small group of subjects in whom no effects had previously been noticed. Four women in the 21–29 age group and four defective boys in the 10–14 age group were selected, and were given a sludge of glutamic acid powder in water, starting with 24 g. per day in divided doses and increasing by 12 g. per day. On the first and second days, due to a misunderstanding, the four boys each received 72 and 108 g., without showing any consequences save a slight feeling of nausea. After reverting to the planned dosage, they and the four adult subjects continued to have increasing doses until each was having 96 g. of glutamic acid per day. After one week no effects had been noticed in the adults, but two of the children were more active and excitable.

The Tests

For the defective adults Group I, the defective children, and the normal boys junior group, the following battery, known as "T_1", was used at the beginning of the experiment, before any medication had been started.

- Revised Stanford-Binet, Form L
- Kohs' blocks (Drever-Collins version)
- Manikin and profile object assembly test (Drever-Collins)
- Healy picture completion test No. 1 (Drever-Collins)
- Goodenough "drawing a man" test
- Body sway test of suggestibility

The batteries used for the second and third testing sessions ("T_2" and "T_3" respectively) were the same as the above, except that the static ataxia test was introduced immediately before the body sway test, and that the Binet form M was used in place of form L in T_2. In T_3, form L was used again.

The defective adults Group II, who were, as previously indicated, added when the other defective groups were entering the second phase of the experiment, were given the same battery that has already been described. In their T_1, the Binet form M was used, and form L in their T_2. The static ataxia test was included on both occasions.

The battery used for the normal boys senior group was as follows:

- Wechsler-Bellevue intelligence scale I
- Goodenough "drawing a man" test
- Static ataxia test (in T_2 and T_3)

Body sway test
Thurstone primary mental abilities group test
Rorschach group test (using lantern-slides of the ink-blots)
Maudsley letter series group test

After the rejection of those subjects who failed to satisfy the criteria previously stated, a few more were lost from each group, either because they could not be matched sufficiently closely, or because of refusal to take the mixture, or because of unexpectedly becoming unavailable within a few days of the completion of T_1. All who were rejected for any of these reasons were classified as " not used ", and their mean I.Q.s have been compared with the mean I.Q.s of those who were used. Table I shows the results of these comparisons.

In the case of the defective children, where a highly significant difference occurs, the reason for the rejection of all three subjects was inability to match.

At later stages in the experiment a few more subjects were lost for reasons beyond the control of the experimenters, and it also happened occasionally that a subject was not able to take all the tests in the battery. Due to the disturbance of the matching thus entailed, a modification was introduced into the statistical treatment of the data.

The Statistical Technique

The hypothesis to be tested was that the mean improvement of the experimental section on any test would be significantly greater than the corresponding improvement of the control section.

If G represents the effect attributable to glutamic acid, and P the effect attributable to the placebo, it is possible to derive from the test scores obtained in this type of experiment an estimate of the difference G–P, representing the excess of the improvement due to glutamic acid over that due to the placebo, and to test the significance of this estimate. Since the sign of this difference is specified by the hypothesis (positive in all cases except that of the static ataxia and body sway tests, where the sign is negative), the significance of the estimated difference can be tested by the one-tail t-test when the difference is in the predicted direction. Those differences which are not found to be in the predicted direction show immediately without testing that the hypothesis is not confirmed.

The procedure adopted was as follows. From the score of each subject on a test in T_2 was deducted his score on that test in T_1. The resulting difference, D, with its sign, represented the subject's improvement (or otherwise) during the first period of the experiment. If the mean improvement of the experimental subjects is written $\bar{D}_e$, and the mean improvement of the controls is written $\bar{D}_c$, then the expression $\bar{D}_e-\bar{D}_c$ represents that function of the test scores which was taken as the estimate of G–P. The effects of growth, practice and errors were assumed to be equal, on the average, between the experimental and control sections of the group tested.

The same procedure was adopted in the treatment of the scores made on the tests in T_3 and T_4, which were administered at the beginning and end respectively of the second period of the experiment. In this case, D^1 represents the score on a test in T_3 minus the score on the same test in T_2, and it can be shown algebraically that the function $\bar{D}^1_e-\bar{D}^1_c$ is an estimate of twice the difference G–P, on the same assumption that the effects of growth, practice, and errors cancel out.

The technique of analysis of variance, of which the ordinary t-test is the simplest case, was used for the treatment of the data. It was originally intended to use that version of the technique which has been devised for data obtained from matched groups, such as were arranged for this experiment, but owing to the loss of some of the subjects after the matching had been carried out, and to the already small numbers of subjects in the groups, it was decided, in consultation with A. Lubin, to disregard the matching entirely, and to apply the analysis of variance technique in its ordinary form. There was no reason to suppose that this introduced any bias. Fisher states (1941) that

> " In cases in which each observation of one series corresponds in some respects to a particular observation of the second series, it is always legitimate to take the differences and test them ... as a single sample ; but it is not always desirable to do so. A more precise comparison is obtainable by this method only if the corresponding values of the two series are positively correlated, and only if they are correlated

TABLE I

Group	Test	Numbers		Mean I.Q.s		Variances		Significance of Difference
		Used	Not Used	Used	Not Used	Used	Not Used	
Defective adults I ..	Binet	26	9	58·4	65·0	72·2	152·0	—
Defective adults II ..	Binet	12	2	57·8	61·5	163·4	0·5	—
Defective children ..	Binet	16	3	49·2	61·7	51·8	369·3	·1%
Senior normal boys ..	Wechsler	61	16	88·1	86·3	147·7	169·2	—
Junior normal boys ..	Binet	24	4	95·0	80·0	142·1	86·0	2·5%

to a sufficient extent to counterbalance the loss of precision due to basing our estimate of variance upon fewer degrees of freedom."

In the present case, using the method for matched groups (i.e. the first method described by Fisher) would have entailed the loss of unmatched subjects as well as half the total number of matched subjects in each group, for the purpose of computing the number of degrees of freedom available. It should be noted that the two "series of observations" which were compared were not the actual scores on the tests, but the differences between the scores on successive administration of the tests.

The Results

Table II shows the results obtained from the test scores made by members of four of the groups, and the results from the fifth group are shown in Table III. The "drawing a man" test, and the group Rorschach and Maudsley letter series have not yet been scored, and their results will form the subject of a later report.

Inspection of the cognitive test results in Table II shows that in no case where $\bar{D}_e-\bar{D}_c$ (i.e. the estimate of G–P) is positive, is it significant. In fact, t was found to be less than unity in every such case. For the static ataxia and body sway tests a negative value of G–P was predicted, since improvement in personality would be expected to be associated with decreased sway under the conditions of these tests. Negative values of $\bar{D}_e-\bar{D}_c$ occur seven times in Table II, and of these seven negative values, one is significant at the 5% level and one at the 1% level, by the one-tail t-test. In four of the remaining five cases, t was less than unity.

Table III shows the results obtained from the analysis of the scores made by members of the normal senior group on the ten tests which constitute the Wechsler scale I, the five tests of which the P.M.A. group test is comprised, and the static ataxia and body sway tests. Of the three values for $\bar{D}_e-\bar{D}_c$ provided by the latter, only one is negative as predicted, and it is not significant. Twenty estimates of G–P are provided by the Wechsler results, and of these 20, one is significant at the 5% level, three at the 2½% level, and one at the 1% level. In only one other case was t greater than unity. By chance alone, one result out of 20 would be expected to be significant at the 5% level.

TABLE II

Group	Statistic	Binet Mental Age (Months)		Kohs' Blocks (Raw Scores)		Object Assembly (Raw Scores)		Picture Completion (Raw Scores)		Static Ataxia (Half-inches)		Body Sway (Half-inches)	
		E	C	E	C	E	C	E	C	E	C	E	C
Defective Adults Group I	First Period:												
	Number ..	14	12	14	12	14	12	14	12			12	12
	Mean Gain ($\bar{D}$)	9·21	14·83	2·71	3·00	1·00	1·92	1·36	1·75	—		−0·33	−0·08
	Variance ..	75·26	63·61	1·91	26·36	19·38	26·99	14·71	8·02			2·24	16·45
	Significance ..	—		—		—		—		—		—	
	Second Period:												
	Number ..	10	13	10	13	10	13	10	13	10	13	8	13
	Mean Gain ($\bar{D}'$)	−4·20	0·69	0·60	0·85	0·00	1·31	1·40	1·38	0·40	−0·23	−1·12	0·85
	Variance ..	52·18	39·90	6·93	9·81	14·00	8·90	4·93	10·09	3·38	1·69	2·70	1·97
	Significance ..	—		—		—		—		—		<1%	
Defective Adults Group II	Only Period:												
	Number ..	6	6	5	6	6	6	6	6	6	4	6	4
	Mean Gain ($\bar{D}$)	3·33	−1·17	1·60	2·33	1·83	2·00	1·67	2·50	−0·33	2·75	−0·54	0·50
	Variance ..	98·67	125·77	0·80	16·27	10·57	11·20	14·67	4·70	3·47	2·92	16·57	0·33
	Significance ..	—		—		—		—		<5%		—	
Defective Children	First Period:												
	Number ..	8	8	8	8	8	8	8	8			8	7
	Mean Gain ($\bar{D}$)	1·25	3·37	1·50	1·25	0·25	−0·25	2·25	3·00	—		1·75	2·14
	Variance ..	4·50	21·98	5·43	3·36	3·14	14·86	21·93	12·86			11·07	4·48
	Significance ..	—		—		—		—		—		—	
	Second Period:												
	Number ..	8	8	8	8	8	8	8	8	7	8	7	8
	Mean Gain ($\bar{D}'$)	1·75	0·25	−0·50	0·25	3·37	1·50	0·87	1·62	0·14	−1·00	−0·86	−1·75
	Variance ..	4·96	11·64	2·75	3·44	18·55	15·71	26·41	4·84	1·48	2·86	5·81	14·50
	Significance ..	—		—		—		—		—		—	
Normal Boys Junior Group	First Period:												
	Number ..	12	12	12	12	12	12	12	12			12	11
	Mean Gain ($\bar{D}$)	3·83	2·50	1·75	1·67	1·58	0·67	1·67	1·42	—		−1·25	−1·00
	Variance ..	93·61	101·36	0·75	1·33	10·81	4·24	13·33	19·90			4·85	15·09
	Significance ..	—		—		—		—		—		—	
	Second Period:												
	Number ..	11	10	11	10	11	10	11	10	10	10	10	10
	Mean Gain ($\bar{D}'$)	5·45	5·10	−0·36	1·20	1·64	0·00	2·64	3·20	−0·20	−0·30	0·50	0·60
	Variance ..	102·47	21·88	4·65	6·18	17·65	17·78	8·25	7·73	7·73	8·46	0·94	3·38
	Significance ..	—		—		—		—		—		—	

TABLE III

Normal Boys: Senior Group		Test	First Period							Second Period						
			Numbers		Mean Gains		Variances		Significance	Numbers		Mean Gains		Variances		Significance
			N_e	N_c	$\bar{D}_e$	$\bar{D}_c$	V_e	V_c		N_e	N_c	$\bar{D}_e$	$\bar{D}_c$	V_e	V_c	
Wechsler (Raw Scores)	Verbal	General comprehension	30	31	1·17	0·64	4·97	3·97	—	27	29	0·22	−0·79	3·03	2·60	2·5%
		Arithmetic	30	31	0·97	−0·03	1·55	2·77	1%	27	29	0·00	−0·14	2·69	1·12	—
		Similarities	30	31	1·63	1·32	5·21	5·16	—	27	29	0·41	0·52	4·63	3·83	—
		Digit repetition.. ..	30	31	0·30	−0·13	2·15	2·05	—	27	29	0·48	0·31	1·26	1·29	—
		Vocabulary	30	31	0·35	1·00	2·09	3·25	—	27	29	0·00	0·40	2·92	1·93	—
	Performance	Picture arrangement ..	30	31	2·10	1·58	10·44	6·92	—	27	29	1·18	1·45	6·93	14·47	—
		Picture completion ..	30	31	1·50	1·29	4·67	1·95	—	27	29	0·63	0·28	1·24	2·99	—
		Object assembly.. ..	30	31	1·77	2·26	3·23	15·20	—	27	29	2·11	1·59	9·64	8·82	—
		Block design	30	31	3·37	3·26	15·76	14·60	—	27	29	3·07	0·86	18·69	8·77	2·5%
		Digit symbol	30	31	5·32	2·81	37·04	26·73	5%	27	29	2·63	0·19	12·47	17·56	2·5%
P.M.A. (Raw Scores)		Word meaning	27	30	1·18	3·40	15·00	11·08	—	26	25	2·04	0·88	22·44	31·53	—
		Space	30	31	7·60	6·26	63·90	91·73	—	27	28	2·41	2·86	27·25	65·24	—
		Reasoning	29	30	1·76	1·00	11·62	14·90	—	26	27	0·69	0·37	9·82	26·63	—
		Number	30	31	1·77	0·35	20·05	17·70	—	27	28	1·37	−1·71	21·63	31·47	2·5%
		Word fluency	30	31	1·53	0·71	22·46	31·88	—	26	27	3·04	1·33	21·96	21·69	—
Static ataxia (half-inches)			—	—	—	—	—	—	—	26	29	1·31	0·55	3·82	5·68	—
Body sway (half-inches)			28	26	0·93	0·04	9·70	9·08	—	26	29	0·00	0·14	3·36	8·91	—

Of the ten estimates of G–P derived from the P.M.A. scores, one is significant at the 2½% level. By chance, one out of ten would be expected to be significant at the 10% level. Values of t greater than unity were found in two other cases in the P.M.A. results.

It will be observed that, whereas in Table II 16 of the 28 values for $\bar{D}_e-\bar{D}_c$ derived from the cognitive test scores are negative, Table III shows only seven out of 30 values for the same function to be negative.

Discussion

It seems fairly clear that the cognitive test results in Table II (i.e. those of the three defective groups and the junior normal group) afford no evidence whatever in favour of the hypothesis. Not only is none of the positive differences significant, but there are approximately as many negative as positive differences, which is in accord with chance expectation.

The results of the ataxia and suggestibility tests are more difficult to interpret. There are 14 such results altogether, including those in Table III, as the tests were exactly the same for the senior normal group. Eight of these, that is, just over half of them, are in the predicted direction, which, again, is in accordance with chance expectation; but two of them are significant, one at the 1% level, and one at the 5% level. Strictly speaking, this is not what would be expected on a chance hypothesis, but the departure from a chance prediction is scarcely large enough to be regarded as affording any but the slenderest evidence in favour of the hypothesis of personality improvement.

The results obtained from the Wechsler scores of the senior normal group (Table III) show some departure from the general pattern of the other results. These scores provide 20 estimates of G–P or, more precisely, ten of G–P and ten of 2(G–P): the distinction is immaterial in the present context. Fifteen of these estimates are in the predicted direction, and five of them are significant. On a chance hypothesis, ten would be expected to be positive, and one to be significant at the 5% level. It does appear, therefore, that in this case there is some slight evidence in favour of the main hypothesis. A difficulty arises, however, from the fact that the tests used are not independent. The tests which yielded the significant differences are the digit symbol (5% and 2½%), block design (2½%), general comprehension (2½%), and arithmetic (1%). Examination of the table of inter-test correlations given by Wechsler (1944), based on 355 cases in the 20-34 age group, discloses that the inter-test correlations under consideration range from 0·429 to 0·538 The table shows eight inter-correlations higher than 0·538 and 17 lower than 0·429. The highest in the table is 0·721, the median is 0·456, and the lowest is 0·155. If these published (American) inter-correlations are accepted as a basis for comparison in the present case, the objection that the tests are not independent appears to have force.

An alternative approach is to consider the character of the tests which have yielded significant results. In all except one (general comprehension) the score of the subject depends partly on his speed of performance. This suggests that the observed effect of glutamic acid on the test scores may be explicable in terms of a general improvement in mental and physical alertness, or of a raising of the general activity level, as reported by other investigators. If this were so, then significant differences would be expected in the P.M.A. scores, since the

five tests in this battery are all rigorously timed. Table III shows that eight of the ten estimates of G-P [or 2(G-P)] derived from these scores are in the predicted direction, and one of them is significant at the 2½% level. Although this is slightly above chance expectation, it is less so than in the case of the Wechsler scores. It is unlikely, therefore, that improvement in alertness, activity, or speed because of glutamic acid accounts for the improvement in the Wechsler scores.*

The general conclusion must be that the changes in the test scores of the subjects who were fed with glutamic acid in this experiment have provided little or no evidence in favour of the hypotheses which were to be tested.

The glutamic acid was administered in this experiment as a solution, neutralized with caustic soda: the New York investigators administered it suspended in water. As there is a possibility that the effects of these two modes of administration might be different because of the large amount of alkali received in the former, a subsidiary experiment was carried out by Dr. D. L. Davies. Since it was not practicable to determine the glutamate level in the blood at regular intervals after ingestion, because of the large quantity of blood required for each estimation and the uncertainty as to the range of individual variation, it was decided to measure the total acidity of the urine, and the serum alkali reserve after glutamic acid and after sodium glutamate, in order to test the correctness of an assumption that on an ordinary mixed diet no difference in the effect upon alkali reserve would be found. Two patients were studied. Patient A, aged 27, was observed over a period of five weeks in hospital. His diet was the ordinary hospital fare. During the first week he received no drugs. During the second week he received 10 g. of sodium glutamate b.d. for the first five days; on the sixth day the morning dose was doubled and on the seventh day both doses were doubled. During the third week no drugs were administered. During the fourth week he received 10 g. of glutamic acid powder for the first five days; on the sixth day the morning dose was doubled and on the seventh day both doses were doubled. During the fifth week no drugs were administered. Throughout this period 24 hour specimens of urine were collected, checked by the estimation of total creatinine, and subjected to titration of total acidity by Folin's method, the results being expressed in ml. $\frac{N}{10}$ alkali. Every Monday, Wednesday, and Friday morning at 11 a.m., with the patient resting in bed for at least 30 minutes, venous blood was taken for the estimation of serum alkali reserve by Van Slyke's titration method, the result being expressed in vols. of CO_2/100 ml. Patient B, a man aged 39, suffering from a chronic anxiety state, was subject to the same procedures except that he received glutamic acid on the days when Patient A received sodium glutamate, and vice versa. This was to remove any subjective bias in the technical assistants who did not know which drug the patients were receiving. In neither patient was there evidence of impairment of kidney or liver function. No symptoms suggestive of acidosis or alkalosis were observed. The results are shown in Table IV on page 54.

Although the expected shifts in the total titratable acidity of the urine occurred, the fluctuations in the alkali reserve followed no such clear-cut trend. Indeed the lowest reading in Subject A was obtained in the initial control period after he had recently finished strenuous physical exercise. The changes in the level of the alkali reserve were evidently well within the normal physiological range. Any difference between the effects obtained after glutamic acid and those obtained after sodium glutamate cannot be attributed to different effects upon the alkalinity of the serum.

Summary

Two groups of mentally defective adults, one group of mentally defective children, and two groups of normal boys, were divided each into an experimental and a control section. Before and after treatment with glutamic acid or with an indifferent substance, each subject was given verbal, performance, and personality tests. After the second test administration each subject was transferred to the opposite section for a further period of treatment, at the end of which the tests were administered for a third time. The results of the cognitive tests provided no evidence in favour of the hypothesis that glutamic acid improves cognitive functioning, except in one group of normal boys, whose findings yielded slight but equivocal evidence in favour of the hypothesis. For the additional hypothesis, that scores on the personality tests would be improved, there was no evidence.

We wish to record our indebtedness to Dr. H. E. Magee, of the Ministry of Health, for advice regarding the choice of a home for normal children, and to Dr. Gilmore and the authorities of Dr. Barnardo's Homes for permission to conduct part of the trial in New Lodge, Windsor; to the Superintendent, Mr. Mace, and to Mrs. Mace and the staff of the New Lodge, Windsor, to

* A small experiment, designed similarly to that described in this paper, was conducted by Dr. B. H. Kirman and Miss S. M. Cox at the Fountain Hospital, Tooting. The subjects were three pairs of children, with I.Q.s ranging from 36 to 58 (Stanford-Binet), and the test battery consisted of a variety of standard verbal and performance tests. The period of administration was three months, which was repeated with the subjects in reversed roles. No significant evidence of improvement due to glutamic acid was obtained, but the biggest increases observed in the experimental subjects were those in the Porteous maze test.

TABLE IV

	Patient A		Patient B	
	Total Titratable Acid in ml. N/10 alkali (24 hour excretion)	Serum Alkali Reserve Vol. CO_2/100 ml.	Total Titratable Acid in ml. N/10 alkali (24 hour excretion)	Serum Alkali Reserve Vol. CO_2/100 ml.
Week 1	(No drugs)		(No drugs)	
8-9 March	217	76	298	71
9-10 "	267	—	163	—
10-11 "	194	66*	240	70
13-14 "	182	75	158	72
14-15 "	272	—	209	—
Week 2	(Sodium glutamate)		(Glutamic acid)	
15-16 March	144	79	269	69
16-17 "	139	—	261	—
17-18 "	141	74	365	67
20-21 "	128	80	197	76
21-22 "	194	—	310	—
Week 3	(No drugs)		(No drugs)	
22-23 March	140	71	232	71
23-24 "	168	—	170	—
24-25 "	206	70	246	64
27-28 "	266	69	230	69
28-29 "	246	—	166	—
Week 4	(Glutamic acid)		(Sodium glutamate)	
29-30 March	203	69	81	77
30-31 "	306	—	72	—
31 March-1 April	217	66	69	75
3-4 April	240	72	107	82
4-5 "	316	—	97	—
Week 5	(No drugs)		(No drugs)	
5-6 April	192	70	103	69
6-7 "	233	—	92	—
7-8 "	256	71	329	70
10-11 "	331	—	249	—
11-12 "	196	70	157	69
12-13 "	205	—	225	—

* This reading was obtained within 30 minutes of finishing physical exercise.

Dr. H. T. McMahon and the pharmacist and other members of staff of the Manor Hospital, Epsom, and to Dr. L. T. Hilliard and the pharmacist and other staff of the Fountain Hospital, for their willing cooperation; and to Mr. A. Lubin for his guidance in the statistical analysis. The Ministry of Health, by arrangement with Professor Aubrey Lewis, provided the glutamic acid and defrayed part of the expenses.

REFERENCES

Albert, K., Hoch, P., and Waelsch, H. (1946). *J. nerv. ment. Dis.*, 104, 263.
Albert, K. E., and Warden, C. J. (1944). *Science*, 100, 476.
Ewalt, J. R., and Bruce, E. I. (1948). *Tex. Rep. Biol. Med.*, 6, 97.
Eysenck, H. J. (1947). "Dimensions of Personality." London.
Fisher, R. A. (1941). "Statistical Methods for Research Workers", 8th ed. Edinburgh.
Gasorek, K. A. (1948). *Amer. Psychologist*, 3, 253.
Hamilton, H. C., and Maher, E. B. (1947). *J. comp. physiol. Psychol.*, 40, 463.
Harney, M. (1950). *Stud. Psychol. Psychiat. Cathol. Univ. Amer.*, 8, 1.
Kitzinger, H., DeVere, G. A., Cartwright, R. W., and Shapiro, D. (1949). *Rorschach Res. Exch. J. Proj. Tech.*, 13, 210.
Marx, M. H. (1948). *J. comp. physiol. Psychol.*, 41, 82.
Price, J., Waelsch, H., and Putnam, T. (1943). *J. Amer. med. Ass.*, 122, 1153.
Stellar, E., and McElroy, W. D. (1948). *Science*, 108, 281.
Waelsch, H., and Price, J. (1944). *Arch. Neurol. Psychiat., Chicago*, 51, 393.
Wechsler, D. (1944). "The Measurement of Adult Intelligence", 3rd ed. Baltimore.
Zimmerman, F. T., Burgemeister, B. B., and Putnam, T. J. (1946). *Arch. Neurol. Psychiat., Chicago*, 56, 489.
Zimmerman, F. T., Burgemeister, B. B., and Putnam, T. J., (1947). *Psychosom. Med.*, 9, 175.
——, ——, ——, (1948). *Amer. J. Psychiat.*, 104, 593.
——, ——, ——, (1949a). *Arch. Neurol. Psychiat., Chicago*, 61, 275.
——, ——, ——, (1949b). *Amer. J. Psychiat.*, 105, 661.
——, and Ross, S. (1944). *Arch. Neurol. Psychiat., Chicago*, 51, 446.

[6] Cohen, A. I., *Amer. J. Anat.*, 120, 319 (1967).
[7] Olney, J. W., *J. Neuropath. Exp. Neurol.*, 28, 455 (1969).
[8] Olney, J. W., *Science*, 164, 719 (1969).
[9] Redding, T. W., and Schally, A. V., *Fed. Proc.*, 29, 755 (1970).
[10] Olney, J. W., and Sharpe, L. G., *Science*, 166, 386 (1969).
[11] Gerber Products, Inc., *Hearings before the Select Committee on Nutrition and Human Needs of the US Senate*, 13A, 4170 (July 1969).
[12] Lowe, C. U., *Science*, 167, 1010 (1970).
[13] Blood, F. R., Oser, B. L., and White, P. L., *Science*, 165, 1028 (1969).
[14] Curtis, D. R., and Crawford, J. M., *Ann. Rev. Pharm.*, 9, 209 (1969).

Monosodium Glutamate and the Chinese Restaurant Syndrome

It has been suggested[1-4] that monosodium glutamate (MSG) is responsible for the "Chinese restaurant syndrome"—a burning sensation in the back of the neck spreading to the forearms and to the anterior thorax, accompanied by a feeling of infraorbital pressure, tightness and substernal discomfort. But no study of this phenomenon involved a double blind technique, or any other experimental condition which can be used to assess the significance of subjective reactions.

We have carried out a study on twenty-four healthy volunteers (seventeen males and seven females) aged between 18 and 34 (mean 25), using double blind and crossover techniques. MSG was administered at lunch time in doses of 3 g/subject in 150 ml. of beef broth. This was followed by other dishes (meat, vegetables and fruit).

Subjective and objective evaluations were carried out every 20 min during the following 3 h. The subjects, randomly divided into two groups, were told they were going to receive three different kinds of broth, one of which would contain MSG (the L isomer). Observations were carried out in two experimental sessions. In the first, eight subjects received MSG in the broth and sixteen had normal beef broth—in the second session, two days later, the eight subjects who had previously received MSG were given normal beef broth, and the other sixteen were given MSG in the broth.

The subjects were asked to specify every twenty minutes any particular feeling or sensation they were experiencing. They were supplied with a form which listed possible symptoms (headache, burning feeling, flushing, facial pressure, chest pressure, weeping, gastric distress, nausea, perspiration, tiredness), and there was space to record additional symptoms. The symptoms had to be scored as mild, moderate or severe. These parameters were considered to be subjective symptoms. At the same time, arterial blood pressure (max and min), pulse and respiration rate were recorded. These parameters were considered to be objective symptoms. The data were subjected to the χ^2 test, Bartlett-box test and variance analysis.

No differences in subjective symptoms were observed between the subjects given MSG and those who had normal beef broth (Tables 1 and 2). There was also no significant difference in the number of times each single symptom occurred in the 3 h period (Table 1) nor in the number of subjects who experienced symptoms at each time (Table 2). There was a tendency in both groups to experience more symptoms during the first hour.

It should be emphasized that nobody, either in the control or in the MSG group, experienced the burning feeling which is typical of the Chinese restaurant syndrome. Also, there was no difference in the intensity of the symptoms experienced by each group. Only one subject, a female 24 years old, experienced a panic-like syndrome after being given MSG. This lasted for about 20 min, but it was not associated with any significant modification of objective parameters such as arterial pressure, pulse or respiration rate.

There was no significant variation in objective parameters ($P > 0.05$). There was some tendency towards an increase in maximum blood pressure in those given MSG, but there was no variation in minimum values (Table 3). In fact, by variance analysis, there was a significant difference ($P < 0.01$) in the overall variation. It should be noted that all the blood pressure variations are within the physiological modifications which take place during a normal digestive process. Previous authors[3] have reported that MSG is responsible for the Chinese restaurant syndrome in a very small number of subjects. The same authors later studied the acute pharmacology of MSG using an experimental procedure which was not fully described[4]. No double blind trials or statistical analysis were reported. The high dose used and the route of administration were also very questionable.

From our observations, there is no evidence that Chinese restaurant syndrome follows ingestion of MSG in a beef broth. The only subject who described a panic-like syndrome did not experience any burning feeling. Moreover, many of the subjective symptoms considered to be part of the Chinese restaurant syndrome may also be present during a normal digestive process. We therefore believe that MSG administered orally in relatively high doses does not provoke any symptoms of Chinese restaurant syndrome.

We thank Dr S. Paglialunga for the statistical

Table 1. INCIDENCE OF THE VARIOUS SUBJECTIVE SYMPTOMS IN THE 3 H OBSERVATION PERIOD

Symptoms	Number of subjects experiencing the symptom		Number of times (observations) in which the symptom was experienced in the three hours	
	Control	MSG	Control	MSG
Headache	1	2	3	5
Burning feeling	0	0	0	0
Flushing	3	1	4	2
Facial pressure	0	1	0	2
Chest pressure	4	2	10	3
Weeping	0	1	0	1
Fainting feeling	1	2	2	4
Gastric distress	2	2	6	3
Nausea	0	1	0	2
Perspiration	1	1	1	2
Tiredness	5	4	15	17
Total	17	17	41	41

No difference between control and MSG groups was statistically significant ($P > 0.05$).

Table 2. INCIDENCE OF SYMPTOMS AT VARIOUS TIMES AFTER ADMINISTRATION

Time (min)	Number of subjects experiencing symptoms		Total number of symptoms experienced	
	Control	MSG	Control	MSG
20	5	6	7	14
40	6	5	7	7
60	5	4	7	5
80	4	1	6	1
100	4	1	4	1
120	2	2	3	1
140	2	1	1	1
160	1	0	2	0
180	1	0	1	0
		Total	38	30

No difference between control and MSG groups was statistically significant ($P > 0.05$).

Table 3. CHANGES IN BLOOD PRESSURE AFTER MSG

Time (min)	Maximum values		Minimum values	
	Control	MSG	Control	MSG
20	−1·54±1·50	+0·67±1·61	+2·06±1·92	+1·71±2·18
40	−3·29±1·90	+1·08±2·02	+1·58±2·49	+4·00±2·82
60	−5·71±2·34	−0·87±2·01	+0·79±2·58	+0·96±3·22
80	−4·38±2·38	−1·42±1·83	−3·67±2·52	−1·04±2·92
100	−5·29±2·45	−2·29±2·15	−6·83±2·56	−0·67±3·06
120	−5·25±1·91	−0·37±2·16	−6·08±2·36	−3·12±2·96
140	−3·79±2·15	−1·54±2·48	−4·79±1·98	−3·83±3·04
160	−3·75±2·22	−3·92±1·62	−0·17±2·03	−0·67±3·30
180	−4·83±1·82	−0·79±1·72	−2·50±2·24	−1·04±3·05

The figures show the mean differences (mm Hg) ± s.e. from the basal values of blood pressure. Basal values in control session = 114 ± 2 (max); 67 ± 1 (min); basal values in MSG session = 111 ± 2 (max); 69 ± 2 (min).

evaluation of the results. MSG was kindly supplied by COFAG (Paris).

P. L. MORSELLI
S. GARATTINI

Istituto di Ricerche Farmacologiche,
"Mario Negri", Via Eritrea, 62,
20157 Milano, Italy.

Received March 24, 1970.

[1] Man-Kwdk, R. Ho, *New Engl. J. Med.*, 278, 706 (1968).
[2] Schaumburg, H. H., and Byck, R., *New Engl. J. Med.*, 279, 105 (1968).
[3] Abos, M., Leavitt, M. R., Marmorek, L., and Wolschina, S. B., *New Engl. J. Med.*, 279, 105 (1968).
[4] Schaumburg, H. H., Byck, R., Gerstl, G., and Mashman, J. H., *Science*, 163, 826 (1969).

Progressive Neuromuscular and Skeletal Disorders in a Male with an Acrocentric Supernumerary Chromosome

A CONNEXION between sex chromosome abnormalities and a widespread defect of either connective or nervous tissues has recently been discussed[1-8]. Several clinical features, such as presenile cataract, have been found to be common to individuals with *XYY*, *XXY* and other sex chromosome polysomes, as well as to those with progressive myopathies[9-11] and hereditary errors in connective tissue. The transitional forms of these familial diseases have also been reported in association with supernumerary chromosomes of a different nature[12-15].

A generalized defect in both tissues, as we have found in connexion with an acrocentric supernumerary chromosome, indicates a common aetiological factor which probably interferes with the linking biochemical systems[6,7].

A 67 yr old white male, 5 feet 2 inches tall, was admitted to St Elizabeth's Hospital, Washington DC, in 1947 for committing antisocial offences against uniformed women. On clinical examination, partially removed bilateral cataracts, hypogonadism, premature baldness and mental inadequacy were noted. Except for generalized awkwardness, there were no neurological findings.

At present he has several clinical features which were detected and recorded during his 23 yr in this hospital. The pedigree does not give any indication of hereditary disorder (Fig. 1).

His face is typically myopathic. The left ear is prominent and slightly lower than the right. A champagne bottle deformity and the webbing of his neck which were present on admission have become more pronounced in recent years. Also, the sternocleidomastoid muscles are barely palpable and there is weakness of inflexion. In addition, the patient has a pectus excavatum and a genu deformity of both elbows. The little finger of each hand shows an inward curve.

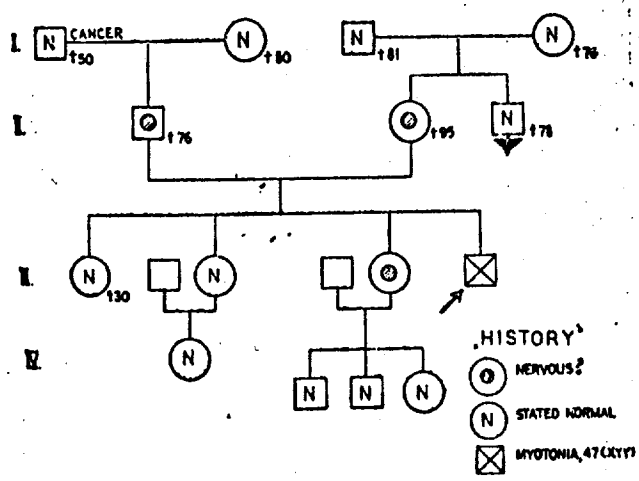


Fig. 1. The pedigree of the case described.

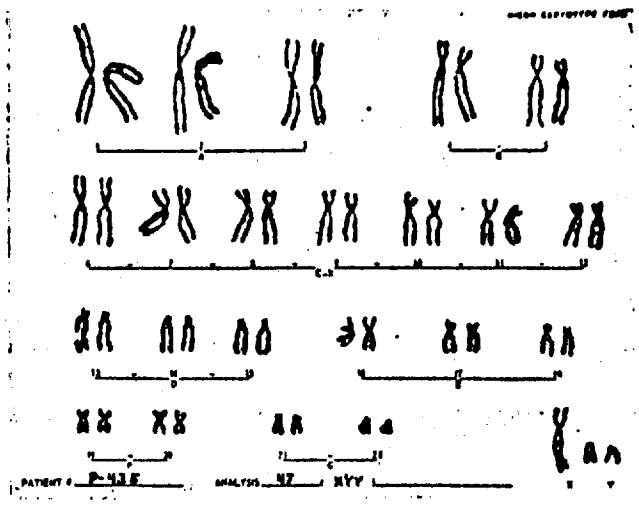


Fig. 2. The karyotype of the case described, showing a slight structural difference between the presumed *Y* chromosomes.

An umbilical hernia and bilateral hydroceles were first detected in 1961 when neurological defects began to manifest themselves with extraordinary hypotonia of the entire body. Although a progressive generalized muscle weakness slowly increased, symptoms such as hyperreflexibility of the lower limbs and the bilateral positive Babinski remained unchanged.

No myotonic response could be elicited in any muscle. Electromyograph (EMG) tests gave results consistent with myopathy; however, no myotonic response was elicited. All the other routine analyses were within normal limits. The electrokinetogram (EKG) showed a right bundle branch block. The electroencephalograph (EEG) revealed 2·25–6 cycles per second generalized slow waves and associated sharp discharge.

The slight mental deficiency we have mentioned has changed to an extremely rapid deterioration during the past 2 yr.

The modal chromosomal number was consistently 47 with the presence of an extra subterminal chromosome larger than the *G* autosomes in both blood and skin (Fig. 2), as well as in fibroblast cultures from the testicular tunica[16]. The diakinetic stages contained only 23 bivalents. No extra genetic material could be located in testicular preparations or in spermatogonial squashes from which the supernumerary chromosome or fragment was consistently absent.

Autoradiographic investigation of the DNA replication pattern revealed late labelling with asynchrony among the two non-*G* group acrocentrics. The larger of the pair (*M*) was later replication than the more compact (*Y*) acrocentric.

In most metaphase spreads, only one presumed male sex chromosome (*Y*) could be identified by its parallel long arms with distal light staining and non-satellited short arms. These findings are suggestive of a possible Cp-fragment from the long arm deletion of a group *C*+*X* chromosome.

The communications reporting sex chromosome anomalies in association with those genetic disorders which include presenile lenticular opacities and specific musculoskeletal defects have not yet provided sufficient evidence to postulate a causal relationship between both events[6-9,12,14].

The case described here has particular relevance in the interpretation of findings by authors who emphasize the possibility that supernumerary chromosomes contribute myopathies[9-11], as well as several other neurological dysfunctions[17].

Several clinical signs recorded in this case history, such as the normal EMG, the positive Babinski reflexes and the

Am. J. Psychol. 65, 573-579 (1952)

THE EFFECT OF MONOSODIUM GLUTAMATE ON ACUITY TO THE PRIMARY TASTES

By James N. Mosel, George Washington University, and
Gerald Kantrowitz, USAF Human Resources Research Laboratory

In recent years food technologists have displayed increasing interest in certain unusual taste properties which have been claimed for monosodium glutamate (*MSG*). Foremost among these properties is the ability to enhance considerably the palatability of a wide variety of foods. The substance is now available to the consumer under such trade names as 'Ac'cent,' and 'Zest,' and is apparently coming into general use by dieticians and food manufacturers. While there are numerous reports of its dramatic effects, rigorous experimental evidence of its effect on *measured* preference is lacking. Nevertheless, the unanimity of reports from various sources strongly supports the conclusion that *MSG* can cause definite changes in the flavor of many foods and that these changes usually occur in the direction of increased palatability.[1]

MSG is especially interesting to psychologists not only because changes in palatability are credited to it but also because of the mechanism reputed to underlie its action.

The hypothesis generally held by food technologists and manufacturers of the substance is that *MSG* acts, not by imparting a taste of its own (as would a condiment or seasoning), but by serving as a stimulant which increases the acuity of the taste receptors and thus accentuates the natural taste characteristic of the food.[2] The statement by Melnick exemplifies this point of view: "the advantage of adding glutamate to food products [is] not for imparting a flavor of its own but for physiologically promoting and prolonging sensory acuity . . . for the natural flavor."[3] Logan similarly states that "[MSG] has the unique and unexplainable ability to create a keener sense of taste in the human taste buds."[4]

It is further asserted that *MSG* suppresses acuity to certain undesirable flavors naturally present in some foods, *e.g.* sharpness in onion flavor, rawness in many vegetables, a fishy taste in Lima beans, and bitter tastes present in a few freshly opened vegetables.[5]

This appears inconsistent with the hypothesis discussed above. If *MSG* acts on

[a] Accepted for publication December 3, 1951.

[1] *Monosodium glutamate: A symposium.* Chicago Quartermaster Food and Container Institute and Associates Food and Container Institute, 1, 1948, 25-31, 32-38.

[2] *Loc. cit.*

[3] D. Melnick, Monosodium glutamate: Improver of natural food flavors, *Scient. Mo.*, 70, 1950, 199-204.

[4] Paul Logan, in *National Restaurant Association News Bulletin*, June, 1949.

[5] S. E. Cairncross, Effect of *MSG* on food flavor, In *Monosodium glutamate: A symposium, op. cit.*, 32-38.

taste acuity, it is difficult to see how it could increase sensitivity when the original taste is pleasant and yet depress it when the original taste is unpleasant. What is probably meant by such reports is that for certain foods the increase in sensitivity serves to occlude certain taste qualities which were previously more perceptible because of the lower acuity to competing qualities. In any case, however, the commonly accepted explanation is that taste acuity is in some manner changed.

Pure *MSG* by itself produces very distinctive taste and mouth sensations but no odor. Introspection reveals all four primary tastes, especially salty and sweet. The effect is pleasant and mild, and attended by definite tactile sensations: a slight tingling or furriness on the tip of the tongue and mild stimulation in the throat and back of the mouth. Both taste and tactile sensations are very persistent, lasting several hours after exposure. The total effect is a feeling of mouth satisfaction, *Vollmündigkeit*, as though the mouth had been occupied with much eating.[6]

The hypothesis that enhanced palatability is due to the taste of *MSG* itself has been rejected for the following reasons: First, the glutamate taste itself is not detectable in introspective analysis of the enhanced flavor; the result of its addition seems rather to be an intensification of the existing flavor.[7] Secondly, even subliminal concentrations of *MSG* are reputed to enhance palatability.[8] Thirdly it is possible, according to Crocker and Henderson,[9] to 'match closely' the glutamate taste by a proper synthesis of sweet (sucrose), salty (sodium chloride), sour (tartaric acid), and bitter (caffeine), but the synthetic taste fails to enhance the flavor of natural foods.[10]

The first reason for rejection does not, however, exclude the possibility that enhancement may in part be due to the impartment of tactile sensations. Furthermore, the claim that enhanced palatability is a matter of intensification of the original characteristic flavor needs further verification. While this contention is reiterated throughout the literature, it is evidently based on casual observation rather than on rigorous introspective analysis.

Evidence for the second reason rests upon Cairncross' report. He, however, gives so few details about his work that his results must be corroborated before they can be accepted. In regard to the third reason, it should be noted that Sanders failed with 10 *Os* to reproduce the glutamate taste by synthesis.[11] Crocker and Henderson commented that their synthesis was "distinguishable from the glutamate but was very similar."[12] They do not, however, specify statistically what they meant by "matched very closely." (This could have easily been done by an adaptation of the method of average error used by Lichtenstein in matching sugars for sweetness.[13])

---

[6] E. C. Crocker and L. B. Sjöström, Observational study of monosodium glutamate, *Food Research*, 13, 1948, 450-455.

[7] Cairncross, *op. cit.*, 32-38; Cairncross and L. B. Sjöström, What glutamate does in food, *Food Industries*, 20, 1948, 982 ff.; Melnick, *op. cit.*, 199; MSG, a matter of taste, *Consumer Reports*, 14, 1949, 64-65.

[8] Cairncross, *op. cit.*, 32-38; Cairncross and Sjöström, *op. cit.*, 983.

[9] E. C. Crocker and L. Henderson, The glutamic taste, *Amer. Perfumer Essent. Oil Rev.*, 27, 1932, 156-158.

[10] Melnick, *op. cit.*, 202.

[11] Rosaltha Sanders, The significance of thresholds of taste acuity in seasoning with glutamate. In *Monosodium glutamate: A symposium*, *op. cit.*, 70-72.

[12] Crocker and Henderson, *op. cit.*, 158.

[13] P. E. Lichtenstein, The relative sweetness of sugars: sucrose and dextrose, *J. Exper. Psychol.*, 38, 1948, 578-586.

In view of the doubtful success in synthesizing the glutamate taste, the failure of the synthetic taste to enhance palatability is not a crucial reason for rejecting the theory that enhancement is due to the addition of the glutamate taste rather than to effects on taste acuity.

These arguments, then, do not appear sufficiently valid for discarding completely the hypothesis that *MSG* acts by imparting its own characteristic taste. Actually, the only thing that seems certain from existing data is that *MSG* can change the flavor of food, usually in the direction of increased palatability. Whether this can be attributed to changes in taste acuity or to the addition of the glutamate taste or tactile sensations is still to be convincingly demonstrated.

The hypothesis of 'increased acuity' is thus largely an inference from observed changes in palatability, rather than the result of direct study of receptor processes. As far as the present writers can learn, there are no published accounts of definitive studies of the specific effects of *MSG* on taste acuity *per se*. Cairncross and Cairncross and Sjöström come closest to providing pertinent data on this problem. They report, without describing their procedures, that *MSG* accentuates sweet and salty tastes in food when they are present in near optimal amounts, but if these tastes are greatly below the amount required for optimal palatability, the needed remainder will not be supplied. They also state that threshold solutions of salt are increased in saltiness by the addition of *MSG* but supraliminal salt concentrations are not greatly increased. Incidentally, the above findings contradict the contention that *MSG* serves simply to intensify the original natural flavor, since a differential modification of acuity would most certainly change the configuration or relative balance of the original taste components.

The statements of Cairncross and Sjöström also illustrate the current confusion between palatability and acuity. What these investigators really found was that if a food is slightly deficient in the amount of saltiness required for optimal palatability, *MSG* could supply the remainder; but if the deficiency were great, glutamate could not adequately overcome the difference. Actually, it seems likely that there was an increase in saltiness and sweetness even when these tastes were originally greatly below the optimal amount, but because their original intensity was very low, the increase was not sufficient to reach optimal palatability. The use of palatability as a reference point creates confusion and needlessly complicates generalization by adding an unnecessary intermediate condition. Cairncross' criterion of 'accentuation' is really an all-or-none achievement of a change in palatability rather than a change in intensity. There may well be increases in sensory intensity without significant changes in palatability.

The purpose of the present investigation was to test the hypothesis that *MSG* modifies taste acuity or, more specifically, to determine the effect of this substance on the absolute thresholds of the primary tastes.

Method and Procedure

Essentially the procedure consisted of measuring the absolute thresholds of sweet, salty, sour, and bitter solutions before and after exposure to *MSG*. Four graduate students in psychology, all but one with previous

experience in taste experiments, served as *Os*. The following procedures were used for each primary taste in turn.

Pre-glutamate threshold determination. Thresholds were first determined separately for the primary tastes by a modification of the Method of Limits in which constant stimuli were used. For each taste, a series of solutions, the first of which was always tap water, was presented in ascending order of concentration until *O* reported the taste characteristic of the substance at moderate supraliminal concentrations. No descending series or random order presentation could be used because the stronger solutions would occlude the taste of subsequent weaker solutions. Interpresentation rinses are of no assistance here. Dallenbach and Dallenbach[14] and Cameron[15] report that interpresentation rinses were more disturbing to the judgmental task than the benefits warrant.

Os were instructed to judge whether each solution was water or tasted of the primary taste in question. They were cautioned against reacting to mouth sensations other than the specific taste which characterized the substance at moderate supraliminal concentrations. This is in accordance with the results of Mosel and Kantrowitz which show a difference between the 'distinction'-limen (different from water judgment) and the 'recognition'-limen (specific taste of substance in supraliminal concentrations).[16] The present instructions were thus designed to yield a recognitive threshold. Unannounced presentations of tap water were inserted as *Vexierversuche* to diminish errors of expectation. The *Os* were also unaware that the sequence of solutions was increasing in concentration. When *O* reported the taste on two consecutive presentations, the series was terminated.

O held 15 cc. of each solution in his mouth for 5 sec., with 45 sec. between presentations. According to the experience of Dallenbach and Dallenbach these time allowances should be adequate to prevent adaptation and still provide opportunity for a reliable report.[17] Preliminary experimentation in the present study concurred in this recommendation. The amount of taste solution was thought to be adequate in view of Brown's finding that the amount of solution is unimportant as long as there is enough so that a "slight movement of the tongue will bring it into equal contact with all sensitive parts at once."[18] Drop and punctate methods of stimulation were eschewed because they are known to give less stable values of the threshold.[19]

Ten series were given each *O*. The interval between series was 2 min., during which time *O* rinsed his mouth three times with tap water and dried his tongue

---

[14] J. W. Dallenbach and K. M. Dallenbach, The effects of bitter-adaptation on sensitivity to the other taste qualities, this JOURNAL, 56, 1943, 21-31.

[15] A. T. Cameron, The taste sense and the relative sweetness of sugars and other sweet substances, *Scientific Reports of the Sugar Research Foundation*, New York, No. 9, 1947, 23.

[16] J. N. Mosel and G. Kantrowitz, Absolute sensitivity to the glutamic taste, *J. Gen. Psychol.*, (in press).

[17] Dallenbach and Dallenbach, *op. cit.*, 25.

[18] W. Brown, The judgment of very weak sensory stimuli, *Univ. Calif. Publ. in Psychol.*, 1, (No. 3), 1914, 260.

[19] Cameron, *op. cit.*, 22; C. P. Richter and A. McLean, Salt-taste thresholds of humans, *J. Physiol.*, 126, 1939, 1-6.

with filter paper. All solutions and rinses were presented at room temperature and were expectorated.

The substances used for each primary taste were of *USP* purity: sodium chloride (salty), sucrose (sweet), quinine hydrochloride (bitter), and tartaric acid (sour). The range- and step-intervals of the concentrations are shown in Table I. These values were determined through preliminary experimentation and were designed to accommodate all *Os*.

All solutions were stored in cotton-stoppered flasks (to prevent evaporation and contaminating odors) at room temperature (about 20°C). Tap water was used as the solvent since it is generally known that distilled water is not tasteless but possesses a flat or insipid quality.[20] Fresh solutions were prepared daily except for sucrose whose solutions were allowed to stand for at least 16 hr. to permit the

TABLE I

MOLAR CONCENTRATION OF TEST SOLUTIONS FOR EACH PRIMARY TASTE

Sodium chloride	Sucrose	Quinine hydrochloride	Tartaric acid
0.0000	0.0000	0.000000	0.0000
0.0025	0.0050	0.000001	0.0003
0.0050	0.0100	0.000011	0.0006
0.0100	0.0150	0.000021	0.0009
0.0150	0.0200	0.000031	0.0012
0.0200	0.0250	0.000041	0.0015
0.0250	0.0300	0.000051	0.0018
		0.000061	

establishment of a mutarotational equilibrium. Sucrose solutions not in equilibrium have a higher alpha-glucose content and are distinctly sweeter in taste.[21]

Post-glutamate thresholds. Thresholds were redetermined by the same method but this time each series was run 5 min. after exposure to a strong (0.0010 mol.) solution of 99.9% pure *MSG*. Previous study had found the absolute recognition-threshold of *MSG* to be about 0.00085 mol.;[22] the stimulus-solution was therefore approximately a 5-threshold solution. A small amount (15 cc.) of this solution was worked well about the mouth for 7 sec. and then expectorated, after which *O* rinsed his mouth three times thoroughly with tap water and dried his tongue with filter paper. No more than one series was run per day for any *O*. All determinations were made at least 1 hr. after eating.

This procedure was followed in preference to redetermining the thresholds in the actual presence of a *MSG* solution since mixing the taste substances in solution with *MSG* would have made accurate specification of their concentrations difficult. Furthermore, to encourage a maximal operation of any glutamic effect, stimulation with *MSG* had to be strong; the glutamate taste would then have obscured any threshold changes in the primary tastes. Since the effects of strong glutamate stimulation are knows to last for several hours, the 5-min. interval between stimu-

[20] A. Kopera (Untersuchungen über die Unterschiedsempfindlichkeit im Bereiche des Geschmacksinns, *Arch. f. d. ges. Psychol.*, 82, 1931, 273-307) reports no difference in differential sensitivity if solutions are prepared with tap water instead of distilled water.

[21] Cameron, *op. cit.*, 43-11.

[22] Mosel and Kantrowitz, *op. cit.*

lation and threshold determinations was well within the period of the glutamate effect.

Results

The pre- and post-glutamate thresholds (recognitive) of each primary taste, computed as the median by linear interpolation, are given in Table II. Because the *Os* showed very little individual difference in their limens for salty, sweet, and sour, their responses were pooled for each of these tastes into a single distribution to yield the group-limens. For bitter complete pooling was not possible. Guided by *t*-tests for the difference between medians, responses were pooled separately in pairs, the limens being extremely similar within a pair but differing widely between pairs.

The pre- and post-glutamate thresholds for salty and sweet remained unchanged for all *Os*. In the case of sour, and especially bitter, however,

TABLE II

Absolute Thresholds of the Primary Tastes Before and After Stimulation by Monosodium Glutamate

(Four *Os*; and 10 determinations per *O* for every taste quality.)

Taste quality		Pre-glutamate		Post-glutamate	
		Molarity	%	Molarity	%
Salty		.0075	.0438	.0075	.0438
Sweet		.0175	.5985	.0175	.5985
Sour		.00075	.01125	.00045	.00675
Bitter*	*A & B*	.0000150	.000539	.0000005	.000036
	C & D	.0002460	.001643	.0000260	.000935

* Responses of *Os A* and *B*, and *C* and *D* were combined separately because of the large differences in original sensitivity between these two groups.

there was a definite lowering of threshold for all *Os*.[23] In terms of relative increase in acuity, the group-limens in Table II show that sensitivity to sour was increased by a factor of 1.7; for bitter by a factor of 30 for the more sensitive pair of *Os* and by 1.8 for the less sensitive pair. The effect on bitter acuity was further attested by the comment from several *Os* that after exposure to *MSG* cigarettes tasted decidedly bitter for the rest of the day.

Discussion

These results are quite inconsistent with Cairncross' assertions that *MSG* accentuates sweet and salty tastes and depresses sour and bitter.

[23] It is impossible to test accurately the statistical significance of the difference between medians (limens) from nonindependent samples because the correlational term is unknown. Omitting the correlation term, and thus underestimating the significance, yields, however, a *t* which is significant for both sour and bitter beyond the 1% level.

The explanation may partly lie in the fact that his observations were made on changes in supraliminal taste intensities. It is conceivable that changes in the absolute threshold may not be directly and equivalently reflected in the difference limen. Lichtenstein, for instance, found that differences in the absolute threshold of various sugars gave an inadequate picture of relative sweetness at supraliminal concentrations because sweetness varied considerably as a function of concentration.[24] It is possible that *MSG* may induce changes at certain supraliminal concentrations but fail to do so at others or at the threshold. Apparently very little is known for any sensory modality concerning the relationship between absolute and differential sensitivity, although certain relationships are predictable from theory. This would imply that *MSG* has different effects depending upon the relative size of the *JND*. An increment in intensity might well be noted at low subjective magnitudes but the same increment, due to Weber's Law, may not be perceived at higher magnitudes.

These possibilities should be put to experimental check. In any event, future experimentation on the supraliminal intensive effects of *MSG* must certainly take into account the original intensive level of the affected taste.

Summary and Conclusions

To test the widely held hypothesis that monosodium glutamate enhances the palatability of foods by increasing taste acuity, the absolute thresholds of four *Os* to salty, sweet, sour, and bitter were determined before and after exposure to a 5-threshold solution of *MSG*. Results showed that acuity to salty and sweet was totally unaffected; acuity to sour was somewhat increased, while acuity to bitter was greatly increased. In the latter case, *Os* with greater original sensitivity showed larger gains than did the originally less sensitive. The number of *Os* is too small, however, to warrant generalization.

The discrepancy between these results and other reports may possibly lie in the fact that other investigators worked with supraliminal concentrations and made their interpretations with reference to changes in palatability rather than to changes in intensity, and also that they failed to relate their observations to the specific intensity-level of the taste quality affected.

---

[24] Lichtenstein, *op. cit.*, 578-586.

ACTA ENDOCRINOLOGICA
75 (1974) 249-259

Pharmacology Division, National Cancer Center Research Institute,
Tsukiji 5-1-1, Chuo-ku, Tokyo 104 and
*Department of Biology, School of Education,
Waseda University, Totsuka, Shinjuku-ku, Tokyo 160

IRREVERSIBLE INHIBITION OF PITUITARY PROLACTIN AND GROWTH HORMONE SECRETION AND OF MAMMARY GLAND DEVELOPMENT IN MICE BY MONOSODIUM GLUTAMATE ADMINISTERED NEONATALLY

By

*Hiroshi Nagasawa, Reiko Yanai and Sakae Kikuyama**

ABSTRACT

A single subcutaneous injection of 4 mg monosodium glutamate (MSG) to neonatal female mice resulted in obesity when the mice became adult. The MSG-treated mice had significantly smaller contents of prolactin and growth hormone (GH) in the pituitary, as compared with the controls at 60, 150 and 420 days of age except for the content of prolactin at 60 days of age. The development of normal and pre-neoplastic mammary systems was markedly suppressed in the MSG-treated mice at 150 and 420 days of age. The oestrous cycles of the MSG-treated mice showed longed periods of oestrus and shorter periods of diocstrus than those of the controls. The pituitary weight was significantly smaller in the MSG-treated mice than in the controls at 60 and 150 days of age. The weights of several other organs showed no difference between the groups at any of the ages examined. These results indicate that MSG inhibits the pituitary secretion of prolactin and GH through its deleterious effects on the hypothalamus and that the effects are irreversible.

The administration of monosodium glutamate (MSG) and related substances has been shown to induce acute degeneration of hypothalamic neurons in neonatal and foetal rodents (*Olney* 1969, 1971; *Olney & Ho* 1970; *Olney et al.* 1972*a*) and in infant monkeys (*Olney & Sharpe* 1969; *Olney et al.* 1972*b*). It is well known that the hypothalamus secretes several releasing or inhibiting neurohormones which regulate the synthesis and release of specific anterior pituitary hormones. Thus, it is plausible that MSG suppresses the secretion of pituitary hormones. However, information on this problem is scanty. *Redding et al.* (1971) reported that daily injections of MSG beginning on the 2nd day of life resulted in a reduction of the pituitary contents of growth hormone (GH) and luteinizing hormone in rats at 40 days of age.

In the present experiment, the effects of a single neonatal administration of MSG on the pituitary contents of prolactin and GH, the growth, the organ weights, the oestrous cycle and the development of normal and pre-neoplastic mammary systems were investigated in mice at 60, 150 and 420 days of age in order to study the irreversibility of the deleterious effects of MSG on the pituitary secretion of prolactin and GH through its action on the hypothalamus.

MATERIALS AND METHODS

The C3H/He strain of virgin mice was used. At 0 (day of birth) or at 1 day of age, half of the female litter received subcutaneously a single injection of 4 mg MSG dissolved in 0.01 ml physiological saline. The remaining animals of each litter were given only the vehicle and served as controls. When weaned at 20–23 days of age, each litter was kept in a teflon cage (30 × 18 × 15 cm) and maintained in an air-conditioned (24 ± 0.5°C in temperature and 65–70 % in relative humidity) and artificially illuminated (14 h light from 07.00 to 21.00 h) animal room. They were provided with a commercial diet (CE-2: CLEA Japan Inc., Tokyo) and water *ad libitum*. Their body weights were measured every 10 days after 20 days of age and vaginal smears were taken every morning during 140–170, 360–390 and 400–430 days of age.

At 60 and 420 days of age, mice from each group were killed and the anterior pituitary, adrenals, thyroids, ovaries, kidneys, spleen and liver were removed, carefully cleaned and weighed on a torsion balance. The naso-anal length was also measured for calculation of 'Lee index', an indicator of obesity (*Bernardis & Skelton* 1967; *Bernardis & Patterson* 1968). The weights of the pituitary, adrenals and ovaries only were measured at 150 days of age. Prolactin and GH in the pituitary were separated by disc electrophoresis using 10 % polyacrylamide gel and the contents were determined densitometrically as described previously (*Yanai & Nagasawa* 1971*a*). The oestrous stages were not taken into consideration when killing the mice to determine the pituitary contents of the hormones, since the pituitary contents and their synthesis and release were not different with regard to oestrous stages in this strain (*Yanai & [illegible]* 1971*b*, 1972). The third right thoracic mammary gland was removed, spread on cork board, fixed in Bouin's solution and stained with haematoxylin for whole mount evaluation. The degree of development of the normal mammary system was

rated from 1 to 7 in increments of 1 by the standard of *Wrenn et al.* (1966) with a little simplification. The number and size of mammary hyperplastic nodules (HN), a pre-neoplastic state of mammary cancer in mice (*Bern & Nandi* 1961), were also measured. The size of each nodule was expressed as the mean of the 2 major diameters. The mammary gland was examined under ten-fold magnification.

Three mice at 0 day of age were killed 4 h after the injection of 4 mg MSG. The hypothalami were fixed in Bouin's solution, embedded in paraffin, cut at 6 μm in thickness and stained with Mallory II and acid alizanine blue to ascertain the acute effect of MSG on the hypothalamus. Four specimens of thyroid from both the MSG-treated and control mice at 42 and 360 days of age were also prepared for histological observation by the same procedure as described above.

RESULTS

Body weight change (Fig. 1)

The body weight increased similarly in both groups until about 80 days of age. Thereafter the rate of increase was much more marked in the MSG-treated mice than in the controls. While the weights of controls reached a plateau after 150 days of age, those of the MSG-treated mice continued to increase until about 350 days of age. The difference between the groups were statistically highly significant after 100 days of age ($P < 0.01$ or 0.001) (see also Table 1).

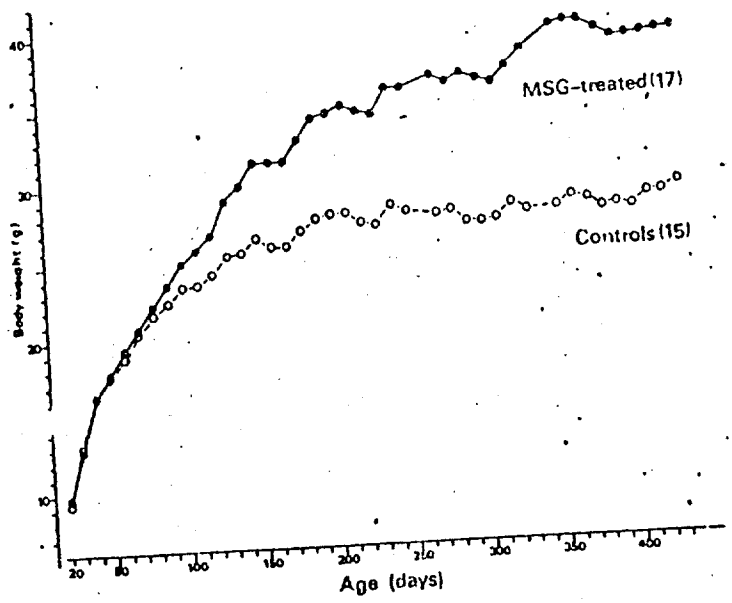


Fig. 1.
Change in average body weight in each group. The number of mice is indicated in brackets.

Table 1.
'Lee index' and organ weights in each group at respective ages (means ± SEM).

Age (days)	Treatment	Body weight (g)	'Lee index'[1]	Organ weight (mg)						
				Anterior pituitary	Thyroid	Adrenal	Ovary	Spleen	Kidney	Liver
60	Controls	20.9 ± 0.5 (8)[2]	0.350 ± 0.001 (14)	1.59 ± 0.09 (8)	1.64 ± 0.15 (4)	5.81 ± 0.17 (6)	7.26 ± 0.53 (6)	187 ± 37 (6)	328 ± 11 (6)	1123 ± 49 (6)
	MSG-treated[3]	21.2 ± 1.1 (8)	0.355 ± 0.003 (15)	1.21 ± 0.09 (8)	1.70 ± 0.13 (5)	4.97 ± 0.33 (6)	6.55 ± 0.89 (6)	172 ± 29 (6)	320 ± 8 (6)	1271 ± 15 (6)
	P[4]	NS	NS	0.01	NS	NS	NS	NS	NS	NS
150	Controls	28.0 ± 0.7 (14)	–	1.83 ± 0.07 (10)	–	6.00 ± 0.20 (10)	13.11 ± 0.58 (10)	–	–	–
	MSG-treated	32.7 ± 0.9 (14)	–	1.38 ± 0.07 (11)	–	5.60 ± 0.21 (11)	11.76 ± 0.47 (11)	–	–	–
	P	0.001		0.001		NS	NS			
420	Controls	28.8 ± 0.8 (17)	0.341 ± 0.004 (12)	1.86 ± 0.07 (17)	3.60 ± 0.51 (6)	4.92 ± 0.31 (6)	7.02 ± 0.71 (6)	100 ± 6 (6)	384 ± 13 (6)	1486 ± 92 (6)
	MSG-treated	41.5 ± 1.1 (15)	0.369 ± 0.006 (13)	1.73 ± 0.05 (15)	4.28 ± 0.30 (6)	4.88 ± 0.43 (6)	6.75 ± 0.68 (6)	137 ± 8 (6)	428 ± 12 (6)	1858 ± 144 (6)
	P	0.001	0.001	NS	NS	NS	NS	NS	NS	NS

1) 'Lee index' = $\sqrt[3]{\text{Body weight}}$/naso-anal length.
2) Number of mice.
3) Each mouse received a single subcutaneous injection of 4 mg MSG at 0–1 day of age.
4) Probability as determined by Student's t-test.

'Lee index' and organ weight (Table 1)

Whereas 'Lee index' was similar in both groups at 60 days of age, it was significantly higher in the MSG-treated mice than in the controls at 420 days of age ($P < 0.001$). The anterior pituitary weight was significantly smaller in the MSG-treated mice than in the controls at 60 and 150 days of age ($P < 0.01$ or 0.001), but not at 420 days of age. There were no significant differences between the groups in the weights of the other organs at any of the ages examined. Autopsies at 150 and 420 days of age revealed a massive accumulation of adipose tissue in both subcutaneous and body cavity depots in the MSG-treated mice.

Anterior pituitary contents of prolactin and GH (Table 2)

The contents of both prolactin and GH in the anterior pituitary were significantly lower in the MSG-treated mice than in the controls at all the ages examined ($P < 0.01$ or 0.001) except for the content of prolactin at 60 days of age which was slightly different between the groups. A representative disc electrophoretic pattern of the anterior pituitary in each group at 150 days of age is shown in Fig. 2. Both prolactin and GH bands were stained much more deeply in the controls than in the MSG-treated mice. No differences were observed between the groups in the concentration of each hormone per unit weight of pituitary at any of the ages examined.

Table 2.

Anterior pituitary contents of prolactin and growth hormone (GH) in each group at respective ages (means ± SEM).

Age (days)	Treatment	No. of mice	Prolactin (No. of counts)[1]	GH (No. of counts)[1]
60	Controls	8	11.1 ± 1.6	33.9 ± 4.2
	MSG-treated[2]	8	9.3 ± 1.4	23.4 ± 2.1
	P[3]		NS	0.001
150	Controls	9	19.2 ± 0.8	48.7 ± 3.5
	MSG-treated	11	12.9 ± 1.3	30.2 ± 1.9
	P		0.001	0.001
420	Controls	17	14.5 ± 0.7	28.4 ± 1.1
	MSG-treated	15	11.9 ± 0.9	24.4 ± 1.5
	P		0.01	0.01

1) Canalco microdensitometer (Model E) counts every 20 mm^2 of area bounded by the tracing line and the horizontal line.
2) Each mouse received a single subcutaneous injection of 4 mg MSG at 0–1 day of age.
3) Probability as determined by Student's *t*-test.

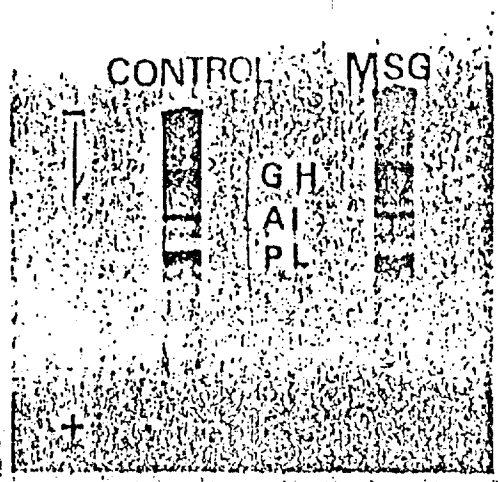


Fig. 2.

Representative disc electrophorectic pattern of anterior pituitary in each group at 150 days of age. One anterior pituitary was employed per column. GH: growth hormone, Al: albumin, PL: prolactin. Both prolactin and GH bands were stained much more deeply in the controls than in the MSG-treated mice.

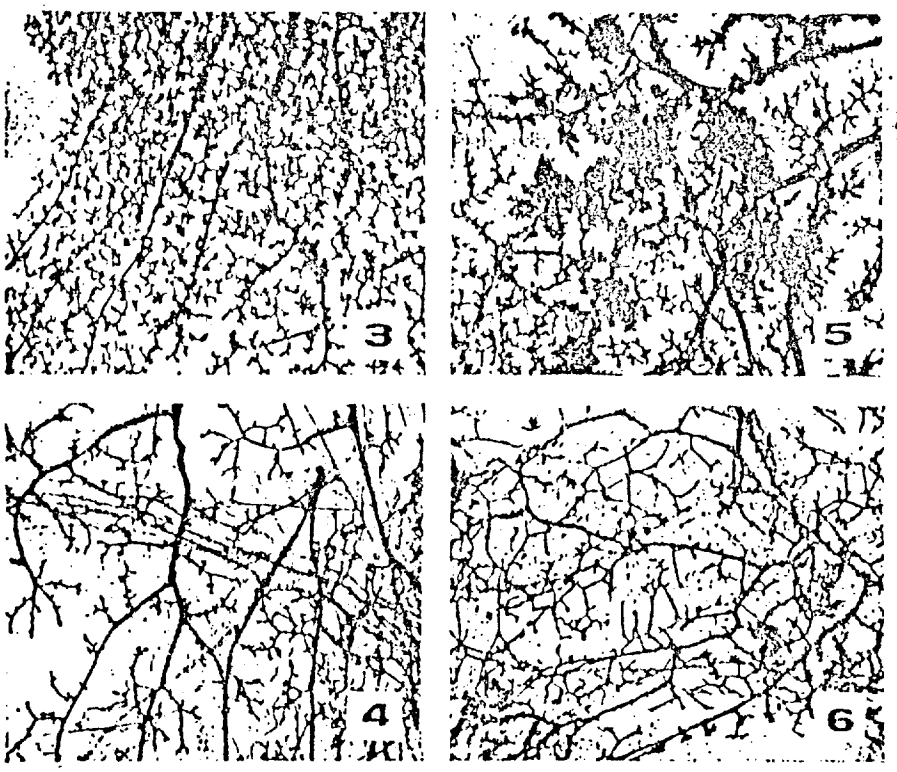


Table 3.

Normal and pre-neoplastic development of mammary gland in each group at respective ages (means ± sem).

Age (days)	Treatment	No. of mice	Mammary rating	No. of mammary hyperplastic nodules (HN)	Av. size of HN (mm)
60	Controls	8	1.6 ± 0.4	0	–
	MSG-treated[1]	5	1.0 ± 0.0	0	–
	P[2]		NS	NS	
150	Controls	14	2.6 ± 0.3	1.3 ± 0.3	0.49 ± 0.04 (18)[3]
	MSG-treated	14	1.4 ± 0.1	0	–
	P		0.001	0.001	
420	Controls	17	1.8 ± 0.2	0.3 ± 1.3	0.90 ± 0.04 (155)
	MSG-treated	15	1.0 ± 0.0	2.5 ± 0.4	0.76 ± 0.06 (38)
	P		0.001	0.001	NS

1) Each mouse received a single subcutaneous injection of 4 mg MSG at 0–1 day of age.
2) Probability as determined by Student's *t*-test.
3) Number of HN measured.

Development of normal and pre-neoplastic mammary system (Table 3)

The mammary rating as an index of development of normal mammary system was only slightly different between the groups at 60 days of age, but it was significantly lower in the MSG-treated mice than in the controls at both 150 and 420 days of age ($P < 0.001$). While no HN were found in the glands of the MSG-treated mice at both 60 and 150 days of age, most of the glands of the controls had 1 to 4 HN at 150 days of age. At 420 days of age,

Figs. 3–6.

Whole mount preparations of the right third thoracic mammary glands in each group at 150 and 420 days of age.

3 The gland of the control at 150 days. Numerous ducts and branches with well developed end-buds and a few pre-neoplastic mammary hyperplastic nodules (HN) are seen.

4. The gland of a MSG-treated mouse at 150 days. The duct system with only slightly developed end-buds is seen.

5. The gland of the control at 420 days. Well developed mammary system with several large HN is seen.

6. The gland of the MSG-treated mouse at 420 days. Only duct system with small HN is seen.

the number of HN per glands was significantly smaller in the MSG-treated mice than in the controls ($P < 0.001$). The controls were much higher than the MSG-treated mice with regard to the average size of HN at 420 days of age, although the significance of difference between the groups was marginal. The mammary glands of the controls consisted of numerous ducts and branches with well developed end-buds (Figs. 3 and 5) and several HN were observed at 420 days of age (Fig. 5). Meanwhile, the glands of MSG-treated mice were mostly limited to the duct system (Figs. 4 and 6) and HN were found only sporadically even at 420 days of age (Fig. 6).

Oestrous cycle (Fig. 7)

The oestrous and metoestrous periods were longer and the frequency of continued dioestrous periods often seen in this strain (*Nagasawa et al.* 1967) was decreased in the MSG-treated mice when compared to the controls during 360–390 and 400–430 days of age.

Histological observation of hypothalamus and thyroid

In the arcuate region of the hypothalamus of new born mice 4 h after the treatment with MSG, the neurons were necrotic with cytoplasmic swelling and nuclear picnosis, confirming the results of *Olney* (1969, 1971).

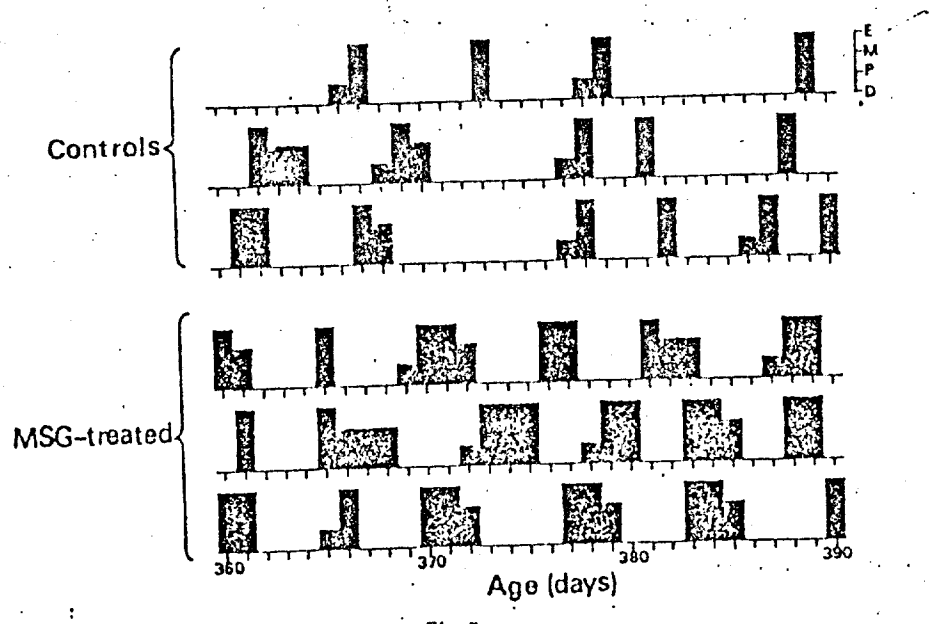


Fig. 7.
Representative patterns of oestrous cycles in each group during 360–390 days of age. O: oestrus, M: metoestrus, p: pro-oestrus, D: dioestrus.

mone (TSH) content in the pituitary and the uptake of labelled iodine by the thyroid in the MSG-treated rats were normal at 40 days of age (*Redding et al.* 1971). The weights and the histological structures of the thyroid were only slightly affected by MSG in the present experiment. These results suggest that TSH releasing hormone may be produced in a rather diffuse portion of the anterior hypothalamus and thus may have escaped the damage by MSG (*D'Angelo* 1963). It is, therefore, unlikely that the decrease in the pituitary secretion of prolactin and GH by MSG is due to hypothyroidism.

All the results obtained in the present experiment clearly demonstrate that the deleterious effects of neonatally administered MSG on the hypothalamus are persistent and irreversible. Neuronal necrosis could be induced in the immature mouse by a very low dose of MSG (0.5 mg/g body weight) (*Olney* 1969). Acute brain damage resulted from the subcutaneously administered MSG in infant monkeys (*Olney & Sharpe* 1969). These findings may warrant more specific precaution against taking any risk to the developing human nervous system due to the maternal use of MSG during pregnancy (*Olney* 1969). Incidentally *Stegink et al.* (1972) reported that little, if any, MSG was concentrated in the milk of lactating women following the ingestion of a single load of MSG.

ACKNOWLEDGMENTS

Our sincere thanks are due to Associate Professor John W. Olney, Department of Psychiatry, Washington University, School of Medicine, St. Louis, Missouri, USA, for his interest and valuable suggestion.

We also wish to thank Messrs. M. Miyamoto and H. Horie for technical assistance.

This work was supported in part by a grant-in-aid for Cancer Research from the Ministry of Education, Japan (Nos. 90183 and 90393).

REFERENCES

Bern H. A. & Nandi S.: Progr. exp. Tumor Res. *2* (1961) 90.
Bernardis L. L. & Patterson B. D.: J. Endocr. *40* (1968) 527.
Bernardis L. L. & Skelton F. R.: J. Endocr. *38* (1967) 351.
D'Angelo S. A. In: Nalbandov A. V., Ed. Advance in Neuroendocrinology, University of Illinois Press, Urbana (1963) 158.
Frohman L. A., Bernardis L. L., Schmatz J. D. & Burek L.: Amer. J. Physiol. *216* (1969) 1496.
Nagasawa H., Fujimoto M., Iwahashi H. & Kuretani K.: GANN *58* (1967) 115.
Olney J. W.: Science *164* (1969) 719.
Olney J. W.: J. Neuropath. exp. Neurol. *30* (1971) 75.
Olney J. W. & Ho Oi-Lan: Nature (Lond.) *227* (1970) 609.
Olney J. W., Ho Oi-Lan & Rhee V.: Exp. Brain Res. *14* (1971) 61.

Olney J. W., Ho Oi-Lan, Rhee V. & Schainker B.: Brain Res. *45* (1972*a*) 309.
Olney J. W., Lawrence G., Sharpe L. G. & Feigin R. D.: J. Neuropath. exp. Neurol. *31* (1972*b*) 464.
Olney J. W. & Sharpe L. G.: Science *166* (1969) 386.
Redding T. W., Schally A. V., Arimura A. & Wakabayashi I.: Neuroendocrinology *8* (1971) 245.
Stegink L. D., Filler L. J. & Baker G. L.: Proc. Soc. exp. Biol. (N. Y.) *140* (1972) 836.
Wrenn T. R., Bitman J., DeLander W. R. & Mench M. L.: J. Dairy Sci. *49* (1966) 183.
Yanai R. & Nagasawa H.: J. nat. Cancer Inst. *45* (1970) 1105.
Yanai R. & Nagasawa H.: Hormone and Behavior *2* (1971*a*) 73.
Yanai R. & Nagasawa H.: Jap. J. zootech. Sci. *42* (1971*b*) 81.
Yanai R. & Nagasawa H.: J. nat. Cancer Inst. *46* (1971*c*) 1251.
Yanai R. & Nagasawa H.: Endocr. jap. *19* (1972) 185.

Received on June 4th, 1973.

The thyroids consisted of well developed follicular cells and partially vacuolated colloid was found in the follicles in both the MSG-treated and control mice at 42 days of age, little differences being detected between the groups. Similarly, no differences between the groups were observed in the structures of the thyroid at 360 days of age.

DISCUSSION

The degree of obesity in adult mice induced by MSG varies according to the dose, the time and the period of treatment (*Olney* 1969 and personal communication). Rather smaller dose and the shorter period of treatment were used in the present experiment than in the previous experiments. The significantly larger body weight and 'Lee index', little differences in the weights of several organs as compared to the controls and a striking increase in fat deposit both in the subcutaneous and body cavity depots were observed in the MSG-treated mice. This would suggest the possibility that MSG can induce obesity at fairly low doses.

One of the main causes of obesity is the metabolic imbalance associated with the reduction of the pituitary secretion of GH, which was strongly indicated by the decrease in the pituitary content of the hormone in the MSG-treated mice at any of the ages examined. In a study on weanling rats with electrolytic lesions in the ventromedial nucleus, *Frohman et al.* (1969) have shown an increase in plasma triglycerides up to a value nearly double that of the controls, associated with decrease in the pituitary and plasma GH levels.

The pituitary content of prolactin decreased significantly in the MSG-treated mice at 150 and 420 days of age. The development of both a normal mammary system and a pre-neoplastic HN which are principally dependent upon prolactin (*Yanai & Nagasawa* 1970, 1971*b,c*) was overtly inhibited in the MSG-treated mice. Moreover, the oestrous cycles of the MSG-treated mice showed a more prolonged oestrus and a shorter dioestrus than in the controls. All these results indicate that the pituitary secretion of prolactin is chronically suppressed by a single neonatal treatment with MSG.

These impairments in the pituitary secretion of prolactin and GH are probably attributable to the subnormal rate of production and/or transportation of neurohormones by the deleterious effects of MSG on the hypothalamus involving the arcuate region, since there is now good evidence that the secretion of the pituitary hormones is primarily controlled by the specific releasing or inhibiting neurohormones.

Hypothyroidism decreased the pituitary synthesis and release of prolactin and GH in rats (*Kikuyama et al.*, unpublished). The thyroid stimulating hor-

円フロートで輸出見通し暗い今年のグル曹産業

輸出減の処理、操短、製品値上げ調整が今年の課題

（グ）ルタミン酸ソーダ業界は，3年前から需要に見合った生産の自粛によって，採算維持の経営をおこなっている。だがスミソニアン体制による円切上げに続いての今年の円フロートによって輸出の見通しが暗くなった。一方原料，人件費の高騰と公害投資はコストの急上昇をさねいている。輸出減の処理，操短，製品値上げをどのように調整するかが今年の課題だ。

目標を下まわる47年度の生産・輸出市場

（47）年度は，45年秋から実施した国内業務用引締め策が成功しているので，46年12月の円切り上げ（スミソニアン体制）による輸出の停滞や円手取額の減少を見込んでも，さらに一段の国内業務用価格の引上げが可能であるとのやや明るい見通しでスタートした。

これを反映して，需給目標も前年比（4～3月）で生産を0.9%減の9万9,400トン，輸出を2.1%増の1万9,800トン，国内向けを3.9%増の7万9,600トンとし，昨年に続いて採算向上を基本戦略として打出した。

ところが，明るくスタートしたこの"採算向上航路"の途上に大きなアクシデントがおきてしまったのである。その一つは47年夏に表面化した米国での中華料理店症候群，日本での結びコンブからの同症状などの有害説である。これがやがて適正使用量表示問題までに発展し，消費者の不安を呼び起したこと。二つ目は円フロート（48年2月14日）以降の円相場の急騰による輸出見通しが難しくなってきたことである。

前者は，一般家庭用への影響は少なく，心配された歳暮需要にもあまり影響はなかったものの，業務用の多量使用業種では需要減要因になったことは否めない。後者の場合は，今年の円フロートによる見通し難は，48年度以降になるが，スミソニアン体制の円切上げの影響が当初予想よりも強く，円建ての値上げは，切上げ巾の2分の1程度しか乗り得ず，輸出目標の達成もむずかしいものとなってきた。

表①　グルタミン酸ソーダの年次別需給

日刊経済通信社調

年次別	生産		輸出		国内消費		国民一人当りの消費量
	数量(t)	指数	数量(t)	指数	数量(t)	指数	(g)
43年	96,493	100	20,994	100	75,499	100	744.5
44	101,483	105.2	21,743	103.6	79,740	105.6	781.0
45	90,576	93.8	17,855	85.0	72,721	96.3	705.5
46	100,317	104.0	19,397	92.4	80,920	107.2	775.0
47(見込)	96,000	99.5	18,000	85.7	78,000	103.3	732.8
48(予想)	95,000	98.5	17,000	81.0	78,000	103.0	732.8

（注）①年度は4～3月。②47年度の見込みは1～12月の実績から推定。③48年度の予想は生産力，需給動向などから推定した。④国内消費量は（生産量－輸出量）⑤国民一人当りの消費量は（国内消費量÷総人口で算出。⑥総人口は総理府の「人口推計月報」による。48年度の増加率を1.0%とみた。

47年1～12月の実績は生産が9万5,450トン，輸出が1[illegible],600トン（いずれも出荷ベース），国内7万7,35[illegible]トンであるから，4～3月でもこれに近い需給量となる[illegible]年初の生産輸出目標を下まわることは確実と言ってよいだろう。

[illegible]なかで一つの好材料が残っている。国内業務用の建値が45年から通算すると3～4段上げをしており，47年には建値を二次にわたって上げ，一次分のkg当り30～40円上げが実現し，二次分の引上げを48年上半期中におこなう気配にあることだ。

これは，44年までの需要上伸時代の乱戦によって業界の再編成が進み，それ以降メーカーが，味の素，旭化成，協和，武田のビッグ4社の他に新進食料を加えて5社で98.1%の生産集中度に達するほど寡占化されたことが，採算重点への転換を可能にしたからである。（表①～表②）

市場引締め可能な高度寡占体制

（か）つて，わが国グル曹業界の政策はビッグ4社の姿勢によって決まるといってよい。

メーカーの少ないことは，業界の話し合いと協調をよくする好例でもある。

さきにも触れた通り，47年末で実際に生産，販売をしている企業は7社であるが，味日本と料理の素は，大手から半製品または製品の供給をうけており，実質的なメーカーは5社ということになる。その集中度は1位の味の素が58.1%，以下旭化成14.0%，協和13.4%（味日本を含む），武用12.3%，新進2.2%となっている。しかも，この寡占化の内容がかつての味の素中心のガリバー形寡占から複数の高度寡占を形成してきており，このことが市場の引締めを可能にしている。（表②）

実際に，48年3月末の公称月産能力1万1,380トンに対する，47年12月の月産推定量を7,840トンとみると，68.9%の操業率となり，年産公称能力約13万4,000トンに対して，47年度の生産量を9万5,000トンと仮定すると操業度は71.7%ということになる。（表③）

この操業短縮のトレンドは，世界の生産力の2分の1以上を占めるわが国グル曹業界にとっては内外戦略上賢明な企業姿勢といえる。だがその背景には世界の経済，社会環境への企業の対応策，特に公害対策と週5日制移行などが大きな要因となっていることが注目される。

円再切り上げで苦しい輸出

（で）はこうした操短のトレンドがどのような需要との見合いからきているのだろうか。

生産は44年度をピークに，45年度以降は需要予測に見合った生産をしているといえる。（表①）

表②　生産集中度と輸出占拠率　日刊経済通信社調

会社名	生産集中度（%）				輸出占拠率（%）			
	45年末	46年末	47年末	累積集中度	45年末	46年末	47年末	累積集中度
味の素	56.6	60.1	58.1	58.1	70.8	70.3	75.0	75.0
旭化成	15.5	13.4	14.0	72.1	16.3	15.6	13.8	88.8
協和醗酵	11.8	11.6	11.5	83.6	10.6	14.0	7.6	96.4
武田薬品	11.8	11.4	12.3	95.9	2.3	0.1	3.6	100.0
新進食料	2.8	2.3	2.2	98.1	—	—	—	
味日本	1.4	1.2	1.9	100.0	—	—	—	
鶴見曹達	—	—	—		—	—	—	
味の世界	—	—	—		—	—	—	
料理の素	0.1	—	—		—	—	—	
大阪食品化学	—	—	—		—	—	—	
江崎グリコ	—	—	—		—	—	—	
計	100.0	100.0	100.0		100.0	100.0	100.0	

表③　昭和46年度の工場別月産能力（推定）　日刊経済通信社調

会社名	工場および製法	47年3月末月産能力（t）	48年3月末月産能力（t）	47年12月の生産量（t）	48年度の設備投資
味の素	川崎（発酵法）	2,650	2,650	2,000	30億（含公害投資）
	九州（〃）	2,500	2,500	1,750	
	東海（合成法）	1,300	1,300	800	
旭化成	延岡（発酵法）	1,500	1,500	1,100	4億（含公害投資）
協和発酵	防府（〃）	1,500	1,500	900	7億（内公害4億）
武田薬品	高砂（〃）	1,500	1,500	960	7億（含公害投資）
三楽オーシャン	八代（〃）	(300)	(300)		味の素㈱へ全量供給
新進食料	前橋（〃）	220	220	170	300万円（公害投資）
味日本	広島（—）	250	200	150	
味の世界	姫路（—）	—	—	—	
江崎グリコ	京都（—）	—	—	—	
大阪食品化学	大阪（—）	—	—	—	
料理の素	名古屋（精製のみ）	15	15	4	月間95～100t程度販売か
ヤマサ醤油	銚子（パッケージのみ）	—	—	—	
計		11,435	11,385	7,834	

生産，輸出，国内向けの数字をみれば判るように，43年いらい生産は9万5,500トン～10万トンの間にあり，輸出は1万8,000トン～2万1,000トンを上下し，国内向けは7万5,000トンから8万トンの間で動いている。

これは，輸出のいかんによって生産が左右され，国内向けは需要が横這い傾向にあることを表わしており，もし輸出規模に生産をスライドせずに増産するとたちまち，国内の業務用市場を圧迫することになるわけだ。

需給のポイントを握る輸出は，46年8月のドルショックいらい，苦しい環境のなかにある。スミソニアン体制の16.88%の円切り上げとワイダーバンドを見込めば20%の円手取額の減少となる。これに対し業界は年平均して10%の値上げを目標にしていた。しかし事態はさらに悪化し，48年2月14日に円が高騰して，変動相場制に移り，その後東京外為市場の閉鎖にまでにいたり，直物円相場はさらに対ドル16%以上をとなえている。

47年度の見込も，48年度の予想も再度の円建て価格の引上げが必要で極めて難しい局面にあるわけだ。

そのほかに，醗酵法の世界的普及と日本資本の現地生産進出によって，海

外の自給率が高まっていることも，日本の輸出を停滞させる原因である。

表①で44年以降の地域別の輸出動向を見ると，日本資本の海外投資，現地生産をおこなっている東南アジア，欧州への輸出は年々減少し，日本の海外投資をしていない北米，カナダ，中南米，中近東，南亜向けの輸出が増えている。

ところが，最も輸出の多い北米，カナダ，などが円フロート率が高いドル圏内であるから，輸出の見通しが難しいわけである。

通関実績（1～12月）で，44年2万1,803トン，45年1万8,974トン，46年2万108トン，47年1万7,605トンとなっているが，48年2月以降の輸出は，各地域の為替レートの変動中や需要調査をみないと，ハッキリとした輸出見通しはたてられないだろう。また，海外投資企業からの振替輸出は，現地工場の生産力がないのが実情であろう。

国内の需要動向をみる

（次）に国内の需要動向をみよ[illegible]

表①および表⑦でわかるよう[illegible]の需要（生輸一出産）は7万6,000トン～[illegible]900トンの間と見られる。従ってこの量を超えた供給をおこなうと，メーカー在庫あるいは市中在庫が増え，業務用の市況が乱れることになる。さきにも触れた通り国内需要は横這いで，老齢商品化しているからだ。

ただ，この在庫がグル曹単体でなく複合調味料や〝だしの素類〟などのような二次製品化されている場合は直接業務用市況の圧迫材料にはならないことは勿論だ。

47年度の国内向けが7万8,000トン程度と見込まれているが，毎年3,000トン～4,000トンのランニングストックがあるとも言われているから，あるいは実需はもっと少ないかも知れない。

これを業務用と家庭用に分ければ，表⑦の通り，業務用4万5,100トン，家庭用3万2,900トンで，46年より少ない。業務用市況の引締まりは供給量の削減によることを物語っている。

家庭用も複合調味料との競合，中元需要の停滞，有害説などから若干減少したものと見られよう。

(一) 業務用消費の動向

44年ま[illegible]向けと自家加工向け需要[illegible]れて需要が[illegible]需要[illegible]下降傾向さ[illegible]表⑨)

これ[illegible]加工向け需要のなかの大量消費部門であったラーメン，水産煉製品の生産停滞のほかに，料飲店向けの需要増が鈍化したこと，さらには食品加工向け，料飲向けともに，消費態様別のアミノ酸系天然調味料が開発，発売されたことなどが原因とみられる。

このように，グル曹使用商品部門の成長率の鈍化とこれに代わる別の調味料の開発は，業務用グル曹の需要停滞の原因となっているが，45年と47年は，これに有害説，大量使用有害説などからユーザーの使用度合が低下しているものとみられる。

45年からおこなわれている操短はこうした業務用需要の停滞が背景にあるわけだ。その結果はさきにも触れた通り，45年秋いらい，数回にわたる値締め政策となって表われているわけだ。

その実態は，小口扱いものの市況がkg当りで45年春の320～340円に比べて48年春は100円以上高の420円～450円となり，47年春の市況に比較しても30～40円高となった。

48年上期中にはさらに20円上げ，下半期には需要期入りとともにさらに20円～30円上げの460円～500円に乗せなければ，[illegible]補填やコストアップ吸収の方法がないだろう。

[illegible]理て直しは，いうまでもなく複合調味料を含[illegible]体の総合的な市況対策が必要なことは論を[illegible]

表④　主要仕向地別輸出状況（1～12月）

日刊経済通信社調

地区別	昭和44年 数量	昭和44年 シェア	昭和45年 数量	昭和45年 シェア	昭和46年 数量	昭和46年 シェア	昭和47年 数量	昭和47年 シェア
	t	%	t	%	t	%	t	%
東南アジア	6,105	28.0	3,815	20.1	3,267	16.2	2,518	14.3
大洋洲	852	3.9	691	3.6	640	3.2	935	5.3
中近東，南亜	280	1.3	207	1.1	227	1.1	352	2.0
欧州	5,190	23.8	6,226	32.8	5,040	25.1	4,213	23.4
中南米	3,171	15.9	3,197	16.8	4,974	24.7	4,625	26.3
北米，カナダ（含ハワイ）	5,671	26.0	4,834	25.5	5,960	29.7	5,052	28.7
韓国	20	0.1	4	0.0	東南アに組み入れ		—	—
その他	220	1.0	—	—	〃			
計	21,803	100.0	18,974	100.0	20,108	100.0	17,605	100.0

（注）各年ともに通関実績。

表⑤　グルタミン酸ソーダの価格変遷

日刊経済通信社調

年別	内需向け生産者出し値 金額（円）	内需向け生産者出し値 指数	平均輸出価格 金額（円）	平均輸出価格 指数	備考
43年	450	100	307.40	100.0	①国内業務用向け市況は，47年春いらい二回の値上げをおこない，48年春には kg当り380～390円。②国内向け家庭用は，平均価格をkg当り810～820円とみた。③47年以降の輸出価格の低下は円切上げによる手取額の減少である。
44	450	100	290.89	94.6	
45	460	102	274.40	89.3	
46	480	107	264.03	85.9	
47	490	109	258.14	84.0	
48. 2年	510	113	258.61	84.1	

注）平均輸出価格は1～12月通関実績から。（FOB）

さいわいに48年度は，47年秋から核酸調味料（イノシン酸，グアニル酸）が一部メーカーの原料手当難から品薄商状にあり，4月頃からkg当り1,000円程度の値上げをせざるを得ない状態である。

従って，IG，複合調味料の市況軟化が例年のようにグル曹建て値引上げの足を引張る要因は少ないと見られる。

おそらく，48年度のkg40～50円値上げは余程のアクシデントでもない限り実現は可能と見たい。

(二) 家庭用消費の動向

年間需要量は3万トン～3万3,000トン程度とみられるが，容器別区分の仕方では3万6,500トン程度とみることもできよう。また複合に変身した分が各社各様に計算がことなるからでもある。（表⑦～⑨，表⑪）

何れにしても，家庭用も業務用と同様，46年頃までは2～3％の年増加率があったが，47年から全く横這いとなった。

家庭用消費の伸びが鈍化した理由をあげてみるとおよそ次のようなものだろう。

①複合調味料に変身している，②贈答品の高級化，多様化によって，贈答用グル曹の進物ものにおける地位が低下してきている，③〝だしの素類〟〝天然調味料〟をグル曹メーカーをはじめ他の調味料メーカーが発売した，④香辛料，マヨネーズ，ドレッシング類などの発達によって，調味料食品の消費パターンが多様化し，変化してきている，⑤家庭用グル曹に核酸系調味料が1.5％含有され，複合化し呈味性が高まってきていることなどがあげられる。

実際，47年（1～12月）の推定でも家庭向けに消費されたグル曹のうち，単体で消費されたものが約297トン，1.5％～2.0％のIH含有ものが1万9,000トンの54.6％，複合が1万6,450トンの49.5％にも達しているものとみられる。

また〝風味だしの素類〟も46年が約8,500トンの68億円，47年が1万3,600トンの110億円，48年が1万8,800トンの150億円市場になるとの見方もある。

表⑥　グルタミン酸ソーダの業務用市況（kg当り）

日刊経済通信社調

年別	扱数量別	標準建値	安値もの	備考
45年春	中口扱い	320～340	—	①44年春＝味の素㈱と武田薬品が原料を酢酸に切替えを開始。
〃	小口扱い	310～370	320～340	
〃 秋	中口扱い	310～360	—	
	小口扱い	360～390	320～340	②44年秋＝大手4社が能力を拡張，月産で味の素㈱が，5,960t，旭化成，協和，武田が各1,500tとなる。
46年春	中口扱い	340～360	—	
	小口扱い	370～400	320～340	
〃 秋	中口扱い	340～370	—	
	小口扱い	370～400	330～350	
47年春	中口扱い	340～370	—	③建値の取扱いは，メーカーとユーザーとの直接契約のため除いた。
	小口扱い	370～410	340～360	
〃 秋	中口扱い	360～380	—	
	小口扱い	390～420	370～390	
48年春	中口扱い	370～390	—	
	小口扱い	420～450	390～400	

(三) 需要鈍化だが販売戦激化

以上のように業務用，家庭用の何れをとわず，国内の需要に鈍化傾向が表わされていることは老齢化商品とも言えようが，これを一層拍車しているのは，グル曹企業が，グル曹使用の2次，3次加工食品を開発し，またアミノ酸系天然調味料，風味だしの素類などを開発して，より付加価値の高い商品を販売していることが大きな原因の一つであることに注目する必要がある。

複合調味料をはじめ，味の素㈱が，ねり味，アジメート，ビーフレンド，ほんだし。協和醗酵工業㈱が，AM，HPP，風味だし（雪印と提携）。武田がプレミックス，ウマミックス，タケダだし。旭化成工業㈱がエキストラート，サンバーグ。新進食品工業㈱のミラクレックス。ヤマサ醤油のヤマサだしなどがその好例だ。

各社とも品揃えが完了し，販売線は一層白熱化の様相を呈している。

いうなれば，グル曹業界は蛋白～アミノ酸体系のなかで商品の総合化と多角化によってより付加価値を高める競争をおこなっており，グル曹はその一環として，母体的役割を果しながら，そのウエイトが徐々に低下する運命にあるといえよう。

表⑦　国内消費の内訳

日刊経済通信社調

年度別	国内向け供給料 数量	前年比	業務用 数量	構成比	家庭用 数量	構成比
	t	%	t	%	t	%
43年	75,500	—	45,149	59.8	30,351	40.2
44	79,500	106.0	46,031	57.9	33,469	42.1
45	73,000	91.8	41,902	57.4	31,098	42.6
46	80,000	109.5	46,500	58.1	33,500	41.9
47(見込)	78,000	97.5	45,100	57.8	32,900	42.2
48(推定)	78,000	100.0	45,100	57.8	32,900	42.2

（注）年度は4～3月

表⑧　昭和47年（1～12月）の各社内販推定量

日刊経済通信社調

会社名	46年の国内向販売量	47年の国内向販売量	業務用 数量	構成比	家庭用 数量	構成比
	t	t	t	%	t	%
味の素	45,156	43,927	20,646	47	23,281	53
旭化成	10,010	9,630	7,415	77	2,215	23
協和醗酵	9,205	9,004	7,203	80	1,801	20
武田薬品	11,289	11,354	6,126	54	5,228	46
新進食料	2,280	2,147	1,997	93	150	7
味日本	1,170	1,750	1,663	95	87	5
ヤマサ醤油	(1,000)	(1,100)	(1,100)	(100)	—	—
料理の素	43	38	38	100	—	—
江崎グリコ	—	—				
大阪食品	—	—				
味の世界	—	—				
計	80,153	77,850	45,088	57.9	32,762	42.1

注）ヤマサ醤油は「協和」から「日東味の精」を供給

採算向上が緊急課題となったグル曹

(ヒ) ッグ4社の経営者たちが，かつてのグル曹販売大戦から〝勝利なき平和〟への政治路線をなぜ布

いたかは，グル曹それ自体の商品の運命ばかりでない。上述のような理由のほかに，蛋白～アミノ酸体系のサイエンスのなかで，さらに進んで，ファインケミカル，ライフサイエンスを指向することの重要性を認識したからであろう

とはいえ，総合多角化のなかで母体的役割を持つグル曹それ自体の採算の悪化も〝勝利なき平和〟への大きな動起となったことはいうまでもない。

①総合多角化を求めながらもグル曹の採算悪化は装置産業だけに企業にとっては重大なことでてる，②原料面で，糖蜜，酢酸ともに値上がりが著るしく，強くコストを押し上げている，③インフレが高進し，人件費をはじめ間接[illegible]の高騰[illegible]い，④公害投資が緊急を要し[illegible]ップ[illegible]なっている，⑤通貨不安によって輸出の見通しが明るくないこと。

これだけの採算悪化要因が重なれば，値引き競争を止めるのが当然だともいえる。

実際に46年～48年の間の当調味料業界の公害投資は年平均30億円としても，90億～100億円の公害投資をすることになり，この償却費はkg当り10円以上だともいわれている。

さらに原料価格は，48年春に糖蜜が前年比50%程度値上がりし，トン当り2万円にも達したといわれ，酢酸はOPECの再三にわたる原油値上げと需要増から，前年比25%～30%アップのkg当り42～43円[illegible]えたともいわれている。さらに輸出の見通しが[illegible]輸出しても採算が悪かったら，国内業務用の[illegible]て，採算向上[illegible]かることは緊急[illegible]

48年度需給見通しと当面の課題

つぎに，48年度の需要見込みと課題を追ってみよう。

48年度の需給見込

48年度の需給見込みは，日本化学調味料工業協会でも結論がでていない。輸出の見通しが立たないからだ。しかし，年間生産能力13万4,000トン～13万5,000トンは対し，9万6,000トン～9万7,000トン以上の操業度（71%以上）は維持したいところだろう。それ以上の操短はコストアップ率を高めるからだ。

輸出は前述の通り，大半を占めるドル圏向けの値上げは早急にむずかしいだろうし，現行価格ではダンピングのそしりをまぬがれない。他地区への輸出転換も地区毎の需要予測は早急には立てはくい状況にあるようだ。

となると，輸出は希望的観測を入れて1万6,000トン，1万7,000トンを最高線と踏み，国内向けを前年なみの7万8,000トンとすれば，生産は9万4,000トン～9万5,000トン程度となってくる。しかし，これは輸出の状況によって上下することは言うまでもない。

業界では国際通貨情勢に応じて，第1段階，第2段階，第3段階と目標を修正設定するのではなかろうか。

面当の課題

昨年の課題がそのまま今年の課題となってしまった。ただ国内景気が昨年よりもよいので，需要が業務面で多少よくなると考えられる程度である。課題を拾ってみると，①コストアップを合理化によって吸収する余地がないので，業務用，家庭用をとわず製品値上げの必要がある，②円相場上昇による輸出の円建て価格引上げ対策，③輸出地域の転換策，④需要促進とコンシューマリズム対策，⑤海外直

表⑨　昭和44～47年（1～12月）の業務用消費の内訳　日刊経済通信社調

項目	昭和44年 数量（t）	昭和44年 百分比（%）	45年 数量（t）	45年 百分比（%）	46年 数量（t）	46年 百分比（%）	47年 数量（t）	47年 百分比（%）
食品加工原料用	16,200	34.8	15,081	33.0	14,837	32.5	14,500	32.2
飲食店向，医薬その他用	16,200	35.0	16,085	35.0	16,207	35.5	15,890	35.3
自家消費用（含自家加工食品用）		30.2	14,[illegible]	[illegible].8	14,609	32.[illegible]	14,610	32.4
計	[illegible]	[illegible].0	45,70[illegible]	[illegible].0	45,653	100.[illegible]	45,[illegible]	100.0

表⑩　昭和47年[illegible]化学調味料の家庭向供給料（推定）
日刊経済通信社調（単位：トン）

会社名	家庭用の年間供給料	グル曹単体	訳 低核酸複合	訳 高核酸複合	核酸調味料（純品）の生産 月産能力	核酸調味料（純品）の生産 年産	品名	含有率(%)
味の素	23,281	—	(味の素) 1.5% 17,081	6,200	140	110	イノシン酸	100
					25	25	グアニル酸	100
旭化成	2,215	60	(旭味) 1.5% 435	1,720	30	20	イノシン酸	100
							グアニル酸	100
協和醱酵	1,801	—	(キーパー) 2.0% 951	850	30	20	イノシン酸	100
					30	10	グアニル酸	100
武田薬品	5,228	—	(高砂) 1.5% 550	4,678	85	50	リボタイド	
ヤマサ醤油	(3,000)	—		(3,000)	50	30	イノシン酸	100
							グアニル酸	100
新進食料	150	150	—	—	—	—		
味日本	87	87	—	—	—	—		
計	32,762 (35,762)	297	19,017	13,448 16,448	390	265		

接投資企業の管理対策などである。

1.）国内向けの価格は，前にも触れた通り，現実にコストが上っている。

業界では業務用価格のみの値上げを考えているようだが，進物箱などは値上げをして採算を改善することを考えるべきであろう。包装費，同資材費の上昇は人件費とともに進物箱のコストアップ要因として大きいといえよう。

特に業務用価格はkg当り450円～500円であれば，各国の為替レートの変更を見込んでも東南亜ものの上陸する心配がないといわれている。ただ，世界の通貨，通商交渉のあおりを受けての関税の一括引下げなどは，海外よりも現行の輸入関税が5～6％以上も高いので警戒を要する点であろう。

2.）輸出価格の引上げは，スミソニアン体制分の調整が済まないうちに，円の変動相場制がやってきた。

従って，スミソニアン体制にさらに16％前後の切上げとすると，輸出採算をどのようにしてつぢつまを合わせるかを考えた上でないと円建て価格引上げのメドすらつかないのではあるまいか。

3.）輸出地域の転換策は価格の面からいえば，ドル圏を避けて，他の通貨圏に輸出するという理屈にはなるが，欧州，東南亜市場は，現地生産者が多い。アフリカ，中近東地区となると需要予測と開拓に時間がかかるだろう。しかし，これは今年だけの事ではない。ドルの交換性が回復しないで，日本の国際収支黒字が続く限り，長期の対策を必要とする。

とはいえ，これは味の素(株)のほか旭化成と協和醱酵がこれにどう対処するかの問題である。

4.）需要促進とコンシューマリズム対策は，46年～47年と有害説解消に力をとられ，真の需要促進策が打出されていないうらみがある。

48年度は，積極的にコンシューマリズムへのアプローチをおこなうとともに，消費促進策についての新らしいパターンが考えられなければならないだろう。

家庭用，業務用を通じて，受身でない能動的な需要促進策への転換期ではなかろうか。そのためには，新らしい商品化計画が伴なわなければならない。

5.）海外直接投資企業の管理対策は，日本資本に対する現地のアンチ運動は別として，現地の他国資本との競合問題が，激しさを増し，海外市場が乱れるケースがあると聞く。世界のグル曹大国日本の節度を保たないと，海外市場の混乱は生産力の最も多い日本にたちまち響いてくることはいうまでもない。

表⑫でも明らかなように，日本のグル曹生産力は世界のそれの2分の1以上を占め，さらに海外直接投資企業の生

表⑪　食品加工用グル曹の消費推定量（1～12月）

日刊経済通信社調（単位：トン）

商品部門	昭和44年	45年	46年	47年	備考
水産煉製品	2,250	2,260	2,500	2,500	消費標準別調味料もグル曹に換算すると約6,000tとも言われている。
魚肉ハム・ソーセージ	2,000	1,900	2,000	1,950	
缶壜詰・冷食	660	600	600	700	
食肉加工	1,500	1,500	1,400	1,400	
即席カレー	900	900	950	950	
即席ラーメン	2,200	2,200	2,200	2,150	
だしの素類	600	800	900	950	
マヨネーズ	1,170	1,250	1,350	1,400	
スープ類	1,450	1,500	1,500	1,500	包装めん用スープも含む。
ふりかけ・のり茶漬	400	400	400	400	
バーベキューつゆ及びそばつゆ類	550	450	400	350	
煎餅・あられ類	170	170	170	170	
酒類	40	30	25	20	
味つけのり	170	170	160	110	
パン・菓子類	150	150	130	120	
ケチャップ・ソース類	220	200	220	230	
醤油用	190	190	170	160	
佃煮類	1,000	1,000	1,000	990	
珍味類	1,200	1,200	1,100	990	
その他	250	250	200	170	
小計	17,070	17,120	17,375	17,210	
給食用	500	550	550	500	
計	17,570	17,670	17,925	17,710	
複合調味料（高級腐もの）	14,000	14,642	16,336	16,448	
合計	31,570	32,312	34,261	34,158	

表⑫　1973年の世界のグル曹推定生産力

日刊経済通信社調（48．2月末現在）

国別	年産公称能力（t）	年間の輸出量（t）	メーカー数	製造方法	備考（社別月産能力）
日本	134,220	17,000	6	醱酵法・合成法	
米国	24,000	2,000	2	醱酵法・分解法	IMC1,800t CSC200t
台湾	17,280	4,000	6	醱酵法	中国醱酵500t，味全500t，津津300t，東海60t，味冠40t，吉峰40t
イタリー	10,800		2	醱酵法	モンテスシー500t インスッド 850t
スペイン	720		1	〃	ペニペリカ 60t
フランス	12,000		1	〃	オルサン 1,000t
香港	600		1	〃	全厨味精 50t
韓国	22,200		2	〃	味元1,300t，味豊550t
タイ	14,160		4	〃	タイ味の素850t，タイ味精100t，味タイ130t，タイ国開発100t
マレーシャ	3,240		2	醱酵法	マレーシャ味の素200t マレーシヤ味厨70t
フィリッピン	8,500		3	醱酵法	ユニオンケミカル550t，フィリッピン醱酵100t，ピープル・インダストリアル50t
ペルー	1,800		1	醱酵法	ペルー味の素 150t
インドネシヤ	5,280		4	醱酵法	インドネシヤ味の素250t，ササ70t，ミキモト70t，その他50t
中共その他	7,000		4	醱酵法・分解法	
計	261,800	23,000			

産力を合わせると，世界の64%の生産能力を資本支配していることになるのである。

わが国グル曹産業の[illegible]は世界にとっても重要なわけだ。

わが国グル曹産業の今後

（最）後はわが国グル曹産業の今後についてみよう。

日本のグル曹産業は構造的に一つの転換期にさしかかっている。その転換はおよそ次のような方向に傾斜するだろう。

㈠国内の需要，輸出ともに今後大巾に伸びることは期待できないし，メーカーは大手5社で生産集中度が98%を超す寡占化体制が形成されている。他は協和醸酵の傘下に入った味日本以外は殆んど生産をおこなっていない。

一方，大手5社の企業内における売上高に占めるグル曹の比重は，総合多角化によって年々低下している。

つまり大手5社はグル曹を中心とする蛋白～アミノ酸体系のサイエンス以外の他業種も含めて総合企業化してしまったのである。

それだけに，内外におけるグル曹市場の競争を厳しいものにする要因を常にはらんでいることになり，これをコントロールしてゆかなければならない宿命を持っている。

そこに日本のグル曹産業の構造的特質があるといえる。

だが，総合多角化の方向が蛋白～アミノ酸工業から，天然調味料，薬品，飼料，肥料，化成品など醸酵工学の可能なあらゆる分野に発展しうるポテンシャルに富んでいることは，当産業企業の将来を明るいも[illegible]ている。

（[illegible]の海外投資は，原料一次産品産出国に向けて[illegible]われるだろう。

但し，これからの投資は単にグル曹の生産ばかりでなく，さきにも触れた通り，醸酵工学の可能な新らしい商品の開発を含む総合的なプロジェクトの形で海外進出がおこなわれるだろう。

この場合，一次産品産出国のナショナルリズムへの対応策と，今後特恵特品の拡大，貿易の自由化と関税の引下げなどが進んだ場合，海外投資企業から日本への逆輸入がおこるケースなども配慮されなければならないだろう。　　（石母田主幹）

表⑬　日本グル曹企業の海外投資と技術援助の状況　　日刊経済通信社調（48．2月末現在）

会社名	国名	出資割合（%）	資本金（億円）	48.3月末の月産能力（t）	48.3月末の月産量（t）	当面の拡張計画（t）	製造方法	設立年月日
タイ味の素	タイ	味の素70：現地30	4.33	850	800	—	醸酵法	35．4.29
マレーシヤ味の素	マレーシヤ	60：40	4.70	200	200	—	〃	36．7.14
味の素インスッド	イタリー	50：50	14.60	850	750	—	〃	38．6.22
ユニオン・ケミカル	フィリッピン	40：60	4.70	550	550	—	〃	32.11.18
ペルー味の素	ペルー	78：22	3.40	150	130	—	〃	43．2.29
インドネシヤ味の素	インドネシヤ	87：13	5.06	250	230	—	〃	44．7．3
オルサン社	フランス	旭化成が技術輸出		1,000	契約期間は1967年4月1日から1977年3月末まで			38．7．
ペニペリカ	スペイン	味の素が技術輸出		60	契約期間は1979年11月26日まで			39．春
メルク社	アメリカ	協和が技術輸出		[illegible]00	契約期間は昭和33.12.10日から15年間			33.12.10
インターナショナル・ミネラルズ・ケミカル（IMC）	アメリカ	〃		[illegible]	契約期間は昭和40.7.1日から13年間			40．7．1
ママーシャル・ソルベント（CSC）	アメリカ	〃			契約期間は昭和41.1.1日から12年間			41．1．1

×　×　×　×　×　×

×　×　×　×　×

×　×　×　×　×　×

The Use of Monosodium Glutamate in Frozen Foods[a]

KARL B. NORTON,[b] DONALD K. TRESSLER,[c] AND LILLIAN D. FARKAS[b]

(Manuscript received June 12, 1952)

The effect of adding monosodium glutamate to various species of fish, to poultry, to beef, pork sausage, hams and bacons and to selected vegetables prior to packaging and freezing is described. The data show that the use of monosodium glutamate in frozen foods under the conditions employed in this investigation prolongs quality during low-temperature storage; an antioxidant effect—particularly with respect to hams, bacon, pork sausage and fatty fish (salmon and mackerel)—was demonstrated organoleptically.

The value of the use of monosodium glutamate to enhance the flavor and improve the acceptability of many canned foods *(1, 2)*, and also as a seasoning in foods prepared in the kitchen for the table *(3, 4)*, has become recognized by the general public as well as by food processors. All are in agreement that most foods become much more flavorful when this comparatively newly developed food ingredient is used in their preparation *(5, 6, 7)*. Now that monosodium glutamate has become a standard ingredient in many canned foods where it is incorporated in the product at the time it is canned, it was only logical to be curious as to the effect it might have on frozen food when the food was treated with it before freezing. Accordingly, an investigation was undertaken to determine the effectiveness of such treatment and to observe this effect on the frozen product during its storage life at low temperatures. Shucy and Eichenberger *(8)* have shown improvement in the acceptability and flavor enhancemnt in certain frozen vegetables by the addition of monosodium glutamate. The present study includes these and other frozen foods evaluated over an extended storage period.

The products investigated were selected from broad fields of frozen foods, *viz.* fish, fresh and cured meat, poultry and vegetables. Included in the list of fish were shrimp, salmon steaks, mackerel, haddock and rosefish fillets. The fresh meat items investigated were ground beef, cube steak and sausage. Cured meats included ham and bacon. The poultry items were broilers, cut up fowl, and turkey, while the vegetable items fairly well covered the whole field now being frozen commercially including asparagus, peas, green and wax beans, corn, baby and Fordhook lima beans, spinach (both leaf and chopped), broccoli, succotash, cauliflower, squash and brussels sprouts.

In all cases samples were selected from production lines during the regular commercial operations so that the samples would represent as nearly as possible commercial practices and procedures, including freezing and storage at both 0° F. and 10° F. (—17.8° and —12.2° C.). During the storage life of the samples, periodic examinations were made visually before cooking and both visually and organoleptically after cooking.

It was the design and intent of the project to observe only visually and organoleptically the results of the use of glutamate at a predetermined average level rather than to attempt to establish optimum concentrations or follow changes by detailed chemical analyses. In all cases a concentration of about 0.15% glutamate was used, since, in general, this amount gave positive flavor enhancement in the types of products investigated.[d]

SERIES I: SEAFOOD

Procedures

Preparation of samples. *Shrimp.* Fresh shrimp from the New York market were peeled, washed and packed without cooking in aluminum foil packages, 12 oz. each. The packages were then frozen on stationary plates in the cold storage at 0° F. (—17.8° C.). After freezing, half of the samples were glazed by brushing the frozen blocks with 25 ml. of an aqueous solution containing 0.5 g. of monosodium glutamate. The other half of the samples, used for controls, were glazed in a similar manner, but with 25 ml. of plain water. All samples were then packed in moisture-proof cellophane bags in cardboard cartons and placed in storage.

Salmon. Silver salmon from the State of Washington were cut into steaks weighing approximately one-half pound each. The control samples were wrapped in cellophane and frozen directly on the freezer plates, while the other steaks were dusted with 0.35 g. of monosodium glutamate, then wrapped and frozen on the plates. Samples were stored at 0° F. and 10° F. (—17.8° and 12.2° C.).

Mackerel. Mackerel fillets were taken from the production line and packed 3 fillets to a package. Half of the samples were wrapped with cellophane and frozen directly while the others were dusted with 0.2 g. of monosodium glutamate per package, then wrapped and frozen. Samples of each were then stored at 0° F. and 10° F. (—17.8° and —12.2° C.).

Haddock. Haddock fillets from the production line, weighing about 14 oz. each, were dusted with 0.6 g. monosodium glutamate, wrapped in cellophane and frozen. Control samples were similarly wrapped and frozen but without treatment with monosodium glutamate. Samples were stored at 0° F. and 10° F. (—17.8° and —12.2° C.).

Rosefish. Rosefish fillets were packaged 8 oz. per package after dusting with 0.34 g. of monosodium glutamate. Control samples were packaged without treatment. Samples were then frozen and stored at 0° F. and 10° F. (—17.8° and —12.2° C.).

Testing. The frozen shrimp were cooked by placing the sample while still frozen in a pan containing 2 cups of boiling water and then boiled for 15 minutes. All other samples of fish were allowed to thaw at room temperature, then broiled in an electric oven until crisp and done.

Paired samples were examined at various periodic intervals to determine the effectiveness of the monosodium glutamate

---

[a] Presented at the Twelfth Annual Meeting of the IFT, Grand Rapids, Michigan, June 11, 1952.

[b] Karl B. Norton & Associates, Westport, Connecticut.

[c] Formerly of Donald K. Tressler & Associates, presently Scientific Director, Quartermaster Food & Container Institute for the Armed Forces, Chicago, Illinois.

[d] Much credit is due the various companies who cooperated in this project by supplying the product for the storage tests and in whose plants the samples were prepared, *viz.* Armour & Co., Seabrook Farms Co., Atlantic Coast Fisheries Co. and Davis-Cleaver Produce Co.; and to Amino Products Division of International Minerals & Chemical Corp. who suggested and supported the work.

treatment by a trained taste panel with all tests blind.* Table 1 shows in condensed tabular form the results of these storage tests. Since the results at 10° F. (—12.2° C.) storage were of the same general character as those at 0° F. (—17.8° C.) storage

TABLE 1

Comparison of controls with glutamate treated seafood samples held in frozen storage at 0° F. (—17.8° C.)

Months storage time	Control	Glutamate treated
	Shrimp	
1	Excellent somewhat flat taste	Excellent full rounded flavor
5	Very Good but toughened sl.	Excellent sl. more tender than control
7	Very Good about same as five months	Very Good color sl. brighter than control
14	Fair dry and flat but still edible	Good sweeter, better flavor than control
	Salmon	
1	Excellent color and flavor	Excellent color and full flavor
3	Fair color fading	Excellent color and full flavor
10	Poor dry, flavorless, very poor color	Very Good still bright color good flavor
12	Poor practically colorless very poor flavor	Fair still pink color fair flavor
	Mackerel, Haddock, Rosefish	
1	Very Good	Very Good all preferred to controls
3	Good	Good all preferred to controls
5	Fair to Good old flavor	Good all preferred to controls
9	Poor Mackerel—rancid Haddock—strong off flavor Rosefish—rancid off flavor	Poor Mackerel—still edible Haddock—better than control Rosefish—still good

except that the changes occurred sooner, only those at the lower storage temperature and those tests that showed significant changes are tabulated.

RESULTS AND DISCUSSION

Shrimp. Treating green peeled fresh shrimp with monosodium glutamate after freezing but before frozen storage greatly improved and intensified the flavor, and increased the storage life. The treated samples were sweeter and slightly more tender than the control samples, which appeared to become tougher after 5 months' storage. The color of the treated samples was brighter and retained this brilliance even after 7 months' storage. At the end of 14 months' storage, while no rancidity had developed in any of the samples, the flavor, odor and texture of the glutamate-treated samples were superior to the control samples.

Salmon. Although no difference in color appeared in any of the samples during the first 2 months' of storage, it was noted that there was a definite fading of the color of the control samples at the end of 3 months' storage, whereas the samples treated with monosodium glutamate had retained the pink color of the fresh salmon. At the end of 3 months' storage the glutamate-treated samples were still excellent in flavor, whereas the control samples had begun to lose their fresh flavor. At the end of 10 months' storage the control samples were dry, flavorless and very faded in color, while the glutamate-treated samples had still retained very good flavor, texture and a fair color. After one year in storage pink color and a fair flavor existed in the treated samples, but the control samples had lost their flavor and had become practically colorless.

Mackerel. At the end of 3 months' storage there was no difference noted in the samples except that those treated with glutamate were superior in flavor to the control samples. At the end of 5 months' storage those held at 0° F. (—17.8° C.) were still good in both odor and flavor with the glutamate samples slightly superior in these respects. At the close of the 7-months' test period the same conditions prevailed as at the end of 5 months except that they were somewhat worse; the 0° F. (—17.8° C.) samples had begun to show rancidity. At the end of 9 months' storage the glutamate samples stored at 0° F. (—17.8° C.) were still edible; the control had become rancid. Both control and glutamate-treated samples held at 10° F. (—12.2° C.) were discarded as being too rancid for tasting. At the end of 11 months all samples were discarded.

Haddock. No difference was noted in any of the samples during the first 2 months of storage, except that the flavor of the treated samples was preferred. At the end of 3 months the control samples had become tougher in texture than the treated samples. At the end of 5 months all samples stored at 0° F. (—17.8° C.) were superior in flavor and texture to those stored at 10° F. (—12.2° C.). At the end of 9 months all samples had become strong in odor and flavor and had to be discarded.

Rosefish. At the end of 3 months' storage the glutamate-treated samples at both 0° F. and 10° F. (—17.8° and 12.2° C.) were superior in flavor and texture to the control samples at the same temperatures. At the end of 5 months the 10° F. (—12.2° C.) treated sample appeared to have a slightly better texture than the 0° F. (—17.8° C.) sample. The control samples, while of a good texture, were poor in flavor. At the end of 7 months the 0° F. (—17.8° C.) glutamate sample was superior in flavor and texture with the control samples poor in both respects. At the end of 9 months all 10° F. (—12.2° C.) glutamate samples were discarded as being rancid in flavor and odor, but the samples held at 0° F. (—17.8° C.) were still good. The control samples at both temperatures were poor in all respects and discarded. At the end of 11 months the treated samples had become rancid and were discarded.

* The panel was usually composed of five or six experts trained in testing the products under examination; care was exercised to preclude the panel's receiving any information in advance of the tests that might influence their judgment. The panel was asked to evaluate by visual and organoleptic criteria the samples submitted to them.

SERIES II: MEATS

Procedures

Preparation of samples. *Bacon.* Pork bellies from regular production were cured in accordance with regular procedure. The pork bellies were chilled for 5 days and sufficient paired bellies were obtained to fill 2 standard curing boxes, each holding 615 pounds. One lot was rubbed with dry cure containing 24.41 pounds of regular salt cure mixture and 1.17 pounds of monosodium glutamate. The other 615 pounds of bellies were treated with 24.41 pounds of the regular salt mixture according to the usual practice. Both lots of bacon were cured according to standard practice, and were then packaged for storage testing.

Each slab of bacon was cut into three pieces for storage at three different temperatures, 0° F., 10° F. and 34° F. (—17.8°, —12.2°, and 1.2° C.). Each piece was carefully wrapped with cellophane and then placed in an outside stockingette. Corresponding pieces of the slabs were marked and were tested for comparison after storage at the different temperatures.

Ham. Nineteen pairs of fresh hams, each ham weighing 12 to 14 pounds, were obtained from the warm carcasses, numbered and marked "left" and "right" for identification purposes. While the hams were still warm, a 1.5% solution of monosodium glutamate in the standard pickle or brine was pumped into the main artery of each left ham. Each of the right hams was similarly artery-pumped with the same weight of the standard pickle or brine, which, however, did not contain any monosodium glutamate. In both cases the amount of pickle added was 10% of the weight of the fresh ham, the hams being placed on accurate scales and the pickle pumped in until the weight of the ham increased 10%. The hams were then cured and smoked in accordance with standard Armour practice.

Upon arrival at the laboratory, the hams which were to be stored at 10° F. (—12.2° C.) were sawed in half and then wrapped in cellophane and inserted in a stockingette. The other hams which were to be stored at 34° F. (1.2° C.) and 0° F. (—17.8° C.) were left whole and wrapped in cellophane and stockingette for storage.

Ground beef. Top round steak was dusted with 0.15% of its weight with monosodium glutamate and then put through a commercial grinder. The ground beef was then carefully mixed and packaged in Reynolds "Pak" aluminum containers, each containing 14 oz., frozen and stored. The control samples were prepared the same way except that no monosodium glutamate was added.

Cube steaks. Top round steak of choice grade was cut into 8-oz. pieces and then cubed. One half of the pieces were dusted with monosodium glutamate and the other half were left untreated. All samples were wrapped in cellophane, placed in a tray container and then overwrapped with cellophane, frozen and stored.

Sausage. Fresh pork butts from the market were ground fine, the spices and seasoning being ground into the meat at the market. Monosodium glutamate was added to the formula for one half and left out of the formula for the control samples. The following is the formula containing monosodium glutamate.

For each pound of ground pork—

.68 g. monosodium glutamate
1.80 g. salt
1.20 g. sage (green)
.8 g. white pepper

The spices were mixed together and added to the ground pork, then the sausage was packaged, 14 oz. each, in Reynolds "Pak" containers, sealed, labeled, frozen and placed in storage.

Testing. All samples of meat to be tested were thawed at room temperature before cooking. The ground beef was made into 4-oz. patties and carefully broiled, done on the outside and medium rare on the inside. The cubed steaks were broiled to the same doneness as the ground beef. The sausage was made into patties and then broiled to a well-done stage. The bacon was sliced from the slab and broiled. Center slices of the thoroughly defrosted ham were also broiled.

Table 2 shows in condensed tabular form results of storage at 0° F. (—17.8° C.) since flavor differences between control and glutamate-treated samples were more pronounced over the longer storage period at this storage temperature. Similar results occurred at the higher storage temperatures but in a much shorter time.

TABLE 2

Comparison of control with glutamate treated meat samples held in frozen storage at 0° F. (—17.8° C.)

Months storage time	Control	Glutamate treated
Bacon		
1	Very Good	Very Good
4	Good very sl. rancid	Very Good
7	Fair sl. rancid	Good no rancidity
10	Poor very rancid	Poor stale flavor but no apparent rancidity
Ham		
1	Very Good	Very Good preferred to control
4	Good	Good preferred to control
9	Fair sl. stale flavor, color faded	Good still full flavored
14	Rancid	Fair still quite satisfactory
Ground Beef and Cube Steak		
1	Very Good	Very Good flavor preferred to control
6	Good	Very Good flavor preferred to control
8	Fair lacks freshness	Good much more flavorful than control
10	Poor	Fair
12	Very Poor flat, stale, old flavor	Poor stale flavor but preferred over control
Pork Sausage		
1	Very Good	Very Good more flavorful than control
3	Fair flat in flavor	Good more flavorful and tender
5	Poor sl. rancid	Good no rancidity
7	Very Poor rancid and color faded	Fair sl. old flavor
9	Very Poor very rancid	Fair poor flavor but not rancid

RESULTS AND DISCUSSION

Bacon. At the end of one month of storage there was little or no difference between the treated and control bacon samples at all 3 storage temperatures. At the end of 3 months' storage the 0° F. (—17.8° C.) untreated sample was preferred but the treated samples held at the other 2 temperatures were preferred over the controls. At the end of 4 months the 34° F. bacon samples, both treated and untreated, were moldy on outside with a slight off-flavor in the control sample. In

the 0° F. (—17.8° C.) samples a slight rancid flavor appeared in the control and so the treated sample was much preferred. At the end of 5 months all 34° F. (1.2° C.) samples were discarded, the surface being very moldy and texture unsatisfactory. At the end of 7 months the treated samples at 10° F. (—12.2° C.) were much preferred to the control samples. There was a slight rancidity in both control samples at the end of this period. At the end of 9 months' storage the treated bacon samples at 10° F. and 0° F. (—12.2° and —17.8° C.) were still not rancid as was the control. At the end of 10 months' storage the treated samples still were not rancid at either the 10° F. or 0° F. (—12.2° and —17.8° C.) temperatures. However, the color and flavor were poor and the samples had passed their usefulness in testing. The control samples were very rancid at both storage temperatures.

One set of samples was kept in storage for 14 months at 0° F. (—17.8° C.). The treated samples, although poor in color and flavor, was still edible on the inside. The control sample was rancid all the way through.

Ham. At the end of one month all glutamate-treated ham samples stored at the 3 temperatures, 34° F., 10° F., and 0° F. (1.2°, —12.2°, and —17.8° C.) were superior in flavor, color and texture to the control samples. At the end of 3½ months the treated sample and control sample at 34° F. (1.2° C.) were very moldy on the outside and gave off a rancid odor in cooking. The 34° F. (1.2° C.) samples were discarded at the end of 5½ months. The 10° F. and 0° F. (—12.2° and —17.8° C.) treated samples at the end of 7 months were juicy and flavorful. The 10° F. (—12.2° C.) treated sample was preferred over the 0° F. (—17.8° C.) sample as being slightly more tender. There was a slight rancid flavor in both of the control samples when tested at this time. This same condition prevailed throughout the testing until the end of 10 months' storage. At the end of 11 months' storage the treated ham samples at 0° F. and 10° F. (—17.8° C. and —12.2° C.) were not rancid but had lost the full flavor and color of first tested samples. Rancidity was evident in both control samples at the end of this time.

One set of samples remained in storage at 0° F. (—17.8° C.) for 14 months. Although this treated sample was slightly bleached in color, the flavor was fairly good and it was edible. The control sample, however, was also bleached out in color but so rancid that it was not edible.

Ground beef. The treated samples at both temperatures were superior in flavor to the control samples at the end of one month. At the end of 2 months the treated sample stored at 10° F. (—12.2° C.) was superior in flavor and texture to the 0° F. (—17.8° C.) treated sample or to the control sample at either temperature. The same condition prevailed at each testing until after 8 months had elapsed. At the end of 8 months the control sample had acquired an old stale flavor. The 10-months' test showed that the glutamate-treated samples at both temperatures were superior in flavor to the control, but there was little if any difference between the treated samples at 0° F. and 10° F. At the end of 12 months all samples, both glutamate-treated and control, were discarded because of flat, stale flavor.

Cube steak. The same condition was observed in all samples of cube steak as described for ground beef. It was interesting to note that in each case the 10° F. (—12.2° C.) sample was juicier and more tender than those held at 0° F. (—17.8° C.). At the end of 12 months all samples were discarded as they had become stale and flat in flavor.

Sausage. At the end of one month's storage the glutamate-treated samples at both temperatures were preferred over the control sample. At the end of 2 months' storage the treated sample at 0° F. (—17.8° C.) storage was preferred over the 10° F. (—12.2° C.) sample and the control sample. The control sample was lacking in color and was slightly tough. At the end of 3 months' storage the 10° F. (—12.2° C.) treated sample was superior in flavor to the 0° F. (—17.8° C.) treated sample, while the control samples possessed a flat flavor and were not as acceptable as the treated samples. At the end of 5 months the 0° F. (—17.8° C.) treated sample was preferred over the 10° F. treated sample while the 10° F. (—12.2° C.) storage control sample was slightly more rancid than the 0° F. (—17.8° C.) control sample. At the end of 7 months of storage the 10° F. (—12.2° C.) treated sample was more tender than the 0° F. (—17.8° C.) treated sample. The control samples at the end of this time had lost all their fresh natural color and were rancid in flavor. The treated samples too had started to fade in color but the fading was not as pronounced as in the control samples. At the end of 9 months the 0° F. (—17.8° C.) treated sample was still edible, not rancid, but not the good sweet flavor found in previous tests. The treated sample stored 10° F. (—12.2° C.) was slightly rancid in flavor. The control samples were definitely rancid in flavor and the color in all samples was definitely faded and gray. At this time all samples were discarded as being no longer useful for further testing.

SERIES III: POULTRY

Procedures

Preparation of samples. *Fowl.* Freshly dressed eviscerated fowl, about 4 pounds each, were treated with monosodium glutamate by 3 different methods before freezing as follows: while still somewhat warm, 11 of the fowl were stitched with an aqueous 5% solution of monosodium glutamate, each bird receiving 5% of its weight of the solution, using a recirculating stitching pump to force the solution through the stitching needle and into the tissues. The monosodium glutamate solution was injected into each leg, each thigh and on each side of the breast, each carcass receiving six injections. If this stitching did not increase the weight by 5%, additional stitching was done to bring the weight to the desired level. Eleven fowl were similarly stitched with a 5% solution of monosodium glutamate in homogenized milk. Eleven fowl were chilled overnight with crushed ice, then dusted in the visceral cavity, using a salt shaker, with monosodium glutamate up to 0.25% of the weight of the bird. Eleven fowl of the same lot and which had been chilled overnight were not treated but used as control samples.

The dusted fowl and the control samples were disjointed immediately and packaged in Waldorf type cellophane-lined waxed cartons containing a cellophane window on the top face of the carton. The cartons were not overwrapped. The fowl that had

been stitched with the milk and aqueous solutions of monosodium glutamate were chilled overnight with crushed ice and then disjointed and packed the same as the dusted and control samples.

The fowl samples were then frozen and stored at 0° F. (—17.8°C.).

Broilers. Freshly killed and eviscerated broilers were treated with monosodium glutamate by the same 3 methods before freezing as in the case of the fowl. Eight were stitched with a 5% aqueous solution of monosodium glutamate up to 5% of their weight. Eight others were stitched with homogenized milk containing 5% monosodium glutamate up to 5% of their weight. Seven others were chilled overnight with crushed ice. Next morning monosodium glutamate was dusted into the visceral cavity of each chicken using a salt shaker, each one receiving 0.25% of its weight in monosodium glutamate. Seven other chickens from the same lot were retained for control samples and received no treatment.

All samples were wrapped in moisture vapor proof cellophane, placed in stockingettes, frozen and stored at 0° F. (—17.8° C.).

Turkey. Eight turkeys, weighing approximately 22 pounds each after dressing, were eviscerated and cut in two by sawing down the backbone. The halves were numbered and marked R (right) and L (left). While still somewhat warm, the right sides were stitched with a 5% solution of monosodium glutamate in homogenized milk until the weight of the half turkey had increased 5%. The turkeys were then chilled by packing in crushed ice overnight after which the 2 halves of each turkey were placed back together and the whole bird inserted in a "Cryovak" bag which was evacuated, shrunk and sealed, frozen and placed in a fiberboard container. The turkeys were then stored at 0° F. (—17.8° C.).

Testing. All samples of fowl, broilers and turkeys were allowed to thaw completely at room temperature before cooking. The samples of defrosted fowl were stewed separately. The broilers were brushed with a vegetable shortening and broiled in an electric oven until brown and, when pierced with a fork, no juices ran. The turkey halves were rubbed with salt, wrapped in aluminum foil and baked (roasted) in a 350° F. preheated oven, allowing 25 minutes to the pound. During the last half hour of baking, the aluminum covering was removed to allow the turkey to brown.

Coded samples of all of the poultry were examined every 2 months by a trained taste panel with the results reported in Table 3.

RESULTS AND DISCUSSION

Fowl. At the end of second month fowl stitched with milk containing glutamate was perferred as to color, odor, flavor and texture over all other samples, with the water-glutamate solution stitched sample ranking second. The control ranked last but had a very good flavor. The samples ranked in the same order during the first 9 months' storage, but at the end of 11 months a slight rancidity and loss of full flavor had occurred in the milk stitched fowl. At this point the water stitched sample, while it showed some loss of fresh flavor, was superior to both the milk stitched and dusted samples. All glutamate-treated samples were preferred to the control. At the end of the 12-month storage period, the control sample was slightly stale in flavor but without rancidity, whereas rancidity occurred in both the dusted and milk stitched samples. At this point the water stitched sample was very much superior in flavor to all other samples and no rancidity was evident.

Broilers. Throughout 11 months of storage at 0° F. (—17.8° C.) the samples ranked as follows, the milk-glutamate solution stitched samples having a superior flavor, sweetness and tenderness:

TABLE 3

Comparison of controls with glutamate treated poultry samples held in frozen storage at 0° F. (—17.8° C.)

Months storage time	Control	Glutamate stitched milk soln.	Glutamate dusted in cavity	Glutamate stitched water soln.
		Fowl		
1	Very Good poorest	Excellent preferred	Excellent 3rd choice	Excellent 2nd choice
9	Very Good poorest	Excellent preferred	Excellent 3rd choice	Excellent 2nd choice
11	Fair flavorless	Fair 2nd choice	Fair 2nd choice	Fair preferred
12	Poor stale	Poor sl rancid	Poor sl. rancid	Fair no rancidity
		Broilers		
1	Very Good poorest	Excellent preferred	Excellent 3rd choice	Excellent 2nd choice
11	Good poorest	Good preferred	Good 3rd choice	Good 2nd choice
12	Fair poorest	Fair 2nd choice	Fair 3rd choice	Fair preferred
		Turkeys		
1	Very Good	Excellent superior in flavor, juiciness and tenderness		
5	Very Good	Excellent superior in flavor, juiciness and tenderness		
6	Very Good	Very Good less difference		
9	Good	Good little if any difference in preference		

No. 1—Stitched with milk containing monosodium glutamate.

No. 2—Sitched with water containing monosodium glutamate.

No. 3—Dusted in visceral cavity with monosodium glutamate.

No. 4—Control (no treatment).

At the end of 12 months the milk stitched sample had lost some of its flavor and became second choice; however, no rancidity was evident in any of the samples. The ranking at this point was:

No. 1—Stitched with water containing monosodium glutamate.

No. 2—Stitched with milk containing monosodium glutamate.

No. 3—Dusted in visceral cavity with monosodium glutamate.

No. 4—Control (no treatment).

Turkeys. During the first 5 months of storage at 0° F. (—17.8° C.) the sample stitched with milk containing glutamate was superior in flavor, juiciness and tenderness to the control or untreated side. At 6 months it was noted that the distinct difference noted in the previous tests had begun to disappear, but the milk-stitched sample was still preferred. At the end of 11 months, while no rancidity was evident, the difference between the control and treated side had disappeared and no preference could be noted.

SERIES IV: VEGETABLES

Procedures

Preparation of samples. *Asparagus.* Native Connecticut asparagus was cut to size and steam blanched for 3 minutes, chilled in running cold water, drained and packaged in 12-oz. folding cartons, then overwrapped with cellophane. Approximately 6 oz. of asparagus spears were placed in the carton, then sprinkled with 5 ml. of a monosodium glutamate solution; the remaining 6 ounces were placed with the spears running in the opposite direction and sprayed with another 5 ml. of the above solution. The center cuts and open spears were cut in desired sizes, steam blanched, weighed, and sprinkled as above with the glutamate solution, then wrapped as above. Control samples were packaged for comparison in testing without treatment. All samples were then frozen and stored at 0° F. (—17.8° C.).

Peas. The blanched peas were weighed up in 5-pound lots, dusted with 0.15% of their weight with monosodium glutamate. They were well stirred and mixed so that the glutamate would be well distributed throughout the peas. They were then packaged in 10-oz. cartons and overwrapped and frozen. The control samples were given no treatment. All vegetable samples were stored at 0° F. (—17.8° C.) only.

Whole kernel corn. Freshly blanched yellow kerneled corn, cut from the cob, was used for this experiment. The monosodium glutamate was dusted on the wet corn. Fifty-ounce batches were weighed up and dusted with 0.15% of their weight with glutamate. The glutamate was well stirred in and then packaged in 10 oz. Marathon cartons, then overwrapped, frozen and stored at 0° F. (—17.8° C.).

Frozen baby lima beans. The blanched limas were weighed up into 50-oz. lots and dusted with 0.15% of their weight in glutamate. They were then well stirred to distribute the glutamate evenly. The treated limas were then packaged in 10-oz. Marathon cartons and then overwrapped. The samples were then frozen and stored at 0° F. (—17.8° C.).

Succotash. The succotash was processed and packaged by the same procedure used in the freezing of baby lima beans. The pack was made up of 60% baby limas and 40% corn. It was also stored in the same manner.

Fordhook lima beans. The blanched Fordhooks were treated in the same manner as were the baby lima beans.

French cut green beans. The blanched frenched beans were weighed up in 50-oz. lots, dusted with 0.15% of their weight in monosodium glutamate. They were then carefully stirred so as to distribute the glutamate evenly. The treated frenched beans were then packaged in folding Marathon cartons, labeled, overwrapped, frozen and stored at 0° F.

Cut wax beans. The same procedure was used in the packing of the wax beans as was described for frozen frenched green beans.

Cut green beans. The same procedure was employed in the freezing of green beans as that used in freezing the frenched green beans and the cut wax beans.

Leaf spinach. The spinach was treated with monosodium glutamate by spraying 70-oz. lots of blanched spinach with 10 strokes of a 50-gram monosodium glutamate to 1000 ml. water solution. By careful calculation this was found to be comparable to 0.15% dry glutamate used in other vegetable experiments. The blanched spinach was treated first on one side, then turned and treated on the other. The spinach was then packaged in 12 oz. Marathon cartons, overwrapped, frozen and stored at 0° F. (—17.8° C.).

Chopped spinach. The same method was used in packaging the chopped spinach as that used in packaging leaf spinach.

Cauliflower. The cauliflower was treated by spraying it with 10 shots of a 50 g. of monosodium glutamate to 1000 ml. water solution per 70 g. of blanched cauliflower. This is comparable to the 0.15% dry glutamate used in other vegetable experiments. The cauliflower was then packaged in Marathon cartons, overwrapped, frozen and stored at 0° F. (—17.8° C.).

Broccoli. The same method of treating, packaging, freezing and testing was used in freezing broccoli as was used in the freezing of cauliflower.

Brussels sprouts. The same method of processing the blanched brussels sprouts was used as that employed in the freezing of cauliflower and broccoli.

Squash. Freshly prepared Boston Marrow Squash was taken from the processing line, was weighed up in 70-oz. lots and thoroughly mixed with 2.5 g. of monosodium glutamate (0.5 g. per 14 oz. of squash), packaged 14 oz. to the package, in Marathon cartons, overwrapped, frozen and stored at 0° F. (—17.8° C.).

Testing. All vegetable samples when tested were cooked in accordance with accepted directions for each product. For example, in general the package of frozen vegetable was dropped into one-half cup of boiling water containing one-half teaspoon of salt and boiling continued for the length of time required for the vegetable being tested. Immediately after cooking the vegetables were drained and tasted without any additional seasoning. In the case of squash the frozen block of squash was placed in the top of a double boiler and heated over boiling water until thoroughly thawed and ready for tasting. One-half teaspoon of salt was added to each package after thawing.

RESULTS AND DISCUSSION

The flavor of all glutamate-treated vegetables was preferred to the control samples. The preference noted at the first month examination has carried through the 10 months the project has been running with the difference more apparent as the storage period progressed. The glutamate seems to produce a sweet, rounded flavor in the vegetables that is not found in the control samples. Glutamate-treated frozen peas were superior in color and flavor to the control samples at the end of 10 months' storage. The treated samples also appeared more tender. In the treated green vegetables—such as broccoli, peas, green beans and spinach—the green color was more brilliant than it was in the control samples. This color was retained during the 10-month storage period and through subsequent cooking. The color and flavor of treated asparagus was also superior to the control samples. In the case of yellow vegetables, the flavor was enhanced and improved.

SUMMARY AND CONCLUSIONS

Addition of monosodium glutamate to many foods prior to packaging and freezing greatly improves their flavor and acceptability which, in general, becomes more evident during frozen storage. In many instances color also is improved by addition of glutamate.

The pink color of fresh frozen salmon seems to be retained by the use of monosodium glutamate, whereas without such a treatment the pink color is bleached out after a very short time in freezing storage.

Frozen green peeled shrimp treated with glutamate have a brighter color, improved sweeter flavor, and are more tender after cooking than when glutamate is not used.

Other fish such as mackerel, haddock and rosefish treated with monosodium glutamate have a finer flavor for a longer period in frozen storage than the same varieties where glutamate is not used.

All poultry has an improved flavor, is juicier and of better texture where glutamate has been used in their preparation for freezing storage.

Beef, pork sausage, hams and bacon have been much improved in flavor and texture by the use of monosodium glutamate before freezing. These meats as well as fish and poultry have retained their original high quality for longer periods in freezing storage than when glutamate is not used.

Monosodium glutamate added to vegetables before freezing effects a full rounded-out sweet flavor and seems to minimize the raw flavor usually encountered. Color also is improved by the use of glutamate.

The use of monosodium glutamate in frozen foods as described has seemingly prolonged the period of high quality during storage at low temperatures and has demonstrated, organoleptically, that glutamate has some properties of an antioxidant. This point was demonstrated particularly in its effect on hams, bacon, sausage and fatty fish, such as salmon and mackerel.

LITERATURE CITED

1. Buchanan, Ben F. Monosodium glutamate in food processing. *Food Packer*, 29, 10 (1948).
2. *Monosodium Glutamate: A Symposium.* The Quartermaster Food & Container Institute for the Armed Forces, and Associates, Food & Container Institute, Inc. (1948).
3. Isker, Rohland A. Notes on the use and effects of monosodium glutamate. *J. Am. Diet. Assoc.*, 25, 9 (1949).
4. Logan, Paul. Test of monosodium glutamate. *News Bulletin* (National Restaurant Association) 30, 6 (1949).
5. Melnick, Daniel. Science on the march. *Sci. Monthly*, 70, 3 (1950).
6. Sjostrom, L. B., Crocker, E. C. The role of monosodium glutamate in the seasoning of certain vegetables. *Food Technol.*, 2, 4 (1948).
7. Peret, Helen. Taste test panels. *Nat. Provisioner*, 121, 10 (1949).
8. Shuey, G. A., Eichenberger, W. R. Monosodium glutamate sharpens flavor of quick-frozen foods. *Quick Frozen Foods*, 13, 12 (1951).

MONOSODIUM GLUTAMATE

By.

HARUOMI OEDA

(AJINOMOTO CO., INC.)

REPRINT

From

KIRK-OTHMER

ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY

SECOND EDITION VOLUME TWO

1963

MONOSODIUM GLUTAMATE

Monosodium glutamate (MSG), $C_5H_8NO_4Na \cdot H_2O$, the monosodium salt of the naturally occurring L form of glutamic acid, has been used the world over for more than fifty years as a seasoning to enhance the natural flavor of the foodstuff to which it is added.

Monosodium glutamate can be tasted at an aqueous dilution of 1:3000 (about 30 mg/dl) and thus is far more sapid than table salt which has a minimal taste dilution of 1:500 (200 mg/dl). The optimum salt concentration in soup is around 1.0 g/dl with 0.8 g/dl giving a flat taste and 1.2 g/dl too salty. This narrow range of concentration is not true for MSG. A minimum concentration of MSG is 0.1 g/dl which is one tenth of the standard concentration of table salt. Although MSG is usually used at 0.2–0.3 g/dl, it is not too concentrated even at 0.5 g/dl. Such a wide range of concentration is a characteristic feature which is not found with other seasonings.

The taste of MSG, however, defies accurate description, being entirely different from that of any of the well-known essential taste sensations, such as the sweetness of sugar, the salinity of table salt, the pungency of mustard, or the bitterness of quinine. Together with succinic acid and inosinic acid, MSG represents another palatable savor. MSG also has the ability of enhancing natural taste. For instance, it is added to Japanese synthesized sake (wine), in concentrations of about 20 mg/dl with effective improvement of the quality of the sake.

The use of MSG as a seasoning was discovered in 1908 by Kikunae Ikeda, professor of physical chemistry with the Faculty of Science of the Imperial University of Tokyo (1). Ikeda found that the meatlike flavor imparted to soup stocks made from the seaweed *Laminaria japonica* was due to its content of glutamic acid. Although glutamic acid has been studied chemically since its discovery in 1866, no one was aware of its taste prior to Ikeda. Cohnheim stated in 1911 in his book (2) that "die Glutaminsäure schmeckt nicht süss, sondern fade und nur schwach sauer."

In addition to glutamic acid, which Ikeda had earlier isolated from *Laminaria*, his associate, Kodama, isolated 5′-inosinic acid from dried bonito meat and concluded that the flavor from the bonito was due to both glutamic acid and inosinic acid (3). In contrast to MSG, the mass production of inosinic acid was not considered, but its extraction from dried sardine (another raw material for soup stocks in Japan) has been tried on a small industrial scale. Recently, however, a process using nucleic acid was developed to produce inosinic acid. It was also found by Sakaguchi that 5′-inosinic acid and 5′-guanilic acid have a strong synergistic effect with MSG (3a). These findings have stimulated interest in this field and a new MSG seasoning, which gives a meaty flavor, in which a certain amount of sodium 5′-inosinate (or its mixture with 5′-guanylate) is coated on MSG was placed on the Japanese market by three companies. Yamasa Brewery and Takeda Pharmaceutical produce these nucleotides from yeast nucleic acid by enzymic cleavage and Ajinomoto produces the 5′-inosinate by chemical phosphorylation of inosine which is manufactured by fermentation.

Properties

The monohydrate of MSG (molecular weight, 187.13) occurs in colorless crystals. Crystallized as rhombic prisms from warm aqueous solutions, monosodium L-glutamate monohydrate conforms to the following unit cell (4):

$$a:b:c = 5.54\ A:17.86\ A:15.30\ A$$

The solubility of L-MSG in aqueous solution is given by the following (5):

$$S = 35.30 + 0.098t + 0.0012t^2$$

where S is the percentage of anhydrous MSG dissolved in the saturated solution at t°C. (Solid phase is MSG monohydrate.)

The specific rotations, $[\alpha]_D^{20}$ and $[\alpha]_{5461\,A}^{25}$, of pure monosodium L-glutamate monohydrate, indicated in Table 1, are affected by temperature and the concentrations of the solvent, the glutamate (glutamic acid) ion itself, as well as any salts present. Therefore, the optical purity of a sample should not simply be compared with a measured specific rotation of any reported value, which may not have been measured under the same conditions. For the precise determination of optical purity, the specific rotation of a sample must be compared with that of an authentic sample under strictly comparable measuring conditions.

Table 1. Specific Rotation of L-MSG Monohydrate

	$[\alpha]_D^{20}$	$[\alpha]_{5461\,A}^{25}$ [b]
wavelength, A	5891[a]	5461
temperature, °C	20[a]	25
concentration of MSG, g/100 ml	10[a]	15
solvent	2N HCl[a]	2.3N HCl[c]
specific rotation, degrees	+25.16	+30.12

[a] Measuring conditions conform to the Japanese Industrial Standards.
[b] See ref. 6.
[c] 0.72M excess HCl beyond that required to convert MSG to glutamic acid hydrochloride.

Manufacture

The various current methods of MSG production all funnel through the isolation of optically active L-glutamic acid. The classical method involved separating glutamic acid from natural sources, either from proteins which contain glutamic acid as peptides (vegetable proteins were adopted as raw materials because of their high content of glutamic acid and their low cost), or from beets which contain glutamine, the monoamide of glutamic acid ($NH_2OCCH_2CH_2CH(NH_2)COOH$). During processing in a sugar refinery, glutamine converts into pyroglutamic acid with ring closure. Thus in beet molasses, as well as in vinasse (see under Raw materials), over 90% of total glutamic acid exists in this form, and the remainder is glutamic acid with no detectable glutamine left.

After World War II, the demand for MSG was stimulated all over the world, and Japanese manufacturers were barely able to secure enough materials to meet the increased demand, since the above mentioned raw materials had to be procured overseas. To avoid these difficulties two new processes were developed in Japan.

Since 1959, fermentation processes have been employed in Japan, and three companies, Ajinomoto Co., Kyowa Fermentation Industry Co., and Asahi Chemical Industry Co., are operating on this basis. In the U.S., Merck & Co. and Chas. Pfizer also manufacture MSG by this method and a few more companies plan to go into such production soon, while in Formosa this process has already begun.

A completely synthetic process, using acrylonitrile as a starting material, was developed and put into production by the Ajinomoto Co. in Japan.

EXTRACTION PROCESS FROM NATURAL SOURCES

Raw Materials. At first, wheat gluten from flour was used as a raw material for the industrial manufacture of MSG. Defatted soybean was used subsequently, and was followed by other materials cited in Table 2. Since few vegetable proteins exist naturally in an almost pure state, it is necessary to select suitable vegetable seeds as the primary raw materials and to extract proteins from them in as high purity as possible, usually giving proteins of 50 to 75% purity as secondary raw materials. In Table 2 the glutamic acid contents in such proteins are cited, and typical processes employed to obtain these secondary raw materials are mentioned.

Table 2. Natural Raw Materials Used in MSG Manufacture

Primary raw material	Typical treatment	By-product	Secondary raw material	Nitrogen content, %	Glutamic acid content, %
flour	Martin method	starch	wheat gluten	12	25
corn	wet method	corn starch	corn gluten	13	20
soy bean	extraction with hexane	soybean oil	defatted soy bean	8	9
peanut	pressing	peanut oil	peanut meal	10	12
beet molasses	Steffen's method	beet sugar	concentrated Steffen's waste		5–12 (wet basis)[a]
beet molasses	alcohol fermentation	alcohol	concentrated vinasse		10–13 (wet basis)[a]

[a] Contains 30% water.

Secondary raw materials from beet molasses are prepared by two methods. In the U.S., Steffen's waste is used for the raw material; it is derived by the recovery of sugar from beet molasses by the addition of lime. The use of barium oxide instead of lime to recover the sugar is also known, and the waste derived from the barytation method may also be used as raw material for the production of MSG if racemization due to the alkali treatment at high temperature is carefully prevented in a suitable way.

In France, on the other hand, industry produces alcohol from beet molasses by fermentation. The distillation residue from this fermentation product is called vinasse, and is used as raw material for MSG. In Italy, both Steffen's and fermentation methods are applied in processing beet molasses as a source of MSG. However, since the glutamine content in sugar beets varies greatly with the cultivating region as well with the species of beet, not all of the beet waste is favorable as raw material for MSG. Hence, the sugar beets of countries north of Germany cannot be used for MSG manufacture because of their low content of glutamine.

Hydrolysis. Acids, alkalies, or enzymes can be used for the hydrolysis of vegetable proteins, but only acids are used in the MSG industry because alkalies cause racemization of amino acids and complete hydrolysis cannot be expected with enzymes. At present hydrochloric is the acid of choice for this purpose in Japan. In the case of beet sugar wastes, most of the glutamine exists in the form of pyroglutamic acid, as mentioned above; alkalies are generally used in the U.S. and Europe as the hydrolyzing agent, but acid is used in Japan to hydrolyze concentrated vinasse imported from France (see under Dehydration reaction of glutamic acid).

When glutamic acid is extracted from the hydrolyzate of vegetable proteins, a mother liquor containing many other amino acids is left as a by-product. In Japan, after adjusting the content of glutamic acid in the mother liquor from soybeans, this solution is supplied as a blending material to soy sauce makers all over the country. One third of the total nitrogen in Japanese-made soy sauce is supplied in this way. The mother liquor from hydrolyzed proteins is also utilized in the U.S. and Europe, where it is sold as hydrolyzed vegetable protein.

In the case of beet materials no comparable utilization of extraction by-products can be expected, as few amino acids are left. The betaine in the residue is utilized in the manufacture of animal feedstuffs in the U.S., while in Japan, the residue is used as fertilizer.

The concentration of hydrochloric acid is maintained at 20% during the hydrolysis of protein, taking into account the water content of the raw material used. The equivalent ratio of hydrochloric acid to total nitrogen of raw material (HCl/N) is best kept at greater than 1.5. Closed tanks with rubber or glass lining are suitable for the reactor. The hydrolysis is completed in about 24 hr at a temperature of 110°C and a pressure of about 1 atm. The hydrolyzate is then cooled under reduced pressure and filtered to separate decomposition products (humus). The filtrate is concentrated in a vacuum evaporator to obtain a concentrate with 50% of solid matter. This concentration may be varied according to the content of glutamic acid as well as the impurities therein. If necessary, hydrochloric acid may be added to raise the concentration of the acid.

Crystallization of Glutamic Acid Hydrochloride. The concentrate is then cooled to crystallize out the hydrochloride. The solubility of glutamic acid at various temperatures and concentrations of hydrochloric acid is shown in Table 3 (7). The time required for crystallization depends on the extent of supersaturation, which is related to impurity, cooling temperature, and stirring speed. It is desirable, if possible, to crystallize out easily separable crystals.

Table 3. Solubility of Glutamic Acid Hydrochloride

Temp, °C (±0.5°)	Glutamic acid hydrochloride, g/(100 ml 20% HCl solution)	Glutamic acid hydrochloride, g/(100 ml 30% HCl solution)
3	1.15	0.38
7	1.31	0.44
10	1.48	0.47
25	2.60	1.16
35	5.29	1.19
50	8.42	2.68

The hydrochloride crystals are separated from the mother liquor by filter press or centrifuge. Pure glutamic acid content in the wet crystals thus obtained is about 30%.

Of the 17 or 18 amino acids derived from vegetable proteins in this process, only glutamic acid is practically insoluble as the hydrochloride in concentrated hydrochloric acid so that it is possible to separate glutamic acid easily from all other amino acids. Although there is another way to obtain glutamic acid directly from the hydrolyzate without precipitating hydrochloride (tyrosine and leucine come out at pH 4–5, followed

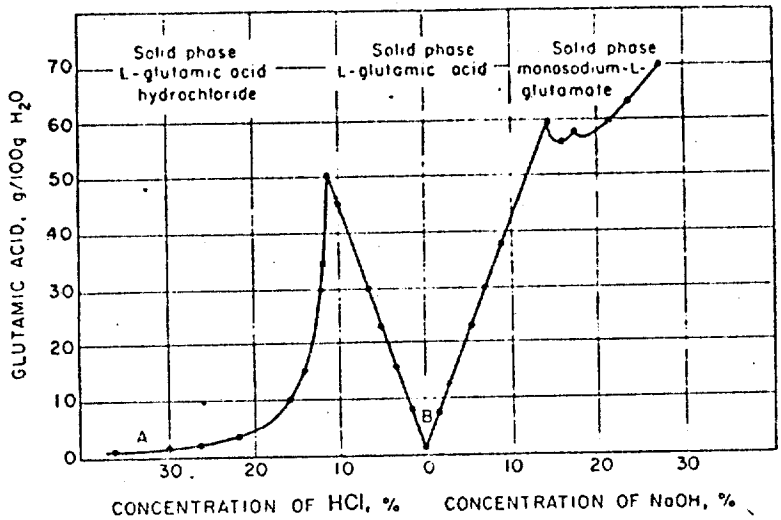


Fig. 1. Solubility behavior of glutamic acid in the presence of acid or alkali of different concentrations at 35°C.

by precipitating out glutamic acid at its isoelectric point, 3.2) it is not applied generally because of the low yield of glutamic acid.

When beet sugar wastes are used as starting materials, almost the same procedure can be applied to obtain glutamic acid hydrochloride by acid hydrolysis. Special devices must be employed, however, to separate glutamic acid hydrochloride from betaine hydrochloride and potassium chloride since they are crystallized out simultaneously.

Preparation of Crude Glutamic Acid. The crude hydrochloride is dissolved in warm water and filtered to remove insoluble matter. The pH of the filtrate is raised to 3.2 by the cautious addition of sodium hydroxide or ammonia in order to crystallize

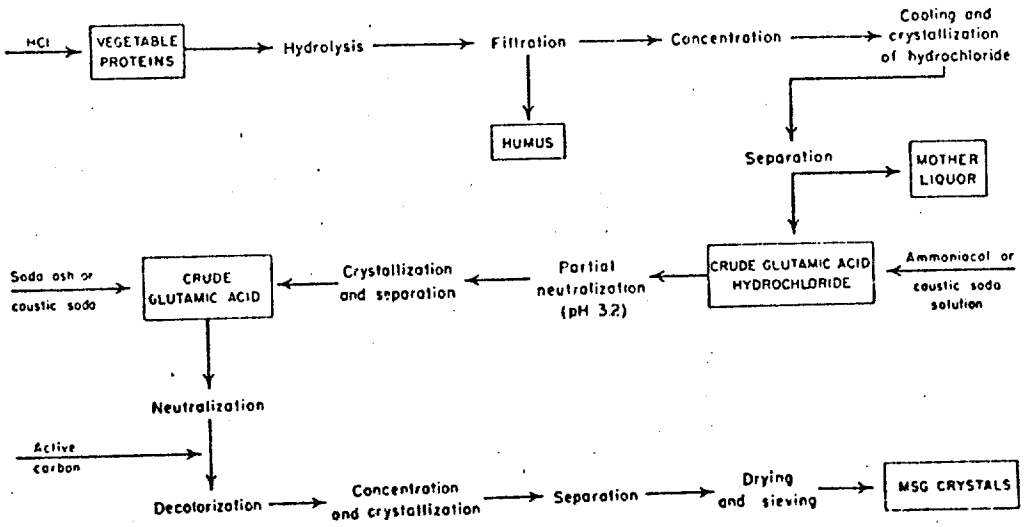


Fig. 2. Flowsheet for the extraction process.

out the glutamic acid. The time of crystallization is shorter than that required for the hydrochloride. The concentrated slurry precipitated is then centrifuged; the purity of the wet crystals is about 85%.

The crystallizations of glutamic acid hydrochloride, glutamic acid, and its monosodium salt are based on their solubility behavior in acidic and neutral solutions. In Figure 1, point A indicates the separation point for the hydrochloride, and point B shows the separation point for glutamic acid (5). The two compounds have their minimum solubilities at these points. Figure 2 illustrates the complete industrial process for the production of MSG from vegetable proteins.

FERMENTATION PROCESS

In 1956 Kyowa Fermentation Industry Co., Ltd., announced their success in producing glutamic acid directly from glucose by fermentation, and this method has quickly developed in Japan in only a few years to compete with the extraction process. In 1957 Kinoshita et al. of Kyowa Fermentation patented the fermentation conditions and mechanisms of formation of glutamic acid by *Micrococcus glutamicus* (8); Tada et al. of Tamagawa College had been studying the same problem independently (9). Papers have since been published by many investigators, but these are chiefly biochemical or microbiological studies; few chemical engineering or mechanical research studies have been published to date. Outlines of various investigations may be found in back issues of the *Symposia on Amino Acid Fermentation*, which is published twice a year by the Association of Amino Acid Fermentation, Japan.

Microorganisms which produce glutamic acid directly from glucose are distributed widely in nature. Numerous university and company laboratories throughout the world are presently studying and isolating glutamic-acid-producing strains, either of natural origin or induced mutants. Also under investigation are procedures to stabilize bacterial activity and to fix fermentation conditions. Research on processes for the broth treatment as well as the crystallization process of MSG are in progress. Figure 3 shows the manufacturing steps used in the fermentation process.

Raw Materials. The main raw materials required in the fermentation process are a carbon source to produce the carbon frame of glutamic acid and a nitrogen source for the amino group. Inorganic salts and growth factors for the nutrition of the microorganism as well as neutralizing reagents and a defoamer are needed as auxiliary materials.

Glucose preparations produced by acid or enzymic hydrolysis of starches from sweet potato, potato, wheat, tapioca, or corn are generally used as a carbon source, although starch hydrolyzates or commercial crystalline glucose can also be used. "Hydrol," a by-product of crystalline glucose manufacturing, can replace glucose if its quality is satisfactory; even crude sugar or molasses obtained from cane and beet may be as good a starting material as pure glucose, if the required purification is carried out. Wood saccharification products may become a new source of raw material in the future.

Urea, liquid ammonia, and aqueous ammonia are excellent nitrogen sources, but generally, inorganic ammonium salts, such as ammonium chloride and ammonium sulfate, are used. Potassium phosphate and magnesium, manganese, and ferric sulfates are also necessary as inorganic salts; a total concentration of 0.2 % is adequate as nutritional factors. Selection of the growth factors and the quantities to be added is very important, because these variables affect the fermentation yield to a great

degree. Natural substances such as corn steep liquor, yeast extract, peptone, casein hydrolyzate, koji extract (saccharified rice malt), protein hydrolyzate, etc, are generally used, but it is advisable to use purified vitamins and amino acids instead. D-Biotin, desthiobiotin, 7,8-diaminopelargonic acid, and thiamine may be used as the vitamin. Plant hormones, such as 2,4-dichlorophenoxyacetic acid, indoleacetic acid, and gibberellin, may be substituted for biotin.

As a neutralizing agent, either urea or ammonia (gaseous or aqueous) is convenient because they also serve as nitrogen sources. Sodium hydroxide or calcium carbonate may be used, but for neutralization only. Vegetable oil or silicone oil is used as defoamer.

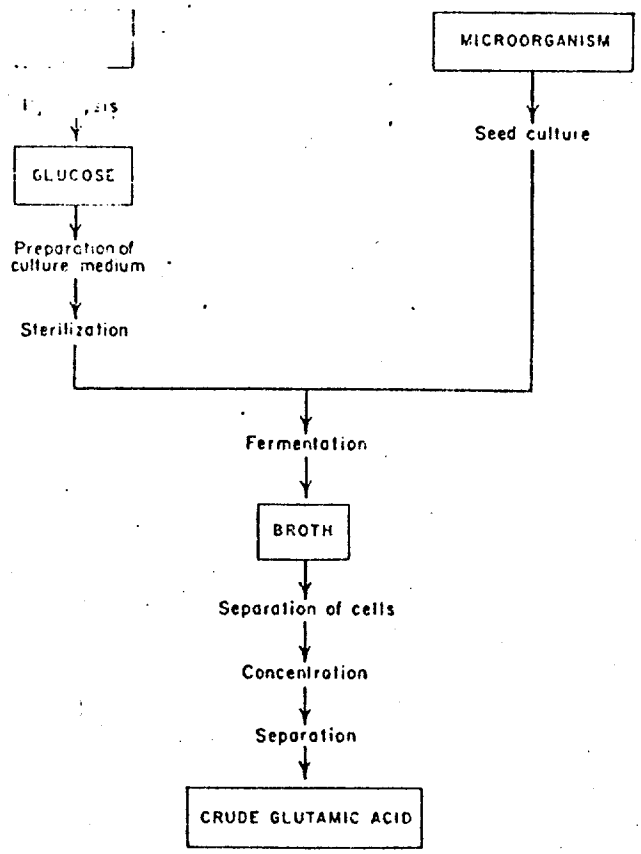


Fig. 3. Flowsheet for the fermentation process.

Microorganisms. Although yeasts, molds, and actinomyces have heretofore been reported to produce glutamic acid, only bacteria are known to produce glutamic acid in a yield greater than 40% (on glucose) with an initial concentration of more than 10% of glucose in the medium. Industrial strains used in Japan belong to the genera *Micrococcus*, *Brevibacterium*, *Microbacterium*, or *Corynebacterium*, i.e., bacteria which are Gram-positive, nonmotile, aerobic, and, except for *Micrococcus*, rod-shaped. In the presence of only a trace of suitable growth factors, these bacteria provide a maximum yield of glutamic acid, more than 50 g/liter.

Seed Culture and Fermentation. Seed culture and fermentation can be performed in the same equipment and under the same process controls as used for antibiotic fermentation. Seed for the main fermentation is also prepared by aerobic cultivation with stirring.

A number of essential points relating to process control are mentioned below.

(a) Careful selection of the components and of the concentration of components in culture medium suitable for the strain to be used is required for maximizing the yield.

(b) Strict sterilization of culture medium and fermenter is necessary. Exposure to steam at too high a temperature should be avoided, not only for the sake of heat economy but also to prevent the denaturation of the culture medium, which would increase the fermentation time and decrease the yield. It is desirable to use a sterile glass wool filter to eliminate contamination from the air, which should always be kept free from water droplets. An adequate rate of aeration must be chosen for the growth and the accumulation periods.

(c) The optimum fermentation temperature, which is usually between 30 and 37°C, must be maintained within the narrow range characteristic for the strain used; hence, a sensitive temperature controlling device is necessary.

(d) Operation standards should be strictly established in order to minimize idle time. The standards relating to the seed culture must be set up most carefully.

(e) Automatic controls of temperature as well as of liquid and gas flows are advisable.

(f) Care must be taken to prevent infection by bacteriophages. Keeping many good strains in reserve, adhering to strict sterilization, and being cautious not to leave any broth in tanks and pipes after fermentation are effective precautions.

Treatment of Broth. The finished fermentation solution, called "broth," contains a fairly high concentration of ammonium glutamate. After whole bacterial cells are removed by centrifuging, the broth is concentrated and hydrochloric acid is added. The mixture is heated to decompose the residual glucose and other materials and filtered. Glutamic acid hydrochloride is then crystallized from this filtrate. Glutamic acid can be obtained directly from the broth, however, since in the fermentation process it is unnecessary to crystallize glutamate as the hydrochloride in order to separate it from other amino acids (10).

SYNTHETIC PROCESS

Although several synthetic methods for glutamic acid were published before World War II, they could only be used on a laboratory scale because of unsatisfactory starting materials or reagents, and low reaction yield. Thus, no industrial production competitive to the extraction method could be established.

During World War II, however, a new synthetic method for preparing aldehydes (11), the oxo process (qv), was developed in Germany (12); at the same time, it was found that acrylic ester could also be synthesized easily from acetylene. These two discoveries provided the impetus to the synthesis of glutamic acid on an industrial scale (13). As with all synthetic processes, however, the product is inevitably a racemic mixture, in this case DL-glutamic acid, containing 50% of the tasteless D isomer. Therefore, it was impractical to manufacture MSG by synthesis for use as seasoning until optical resolution and racemization processes were established. In other words, the D isomer must be efficiently racemized into the DL form and then subjected to

optical resolution again so that all of the DL form can be converted into the biologically active L form. Asymmetric synthesis, which would produce only L or D isomers specifically, cannot yet be accomplished on an industrial scale.

At present, only Ajinomoto Co., Inc., manufactures MSG by the synthetic process. After ten years of research work and two years of pilot-plant operation, Ajinomoto put its commercial plant into production in the spring of 1963. The plant has a capacity of 350 tons of MSG per month.

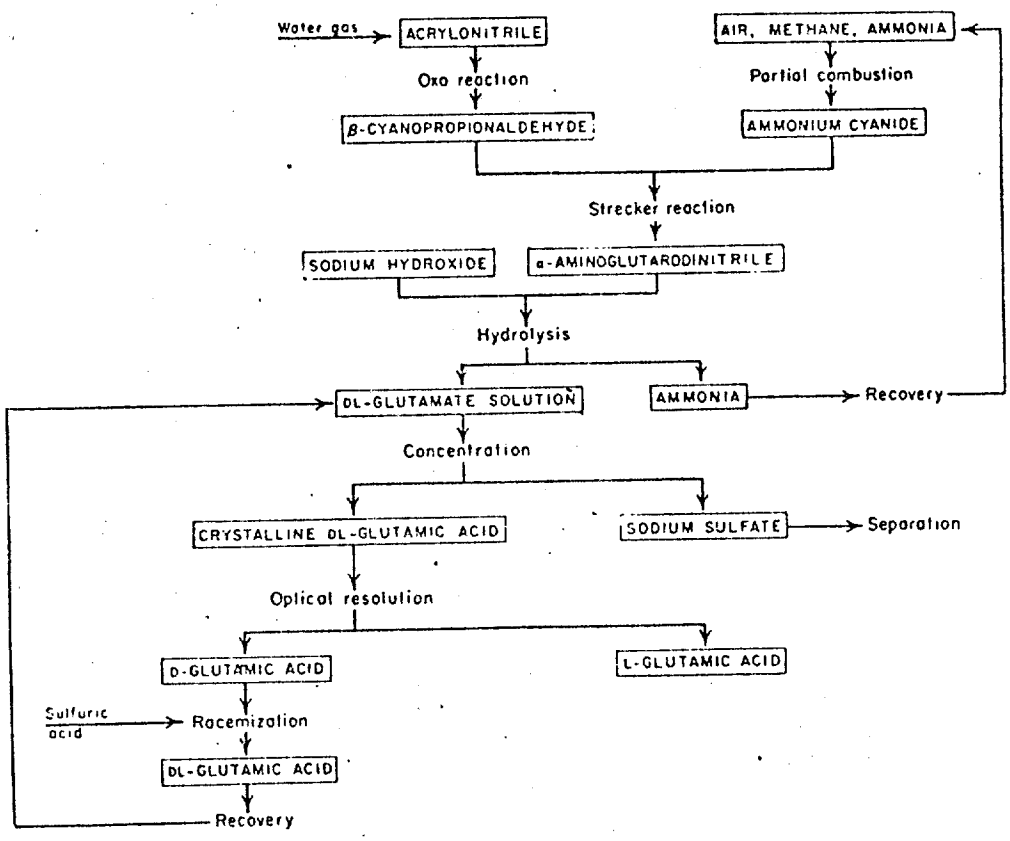


Fig. 4. Flowsheet for the synthetic process.

Figure 4 shows a simplified scheme for the synthetic process, including the optical resolution process. Acrylonitrile is used as a starting material. Processes using acrylate, furfural (14), or cyclopentadiene (15) as starting materials were abandoned as less favorable. In the present synthesis, the whole process consists of automatically controlled continuous flow, which reduces the labor requirement. The oxo reaction consists of the hydroformylation of olefins with hydrogen and carbon monoxide at high pressure and moderately high temperature, but the application of this reaction to acrylonitrile is new. It leads to β-cyanopropionaldehyde.

$$NCCH{=}CH_2 + CO + H_2 \rightarrow NCCH_2CH_2CHO$$

Characteristic features which had to be solved in this process were the choice of an efficient catalyst (from among cobalt compounds), its mode of recovery, and the treatment of β-cyanopropionaldehyde whose properties were little known.

In the Strecker reaction, ammonium cyanide, obtained by partial combustion of methane and ammonia (see Cyanides), is reacted with the β-cyanopropionaldehyde.

$$NCCH_2CH_2CHO + NH_4CN \rightarrow NCCH_2CH_2CH(NH_2)CN + H_2O$$

The process has been simplified in comparison with the usual procedure. Ammonia, utilized in the formation of ammonium cyanide, is recovered after hydrolysis of the dinitrile. Only a makeup for its inevitable loss during the operation is needed. It may be noted that the nitrogen for the amino group in the glutamic acid molecule actually comes from ammonium cyanide; the nitrogen in the acrylonitrile is returned as ammonia to make more ammonium cyanide.

Of the five carbon atoms of the glutamic acid molecule, three are supplied from acrylonitrile, one is derived from methane, and the last from carbon monoxide. One of the advantages of this process is that two carbon atoms are supplied by relatively cheap sources.

DL-Glutamic acid is crystallized from the alkaline solution, after neutralization with recycled glutamic acid solution containing an excess of sulfuric acid, at the isoelectric point of this amino acid (pH, 3.2).

Optical Resolution. The process for resolving the mixture of optical isomers of glutamic acid comprises dissolving the racemic glutamic acid at a definite concentration, and crystallizing out L- and D-glutamic acid alternately by seeding with the respective optically active isomers (16).

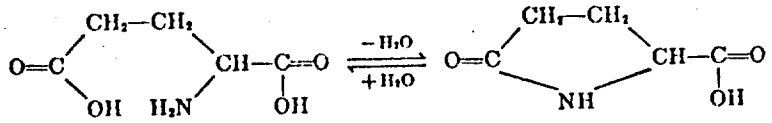
To achieve this process commercially in high yield as well as to obtain a highly uniform optical purity of both isomers, fundamental investigations on the crystal forms by means of infrared and x-ray analysis were required.

The D-glutamic acid must be then racemized by a treatment at high temperature and is subjected again to optical resolution. One disadvantage in the resolution process is the increase in bulk of the materials due to recycling. However, the optical resolution process which is now established has been perfected, so that none of the D isomer is contained in the L-glutamic acid product (see the following section).

CRYSTALLIZATION OF GLUTAMIC ACID

The process of isolating glutamic acid is a common step to all processes of MSG manufacture, be it extraction, fermentation, or the new synthetic process. In order to crystallize favorable MSG crystals, the solution must be fairly pure. In other words, glutamic acid, to be used in this process, must be also of high purity; contamination by other amino acids or inorganic salts must be kept to less than 5% on a dry weight basis. To obtain such pure glutamic acid crystals from various impure solutions, it is important to consider the dehydration reaction as well as the crystallographic problems of glutamic acid.

Dehydration Reaction of Glutamic Acid. Glutamic acid is converted by dehydration into pyroglutamic acid (pyrrolidonecarboxylic acid). This is a reversible reaction, the equilibrium point and rate of reaction of which vary with the change of pH as shown in Table 4 (17).



glutamic acid A — pyroglutamic acid B

Table 4. Dehydration Reaction of Glutamic Acid

pH	Amount in equilibrium	Rate of reaction
strongly acidic	A ≫ B	fast
neutral	A ≪ B	very slow
strongly alkaline	A ≫ B	fast

In order to convert pyroglutamic acid into glutamic acid it must be heated in strongly acidic or alkaline media but not in neutral solution. Hydrolysis is carried out under milder conditions than those for protein hydrolysis. Amino acids formed by alkaline hydrolysis of proteins under vigorous conditions are always converted into racemic forms, but optically active glutamic acid can be obtained by the alkaline hydrolysis of pyroglutamic acid if the hydrolytic conditions are suitably controlled. In the manufacture of MSG from beet sugar wastes in the U.S. and Europe, alkaline hydrolysis of pyroglutamic acid is carried out. If acid hydrolysis is employed with the same raw material, racemization, of course, does not occur.

Crystals of L-Glutamic Acid. Two crystalline forms are known from x-ray analysis for optically active glutamic acid. Their characteristics are as follows:

	a	b	c
orthorhombic α form (18)	7.09 A	10.30 A	8.75 A
orthorhombic β form (19)	5.17 A	17.34 A	6.95 A

A monoclinic form of glutamic acid has been reported but has not been confirmed (20).

In practical processing, two types of crystalline shapes are obtained when L-glutamic acid is crystallized under various conditions. One is a granular or rod-shaped crystal, and the other is a plate form. The former appears to be the α form mentioned above, while the latter is the β form.

The β-form crystals are stable and can be obtained easily under ordinary crystallization conditions, but α crystals are unstable and tend to transform into β especially at temperatures above 30°C. The α form is crystallized below 30°C, and the presence of certain other amino acids in the solution helps to prevent the transformation of α form into β (21).

Table 5 shows the differences in physical properties between α and β forms.

Table 5. Physical Properties of α and β Forms of L-Glutamic Acid (22)

L-Glutamic acid	Specific gravity	Solubility*
α form	1.532	$\log S = -0.377 + 0.0174t$ (0°C < t < 30°C)
		$\log S = -0.328 + 0.0153t$ (30°C < t < 70°C)
β form	1.580	$\log S = -0.467 + 0.0161t$ (0°C < t < 70°C)

* S is the weight, g, of L-glutamic acid dissolved in 100 g of water; t is temperature, °C.

The solubility given by Dalton et al. corresponds to that of the β form, while the α form has higher solubility (23).

The α-form crystals have the more desirable properties for practical purposes, because sedimentation of α-form crystals in suspension is fast and filtration velocity is high so that mother liquor adhering to the separated crystals can be easily removed. Moreover, the apparent density of the dry crystals is greater with the α form than with the β, and the fluidizability is also better.

The β form is crystallized out when a highly concentrated solution is treated at high temperature because of the greater inherent stability of this form. Under conditions where the α form can be easily crystallized out, crystallization of β form therewith must be prevented as far as possible. At any rate, the treatment necessary in crystallization of glutamic acid may be varied with the production methods, ie, whether the extraction, fermentation, or synthetic process is applied.

PREPARATION OF MSG FROM GLUTAMIC ACID

Preparation of MSG. Crude glutamic acid crystals containing about 10% water are neutralized with soda ash or caustic soda to give neutral MSG solution. This solution is decolorized with active carbon (although decolorization is not necessary in the case of the synthetic process). Active carbon is filtered off by vacuum or pressure filters. The decolorization is carried out until the solution becomes almost colorless. Multistage treatment is, of course, preferable in order to save the amount of active carbon.

The decolorized solution is then concentrated in a vacuum evaporator, holding the temperature to about 60°C. MSG is crystallized out when the solution reaches a certain supersaturation, and the crystals are separated from the mother liquor by centrifuging.

The production of MSG from crude glutamic acid is indicated in the lower part of Figure 2, p. 202.

Size of MSG Crystals. Although many crystalline sizes of MSG, to meet various requirements, can be prepared within a certain limit, MSG products on the market are usually of three or four sizes, ranging from 20 to 200 mesh. It is advisable to make a uniform crystal size of MSG by sieving, because size affects the rate of dissolution markedly. For example, standard MSG, "Regular Size," of the Ajinomoto Co. has a crystal size of 30 to 60 mesh and thus is comparable to table salt in its rate of dissolution. Moreover, as the crystal size is closely related to the specific volume of products, it is necessary to make a uniform size for uniform packaging.

Optical Isomers of MSG. MSG obtained by the extraction method is the L somer. Some microorganisms are known to have an enzyme, racemase, which converts L-amino acids into D isomers, though few glutamic-acid-producing bacteria have such a function. During processing in the factory, a part of L-MSG may be attacked by racemase, which is originated from miscellaneous microorganisms, so that the L-MSG produced might be contaminated with D isomer. For example, natto, a daily staple in Japan, a fermented product using *Bacillus subtilis* from cooked soybeans, contains about 2.5% of glutamic acid, of which 16% was found to be D isomer. Mizushima of Tokyo University isolated an enzyme (24) which oxidizes D-glutamic and D-aspartic acids specifically, and which was used to detect D-glutamic acid after aspartic acid had been separated completely from the sample. The reason for investigating the content of D isomer in MSG is that only the L isomer and not the D isomer is responsible for the taste qualities of MSG. In other words, contamination of D isomer diminishes its value as a seasoning. It has been shown by Hara of Tokyo

Medical College, however, that no differences between the L and D isomers can be detected in their pharmacological effects (25).

It is reported that several percent of glutamic acid may be racemized in the course of acid hydrolysis in the extraction process (26–28). In the synthetic process, there is a possibility that D isomer contaminates the L isomer if the optical resolution is not complete. All manufacturing processes may, therefore, involve some risk that the product may be contaminated with D isomer. However, in actual processing, complete removal of D isomer can be easily attained since the D isomer becomes absolutely insoluble by forming DL compound in saturated solutions of monosodium L-glutamate at any temperature. This step of elimination of the D isomer can be easily included in any process if the presence of the isomer is detected. The determination of the D isomer is carried out by the enzyme described above. Thus, no D isomer was detected in any of these products on the market; the lower limit of detection by this method for traces of D isomer is 0.1%.

As described below, determination of specific rotation is prescribed in the standards for MSG in order to check the optical purity of L-MSG. Optical rotation of the purest L-MSG is assumed to be $[\alpha]_D^{20} = +25.2°$ in 2*N* HCl. If a product contains any optically inactive substances such as water or sodium chloride, the specific rotation of the product decreases. Any other amino acids (including D-glutamic acid), which may contaminate the yield of L-MSG, have less specific rotation so that they tend to lower the specific rotation of the product.

All of the products of Ajinomoto Co. manufactured by the three different processes described above have a specific rotation of $[\alpha]_D^{20} = +25.2° \pm 0.1°$ in 2*N* HCl. Accordingly, they cannot be discriminated from each other by their specific rotations.

Analysis and Specification

In the U.S. there are federal specifications on MSG for the quality of monosodium L-glutamate, in which purity and maximum contents of water and sodium chloride are defined as shown in Table 6. In addition, the product must conform to safety test standards of the Federal Food, Drug and Cosmetic Act.

Table 6. Various Standards and Analytical Results of MSG Monohydrate

	Water, %	Sodium chloride, %	Total nitrogen, %	Specific rotation, degrees $[\alpha]_D^{20}$ (in 2*N* HCl)
pure MSG			7.49 (theoretical)	
U.S. Standards	0.25	0.4	>99% of theoretical	[c]
Japanese Standards[a]	a	b	7.41–7.56	24.8–25.3
	[100 − (a + b)] > 99.5			
analytical results in Japan[b]	0.01–0.06	0.01–0.03	7.47–7.49	25.15–25.24

[a] According to the 1963 revision of Japanese Standards.

[b] More than three fourths of the export products have the analytical values in the ranges cited.

[c] Although the U.S. Standards do not give a value for specific rotation, $[\alpha]_D^{23}$ (in 1*N* HCl) = 24.2–25.5° is generally agreed upon by U.S. manufacturers.

In Japan there are the (Japanese) Export Inspection Standards as cited in Table 6. In addition there are two other standards for L-MSG, viz. Standards of Food Additives and the Pharmacopeia which are nearly the same as the Japanese Exp : Insp ction Standards except for the additional regulations for arsenic (less than 2 ppm) and for heavy metals (less than 20 ppm as Pb).

Analyses of MSG for export in Japan are carried out by the Japanese Association of MSG Manufacturers. Their analytical results show that the products not only meet the above standards but also go beyond them, as cited in Table 6.

Economic Aspects

Immediately after the discovery that MSG was a flavorful seasoning, industrial production was begun by Suzuki & Co. Ltd. (the former name of the Ajinomoto Co., Inc.), and its use as a seasoning increased year after year. After seventeen years of sales experience the company tried to establish a joint venture with an American manufacturer for its production in the U.S. (from Steffen's waste from beet sugar), but failed at that time.

The production of MSG in Japan reached a maximum in 1938, but from then on gradually decreased during the Sino-Japanese conflict until it came to a halt during the period of the Pacific War.

In the United States, Japanese MSG was imported before World War II for use in the canning industry, until a domestic industry could be established. In contrast to the decline in Japanese production, however, the outbreak of the Pacific War stimulated this industry in the U.S. The technological knowledge of the Japanese-American venture which failed years before was utilized and the enterprise was merged into the International Minerals and Chemical Corp.

After the war, demand for MSG increased rapidly not only in the United States but also in Europe. Thus, this industry, which was mainly confined to Japan and the Far East before the war, expanded its market over the world after the war. Several U.S. companies are now manufacturing MSG, using the fermentation process.

In Japan the MSG industry was reestablished immediately after the war and exported its whole product in order to gain foreign exchange, and to contribute to the rehabilitation of the Japanese economy.

The expansion in the manufacture of MSG in many countries has resulted in a world production figure during 1961 which is eight times the prewar record.

The production of MSG in 1962 (in part estimated) is indicated in Table 7. These figures show an increase of 30% over the preceding year, due mainly to the

Table 7. Production of MSG in the World

Source	Pre-war maximum, lb	1962 (estimated), lb	
Japan			
Ajinomoto Co., Inc.	8,300,000	56,000,000	
Other manufacturers	1,300,000	26,000,000	
U.S. and Canada	1,800,000	28,000,000	
Europe		19,000,000	
Formosa and other Far East countries		16,000,000	
total			145,000,000

increased capacity of the fermentation process in the U.S., Japan, and Formosa. With respect to starting materials, 60% of the world production of MSG is manufactured by fermentation, 25% is made from vegetable proteins (mainly in Japan), and 15% is derived from beet sugar wastes (in U.S. and Europe). Japan provides about one half of the total world production; one fourth of that is exported. In other words, almost 40% of the total global production is consumed in Japan.

The market price of MSG in the U.S. during the period 1956–1960 settled at about $1.00 per pound in spite of increasing production during this time, but the market price should come down in the near future due to the rapid increase of fermentation facilities.

Bibliography

1. K. Ueda, *Eighth International Congress of Applied Chemistry, Original Communications* **18**, 147 (1912).
2. O. Cohnheim, *Chemie der Eiweisskörper*, 3rd ed., Friedr. Vieweg & Sohn, Braunschweig, Germany, 1911, p. 25.
3. S. Kodama, *J. Chem. Soc. Tokyo* **34**, 751 (1913).

3a. K. Sakaguchi et al., *Bull. Agr. Chem. Soc. Japan* **23**, 239 (1959).

4. S. Hirokawa, *Mem. Defense Acad. Math. Phys. Chem. Eng.* (*Yokosuka, Japan*) **1**, 1 (1960).
5. T. Ogawa, *J. Chem. Soc. Japan, Ind. Chem. Sec.* **52**, 69 (1949).
6. E. Heegaard, International Minerals and Chemical Corp., personal communication.
7. E. Nakamura, *J. Brewing* (*Japan*) **13**, 217 (1935).
8. U.S. Pats. 3,002,889 (Oct. 3, 1961) and 3,003,925 (Oct. 10, 1961), S. Kinoshita et al. (to Kyowa Fermentation Industry Co.). *Paper Intern. Symp. Enzyme Chem., Tokyo, 1957.*
9. Japan. Pat. 258,589, Pat. Publ. 9393 (Nov. 7, 1957), S. Tada et al. (to Ajinomoto Co., Inc., and Sanraku Distillers Co.).
10. U.S. Pats. 3,029,280 and 3,029,281 (Apr. 10, 1962), S. Motozaki et al. (to Ajinomoto Co., Inc.).
11. H. Adkins et al., *J. Am. Chem. Soc.* **71**, 3051 (1949).
12. Ger. Pat. 839,801 (May 26, 1952), H. Nienburg (to I. G. Farbenindustrie A. G.). U.S. Pat. 2,549,456 (Apr. 17, 1951), W. F. Gresham et al. (to E. I. du Pont de Nemours & Co., Inc.).
13. U.S. Pats. 2,978,481 (Apr. 4, 1961), J. Kato et al. (to Ajinomoto Co., Inc.); 3,010,994 (Nov. 28, 1961), T. Yoshida et al. (to Ajinomoto Co., Inc.).
14. U.S. Pat. 2,919,289 (Dec. 29, 1959), M. Murakami et al. (to Osaka University).
15. Japan. Pat. 245,458, Pat. Publ. 3165 (Apr. 25, 1958), Y. Kawai et al. (to Sanko Co.). U.S. Pats. 2,791,606 (May 7, 1957), L. J. Navak et al. (to Commonwealth Engineering Co. of Ohio); 2,833,786 (May 6, 1958) and 2,837,532 (June 3, 1958), J. L. Purvis (to International Minerals and Chemical Corp.).
16. U.S. Pat. 2,940,998 (June 14, 1960), T. Ogawa et al. (to Ajinomoto Co., Inc.).
17. H. Wilson et al., *J. Biol. Chem.* **119**, 309 (1937).
18. J. D. Bernal, *Z. Kristallographie* **78**, 363 (1931).
19. S. Hirokawa, *Acta Crystallographica* **8**, 637 (1955).
20. U.S. Pat. 2,683,739 (July 13, 1954), S. H. Weidman (to International Minerals and Chemical Corp.).
21. Japan. Pat. Publ. 17712 (Sept. 29, 1961), Y. Kawamura et al. (to Ajinomoto Co., Inc.).
22. Y. Sakata, *Agr. Biol. Chem.* (*Tokyo*) **25**, 835 (1961).
23. J. B. Dalton et al., *J. Biol. Chem.* **103**, 549 (1933).
24. S. Mizushima, *J. Gen. Appl. Microbiol.* (*Tokyo*) **3**, 233 (1957); **4**, 12 (1958).
25. S. Hara et al., *J. Tokyo Medical College* **20**, 69 (1962).
26. L. E. Arnow et al., *J. Biol. Chem.* **133**, 765 (1940).
27. O. K. Behrens et al., *Science* **92**, 32 (1940).
28. J. M. Johnson, *J. Biol. Chem.* **134**, 459 (1940).

HARUOMI OEDA
Ajinomoto Co., Inc.

Change of the Plasma Glutamic Acid Levels of Neonatal Rats administered MSG with Milk, and without Milk.

Yuichi O'hara, Yoshisuke Hasegawa
Masamichi Ichimura, Jiro Kirimura
(Central Research Laboratories, Ajinomoto Co., Inc.)

Neonatal rats of 10 days after birth were admistered MSG, in the form of the solution in water and also in the form of the solution in milk, and the difference in the rise of plasma glutamic acid levels was compared.

1. Experimental Method

1) Material; Powdered Milk (Formula P-7 manufactured by Snow Brand Milk Co., Inc.) was used.

2) Animal; Male offspring of Wistar-Imamichi strain (10 days after birth) were served immediately after the separation from the mother.

3) Method of administration

The following three solutions were prepared and administered by a polyethylene-tube at the rate of 0.1ml/10g b.w.

a) MSG 10g in water to 100ml

b) MSG 10g and Powdered Milk 15g in water to 100ml

c) Powdered Milk 15g in water to 100ml

4) Method of collecting blood

Exactly the same procedure as described in Appendix B (Neonates)

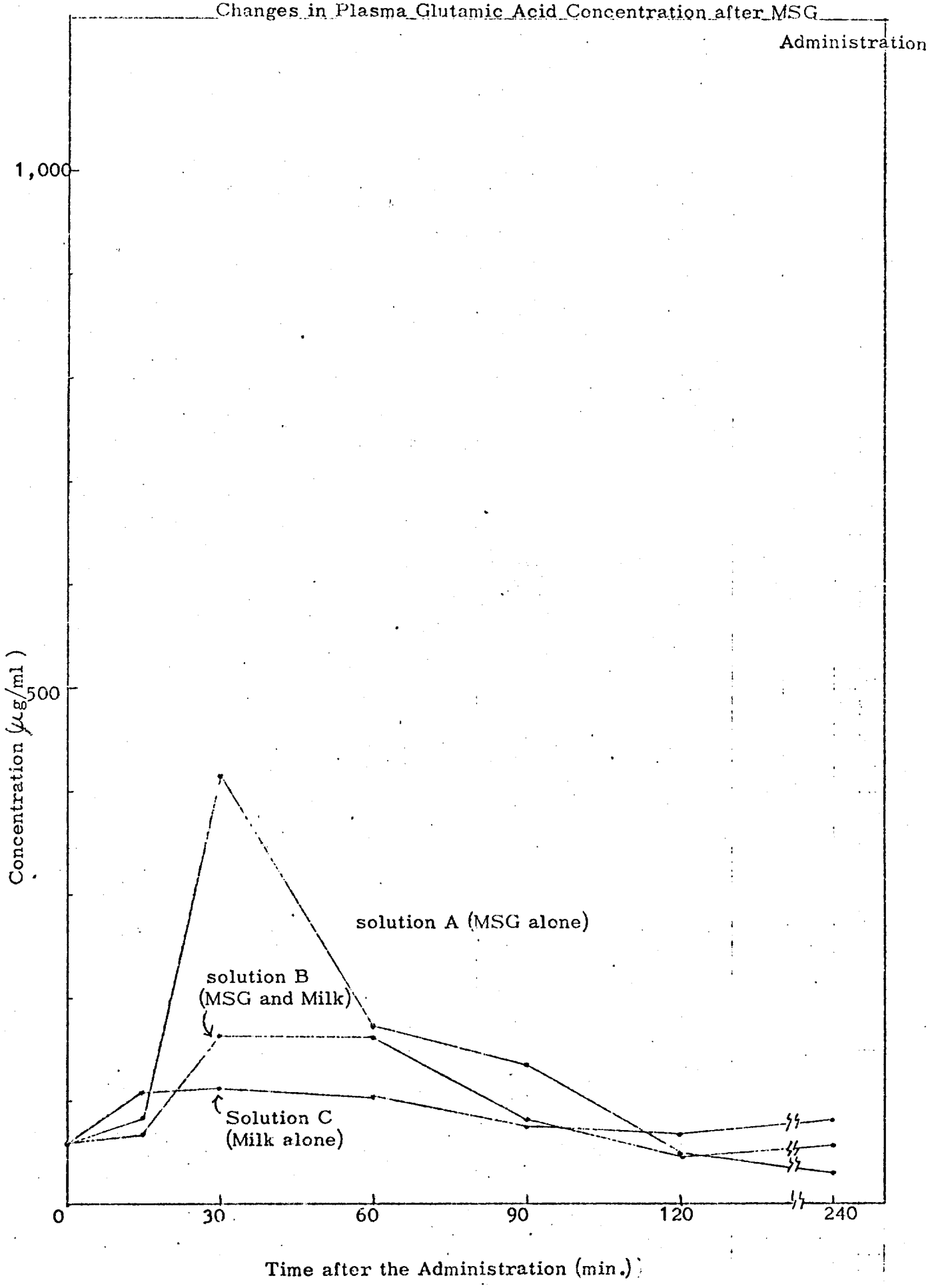
5) Determination of glutamic acid in plasma

Exactly the same procedure as described in Appendix B (Neonates)

2. Results

Experimental resalts are shown in the attached figures.

Changes in Plasma Glutamic Acid Concentration after MSG Administration



GLUTAMATE-INDUCED RETINAL DEGENERATION IN NEONATAL MICE. ELECTRON MICROSCOPY OF THE ACUTELY EVOLVING LESION* †

JOHN W. OLNEY, M.D.

(St. Louis, Missouri)

In 1957, Lucas and Newhouse (1) demonstrated a degenerative lesion in mouse retina following the parenteral administration of monosodium L-glutamate (MSG). Treating the adult mouse produced only a partial lesion and was associated with a high mortality rate, but treating neonatal mice resulted in an extensive and predictable pattern of retinal degeneration with negligible mortality. By light microscopy the lesion was described as involving large numbers of several cell types in the inner retina while sparing cells of the outer retina, particularly the receptors.

Lucas and Newhouse (1) obtained essentially negative results from testing other compounds, either for their ability to produce a similar lesion or to protect the retina against the MSG effect. Vulnerability of neonatal rat retina to the MSG lesion was reported by Freedman and Potts (2) who found altered levels of several enzymes associated with glutamic acid metabolism in retinas studied 2 weeks following MSG treatment. Radioactive uptake studies by the same authors (3) indicated that glutamate uptake by the rat retina was high on the 5th postnatal day but had begun to decline by the 10th day. To obtain a maximal degree of retinal modification for purposes of electrophysiological studies, Potts and colleagues (4) treated neonatal mice with MSG daily for 17 consecutive days. In retinas thus treated only an "a" wave could be recorded in the electroretinogram 3 months following treatment. Cohen (5), who studied mouse retina with the electron microscope, 2 months and 1 year following MSG treatment, obtained the same pattern and extent of retinal modification with only 10 treatments given daily from the 1st to 10th postnatal days. Cohen found in retinas 2 months post treatment that, even though the ratio of receptors to inner neurons was markedly enhanced, residual synaptic relations and certain other aspects of cytoarchitectonics were indistinguishable from normal.

It is estimated that free glutamic acid exists in the central nervous system (CNS) at approximately 10 micromoles/gm (6), a concentration considerably higher than that of any other amino acid found in the CNS. That the sodium salt of this common CNS metabolite, following parenteral administration, induces a striking degenerative response on the part of retinal neurons poses intriguing toxicological questions and, in addition, provides an experimental model of acute neuronal necrosis in the developing CNS that can readily be

* From the Departments of Psychiatry and Ophthalmology, Washington University School of Medicine, St. Louis, Missouri 63110.

† This investigation was supported in part by grants from the United States Public Health Service (NB-01816, MH-7081, MH-5801, MH-38891).

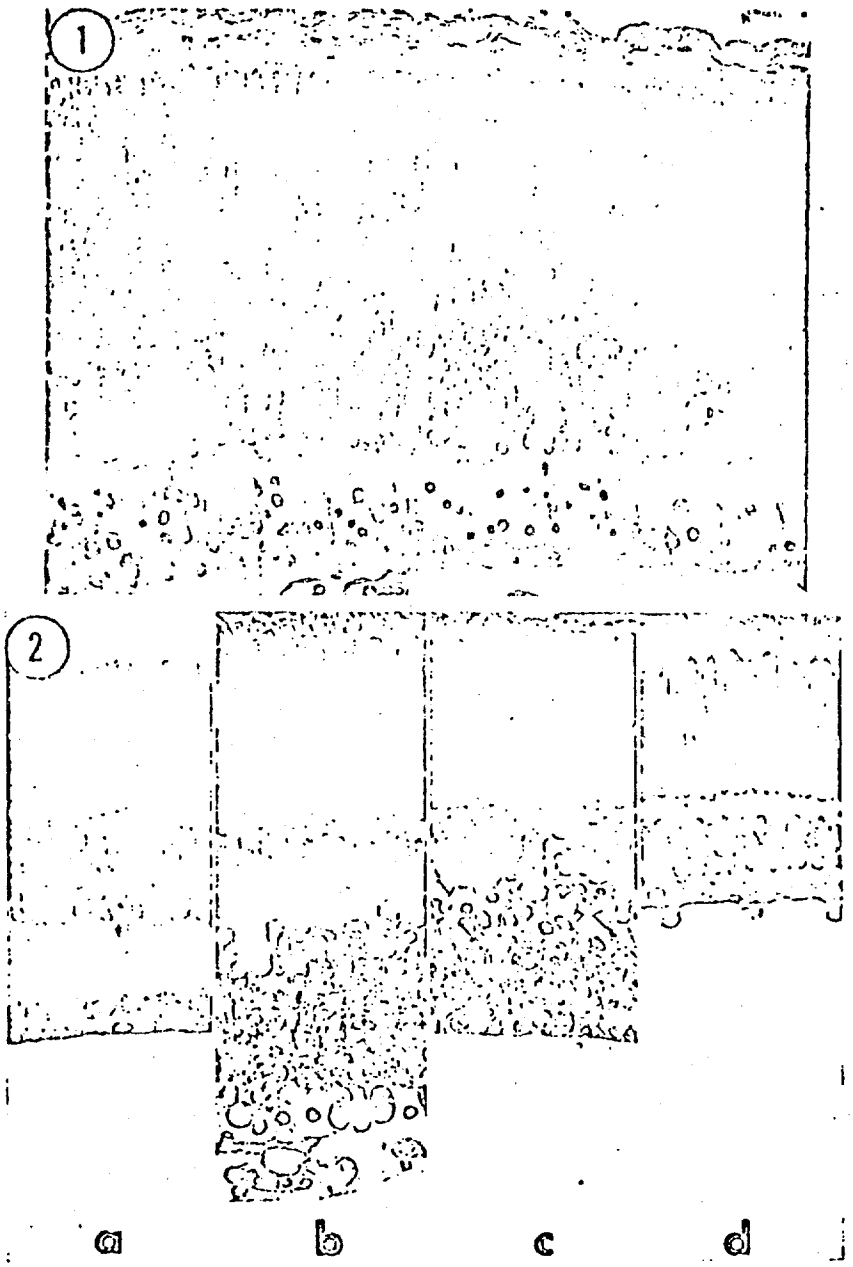


FIG. 1. Light micrograph of 2-day-old mouse retina, 3 hours post treatment, showing pattern and extent of lesion produced by a single subcutaneous injection of MSG, 4 mg/gm. Compare with Figure 2b. Many ganglion cells appear necrotic, but very few cells in the outer mantle are affected. The moth-eaten appearance of the inner plexiform zone is due to dilatation of ganglion cell dendrites. Richardson stain; × 250.

FIG. 2. Series of light micrographs, all from the same litter of mice. Each animal, except the untreated control, received a single subcutaneous injection of MSG, 4 mg/gm, early on the 10th postnatal day. a. Untreated control. Retina is histologically normal with no swelling of plexiform layers or of any cell bodies. b. Litter mate 3 hours post treatment. Marked

reproduced for study. It has not been determined whether there is a particular developmental stage in which the retina is maximally sensitive to MSG treatment nor is it known whether regions of the CNS other than the inner retina are vulnerable. The acutely evolving retinal lesion has not been examined with the electron microscope for ultrastructural clues regarding mechanisms of action. To provide data pertinent to these and related issues a morphological study, employing both light and electron microscopy, has been initiated in which the acute effects of MSG on the entire central nervous system of the neonatal mouse are under investigation. The present report pertains to the acute response of the neonatal mouse retina to parenterally administered MSG. The normal development of neonatal mouse retina was studied with the electron microscope as a preliminary to the present investigation and is reported elsewhere (7).

MATERIALS AND METHODS

In the course of the larger investigation of the effects of MSG on the entire central nervous system, over 200 mice received treatment with MSG according to a variety of treatment schedules. Retinas from approximately half of these animals have been examined to date. Some data presented in the text are based on the larger group of experimental animals, but all illustrations except Figure 1 were specifically taken from 3 litters of Swiss albino mice treated between the 9th and 10th postnatal days with a single subcutaneous injection of MSG at a dose level of 1 mg/gm of body weight. MSG was prepared in sterile distilled water and administered in a volume of approximately 0.2 cc per animal. The batch of MSG used was obtained from Nutritional Biochemical Corporation and contained no impurities which could be detected by thin layer chromatography in our laboratories. It was also established that freshly prepared MSG (by titration of NaOH with glutamic acid just prior to treatment) resulted in the same retinal pathology as when commercially prepared MSG was used. Experimental animals were sacrificed at serial post-injection intervals from 30 minutes to 48 hours and their retinas compared with those of litter mate controls receiving no treatment. All retinas were fixed *in situ* by immersion of the whole eye in 2 per cent osmium tetroxide buffered with 0.1M pyrophosphate. Specimens were dehydrated in graded ethanols, bemisected in absolute ethanol and embedded from toluene in Araldite. Sections approximately 1.5 microns in thickness were cut on a Porter Blum MT-2 microtome and stained by the Richardson method (8) for light microscopy. For fine structural analysis, ultrathin sections were mounted on formvar films over discs with a 1 mm x 2 mm hole, stained with both lead and uranium salts and examined in a Elmiskop 1A electron microscope.

---

tumescence of inner retina with widened, fenestrated inner plexiform layer and hydropic cytoplasm of ganglion cells and neurons in inner tiers of inner nuclear layer. Muller foot processes vitreal to ganglion-cell layer are edematous. Swelling of processes invaginated into receptor bases is suggested by rarefaction of inner regions of the outer plexiform zone. The dark cell phenomenon is evident and in this particular scene affects predominantly ganglion cells. Small increase in thickness of outer nuclear layer is probably due to slight obliqueness of section. c. Litter mate 12 hours post treatment. Generalized tumescence of inner retina is reduced but individual neurons show advanced stages of necrosis, and inner plexiform layer appears degenerate with numerous vacuous compartments. d. Litter mate 48 hours post treatment. The general tissue density has returned to pretreatment appearance, but the inner retina is markedly modified. Ganglion cells are rare, the inner plexiform layer is very thin and the inner nuclear layer is reduced from 6 to 7 tiers of nuclei to 3 to 4 tiers. Some of the nuclei in the inner retina belong to macrophages. A further thinning of the inner nuclear layer to approximately 2 to 3 tiers will occur as macrophages leave and as the retinal circumference expands to mature proportions. Note the several "vitreocytes" lying against the vitreal surface of the retina. Richardson stain; × 290.

RESULTS

In experiments designed to determine whether the sensitivity of the retina to MSG-treatment remains constant throughout the developmental period it was found that a high dose (4 mg/gm) given as a single subcutaneous injection in the early neonatal period (days 1 to 3) tended to produce only a mild lesion essentially limited to one cell type, the ganglion cell (fig. 1). With increasing age, up to approximately the 10th postnatal day, an increasingly severe lesion, involving not only ganglion cells but many cells of the inner nuclear layer, could routinely be induced by a single injection at the same 4 mg/gm dose level (fig. 2b). Although considerable individual and litter variability of response was noted, in general a greater degree of retinal modification resulted from treating the 9- to 10-day-old animal than from treating a younger animal. When daily treatments from the 1st to 10th postnatal days were given, the resulting lesion was usually slightly more severe, but in several retinas was less severe, than was routinely induced by a single injection between the 9th and 10th days. It appeared, therefore, that developmental age and individual or litter variability were more critical determinants of lesion severity than was the number of separate injections given. The MSG effect may also be a dose related phenomenon, but experiments in the present study were not designed to study the dose systematically in relation to other variables. In order to achieve the maximal effect from a single injection, timing was quite critical in that retinal conditions changed abruptly between the 10th and 11th postnatal days. After the 10th to 11th days it was difficult, even with lethal doses of MSG, to produce a significant lesion in the retina. Such lesions as were produced by treatment subsequent to the 10th day showed a spotty distribution usually limited to the region of the ora serrata and to a few cells immediately surrounding the nerve head.

From its onset to resolution the MSG retinal lesion followed a rapid time course as is illustrated light micrographically in the Figures 2a–d. Mild changes were discernible within 30 minutes of a single subcutaneous injection and within 90 minutes an acute progressive swelling phase was well underway which resulted in 40 to 60 per cent increase in thickness of retinas examined in the 3 to 7 hour post treatment interval (fig. 2b). By 12 hours tumescence had subsided (fig. 2c), but necrotic debris remained distributed throughout the inner retina during the 12 to 24 hour period. By 48 hours degenerative elements were almost entirely eliminated and an overall reduction of retinal thickness to approximately 65 per cent of normal was evident (fig. 2d). Thus, an acute degenerative lesion induced in the 10-day-old retina by MSG, involving the death and biological degradation of a vast number of neurons, transpired almost completely in the short time span of 48 hours. Repair and reorganization phenomena, detectable only by electron microscopic examination, continued for several additional days.

In general, the early retinal response to MSG could be characterized as a massive edema formation restricted to the inner compor ts of the retina. By electron microscopy the edema was entirely intracellular, although

in the 12 to 24 hour interval large extracellular spaces appeared, especially in regions of the inner retina adjacent to the vitreal surface (fig. 13).

The light microscopic impression that MSG treatment had no primary effect on photoreceptor cells was confirmed by electron microscopy. The majority of cells in the outer 2 or 3 tiers of the inner nuclear layer also underwent very little acute change. The tips of many processes invaginated into receptor bases, however, began swelling within 30 to 60 minutes of treatment (fig. 3). Most of these acutely swollen processes presumably originated from cells destined to undergo necrosis in the inner tiers of the inner nuclear layer. The majority of these processes rapidly degenerated beyond recognition, leaving many receptor bases devoid of invaginations when examined at 7 hours of treatment.

In cells of the inner nuclear layer which survived treatment, a specific intracytoplasmic organelle, the multivesicular body, became prominent in the post treatment period. This response to MSG treatment was particularly noticeable in that such organelles appeared in the tips of bipolar and/or horizontal cell processes which remained invaginated into receptor bases and were often so large as to fill the entire invagination (fig. 4). Although, in normal developing retina an occasional multivesicular body was encountered in the parakaryal cytoplasm of inner nuclear cells and in larger processes of the outer plexiform layer, they were not normally found in the tips of processes invaginated into receptor bases. The frequent appearance of such figures in invaginated processes of surviving cells suggests a role for these organelles in reversible injury and repair phenomena.

The cell bodies of affected bipolar, amacrine and ganglion cells began to swell within the first 2 hours and some showed frank necrosis in the 3 to 5 hour interval. Although it was difficult in most cases to trace the cellular origins of specific processes in the inner plexiform layer, certain processes having morphological characteristics consistent with those ascribed by Dowling (9) to the ganglion cell dendrite were markedly hydropic within an hour of treatment, whereas others, with morphology characteristic of the bipolar axon, tended to show fewer degenerative changes until a later period, approximately 5 to 7 hours following treatment (fig. 5). A preliminary examination of the ganglion cell axon at fiber tract, nerve head and optic nerve levels revealed a gradually developing reaction of delayed onset in contrast to the rapidly developing changes observed in many ganglion cell dendritic and somal constituents.

Several patterns of degenerative change were observed on the part of inner retinal neurons. In addition to generalized hydropic changes in both the cytoplasm and nucleoplasm, mitochondrial transformations were consistently observed within 30 to 90 minutes of treatment (figs. 6, 7). Bloating initially occurred in many mitochondria, although this sometimes affected only a portion of the mitochondrion. In the swollen portion the matrix appeared obliterated while in other areas of the same mitochondrion a dense matrix sometimes was evident. In a few cells, especially of the ganglion cell layer,

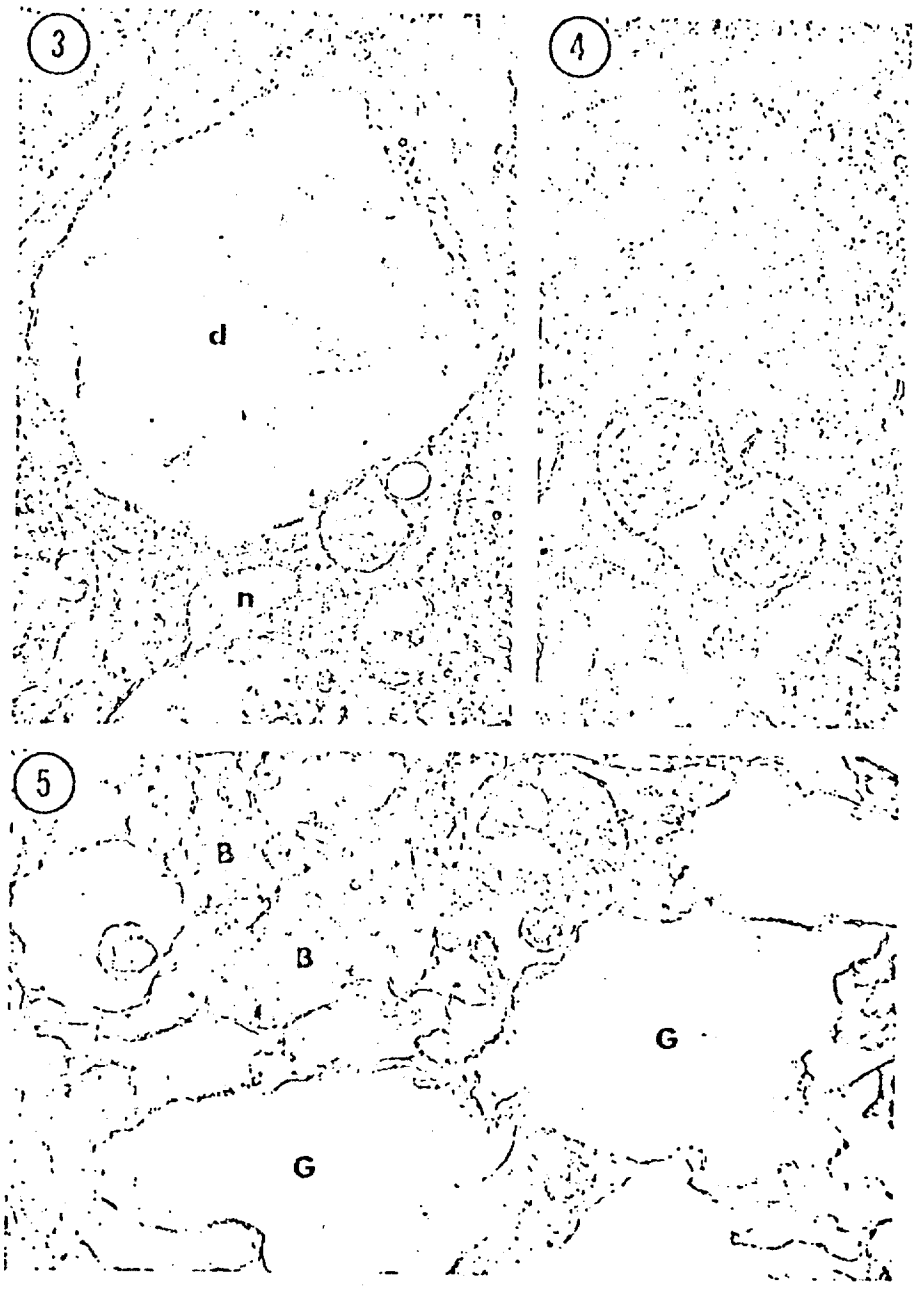
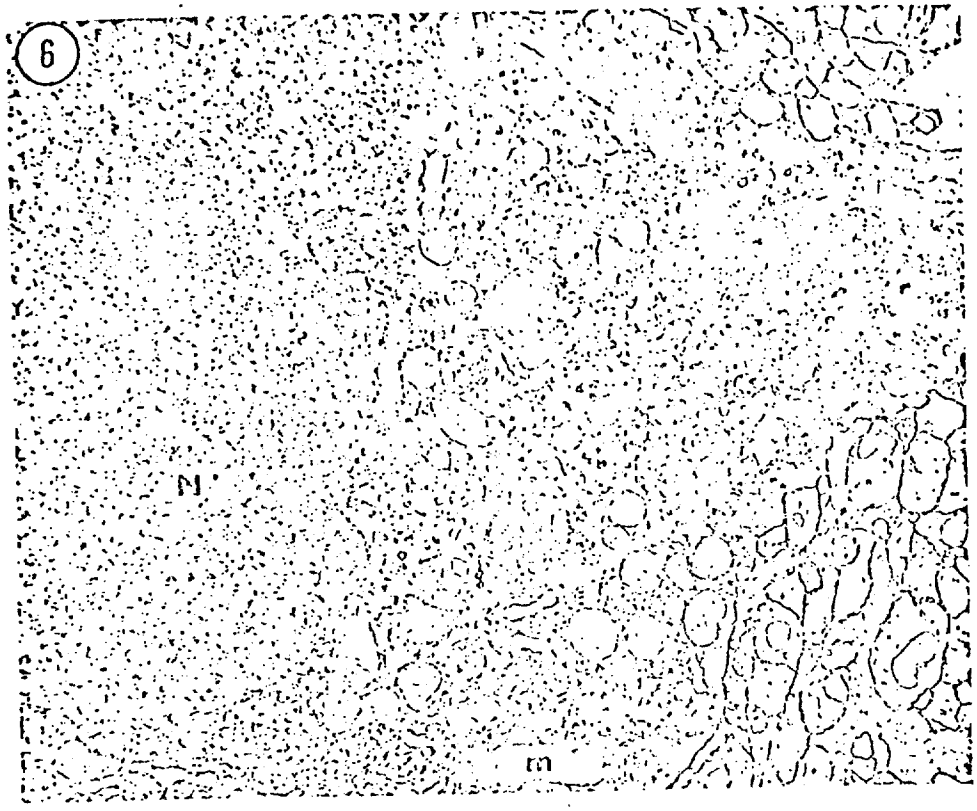


FIG. 3. Outer plexiform zone 2 hours post treatment showing dilated process invaginated into receptor base. A normal or relatively unaffected process invaginates the receptor base at lower center (n) for comparison with dilated invagination (d). Although compressed and distorted by the dilated invagination, the receptor terminal appears otherwise unaffected; × 28,000.

FIG. 4. Photoreceptor terminal, 24 hours post treatment, showing pair of large multivesicular bodies in invaginated tips of inner neuron processes; × 42,000.

FIG. 5. Inner plexiform layer, 3 hours post treatment, with large vacuous processes of presumptive ganglion cell origin (G) in the same field with a pair of bipolar axon terminals (B) identifiable by their vesicle content and small synaptic lamellae. Heterogeneity in size of synaptic vesicles in the bipolar terminal suggests mild early changes in contrast to more extreme changes in ganglion cell dendrites which are hydropic and devoid of content. The markedly dilated process in Figure 3 is probably a bipolar dendrite which reflects a greater degree of injury at 2 hours than is seen in the bipolar axons in Figure 5 at 3 hours; × 28,000.



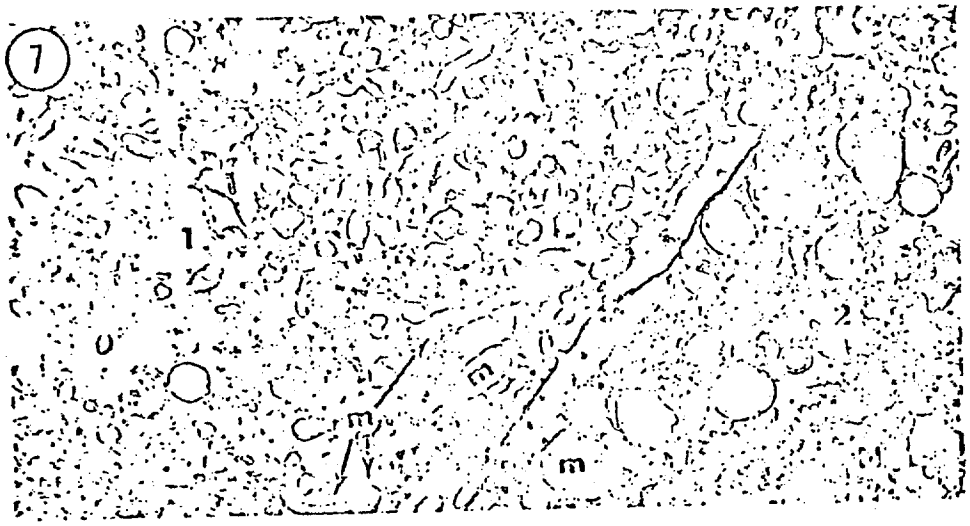


FIG. 6. Ganglion cell from 10-day-old untreated control animal which was a litter mate of animals from which micrographs 7 to 19 were taken. Typical appearance of mitochondrial (M) and other cytoplasmic constituents is illustrated. Note the density of nucleoplasm (n) and cytoplasm. Normal inner plexiform processes are also shown at right, top and bottom; × 11,000.

FIG. 7. Portions of two ganglion cells (1 and 2), 1½ hour post treatment. Magnification is same as in Fig. 6 for direct comparisons. Both cytoplasm and nucleoplasm (N) are of lowered density due to intracellular edema. In the 1st ganglion cell mitochondria (m) are pleomorphic with a few showing bloating but others appearing slim with sundged thickened membranes. Mitochondria in 2nd ganglion cell are markedly bloated; × 11,000.

other mitochondrial alterations could be detected early. In such cells mitochondria were often thinner than normal with internal contents compacted into thick septa surrounding vacuous compartments (fig. 7). A rounding up of mitochondria as is described below in connection with a more advanced stage of cellular degeneration was not seen in this early period.

Following the initial 2 to 3 hour period involving mild changes in many cells, divergent patterns of change became apparent. The most dramatic pattern noted was one suggesting an acute lytic process. In neurons thus affected, the cytoplasm appeared empty except for dispersed particulate debris and several remnants of formed elements such as a degenerate mitochondrion or a few profiles of rounded up endoplasmic reticulum (fig. 8). Nuclear pyknosis accompanied this type of cellular change with the nucleus becoming shrunken and dark. This pattern of cellular necrosis was not limited to a particular cell type. Although it must have represented an advanced state of degeneration, it was seen with greatest frequency in the 3 to 7 hour post treatment interval.

In a second, less acute pattern of cytopathology, which also was not limited to any particular cell type, mitochondria remained quite numerous but became uniformly spherical with a moderately dense matrix (figs. 9, 10). The granular endoplasmic reticulum gradually underwent severe fragmentation which resulted in a dispersion of ribosomes throughout the cytoplasm and was accompanied by a tendency for remnants of reticular membranes to round up into membrane bound vacuoles. In the process of formation some vacuoles appeared to incorporate small portions of cytoplasm (figs. 11, 12). Granulated vesicles (dense bodies) of variable size, resembling those which have been referred to alternatively as lipofuscin bodies (10) or as lysosomal "storage granules" (11) were observed, occasionally, in these cells but there was no suggestion of interaction between such granules and other cytoplasmic constituents. Nuclei underwent a gradual dissociation into 3 components: 1. A fine granular matrix, 2. dense particles or aggregates of particles embedded in this matrix and 3. moderately dense granular clumps (fig. 9). This type of degenerative change was not clearly manifest in any cells until approximately the 5th to 7th hours after treatment. In the 24 to 48-hour interval, macrophages were found containing phagocytized necrotic cells which could be identified by nuclear configuration as having undergone this gradual pattern of cellular necrosis (fig. 18). To facilitate description, this pattern of necrosis and the preceding one have been described as separate and distinct patterns of degeneration. In fact, some overlap between the two patterns occurred and an occasional degenerating cell appeared to have features of both patterns.

While the majority of amacrine and ganglion cells underwent a degenerative process terminating in cellular necrosis and were phagocytized or otherwise degraded and removed from the retina, a few cells of each type not only survived treatment, but showed almost no signs of being affected by the degenerative process. Every retina examined subsequent to the 5th post treatment hour contained a few such cells which, aside from mild mito-

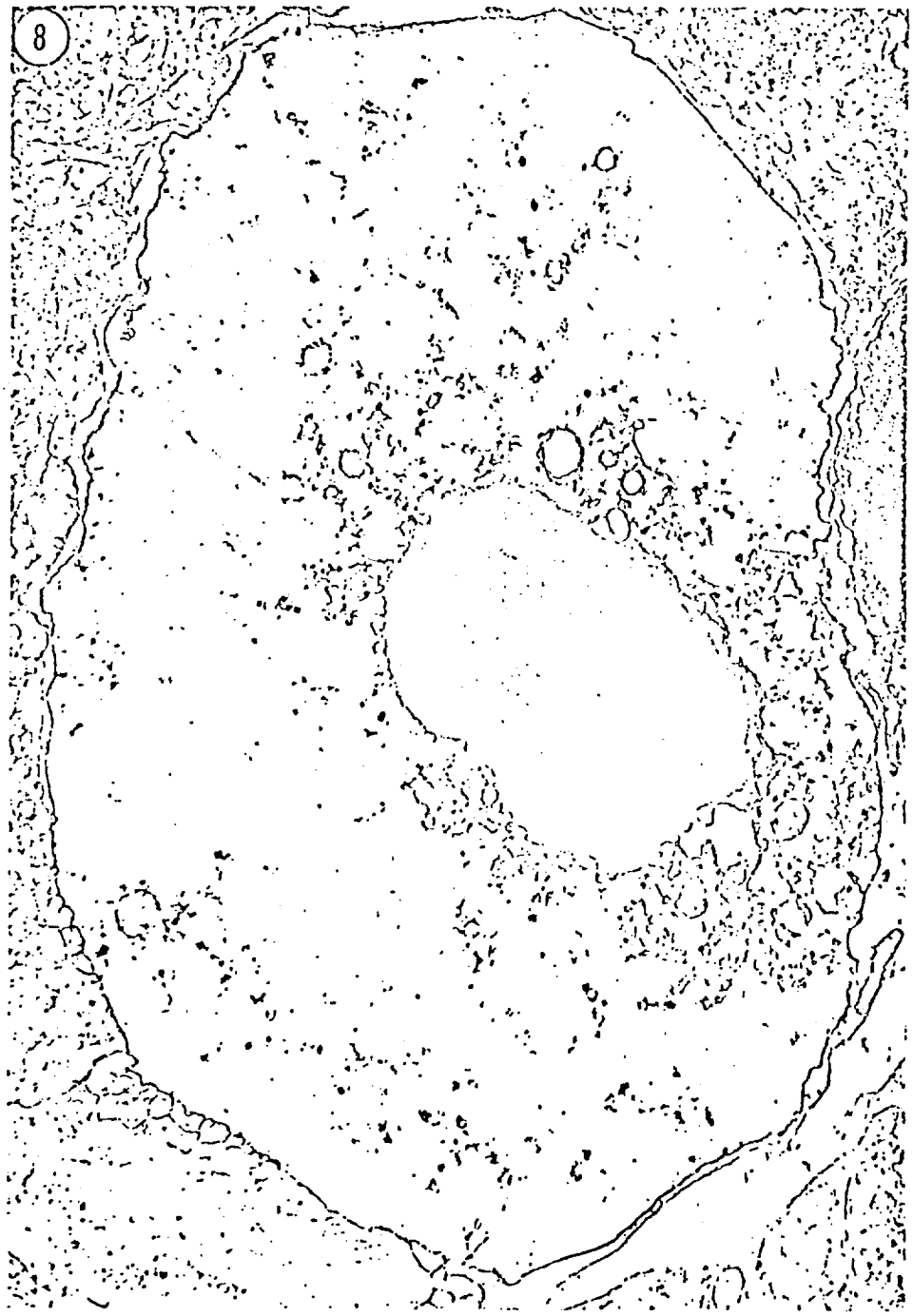


FIG. 8. Retinal neuron from inner nuclear layer 3 hours post treatment showing rapid degenerative process. The cytoplasm is empty in many areas except for particulate debris and a few remnants of formed elements which, in this particular cell, appear to be grouped around the nucleus. Although cytoplasmic structures have undergone a marked degree of autolysis, the plasma membrane appears intact. The nucleus is darker and smaller than normal representing a moderate degree of nuclear pyknosis; × 11,000.

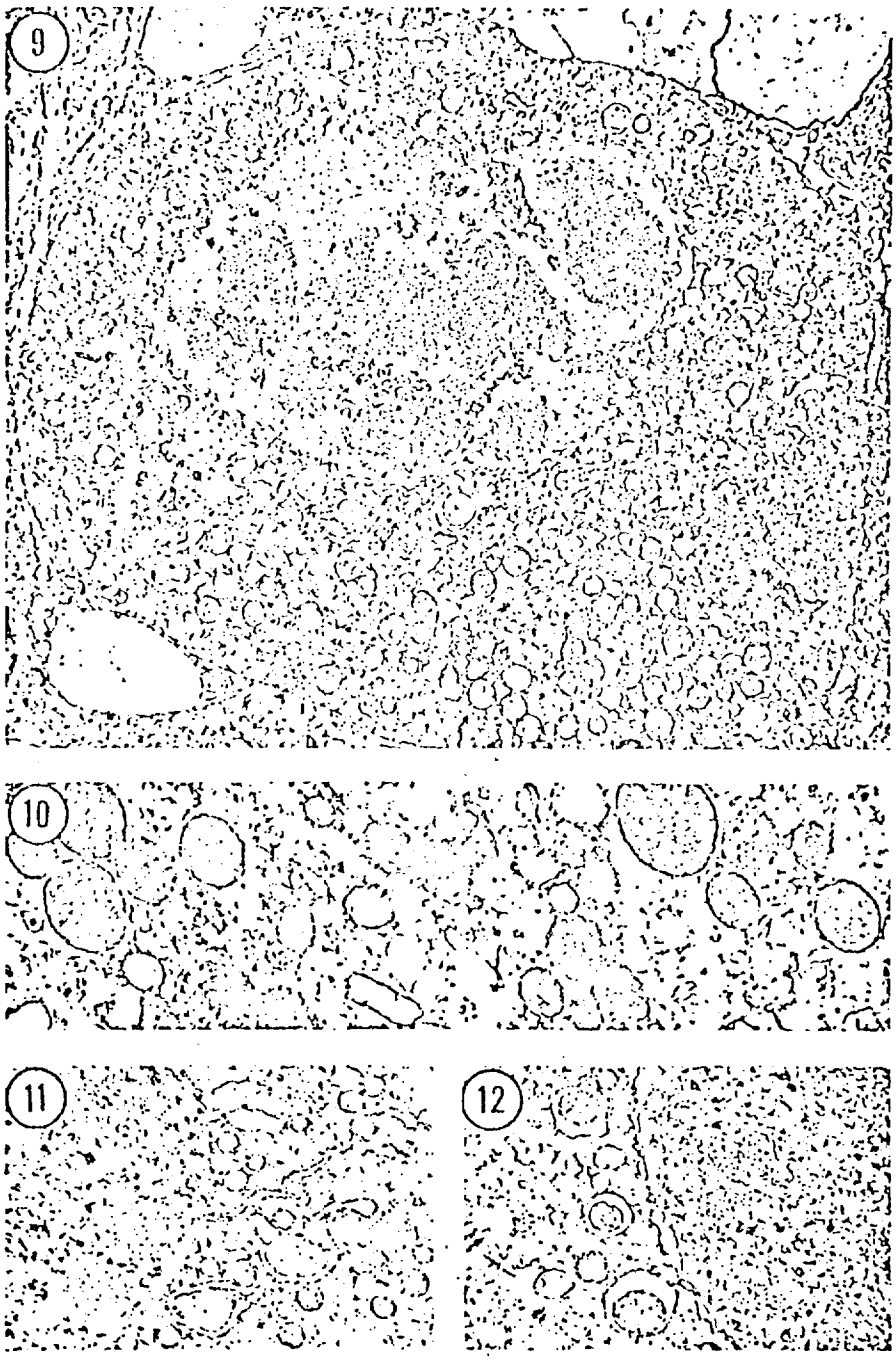


FIG. 9. Ganglion cell, 7 hour post treatment, undergoing gradual degenerative changes. Mitochondria are uniformly spherical and have relatively dense matrices. Endoplasmic reticulum is fragmented with some cisternae transformed into small vacuoles. Ribosomal granules are dispersed throughout the cytoplasm and the nucleus consists of 3 morphologically distinct components: a fine granular matrix; dense particles in clusters or strands; and dense granular clumps; × 12,000.

chondrial bloating, appeared normal in striking contrast to the surrounding field of necrotic debris.

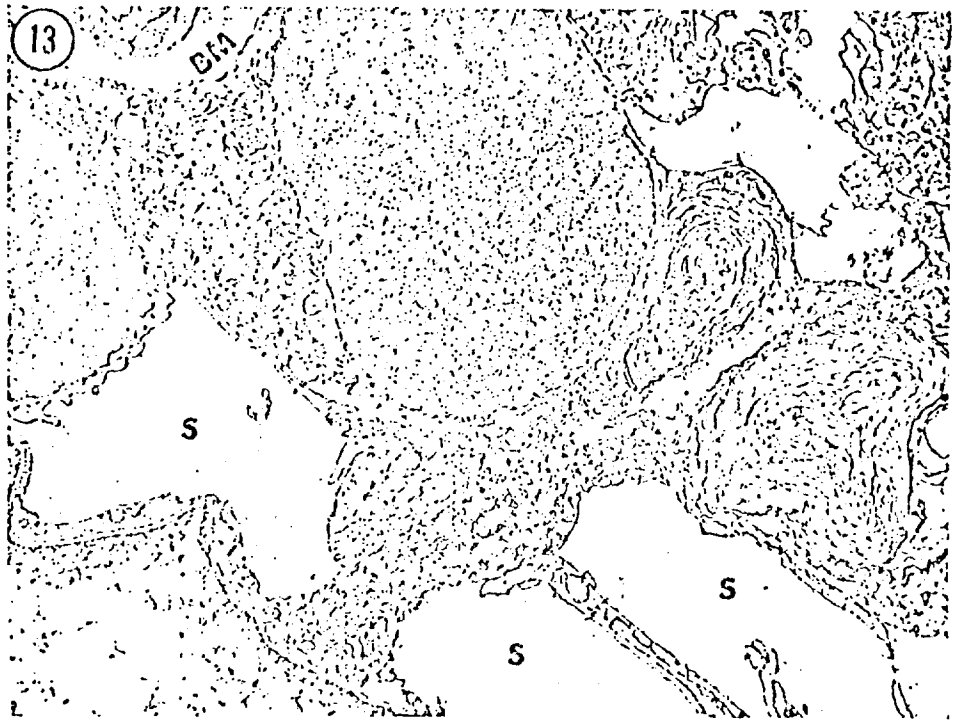
In experimental retinas a dark cell phenomenon became evident 3 hours following treatment (fig. 2b) but was not seen in earlier post treatment intervals nor in any control retinas. No particular pattern of degeneration was a characteristic accompaniment of the dark cell appearance. Approximately ¼ of the cells in the inner retina were affected by this phenomenon which appeared to be a selective cellular dehydration process. In that the process occurred only in treated retinas and only in the region of the evolving lesion it was presumably induced either directly or indirectly by the administered load of MSG. Why it affected some cells but not others and by what specific mechanism it developed is not clear.

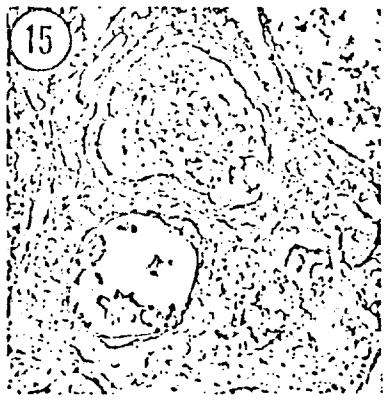
Changes interpreted as reactive rather than degenerative were noted in several non-neuronal cell types. In the vitreally oriented Muller process an increased amount of agranular endoplasmic reticulum was apparent within a few hours of treatment but the extent of increase was difficult to assess due to a variable degree of swelling affecting Muller processes in the early post treatment period. After the 12th hour, as swelling subsided, it was evident that most Muller processes did contain an increased amount of agranular endoplasmic reticulum (fig. 14) and, in addition, a marked tendency for the profiles of endoplasmic reticulum to appear in whorls or in circular concentric configurations became evident in this period (figs. 13, 15). A reduced glycogen content was suggested as an early response in Muller processes although this also was difficult to evaluate due to swelling and to the fact that the concentration of glycogen granules varies considerably in normal retinas from one process to another and from one animal to another. In the late post treatment period an excess accumulation gradually developed so that by 48 hours most Muller processes contained decidedly more glycogen than normal. Phagocytosis or "endocytosis" (12) was observed on the part of the Muller process as early as 3 hours and as late as 48 hours post treatment. Although an attempt to engulf entire cell bodies was not observed, slim pseudopodia extending from the main stalk of the Muller process were frequently seen enveloping less sizeable degenerating structures in the inner plexiform zone. Vacuoles of diverse size, apparently representing fragments of disintegrating neuronal processes, were routinely encountered at various post treatment intervals, incorporated within the

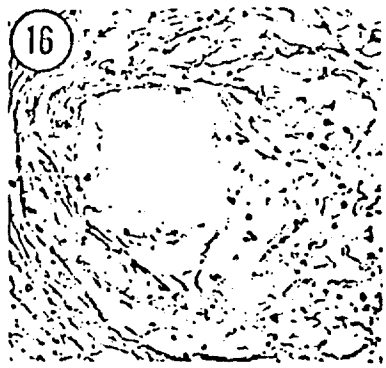
---

FIG. 10. Mitochondria 7 hours post treatment, from cell undergoing gradual degeneration as in Fig. 9. All mitochondria are essentially spherical. Although some appear more degenerate than others, none is being engulfed or acted upon in any recognizable way by other constituents of the cytoplasm. Particles in the cytoplasm are presumably dispersed RNA granules; × 31,000.

FIGS. 11 and 12. Two ganglion cells, 7 hours post treatment, each undergoing a pattern of degeneration similar to cell in Fig. 9. Fig. 11 illustrates vacuole formation by granular endoplasmic reticulum. Cisternae of the granular reticulum are curling upon themselves to form vacuoles which appear to be trapping portions of cytoplasm in the immediate environment. In Fig. 12, vacuoles appear to have already formed with small portions of the cytoplasm possibly enclosed within the vacuole. Whether a limiting membrane completely surrounds these apparent inclusions could only be established by careful serial sectioning which was not undertaken in the present study; × 15,000.







FIGS. 13-16.

main body of cytoplasm of the Muller process (fig. 15). Numerous sizeable fat droplets accumulated in Muller processes of treated retinas (fig. 14) whereas in normal development the Muller process contains only occasional fat droplets. Whorls of agranular endoplasmic reticulum sometimes were closely associated with fat droplets (fig. 16) but often they were not, nor was there any clearly recognizable relation between these lamellar configurations and ingestion vacuoles (fig. 15).

In the post treatment period, many macrophages similar in appearance to the type normally present at various levels of the developing retina (7) gradually became engorged with degeneration products. In the pre-engorged state, macrophages typically contained abundant granulated vesicles (dense bodies) which, except for size variation, were of homogeneous appearance. Engorged macrophages lacked such vesicles but contained instead an assortment of pleomorphic inclusions ranging in content from recognizable formed elements of other cells to an admixture of myeloid figures, dense particles and amorphous debris (fig. 17). With the exception of fat droplets, which increased in size and number within macrophages in later post treatment periods, most inclusions were membrane bound. Some macrophages appeared to incorporate within their cytoplasm entire corpses of necrotic retinal neurons. Polymorphonuclear leukocytes which appeared in degenerating regions within 12 hours of treatment also performed a phagocytic function but on a smaller scale. In fact, several examples were noted in which polymorphonuclear leukocytes appeared to be engulfed along with degenerating neurons by retinal macrophages (fig. 18). As was observed by Weidman (13) in developing rat retina, spontaneous neuronal necrosis occurs to a limited extent as a natural concomitant of retinal development. Such necrotic neurons are routinely removed from developing mouse retina by macrophages similar in appearance to those evacuating debris from treated retina (14). Theories concerning the origin of retinal macrophages, phagocytosis and the degradation and disposal of necrotic debris were well reviewed recently (15). The present study sheds no additional light on the controversial aspects of these issues.

FIG. 13. Scene near vitreal surface, 18 hours post treatment. Large extracellular spaces (S) are present near vitreal surface. Muller process contains whorls of agranular endoplasmic reticulum. Central region of whorl also consists of endoplasmic reticulum which appears vesiculated. The paravascular cell lying in contact with the vascular basement membrane (BM) may be a glial cell. It is commonly seen in the fiber tract region of the developing retina but unlike the pericyte, is not surrounded by basement membrane; × 12,000.

FIG. 14. Muller processes illustrating hypertrophy of endoplasmic reticulum 24 hours post treatment. An accumulation of fat droplets is present at lower left. Glycogen granules are relatively abundant but are rendered inconspicuous by the dense background of hypertrophied reticulum; × 15,000.

FIG. 15. Muller process 12 hours post treatment showing whorl of agranular reticulum adjacent to several vacuoles of heterogeneous appearance. Except for a small fat droplet the whorl contains nothing but vesiculated agranular reticulum and it bears no particular relation to any of the nearby vacuoles; × 10,000.

FIG. 16. Muller process 24 hours post treatment showing layers of agranular reticulum in semicircular configuration appearing to drape around a fat droplet. In that the entire cytoplasm of the Muller process was filled with agranular reticulum, the apparent close association between reticular membranes and the fat droplet may only represent crowding; × 36,000.

FIG. 17. Macrophage in inner plexiform zone, 24 hours post treatment. This macrophage appears quite engorged with ingested materials, some of which are formed elements of degenerating neurons and some of which are broken down beyond recognition. Membrane bound granular vesicles (dense bodies) are a less conspicuous component in the makeup of this cell and fat droplets are more common than in pre-engorged macrophages; × 12,600.

A paravascular cell, similar in appearance to the retinal macrophage, but which in normal development is seen as a "vitreocyte" in close association with vitreal blood vessels, underwent a marked increase in numbers in the 24 to 48 hour interval following treatment. These cells appeared to follow a path of migration from vitreal blood vessels to the vitreal surface of the retina where, by 48 hours, they could be found in large numbers lying against the basement membrane separating the vitreal chamber from the retina (fig. 2d). Since such cells were never seen actually to enter the retina, their specific role in relation to the retinal lesion remains obscure. A hematogenous origin of the vitreocyte is suggested by the fact they increased in number in the post treatment period but were never seen to undergo mitosis.

In the normal development of mouse retina blood vessels migrate from the level of the fiber tract to the inner plexiform level by the 4th postnatal day and are present in the outer plexiform layer by the 8th postnatal day (7). In the present investigation it was observed that these normal vascular developments did not proceed according to schedule while the animal was under treatment with MSG. In animals treated with daily doses of MSG from the 1st to 10th postnatal days, vascularization of the retina was completely inhibited in that blood vessels could not be found beyond the fiber tract level on the 10th day. In retinas from animals similarly treated but examined

FIG. 18. A complex constellation of degenerate and non-degenerate constituents of an indeterminate number of cells. Two nuclei or nuclear lobes (n) which appear mildly condensed are interpreted as belonging to a polymorphonuclear leukocyte. Each nuclear component is surrounded by a clear cytoplasm containing numerous individual particles, presumably glycogen granules, and occasional dense granular vesicles plus accumulations of degenerate elements partially engulfed by the main cytoplasmic mass. The boundaries of the polymorphonuclear plasma membrane are demarcated by single arrows. At upper left is the corpse of a degenerate neuron. Its nucleus (N) is dissociated into 3 components as was illustrated in Fig. 9 as a pattern of gradual neuronal degeneration. Cytoplasmic fingers from the PMN leukocyte embrace but do not surround this neuronal corpse. Surrounding the entire complex is a slim continuous margin of cytoplasm (between single and double arrows), relatively rich in RNA granules, which is interpreted as the cytoplasm of a retinal macrophage in the process of incorporating one whole retinal neuron plus one whole PMN leukocyte plus portions of other retinal neurons which the leukocyte was attempting to phagocytize; × 12,000.

on the 20th day, vascularization was complete to the level of the outer plexiform layer. When a single MSG treatment was given on the 10th postnatal day, a retrogression in vascular development ensued. This appeared to be a delayed response primarily affecting vessels most recently developed,

i.e., those at the outer plexiform level. The walls of outer plexiform blood vessels appeared normal 12 hours following treatment, but a subsequent narrowing of vascular lumen was a common finding and a few vessels appeared completely closed at 24 hours. This occurred by a process of rounding up of the endothelial cell until its cell body fully compromised the vascular lumen. No specific signs of a degenerative process affecting the endothelial cell were detected but vessels were completely absent from the outer plexiform zone at 48 hours of treatment (12-day-old retina). Macrophages, normally seen at this retinal level in close association with the vasculature, remained abundant as wandering histiocytes. In the early post treatment period when neurons were undergoing acute necrotic changes, no signs of vascular disturbance were observed other than a possible dilatation of small vessels which appeared filled with red cells.

DISCUSSION

The finding that increasing numbers of cells and additional cell types became vulnerable to MSG treatment with advancing developmental age suggests that some degree of maturity may be a prerequisite of susceptibility to the MSG effect. Lucas and Newhouse (1) reported an absence of retinal degeneration in offspring of female mice treated throughout gestation with 1 Mg. Gm/day of MSG. Although this might be taken as evidence that the administered lead of MSG was effectively shielded from the fetal circulation by placental barriers, it might also be an indication that neurons in the mouse retina were simply not sufficiently mature prior to birth to be susceptible to the MSG effect. Evidence has been presented (16) that the primate placenta, by active transport mechanisms, maintains a glutamic acid concentration in the fetal circulation which is consistently in excess of that in the maternal circulation. In that the primate retina reaches a higher state of maturity *in utero* than does the mouse, this would be an ideal species to test the hypothesis that increased glutamate intake by the mother during gestation might produce retinal damage in the developing embryo.

The fact that MSG treatment of neonatal mice produces a highly predictable and rapidly developing pattern of retinal degeneration does not necessarily imply a direct cytopathic effect of glutamate on retinal neurons. Several lines of evidence, however, suggest that a relatively direct mechanism may be involved. That neurons are particularly sensitive to glutamate is suggested by iontophoretic studies (17) in which accelerated spike activity can be routinely induced by administration of MSG in the extracellular surroundings of cerebral or spinal neurons. According to Krnjevic and Phillis (18), this excitation effect occurs only when MSG is administered in the region of dendritic and somal constituents but is not obtained from regions concentrated in axons. A selective sensitivity of neuronal cell bodies and dendrites to MSG, as suggested by their data, is consistent with findings in the present study of early swelling and degenerative changes in these components of retinal neurons. *In vitro* studies on both retina (19) and brain

slices (20) have shown that glutamic acid uptake supports a high respiratory rate but leads to a net reduction in high energy stores. Early mitochondrial changes noted in the present study may have been a reflection of this phenomenon *in vivo* in that mitochondrial membranes are considered to be closely associated with enzyme systems promoting cellular respiration and oxidative phosphorylation. The marked intracellular edema which occurred very early may have represented fluid imbibition secondary to uptake of glutamic acid and potassium by the cell as is believed to be the phenomenon occurring under *in vitro* conditions (19). On the other hand, acute swelling is a relatively nonspecific change associated with many types of toxic or other injurious influences on cells and, therefore can be interpreted only with caution as a direct reflection of MSG uptake by the cell. Loss of glycogen by the Muller cell would be consistent with *in vitro* data that glutamate uptake by the retina is associated with glucose utilization (19). Hypertrophy of the agranular reticulum in liver cells of the hamster following the administration of drugs such as phenobarbital has been interpreted as an adaptive response enabling the liver to more efficiently eliminate these drugs (21). A similar interpretation might be placed on the observed hypertrophy and whorl formation of agranular reticulum in the retinal Muller process following administration of MSG. Such changes may also have been in response to degenerative products from dying cells; however, no consistent relation to ingestion vacuoles or other morphologic entities in the Muller process were noted.

For parenterally administered MSG to have a direct effect on retinal neurons, it must first gain entry to the retina. Cunha-Vaz *et al.* (22) place the blood retina barrier at the level of the capillary endothelium and have presented evidence that barriers are intact against trypan blue and carbon particles in the neonatal as well as adult retina of rat, cat and rabbit. Of course, barrier phenomena pertaining to trypan blue and carbon particles may well be irrelevant to the question of barrier permeability to MSG; especially in that glutamate is a naturally occurring substance in the blood stream and CNS. The demonstration by Freedman and Potts (2) of high uptake of isotopically labelled glutamate by the rat retina on the 5th postnatal day indicates, insofar as the early neonatal period is concerned, that glutamate can enter the retina from the blood stream. Decline of glutamate uptake by rat retina on the 10th postnatal day (2) may reflect maturational changes in blood retina barriers to MSG. This in turn, would explain the reduced vulnerability of mouse retina to MSG treatment after the 10th postnatal day, assuming vulnerability to be dependent on glutamate entering the retina. To further investigate the possibility of a direct cytopathic effect of MSG on retinal neurons, blood retina barriers could be circumvented and other variables brought under more precise control by use of *in vitro* retina preparations which could be followed for histological changes to supplement findings of a biochemical nature already available.

An alternative mechanism by which the entire retinal lesion might be

explained, and which would not require the passage of MSG across blood retinal barriers, would be through an impairment of blood supply to the inner retina. It was observed that vascularization of the neonatal retina underwent retrogressive changes following MSG treatment. A similar retrogressive effect on the developing retinal vasculature was demonstrated by Ashton (23) in neonatal mice exposed to increased oxygen tension. Although increased oxygen tension interfered drastically with the process of vascularization in the inner retina, acute neuronal necrosis did not ensue. Thus, an interference by MSG in retinal vascular development would probably not explain the acute degenerative changes noted in retinal neurons.

In some respects the acute retinal pathology associated with MSG treatment resembles the findings in retinal ischemia recently described by Shakib *et al.* (15). These authors induced focal ischemia experimentally by microembolization of arterioles in the inner retina of the pig. The lesion induced by this technique was of rapid onset, and affected the same neuronal cell types as does MSG treatment. Certain mitochondrial changes noted in ischemic neurons were the same as those found in the present study and swelling of many neural components accompanied by shrinkage or cytoplasmic condensation in others was featured in both cases. A relatively early clearing of the area by macrophages was noted in both studies. In several respects, however, the findings in these acutely evolving lesions differ. In the ischemic pig retina it was emphasized that degenerative changes in ganglion cell axons occurred very early (within the first hour), whereas, axonal involvement in the glutamate induced lesion was a relatively delayed phenomenon. No particular response was described for the Muller cell of infarcted pig retina but hypertrophy of the endoplasmic reticulum with whorl formation was a conspicuous change induced in Muller cells by MSG treatment. In the pig retina early narrowing of capillary lumina with absence of red cells was noted in regions undergoing neuronal necrosis. In MSG-treated mice neither capillary narrowing nor a reduced red cell content was detected in early periods when retinal edema and neuronal necrosis were strikingly evident. In a later period (24 to 48 hours post treatment) capillary closure was observed but only in the outer plexiform layer. In that species and age variables could account for some of the differences noted, the similarities between the acute histopathology induced by MSG treatment and the process of ischemic necrosis are perhaps all the more noteworthy. It would be helpful to determine with special dye injection and related techniques such as have been used successfully to study circulation dynamics of the retina (24), whether early vessel changes or altered blood flow to the inner retina accompany the administration of MSG. If further studies fail to support ischemia, some type of lesion affecting vascular function on a molecular level might be considered. A lesion paralysing some vital aspect of metabolic exchange between the vascular compartment and retinal neurons could account for acute neuronal necrosis with only minimal or delayed changes reflected in the endothelium.

SUMMARY

A pattern of degeneration affecting certain cells of the inner retina was induced in 9 to 10-day-old mice by a single subcutaneous injection of MSG. Retinas were studied by electron microscopy at serial post treatment intervals to provide a description of the acutely evolving lesion. Swelling of dendrites and cell bodies with mitochondrial transformations occurred as early changes in all neurons affected. Several patterns of degeneration leading to neuronal necrosis were described, but none was specific to a given type of neuron. The Muller process showed reactive reversible changes consisting of swelling, hypertrophied agranular reticulum, and alterations in glycogen content. Phagocytosis was observed on the part of several cell types including the Muller process, and nearly all products of degeneration were removed from the retina within 48 hours of treatment. An apparent primary effect of MSG on retinal vascular development was noted, the significance of which remains to be clarified. Incidental observations concerning the developmental period of greatest retinal vulnerability to the MSG effect were reported. Possible correlations between observed patterns of response to MSG and mechanisms of pathogenesis were discussed.

Acknowledgment: I wish to thank Dr. A. I. Cohen for valuable advice in connection with the present investigation and for the generosity with which he has made electron microscopy facilities available to me.

REFERENCES

1. Lucas, D. R., and Newhouse, J. P.: The Toxic Effect of Sodium L-Glutamate on the Inner Layers of the Retina. AMA Arch. Ophth., 58: 193, 1957.
2. Friedman, J. K., and Potts, A. M.: Repression of Glutaminase I In Rat Retina by Administration of Sodium L-Glutamate. Invest. Ophthal., 1: 118, 1962.
3. ——, and ——: Repression of Glutaminase I in Rat Retina by Administration of Sodium L-Glutamate. Invest. Ophthal., 2: 252, 1963.
4. Potts, A. M., Modrell, R. W., and Kingsbury, C.: Permanent Fractionation of the ERG by Sodium Glutamate. Am. J. Ophth., 50: 900, 1960.
5. Cohen, A. I.: An Electron Microscopic Study of the Modification by Monosodium Glutamate of the Retinas of Normal and "Rodless" Mice. Am. J. Anat., 120: 319, 1967.
6. McIlwain, Henry: *In* Biochemistry and the Central Nervous System, 3rd Edition, p. 118, Boston, Little, Brown, and Company, 1966.
7. Olney, J. W.: An Electron Microscopic Study of Synapse Formation and Other Aspects of Developing Mouse Retina. Invest. Ophthal., 7: 250, 1968.
8. Richardson, K. C., Jarett, L., and Finke, E. H.: Embedding in Epoxy Resins for Ultrathin Sectioning in Electron Microscopy. Stain Technol., 35: 325, 1960.
9. Dowling, J. E., and Boycott, B. B.: Organization of the Primate Retina: Electron Microscopy. Proc. Roy. Soc. B., 166: 80, 1966.
10. Gonatas, N. K., Terry, R. D., Winkler, R., Korey, S. R., Gomez, C. J., and Steine, A.: A Case of Juvenile Lipidosis: The Significance of Electron Microscopic and Biochemical Observations of a Cerebral Biopsy. J. Neuropath. & Exper. Neurol., 22: 557, 1963.
11. De Duve, Christian: The Lysosome. Scientific Am., 208: 64, 1963.
12. ——, and Wattiaux, R.: Functions of Lysosomes. Ann. Rev. Physiol., 28: 435, 1966.

13. Weidman, T. A., and Kuwabara, T.: Postnatal Development of the Rat Retina. Arch. Ophthal., 79: 470, 1968.

14. Olney, J. W.: Unpublished Data.

15. Shakib, M., and Ashton, N.: Ultrastructural Changes in Focal Retinal Ischemia. Brit. J. Ophthal., 50: 325, 1966.

16. Kerr, G. R., and Waisman, H. A.: *In* Transplacental Ratios of Serum free Amino Acids during Pregnancy in the Rhesus Monkey, in Amino Acid Metabolism and Genetic Variation. Ed. Nyhan, W. L., p. 429, Pub. N.Y., McGraw Hill, 1967.

17. Curtis, D. R., Perrin, D. D., and Watkins, J. C.: The Chemical Excitation of Spinal Neurones by Certain Acidic Amino Acids. J. Physiol., 150: 656, 1960.

18. Krnjevic, K., and Phillis, J. W.: Iontophoretic Studies of Neurones in the Mammalian Cerebral Cortex. J. Physiol., 165: 274, 1963.

19. Terner, C., Eggleston, L. V., and Krebs, H. A.: The Role of Glutamic Acid in the Transport of Potassium in Brain and Retina. Biochem. J., 47: 139, 1950.

20. Woodman, R. J., and McIlwain, H.: Glutamic Acid, Other Amino Acids and Related Compounds as Substrates for Cerebral Tissues: Their Effects on Tissue Phosphate. Biochem. J., 81: 83, 1961.

21. Jones, A. L., and Fawcett, D. W.: Hypertrophy of the Agranular Endoplasmic Reticulum in Hamster Liver Induced by Phenobarbital (With a Review on the Functions of this Organelle in Liver). J. Histochem. Cytochem., 14: 215, 1966.

22. Cunha-Vaz, J. G., Shakib, M., and Ashton, N.: Studies on the Permeability of the Blood-Retinal Barrier. Brit. J. Ophthal., 50: 441, 1966.

23. Ashton, N.: Some Aspects of the Comparative Pathology of Oxygen Toxicity in the Retina. Brit. J. Ophthal., 52: 595, 1968.

24. Dollery, C. T., Henkind, P., Paterson, J. W., Ramalho, P. S., and Hill, D. W.: Ophthalmoscopic and Circulatory Changes in Focal Retinal Ischemia. Brit. J. Ophthal., 50: 285, 1966.

NEUROTOXIC EFFECTS OF GLUTAMATE

To the Editor: In a recent article[1] we stated that several animal species are susceptible to the neurotoxic effects of glutamate and related amino acids. In a simultaneously published editorial,[2] Filer and Stegink commented that "the neurotoxic effects of these amino acids in species other than the mouse are debatable." By keeping open the debate, perhaps we can get out the facts on glutamate.

Filer and Stegink invoke "a null effect that may be strain related" as their argument against susceptibility of the rat. We note that the "null effect" referred to was carefully explored by Burde et al.[3,4] and explained in terms of faulty methodology, not strain specificity. In the world literature a dozen separate laboratories[5-12] have reported the rat susceptible to central-nervous-system lesions or associated neuroendocrine and behavioral disturbances from glutamate treatment. Nonrodent species in which neurotoxic effects of glutamate have been reported include rabbit,[13] chick[14] and rhesus monkey.[15,16] We can add abundant unreported data (Olney, J.W., Ho, O.L., Rhee, V., unpublished) further corroborating glutamate-induced brain damage in rabbits, chicks and three strains of rats.

Shifting next to an unstudied species, Filer and Stegink point out that "no neurotoxic effects of glutamate have been reported in the newborn guinea pig. . . ." Some of us (J.O., T.G., V.R.) recently studied guinea pigs because they resemble primates in having a relatively well developed nervous system at birth. We found that the guinea pig, like the primate, is susceptible to glutamate-induced brain damage (Fig. 1*a* and *b*).

Figure 1. Sections (X200) from Three-Day-Old Guinea-Pig Brains in the Region of Junction (←) of Median Eminence (Bottom) and Arcuate-Periventricular Nucleus of the Hypothalamus (Above).

b, from an animal given sodium glutamate, 1 mg per gram subcutaneously, five hours previously shows numerous necrotic neurons. a, from an animal given sodium chloride, 0.34 mg per gram (molar equivalent to 1 mg per gram of sodium glutamate) five hours previously, appears normal.

Although not denying primate susceptibility, Filer and Stegink are troubled by the way Olney and his colleagues[16,17] describe the glutamate lesion in infant monkeys. They complain that it was initially described as a "massive lesion" resembling "that found in the infant mouse" but later as a "microlesion involving 50 to 90 cells seen only in epon sections." Furthermore, they contend that blood glutamate levels in infant monkeys with "massive" lesions did not differ enough from levels in infants with "micro" lesions to explain the "drastic" descriptive differences. This is not an accurate representation of the reported findings.[16,17] The studies cited[16,17] did include descriptions and illustrations of very large lesions in both mouse and monkey brain. Appropriately, they also included descriptions and illustrations of the opposite extreme — very small lesions involving only a few neurons per section — again, in both mouse and monkey brain. There was no mention of epon sections; in fact, aral-

dite (not epon) was the embedding medium used to illustrate all lesions — large, intermediate and small. The largest lesions in infant monkeys were induced by high subcutaneous doses of glutamate and were accompanied by very high blood glutamate levels (70 to 100 mg per 100 ml); the smallest lesions were induced by low oral doses and were accompanied by relatively low blood glutamate levels (20 mg per 100 ml). For a thoroughly consistent and unambiguous presentation of glutamate neuropathology in infant primates we urge Filer and Stegink to re-examine the cited studies[15-19] carefully.

John W. Olney, M.D.
Oi Lan Ho, M.D.
Vesela Rhee, M.D.
Taisija deGubareff, M.S.
Washington University
St. Louis, Mo.

1. Olney JW, Ho OL, Rhee V: Brain-damaging potential of protein hydrolysates. N Engl J Med 289:391-395, 1973
2. Filer LJ, Stegink LD: Safety of hydrolysates in parenteral nutrition. N Engl J Med 289:426-427, 1973
3. Burde RM, Schainker B, Kayes J: Acute effect of oral and subcutaneous administration of monosodium glutamate on the arcuate nucleus of the hypothalamus in mice and rats. Nature (Lond) 233:48-60, 1971
4. Burde RM, Schainker B, Kayes J: Monosodium glutamate: necrosis of hypothalamic neurons in infant rats and mice following either oral or subcutaneous administration. J Neuropathol Exp Neurol 31:181, 1972
5. Olney JW: Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science 164:719-721, 1969
6. Arees E, Mayer J: Monosodium glutamate-induced brain lesions: electron microscopic examination. Science 170:549, 1970
7. Everly JL: Light microscopic examination of monosodium glutamate induced lesions in the brain of fetal and neonatal rats. Anat Rec 169:312, 1971
8. Redding TW, Shalley AV, Arimura A, et al: Effect of monosodium glutamate on some endocrine functions. Neuroendocrinology 8:245-250, 1971
9. Knittle JL, Ginsberg-Fellner F: Cellular and metabolic alterations in obese rats treated with monosodium glutamate during the neonatal period. Program and Abstracts of the American Pediatric Society, Atlantic City, New Jersey, April 29, 1970, p 6
10. Bhagavan HN, Coursin DB, Stewart CN: Monosodium glutamate induced convulsive disorders in rats. Nature (Lond) 232:275, 1971
11. Johnston GAR: Convulsions induced in 10-day-old rats by intraperitoneal injection of monosodium glutamate and related excitant amino acids. Biochem Pharmacol 22:137-140, 1973
12. Mushahwar IK, Koeppe RE: The toxicity of monosodium glutamate in young rats. Biochim Biophys Acta 244:318-321, 1971
13. Pradhan SN, Lynch JF: Behavioral changes in adult rats treated with monosodium glutamate in the neonatal stage. Arch Int Pharmacodyn Ther 197:301-302, 1972
14. Weiss LR, et al: Effects of prolonged monosodium glutamate and other high-salt diets on arterial pressure and learning ability in rats. Toxicol Appl Pharmacol 19:389, 1971
15. Hansson HA: Ultrastructure studies on long-term effects of MSG on rat retina. Virchows Arch [Zellpathol] 6:1, 1970
16. Hamatsu T: Effect of sodium iodate and sodium L-glutamate on ERG and histological structure of retina of adult rabbits. Nippon Ganka Gakkei Zasshi 68:1621, 1964
17. Snapir, et al: Brain damage in male domestic fowl treated with monosodium glutamate. Poult Sci 50:1511-1514, 1971
18. Olney JW, Sharpe LG: Brain lesions in an infant rhesus monkey treated with monosodium glutamate. Science 166:386-388, 1969
19. Olney JW, Sharpe LG, Feigin RD: Glutamate-induced brain damage in infant primates. J Neuropathol Exp Neurol 31:464-488, 1972

Effect of Amino Acid Intake upon Serum Cholesterol in Man. ROBERT F. OLSON,* MILTON Z. NICHAMAN, JUDITH NITTKA, AND LAKELES DORMAN, Pittsburgh, Pa.

It has been shown that low protein diets (25 g per day) containing 36% of calories from relatively saturated fat induce a fall in serum cholesterol of 20% in human subjects (Olson and associates, Amer. J. clin. Nutr. 1958, 6, 310). Supplementation of this diet with single amino acids produced no consistent response. The effect of various pure 1-amino acid mixtures was, therefore, studied to afford better control of the amino acid intake. For 4 weeks four male subjects were fed a control diet containing 100 g of protein, 36% of calories from fat, and all of the essential nutrients. Linoleate supplied 4% of total calories. Each subject was then given a formula diet containing either all of the 1-amino acids in the same proportions as in the control protein mixture or a diet containing the eight essential amino acids in adequate amounts plus glutamate as a source of nonessential nitrogen for 3 to 4 weeks. The caloric value and fat content of these diets were kept constant. The control diet was refed at the end of the experimental period. We found that when all the amino acids were fed, there was no significant change in serum lipids or beta-lipoproteins from control values. When eight essential amino acids plus glutamate were fed, however, serum cholesterol fell 37 mg per 100 ml, phosphatides 19 mg per 100 ml, and beta-lipoproteins 73 mg per 100 ml. Triglycerides actually increased 49 mg per 100 ml. All subjects remained in nitrogen balance during all experimental periods, showing that the hypolipidemic effect is not due to nitrogen imbalance. These data suggest that the formation of beta-lipoproteins by the liver is a function of the nonessential as well as the essential amino acid nitrogen in the diet.

AMINO ACID CONTENT OF FOODS

by

M. L. Orr and B. K. Watt

Household Economics Research Division

Institute of Home Economics

Agricultural Research Service

U. S. Department of Agriculture

Washington, D. C. December 1957

Home Economics Research Report No. 4

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price 45 cents

CONTENTS

	Page
Sources of data	1
Procedures and problems in compiling data	2
Discussion of data in table 1	6
Discussion of data in table 2	46
Published sources of data used in amino acid tables	66
Literature cited	81

TABLES

Table 1.--Amino acids per gram of total nitrogen in edible portion of foods:

Milk; milk products	8
Eggs	10
Meat; poultry; fish; and their products	10
Legumes (dry seed); nuts; other dry seeds	16
Grains and their products	24
Fruits	32
Vegetables	34
Miscellaneous food items	44

Table 2.--Amino acid content of foods, 100 grams, edible portion:

Milk; milk products	48
Eggs	48
Meat; poultry; fish; and their products	48
Legumes (dry seed); nuts; other dry seeds	54
Grains and their products	56
Fruits	60
Vegetables	60
Miscellaneous food items	64

AMINO ACID CONTENT OF FOODS

The importance of having information on amino acid composition of foods was recognized years ago when investigators pointed out that the quality of a protein was related to the kinds and quantities of amino acids it contained. Before 1943 few figures on amino acids were available because analytical methods were complicated and time consuming and their use required highly specialized training.

With the development of altogether new and less costly methods, the volume of information has increased rapidly. Techniques, although not perfected, are being improved, and the volume and quality of data have increased to such an extent that it is now feasible to compile data and prepare tables of amino acid values for foods and feeds (13, 19 1/).

This publication presents the average values that at present appear to be most suitable for estimating the amino acid content of foods, dietaries, and food supplies. It also shows the extent to which data are available for specific foods, thus indicating those for which more determinations are needed.

The two tables presented here have data for the 18 most frequently occurring amino acids. The first, which is the basic table, gives average, maximum, and minimum amino acid values in grams per gram of total nitrogen in the edible portion of the food. The second, calculated from the average values in the first, has been included to provide data that may be used directly to estimate amino acid content of foods of average protein content; it gives averages in grams per 100 grams edible portion.

Part of the material, including data for 12 amino acids, was prepared in preliminary form (21) for use by a committee on protein requirements of the Food and Agriculture Organization of the United Nations, which met in Rome in October 1955. Although the publication was not intended for general circulation and only a few copies were issued, reference to it has appeared in several places. It is now superseded by this revised and more comprehensive report.

SOURCES OF DATA

The amino acid values shown in the tables in this publication have been developed from a study of original analyses of the amino acid content of foods. Research findings from a great many laboratories in this country and abroad have been used. The data have come from agricultural experiment stations, from colleges and universities, and from numerous private and industry-maintained research laboratories. The studies in which data were located had been undertaken to serve a number of purposes--among them, to obtain information on several amino acids in a series of foods, to study the effect on amino content of various production and processing treatments, to perfect details of analytical procedures as applied to different types of foods.

1/ Numbers in parentheses refer to Literature Cited, p. 81.

Most of the amino acid data used for this publication were based on work reported since 1945--nearly half since 1950. In a few scattered instances, work reported before 1945 has been included. The published reports from which data were used are listed on pages 66 to 80. In addition, unpublished data from a number of sources were made available. Appreciation is expressed to the individuals and their laboratories for the data they so generously supplied.

PROCEDURES AND PROBLEMS IN COMPILING DATA

Summarizing data from work done for a wide range of purposes and from a great variety of sources presents many problems. A search for the more important factors that influence amino acid content of food must be made and judgment exercised in making the various decisions on the handling of data.

Selection of a uniform basis for expressing data. A suitable way of expressing the amino acid values was one of the first problems encountered. Investigators differ in their choice of a basis for reporting amino acid values; before data from various sources could be collated all values had to be converted to uniform terms. After the kind of information available and the uses likely to be made of the published tables were considered, it was decided to express the values as grams of each amino acid per gram of total nitrogen present in the food. This way of expressing values is not new; it was used as early as 1937 by Csonka (9) and later by various other investigators (10, 17, 24).

A 1-gram nitrogen basis has certain obvious advantages. Total nitrogen is directly determined; it is usually reported by the investigator; it avoids the use of nitrogen conversion factors, which further research may change. The use of the nitrogen content of each sample as the basis for expressing amino acid content also eliminates variations among samples of a food due to different levels of other constituents; for example, different moisture or fat content. It was hoped that by reducing all data to this uniform basis the effect of special treatment, such as heating or dehydrating, would be more readily apparent.

Among other possibilities considered for expressing amino acid content of foods uniformly was that of using only protein and amino nitrogen rather than total nitrogen. This was rejected because data from too many good sources would have had to be excluded.

Not all data available could be converted to a common basis and used in the derivations of values presented in these tables. Unfortunately, a few sources of amino acid data did not include information on protein or nitrogen content of the samples analyzed. In some cases this information was obtained by correspondence; the remaining sources had to be omitted.

Some other reports were omitted because no units were designated or because of apparent errors in designations. These problems were encountered more often in work with amino acids than with most other nutrients. Perhaps this situation is partly due to the old procedure of reporting amino acids as "percent of the protein"--a procedure that has received much criticism. As a result a few authors adopted "16 grams of nitrogen" as their basis of reporting. This basis

though clear is not altogether satisfactory, since many specific proteins contain considerably more or less nitrogen than this average amount of 16 percent. Much recalculating would be unnecessary if all investigators would express results on the basis of 1 gram of nitrogen.

Identification of samples analyzed. Accurate identification and description of the sample analyzed is essential for the correct use of data. Common names of some items differ from one locality to another even within one country. When data are compiled from global literature the problems of identification are increased. The scientific name of the item in addition to the local name is needed. For this publication the scientific names when given were checked with Standardized Plant Names (16) and both the scientific name and the corresponding common name used. However, there is some confusion in the scientific nomenclature, and additional checking of terms often was needed.

When the parts of the plant or animal tissue actually analyzed are not described, the compiler of data must decide whether or not to include the values and if they are to be included how best to classify the material. Inadequate description of the form of the food item analyzed is an added complication. Some materials analyzed are described only as fresh or as dried samples but without other specific information either as to maturity or as to proximate chemical composition. The latter if given may serve as a clue to the form of the sample.

Comparison of results obtained by different methods. Evaluating the analytical methods used for the determinations is another problem confronting those who review data in the literature. Since 1943, microbiological methods have, to a great extent, replaced the more difficult early chemical methods. The even newer chromatographic methods appear promising. Although the newer procedures are not so complex as the former chemical methods they are far from simple. At each step they require very careful attention to specific conditions for the particular reaction. Detailed discussion of many methods has been presented by Block and associates (2, 3, 4). A number of review articles on various phases of amino acid analysis have also appeared (8, 11, 12, 25).

Hydrolysis, ordinarily the first step in amino acid analysis, is one of the principal sources of error in the determination. Difficulties regarding hydrolysis have been mentioned repeatedly in the literature, yet few workers have reported any extensive preliminary tests regarding adequacy of the hydrolytic method for the particular foods under study. Some investigators have prepared their samples by chemical hydrolysis, others have used enzymes. A few have applied one of these techniques to one portion of a sample and the other to another portion. It has been suggested that chemical hydrolysis is more nearly complete than enzyme hydrolysis and more suitable for use in preparing a food sample for determination of total amino acid content. Enzyme hydrolysis, on the other hand, has been assumed to more nearly simulate the treatment of foods when digested in the body, and enzyme hydrolysates have been considered more suitable for determination of "available" amino acids. Consideration was given to the possibility of separating data by method of preparation of sample, but after a preliminary trial, this classification was abandoned.

Most of the values in the array of data used in the amino acid tables were based on microbiological analysis of chemical hydrolysates. In the relatively

few instances in which split samples had been hydrolyzed, one portion chemically and the other by enzymes, the results based on analysis of the chemical hydrolysate were usually selected. Sometimes there was good agreement between results. Differences were not consistent in size or direction. If only enzyme hydrolysates had been analyzed by an investigator, results were not necessarily excluded. To have done so might have meant eliminating data showing the effect of some important variables, even omitting an item entirely.

The methods used for analysis of the hydrolysates probably varied widely in degree of accuracy. Many of the amino acid values in the array of data on which the values in this publication were based were from studies in which a large number of foods were analyzed by routine procedures. These procedures may not have been equally well adapted to the different types of food being analyzed. Greater accuracy of results from different studies can be expected with the continued discovery of the more important factors that affect results at various steps in analysis.

Many problems in method have been recognized, among which are those relating to measurement of sensitivity of specific organisms, suitability of media, optimum pH, time and temperature of incubation, formation of humin, interference of carbohydrates, conditions under which hydrolysates were stored, and purity of standards. Many improvements in amino acid procedures are anticipated as an outgrowth of the renewed interest and study of these problems.

Collaborative studies, such as the one on methionine in feeds (1), hasten the development and adoption of satisfactory procedures. Because such studies are time consuming, the results lag far behind the wants of the technician or the research worker needing the improved tools. Some laboratories have set up for their own use an internal standard; with each study of new products they have repeated the analyses of a series of items kept on hand to use as a check.

The values in the two tables should be used with the knowledge that averages have been computed even though for some foods the number of determinations was too small or the range too great to provide a satisfactory basis for an average. Considering both the problems arising from the small number of determinations for some foods and the possibility that different adaptations or modifications of a method may have been used, small differences between averages for different kinds or forms of food may not be important.

Organization of summary tables. The order in which to present the amino acid data for maximum convenience in use was another problem for which there was no clear-cut or obvious best solution. The order finally adopted and shown here has met with fewer objections than any of the many others considered.

Of the 18 amino acids included in the table, those of most importance in human nutrition were placed in the positions considered easiest to use. The essential amino acids of greatest interest appear in the columns closest to the descriptions of the food items. Cystine and tyrosine, the semidispensable amino acids, are adjacent to methionine and phenylalanine, respectively, the amino acids for which they are known to have sparing action (22, 23).

Deficiency of methionine in foods and diets is often reported; therefore special consideration needs to be given to the sparing action of the cystine in the foods. Cystine cannot completely replace methionine but at present information is inadequate for calculating the effective methionine value. On the basis of sulfur content of the molecule, 1.0 gram of cystine would be equivalent to 1.24 grams of methionine. In initial experiments with 3 young men, cystine replaced from 80 to 89 percent of the minimum need for methionine (22). Other replacement factors may be found under different experimental conditions.

In the present table a column showing the total quantity of the sulfur-containing amino acids is included. The values are the straight sums of the averages for methionine and cystine contents. Considerable change is to be expected in this column in future revisions.

The table columns for arginine and histidine follow those for the amino acids known to be essential for the human adult; histidine may be essential for the growing child. Both have been determined in a number of foods. For the remaining amino acids, for which foods were infrequently assayed, an alphabetical order was used.

NOTE

The average values for the amino acid content of foods shown in this publication are tentative. They will need to be revised as more data become available. Revision would be greatly facilitated if reprints of published reports or copies of unpublished reports were made available by the investigators. The authors would very much appreciate receiving such material.

DISCUSSION OF DATA IN TABLE 1

Table 1 is a summary of the data available on 18 amino acids expressed in terms of amino acid content per gram of nitrogen present in the food. For each food the average, the maximum, and the minimum values are shown. This table can be used to best advantage when the nitrogen content is known for the food under investigation.

The number of values selected for use in obtaining the mean is also shown in table 1 and is an indication of the extent of available data. It is obvious that amino acid values for the different foods are not equally representative, nor are the different amino acid values for one food; seldom has one sample been analyzed for all 18 of the amino acids shown in table 1. On the whole, fewer analyses have been made for tryptophan, which presents additional analytical problems, than for the other amino acids considered essential--isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine. Fewer analyses have been reported for cystine and tyrosine content of foods than for arginine and histidine.

The maximum and minimum values shown for each amino acid in a food do not in every case include the extreme values reported. For example, averages of results were used from a large collaborative study (24) in which several laboratories participated in analysis of the same samples. Also averages from replicate determinations from a single laboratory or the preferred values, if indicated by the investigators, were used. Occasionally extreme values were arbitrarily eliminated, where they appeared to be entirely unreasonable in view of reports on other samples of the item. Even so, inspection of the maximum and minimum values shows very wide ranges, indicating the need for further intensive study.

The number of food items listed in table 1, about 200, should not be taken to mean that for only this number of items have there been analyses. If the values for related items in a group of foods were within the same range, all the data were combined. For example, in the case of milk, data were available for several forms: fresh fluid, evaporated, and dried whole and nonfat milk. When the separate ranges and averages for each of these milk items were reduced to 1 gram of nitrogen basis, it appeared that differences among items were more probably due to analytical method than to actual difference in these items. For the present it seemed preferable to derive a single mean per gram of nitrogen based on the total number of analyses rather than to keep the items separate. Likewise, for the meats, many different cuts of beef have been analyzed, but when the results were expressed in terms of grams of amino acids per gram of nitrogen it did not seem worthwhile to show the tabulation by cut. In fact, differences among averages for beef, lamb, pork, and veal were small and even further combinations might be desirable.

A great many determinations have been made on amino acids in fish. The range in values reported for each amino acid is unusually wide and there may be real differences among different species or types of fish. For histidine in particular there seems to be some evidence of species difference (20). Histidine values showed such a wide range, from 0.010 to 0.444 gram per gram of nitrogen, that for this amino acid the average, 0.176 for 153 samples, was purposely omitted. About

one-half of the values were between 0.100 and 0.250 gram of histidine per gram of nitrogen. For cystine, perhaps the data should not have been averaged since cystine may be more concentrated in the skin than in the muscle. Almost no mention was made as to whether skin was or was not included in the portion of the fish analyzed. Presumably it would have been included if the investigator considered it edible. Probably the various kinds of fish could be grouped, but a satisfactory classification from the viewpoint of differences in amino acid content was not readily apparent. In this table data for all fish have been combined.

Very few determinations have been located for amino acids in different kinds of shellfish. Only for shrimp, including prawns, have data been shown separately in this table. For all other kinds of shellfish only a few scattered data on one or more amino acids were located. From the limited data available there is no way of knowing how similar the different kinds of shellfish are in amino acid content. As might be expected, foods known to be important sources of protein of high quality have been analyzed by more laboratories and for more variants than have fruits, vegetables, and nuts. Additional determinations are most needed for bread, flour, and potatoes--low protein foods that are important sources because of the relatively large quantities that are consumed. For wheat flours, for example, only 22 reports were found. Of these, only 12 were well enough defined to use in deriving values for white flour. Only one study was located on flour of intermediate extraction (14) and one study (26) on flours of 95, 70, and 30 percent extraction. Particularly helpful were unpublished data from the American Institute of Baking and a recent study by the Institute of Home Economics (14). In both of these studies amino acid values were determined for breads made from flours and wheats that had been analyzed.

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
MILK; MILK PRODUCTS:								
MILK:								
1 Cow, whole, nonfat, evaporated, or dry:								
Average.....grams..	0.090	0.294	0.407	0.626	0.496	0.156	0.057	0.213
Maximum.....do...	.117	.386	.531	.779	.609	.244	.100	--
Minimum.....do...	.062	.225	.275	.513	.325	.102	.025	--
Samples.....number..	91	96	95	108	104	106	13	--
2 Goat.....grams..	.075	.420	.168	.537	.604	.126	--	--
Samples.....number..	1	1	1	1	1	1	--	--
3 Human:								
Average.....grams..	.103	.284	.344	.567	.413	.128	.125	.253
Maximum.....do...	.194	.400	.475	.676	.510	.249	.250	--
Minimum.....do...	.058	.257	.240	.475	.353	.051	.039	--
Samples.....number..	25	13	11	10	13	19	16	--
4 Indian buffalo, whole or dried:								
Average.....grams..	.090	.322	.310	.639	.503	.170	.088	.258
Maximum.....do...	.113	.382	.376	.698	.582	.197	.118	--
Minimum.....do...	.066	.295	.204	.602	.447	.137	.054	--
Samples.....number..	8	8	8	8	8	8	4	--
MILK PRODUCTS:								
5 Buttermilk:								
Average.....grams..	.069	.301	.399	.633	.530	.149	.059	.208
Maximum.....do...	.096	.372	.471	.784	.628	.176	.059	--
Minimum.....do...	.032	.252	.294	.512	.419	.122	.059	--
Samples.....number..	7	6	6	6	6	6	3	--
6 Casein:								
Average.....grams..	.084	.269	.412	.632	.504	.194	.024	.218
Maximum.....do...	.138	.307	.519	.942	.610	.252	.034	--
Minimum.....do...	.060	.225	.256	.517	.393	.134	.019	--
Samples.....number..	48	42	33	34	40	53	22	--
Cheese:								
7 Cheddar, other ripened cheeses 1/, and processed cheese foods:								
Average.....grams..	.087	.237	.430	.622	.468	.166	.036	.202
Maximum.....do...	.117	.282	.708	.784	.539	.216	.066	--
Minimum.....do...	.069	.152	.309	.457	.343	.123	.014	--
Samples.....number..	27	15	12	12	12	12	7	--
8 Cottage:								
Average.....grams..	.067	.298	.371	.685	.536	.176	.055	.231
Maximum.....do...	.073	.302	.449	.780	.596	.198	.064	--
Minimum.....do...	.064	.294	.302	.592	.472	.161	.046	--
Samples.....number..	3	3	3	3	3	3	4	--
9 Cream cheese:								
Average.....grams..	.057	.289	.368	.654	.511	.162	.060	.222
Maximum.....do...	.067	.314	.476	.686	.571	.181	.095	--
Minimum.....do...	.048	.267	.305	.629	.476	.152	.038	--
Samples.....number..	3	3	3	3	3	3	4	--

1/ Includes such kinds as Blue, Limburger, and Swiss.

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											1
0.309	0.325	0.438	0.233	0.168	0.220	0.465	1.491	0.126	0.709	0.376	Average
.423	.400	.541	.315	.310	.253	.535	2.199	.150	1.224	.545	Maximum
.236	.256	.281	.171	.077	.184	.371	1.337	.107	.448	.266	Minimum
100	47	98	99	98	4	6	8	6	6	6	Samples
.235	--	.269	.336	.131	--	--	--	--	--	--	2
1	--	1	1	1	--	--	--	--	---	--	Samples
											3
.272	.323	.391	.253	.138	.221	.498	1.045	.135	.473	.256	Average
.369	.620	.619	.425	.175	.241	.583	1.184	.143	.500	.286	Maximum
.142	.150	.283	.172	.061	.200	.341	.797	.123	.411	.173	Minimum
13	11	12	15	15	5	6	6	6	6	6	Samples
											4
.269	--	.388	.206	.131	--	--	--	--	--	--	Average
.317	--	.482	.221	.145	--	--	--	--	--	--	Maximum
.181	--	.343	.193	.123	--	--	--	--	--	--	Minimum
8	--	8	5	5	--	--	--	--	--	--	Samples
											5
.338	.249	.477	.306	.180	--	--	1.129	--	--	.242	Average
.347	.333	.510	.644	.216	--	--	--	--	--	--	Maximum
.258	.086	.429	.194	.153	--	--	--	--	--	--	Minimum
6	5	5	6	5	--	--	1	--	--	1	Samples
											6
.339	.366	.465	.256	.190	.211	.465	1.450	.125	.739	.418	Average
.400	.431	.601	.456	.265	.238	.522	1.860	.139	.835	.591	Maximum
.250	.304	.347	.216	.131	.151	.419	1.300	.114	.674	.327	Minimum
37	30	31	32	36	6	5	21	11	10	14	Samples
											7
.342	.305	.458	.233	.208	.179	.372	1.745	.098	.731	.364	Average
.479	.384	.560	.294	.243	.181	.517	1.995	.110	.787	.603	Maximum
.221	.115	.362	.172	.171	.175	.199	1.564	.089	.670	.201	Minimum
12	7	12	12	12	3	3	3	3	3	3	Samples
											8
.344	.344	.367	.301	.206	--	--	--	--	--	--	Average
.362	.399	.367	.312	.229	--	--	--	--	--	--	Maximum
.326	.308	.367	.289	.192	--	--	--	--	--	--	Minimum
3	3	3	3	3	--	--	--	--	--	--	Samples
											9
.388	.289	.381	.222	.197	--	--	--	--	--	--	Average
.391	.314	.457	.257	.257	--	--	--	--	--	--	Maximum
.381	.267	.295	.191	.162	--	--	--	--	--	--	Minimum
3	3	3	3	3	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
MILK; MILK PRODUCTS--Continued								
MILK PRODUCTS--Continued								
10 Lactalbumin:								
Average.....grams..	0.143	0.340	0.403	0.801	0.588	0.146	0.221	0.367
Maximum.....do...	.188	.425	.544	1.166	.787	.162	.252	--
Minimum.....do...	.112	.288	.282	.650	.413	.101	.169	--
Samples.....number..	11	9	7	6	11	10	4	--
11 Whey, dried:								
Average.....grams..	.075	.346	.375	.533	.393	.096	.128	.224
Maximum.....do...	.129	.406	.400	.581	.523	.148	.162	--
Minimum.....do...	.037	.296	.345	.444	.296	.040	.094	--
Samples.....number..	5	4	6	6	5	7	2	--
EGGS, CHICKEN:								
12 Whole, raw or dried:								
Average.....grams..	.103	.311	.415	.550	.400	.196	.146	.342
Maximum.....do...	.129	.456	.525	.606	.488	.288	.184	--
Minimum.....do...	.071	.236	.319	.488	.316	.088	.083	--
Samples.....number..	27	27	16	16	26	34	24	--
13 Whites, raw or dried:								
Average.....grams..	.095	.276	.404	.550	.375	.243	.152	.395
Maximum.....do...	.142	.325	.456	.669	.505	.342	.321	--
Minimum.....do...	.066	.219	.286	.444	.238	.162	.056	--
Samples.....number..	54	22	14	16	56	61	51	--
14 Yolks, raw or dried:								
Average.....grams..	.090	.317	.382	.526	.412	.160	.105	.265
Maximum.....do...	.159	.388	.431	.531	.549	.226	.197	--
Minimum.....do...	.066	.219	.252	.519	.312	.121	.089	--
Samples.....number..	36	6	4	4	39	47	42	--
MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS:								
MEAT:								
15 Beef cuts, 2/ fresh or canned:								
Average.....grams..	.073	.276	.327	.512	.546	.155	.079	.234
Maximum.....do...	.119	.350	.374	.622	.755	.198	.114	--
Minimum.....do...	.040	.230	.175	.411	.436	.118	.052	--
Samples.....number..	71	44	42	46	45	47	29	--
16 Lamb cuts, 2/ fresh or canned:								
Average.....grams..	.081	.286	.324	.484	.506	.150	.082	.232
Maximum.....do...	.100	.330	.393	.535	.586	.163	.196	--
Minimum.....do...	.064	.243	.265	.423	.411	.130	.049	--
Samples.....number..	23	22	17	18	17	21	14	--
Pork cuts:								
17 Fresh pork, 2/ raw or canned:								
Average.....grams..	.081	.290	.321	.460	.513	.156	.073	.229
Maximum.....do...	.088	.336	.395	.540	.616	.214	.098	--
Minimum.....do...	.046	.219	.247	.436	.462	.112	.042	--
Samples.....number..	27	22	17	17	21	24	17	--
Cured pork:								
18 Bacon, raw.....grams..	.065	.210	.274	.500	.403	.097	.073	.170
Samples.....number..	1	1	1	1	1	1	1	--
19 Fatback, raw.....grams..	.010	.226	.176	.588	.508	.088	.069	.157
Samples.....number..	1	1	1	1	1	1	1	--

2/ Based on data from many cuts.

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											10
0.283	0.247	0.369	0.227	0.124	0.412	0.643	1.044	--	0.268	0.268	Average
.350	.321	.425	.275	.144	--	--	1.170	--	--	--	Maximum
.204	.181	.268	.200	.094	--	--	.918	--	--	--	Minimum
8	7	6	8	8	1	1	2	--	1	1	Samples
											11
.165	.067	.327	.120	.081	--	--	.695	--	--	.191	Average
.206	.124	.400	.156	.098	--	--	.760	--	--	.222	Maximum
.120	.037	.280	.080	.074	--	--	.630	--	--	.160	Minimum
6	3	6	5	5	--	--	2	--	--	2	Samples
											12
.361	.269	.464	.410	.150	--	.438	.773	0.221	.265	.525	Average
.500	.372	.549	.606	.228	--	.673	.788	.247	.280	.618	Maximum
.279	.163	.414	.350	.086	--	.356	.744	.162	.256	.469	Minimum
26	18	16	21	26	--	4	4	5	4	10	Samples
											13
.399	.260	.487	.367	.135	.472	.492	.860	.234	.236	.491	Average
.506	.335	.581	.411	.175	.631	.687	1.127	.250	.268	.670	Maximum
.281	.189	.381	.262	.075	.361	.356	.443	.203	.205	.400	Minimum
20	16	16	20	20	4	6	12	6	8	12	Samples
											14
.275	.290	.430	.434	.141	--	.344	.748	.219	.277	.554	Average
.288	.299	.462	.450	.181	--	.350	.762	.225	.281	.566	Maximum
.233	.281	.366	.417	.094	--	.338	.738	.206	.269	.538	Minimum
6	5	4	7	6	--	3	3	3	3	6	Samples
											15
.257	.212	.347	.403	.217	.361	.583	.946	.387	.308	.262	Average
.366	.269	.436	.469	.278	.400	.694	1.070	.536	.402	.300	Maximum
.212	.156	.306	.337	.112	.205	.525	.769	.263	.220	.237	Minimum
46	35	42	48	53	7	25	23	26	23	21	Samples
											16
.254	.217	.308	.407	.174	.349	.576	.948	.365	.289	.250	Average
.306	.306	.346	.474	.214	.388	.731	1.130	.447	.328	.312	Maximum
.230	.179	.286	.374	.141	.300	.463	.800	.212	.256	.229	Minimum
19	13	18	17	21	4	11	11	11	11	11	Samples
											17
.246	.223	.325	.383	.216	.290	.592	.956	.310	.276	.254	Average
.279	.276	.364	.434	.294	.400	.699	1.160	.407	.316	.331	Maximum
.219	.160	.301	.281	.135	.185	.521	.831	.151	.256	.201	Minimum
20	15	17	19	25	5	11	11	12	11	12	Samples
.298	.161	.298	.427	.169	--	.589	.702	.589	.331	.242	18
1	1	1	1	1	--	1	1	1	1	1	Samples
.252	.084	.269	.608	.056	--	--	--	--	--	--	19
1	1	1	1	1	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS--Continued								
MEAT--Continued								
Pork cuts--Continued								
Cured pork--Continued								
20 Ham and other cured pork, raw, cooked, canned:								
Average.....grams..	0.060	0.256	0.311	0.483	0.525	0.152	0.101	0.253
Maximum.....do...	.082	.306	.381	.519	.707	.175	.108	--
Minimum.....do...	.044	.181	.269	.419	.418	.113	.094	--
Samples.....number..	25	25	29	29	27	26	5	--
21 Rabbit, muscle:								
Average.....grams..	--	.304	.322	.487	.541	.161	--	--
Maximum.....do...	--	.312	.325	.494	.551	.169	--	--
Minimum.....do...	--	.300	.318	.477	.523	.156	--	--
Samples.....number..	--	3	3	3	3	3	--	--
22 Veal cuts, fresh or canned:								
Average.....grams..	.082	.271	.330	.458	.522	.143	.074	.217
Maximum.....do...	.106	.319	.394	.505	.601	.152	.095	--
Minimum.....do...	.065	.238	.281	.382	.464	.124	.060	--
Samples.....number..	12	13	8	9	8	7	10	--
POULTRY:								
23 Chicken, muscle without skin:								
Average.....grams..	.076	.266	.330	.452	.549	.163	.084	.247
Maximum.....do...	.094	.293	.356	.469	.622	.195	.089	--
Minimum.....do...	.056	.238	.288	.434	.469	.134	.078	--
Samples.....number..	4	6	6	6	6	6	2	--
24 Duck, muscle without skin:								
Average.....grams..	--	.273	.324	.484	.538	.155	--	--
Maximum.....do...	--	.276	.327	.485	.549	.156	--	--
Minimum.....do...	--	.270	.320	.483	.528	.154	--	--
Samples.....number..	--	2	2	2	2	2	--	--
25 Turkey, muscle without skin:								
Average.....grams..	--	.264	.328	.478	.566	.173	.086	.259
Maximum.....do...	--	.294	.346	.500	.603	.184	.094	--
Minimum.....do...	--	.216	.311	.456	.527	.168	.079	--
Samples.....number..	--	4	4	4	4	4	2	--
FISH AND SHELLFISH:								
26 Fish, raw or canned:								
Average.....grams..	.062	.271	.317	.472	.548	.182	.084	.266
Maximum.....do...	.106	.506	.496	.781	1.238	.292	.215	--
Minimum.....do...	.017	.131	.156	.374	.230	.073	.031	--
Samples.....number..	182	155	133	139	157	185	42	--
Shellfish:								
27 Shrimp, including prawns, raw or canned:								
Average.....grams..	.056	.252	.324	.563	.453	.177	.142	.319
Maximum.....do...	.069	.288	.362	.694	.594	.231	.287	--
Minimum.....do...	.025	.183	.300	.488	.253	.138	.048	--
Samples.....number..	8	6	7	8	12	8	8	--
28 Other shellfish:								
Average.....grams..	.054	.186	.296	.535	.403	.163	.112	.275
Maximum.....do...	.104	.331	.309	.706	.475	.273	.172	--
Minimum.....do...	.022	.120	.269	.449	.329	.106	.090	--
Samples.....number..	7	9	3	3	5	16	8	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											20
0.239	0.241	0.325	0.395	0.201	--	0.561	0.884	0.412	0.313	0.257	Average
.277	.250	.356	.464	.231	--	.632	.988	.477	.318	.272	Maximum
.206	.231	.287	.338	.169	--	.481	.706	.369	.302	.227	Minimum
25	5	29	25	25	--	15	15	15	5	5	Samples
											21
.236	--	.304	.350	.141	--	.633	1.080	.285	--	--	Average
.238	--	.306	.352	.144	--	.659	1.119	.295	--	--	Maximum
.233	--	.301	.347	.136	--	.600	1.060	.267	--	--	Minimum
3	--	3	3	3	--	3	3	3	--	--	Samples
											22
.254	.225	.323	.407	.201	0.371	.616	.975	.299	.252	.275	Average
.276	.304	.358	.471	.226	.394	.694	1.079	.426	.301	.306	Maximum
.244	.182	.281	.327	.149	.338	.567	.833	.256	.218	.225	Minimum
10	8	9	10	10	6	7	7	7	7	7	Samples
											23
.246	.220	.307	.395	.180	--	.614	1.004	.418	--	--	Average
.258	.223	.336	.441	.233	--	.622	1.036	.429	--	--	Maximum
.232	.216	.285	.370	.137	--	.606	.971	.407	--	--	Minimum
6	2	6	6	6	--	2	2	2	--	--	Samples
											24
.246	--	.300	.380	.142	--	.658	1.062	.315	--	--	Average
.247	--	.307	.381	.142	--	.659	1.070	.340	--	--	Maximum
.244	--	.294	.378	.142	--	.657	1.054	.290	--	--	Minimum
2	--	2	2	2	--	2	2	2	--	--	Samples
											25
.250	--	.309	.394	.169	--	.632	1.112	.338	--	--	Average
.274	--	.331	.442	.241	--	.636	1.123	.347	--	--	Maximum
.233	--	.290	.361	.135	--	.629	1.102	.328	--	--	Minimum
4	--	4	4	4	--	2	2	2	--	--	Samples
											26
.232	.169	.333	.352	--	--	.551	.796	.345	.381	.193	Average
.320	.294	.619	.600	.444	--	.638	.950	.469	.411	.195	Maximum
.125	.072	.181	.162	.010	--	.462	.621	.252	.353	.188	Minimum
138	60	139	134	153	--	43	43	43	3	3	Samples
											27
.292	.243	.307	.553	.227	.308	.581	.969	.398	--	--	Average
.400	.320	.331	.653	.540	.360	.606	1.000	.444	--	--	Maximum
.238	.160	.256	.469	.100	.267	.556	.938	.350	--	--	Minimum
11	4	7	13	13	4	3	3	3	--	--	Samples
											28
.210	.183	.448	.388	.090	--	.618	.763	.305	.390	.206	Average
.236	.207	.585	.476	.112	--	.703	.769	.321	.419	.218	Maximum
.169	.158	.181	.329	.075	--	.532	.737	.289	.362	.195	Minimum
3	4	3	6	6	--	2	2	2	2	2	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS--Continued								
PRODUCTS FROM MEAT, POULTRY, AND FISH:								
29 Brains:								
Average.....grams..	0.083	0.297	0.303	0.508	0.457	0.132	0.087	0.219
Maximum.....do...	.102	.349	.350	.594	.581	.166	.102	--
Minimum.....do...	.062	.244	.238	.266	.374	.110	.081	--
Samples.....number..	15	16	12	13	14	18	4	--
30 Chitterlings.....grams..	.068	.209	.224	.332	.487	.140	.079	.219
Samples.....number..	1	1	1	1	1	1	1	--
31 Fish flour:								
Average.....grams..	.062	.360	.348	.509	.607	.166	--	--
Maximum.....do...	.076	.449	.468	.846	1.058	.197	--	--
Minimum.....do...	.054	.188	.280	.392	.493	.143	--	--
Samples.....number..	7	8	7	8	8	8	--	--
32 Gelatin:								
Average.....grams..	.0004	.124	.088	.190	.274	.051	.005	.056
Maximum.....do...	.0020	.169	.136	.250	.380	.079	.006	--
Minimum.....do...	.000	.094	.038	.138	.223	.025	.004	--
Samples.....number..	8	11	13	14	15	15	4	--
33 Gizzard, chicken:								
Average.....grams..	.056	.290	.296	.457	.424	.150	.059	.209
Maximum.....do...	.064	.297	.318	.539	.473	.161	--	--
Minimum.....do...	.049	.283	.275	.375	.375	.138	--	--
Samples.....number..	2	2	2	2	2	2	1	--
34 Heart:								
Average.....grams..	.081	.287	.317	.558	.513	.149	.062	.211
Maximum.....do...	.089	.396	.409	.690	.665	.193	.084	--
Minimum.....do...	.058	.241	.274	.497	.434	.128	.055	--
Samples.....number..	32	29	25	26	27	32	6	--
35 Kidney:								
Average.....grams..	.092	.277	.304	.542	.453	.128	.076	.204
Maximum.....do...	.130	.308	.388	.625	.525	.168	.088	--
Minimum.....do...	.065	.219	.271	.483	.388	.105	.062	--
Samples.....number..	32	22	16	17	18	24	5	--
36 Liver:								
Average.....grams..	.094	.297	.327	.577	.468	.147	.077	.224
Maximum.....do...	.113	.369	.406	.738	.588	.258	.123	--
Minimum.....do...	.046	.206	.250	.471	.345	.121	.062	--
Samples.....number..	43	41	36	36	37	42	7	--
37 Pancreas:								
Average.....grams..	.081	.290	.316	.488	.461	.113	--	--
Maximum.....do...	.101	.364	.383	.531	.512	.120	--	--
Minimum.....do...	.048	.225	.256	.425	.388	.106	--	--
Samples.....number..	4	5	5	5	5	4	--	--
38 Pork and beef, canned:								
Average.....grams..	.066	.270	.319	.520	.588	.143	.114	.257
Maximum.....do...	--	--	--	--	.679	.151	--	--
Minimum.....do...	--	--	--	--	.498	.135	--	--
Samples.....number..	1	1	1	1	2	2	1	--
39 Potted meat, canned.....grams..	.058	.257	.249	.467	.412	.140	--	--
Samples.....number..	1	1	1	1	1	1	--	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											29
0.304	0.260	0.322	0.369	0.167	0.360	0.682	0.875	0.252	0.287	0.380	Average
.364	.319	.388	.412	.194	.412	.747	.977	.300	.305	.454	Maximum
.238	.238	.100	.319	.144	.276	.625	.731	.178	.275	.270	Minimum
16	6	13	16	17	4	4	4	4	3	4	Samples
.261	.166	.336	1.022	.123	--	--	--	--	--	--	30
1	1	1	1	1	--	--	--	--	--	--	Samples
											31
.234	--	.322	.428	.106	--	--	--	--	--	--	Average
.292	--	.396	.500	.150	--	--	--	--	--	--	Maximum
.084	--	.267	.345	.076	--	--	--	--	--	--	Minimum
8	--	8	7	7	--	--	--	--	--	--	Samples
											32
.132	.026	.157	.510	.050	.550	.372	.614	1.478	.893	.214	Average
.158	.051	.214	.625	.062	.624	.388	.653	1.700	1.023	.254	Maximum
.112	.012	.131	.450	.037	.438	.361	.512	1.199	.822	.173	Minimum
15	12	12	16	16	7	6	7	10	6	8	Samples
											33
.262	.184	.302	.471	.130	--	--	--	--	--	--	Average
.326	--	.369	.594	.155	--	--	--	--	--	--	Maximum
.199	--	.236	.347	.106	--	--	--	--	--	--	Minimum
2	1	2	2	2	--	--	--	--	--	--	Samples
											34
.283	.232	.360	.395	.160	.412	.659	.930	.344	.301	.284	Average
.319	.276	.440	.465	.191	.425	.703	1.090	.465	.309	.294	Maximum
.257	.182	.306	.333	.109	.391	.594	.799	.293	.288	.277	Minimum
29	6	26	29	32	3	12	12	12	3	3	Samples
											35
.294	.232	.365	.389	.157	.380	.641	.816	.374	.323	.353	Average
.342	.289	.414	.433	.188	.394	.703	.859	.400	.344	.362	Maximum
.269	.164	.325	.362	.135	.359	.612	.762	.359	.312	.338	Minimum
20	6	17	22	20	3	4	4	4	3	3	Samples
											36
.315	.234	.393	.381	.166	.400	.644	.850	.380	.321	.327	Average
.379	.287	.441	.554	.238	.406	.723	1.027	.596	.362	.344	Maximum
.269	.205	.322	.212	.116	.394	.511	.621	.314	.273	.290	Minimum
41	10	36	35	39	3	15	15	16	4	4	Samples
											37
.260	.273	.335	.357	.123	--	.600	.524	.381	--	--	Average
.288	--	.356	.429	.144	--	--	.644	--	--	--	Maximum
.238	--	.306	.319	.091	--	--	.403	--	--	--	Minimum
5	1	5	5	5	--	1	2	1	--	--	Samples
											38
.253	.242	.354	.459	.201	--	.651	.879	.489	.362	.262	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
1	1	1	1	1	--	1	1	1	1	1	Samples
.249	--	.366	.389	.125	--	.514	.677	.658	.447	--	39
1	--	1	1	1	--	1	1	1	1	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS--Continued								
PRODUCTS FROM MEAT; POULTRY AND FISH--Continued								
Sausage:								
40 Head cheese.....grams..	0.033	0.174	0.212	0.394	0.378	0.104	0.087	0.191
Samples.....number..	1	1	1	1	1	1	1	--
41 Sausage containing liver:								
Average.....grams..	.070	.271	.306	.524	.487	.130	.076	.206
Maximum.....do...	.080	.299	.384	.607	.564	.132	.125	--
Minimum.....do...	.051	.225	.239	.462	.375	.124	.051	--
Samples.....number..	4	4	4	4	4	4	5	--
42 Other sausage:								
Average.....grams..	.053	.256	.303	.448	.503	.132	.078	.210
Maximum.....do...	.066	.327	.343	.498	.629	.162	.106	--
Minimum.....do...	.032	.204	.243	.408	.443	.094	.047	--
Samples.....number..	14	14	12	14	14	12	16	--
43 Tongue, fresh or smoked:								
Average.....grams..	.075	.270	.302	.490	.520	.136	.079	.215
Maximum.....do...	.090	.304	.359	.561	.634	.156	.092	--
Minimum.....do...	.065	.200	.256	.396	.444	.117	.066	--
Samples.....number..	19	10	8	9	9	11	5	--
44 Veal and pork loaf, canned.....grams..	.072	.228	.312	.449	.457	.152	.076	.228
Samples.....number..	1	1	1	1	1	1	1	1
LEGUMES (DRY SEED); COMMON NUTS; OTHER NUTS AND DRY SEEDS; THEIR PRODUCTS:								
LEGUME SEEDS AND THEIR PRODUCTS:								
Beans, includes kidney, navy, pinto, red, others (Phaseolus vulgaris):								
45 Raw:								
Average.....grams..	.058	.271	.355	.537	.464	.063	.062	.125
Maximum.....do...	.102	.335	.420	.825	.594	.115	.126	--
Minimum.....do...	.025	.162	.269	.450	.331	.033	.037	--
Samples.....number..	49	16	14	15	26	25	9	--
46 Baked with pork, canned:								
Average.....grams..	.061	.295	.314	.524	.381	.064	.019	.083
Maximum.....do...	.067	.324	.333	.552	--	.076	.029	--
Minimum.....do...	.048	.276	.295	.495	--	.048	.009	--
Samples.....number..	3	3	3	3	3	3	4	--
47 Baked with tomato sauce, canned:								
Average.....grams..	.056	.261	.275	.464	.311	.047	.025	.072
Maximum.....do...	.067	.275	.317	.550	.342	.058	--	--
Minimum.....do...	.042	.250	.242	.400	.292	.042	--	--
Samples.....number..	3	3	3	3	3	3	3	--
48 Black gram (Phaseolus mungo):								
Average.....grams..	.064	.212	.368	.546	.400	.088	.076	.164
Maximum.....do...	.130	.270	.454	.636	.503	.142	.138	--
Minimum.....do...	.025	.136	.309	.450	.319	.047	.042	--
Samples.....number..	6	6	3	3	6	7	4	--
49 Broad beans (Vicia faba):								
Average.....grams..	.058	.204	.392	.544	.351	.026	.044	.070
Maximum.....do...	.081	.244	.438	.612	.355	.034	.068	--
Minimum.....do...	.019	.165	.346	.475	.346	.019	.021	--
Samples.....number..	5	2	2	2	2	2	2	--

TABLE 1.—AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
0.237	0.237	0.257	0.448	0.116	--	0.556	0.660	0.751	0.506	0.245	40
1	1	1	1	1	--	1	1	1	1	1	Samples
											41
.284	.191	.388	.387	.186	--	.605	.715	.560	.395	.305	Average
.300	.285	.470	.470	.218	--	--	--	--	--	--	Maximum
.274	.128	.342	.346	.155	--	--	--	--	--	--	Minimum
4	4	4	4	4	--	1	1	1	1	1	Samples
											42
.228	.203	.314	.434	.168	--	.589	.854	.524	.356	.256	Average
.283	.248	.306	.488	.188	--	.650	.996	.616	.412	.271	Maximum
.164	.149	.224	.383	.140	--	.560	.764	.430	.324	.241	Minimum
14	14	12	10	12	--	7	7	7	7	7	Samples
											43
.252	.209	.320	.406	.157	0.398	.616	.955	.418	.350	.291	Average
.289	.228	.350	.458	.180	.425	.699	1.100	.469	.368	.325	Maximum
.204	.192	.284	.362	.122	.357	.556	.767	.349	.338	.251	Minimum
11	5	9	11	14	3	5	5	5	4	4	Samples
.225	.170	.348	.333	.141	--	.507	.739	.536	.370	.239	44
1	1	1	1	1	--	1	1	1	1	1	Samples
											45
.345	.241	.379	.376	.178	.356	.419	1.000	.106	.269	.325	Average
.462	.359	.445	.588	.229	--	--	--	--	--	--	Maximum
.206	.131	.300	.294	.094	--	--	--	--	--	--	Minimum
24	19	16	18	16	1	1	1	1	1	1	Samples
											46
.359	.178	.336	.270	.200	--	--	--	--	--	--	Average
.429	.200	.371	.305	.200	--	--	--	--	--	--	Maximum
.324	.162	.305	.219	.200	--	--	--	--	--	--	Minimum
3	3	3	3	3	--	--	--	--	--	--	Samples
											47
.281	.158	.264	.367	.144	--	--	--	--	--	--	Average
.308	.183	.275	.367	.158	--	--	--	--	--	--	Maximum
.267	.133	.250	.367	.133	--	--	--	--	--	--	Minimum
3	3	3	3	3	--	--	--	--	--	--	Samples
											48
.329	.146	.384	.411	.148	.237	.583	.878	.260	.253	.253	Average
.365	--	.433	.456	.171	--	--	--	--	--	--	Maximum
.248	--	.328	.356	.125	--	--	--	--	--	--	Minimum
5	1	4	2	2	1	1	1	1	1	1	Samples
											49
.260	.169	.314	.438	.154	.165	.405	.855	.233	.283	.357	Average
.306	.192	.321	.494	.227	--	--	--	--	--	--	Maximum
.215	.144	.306	.371	.112	--	--	--	--	--	--	Minimum
2	4	2	4	4	1	1	1	1	1	1	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

	ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
							METH-IONINE	CYSTINE	TOTAL
	LEGUMES (DRY SEED); COMMON NUTS; OTHER NUTS AND DRY SEEDS; THEIR PRODUCTS--Continued								
	LEGUME SEEDS AND THEIR PRODUCTS--Continued								
	Chickpeas (Cicer arietinum):								
50	Average.....grams..	0.051	0.222	0.359	0.462	0.431	0.083	0.089	0.172
	Maximum.....do...	.094	.298	.388	.501	.573	.133	.150	--
	Minimum.....do...	.025	.169	.319	.421	.336	.046	.041	--
	Samples.....number..	11	8	4	3	10	11	7	--
51	Cowpeas (Vigna spp.):								
	Average.....grams..	.060	.246	.303	.468	.407	.096	.081	.177
	Maximum.....do...	.098	.284	.325	.501	.533	.203	.117	--
	Minimum.....do...	.037	.203	.290	.431	.232	.043	.043	--
	Samples.....number..	14	8	8	8	14	14	6	--
52	Dolichos, twinflower (Dolichos biflorus):								
	Average.....grams..	.064	.242	.419	.494	.492	.085	.139	.224
	Maximum.....do...	.119	.247	--	--	.519	.165	.193	--
	Minimum.....do...	.034	.238	--	--	.466	.041	.102	--
	Samples.....number..	3	2	1	1	2	3	3	--
53	Lentils (Lens culinaris):								
	Average.....grams..	.054	.224	.329	.440	.382	.045	.051	.096
	Maximum.....do...	.125	.312	.408	.532	.462	.095	.111	--
	Minimum.....do...	.011	.149	.272	.339	.306	.025	.019	--
	Samples.....number..	11	10	7	6	10	10	5	--
54	Lima beans (Phaseolus lunatus):								
	Average.....grams..	.059	.296	.362	.520	.416	.100	.094	.194
	Maximum.....do...	.071	.298	.374	.529	.474	.121	.115	--
	Minimum.....do...	.044	.295	.349	.511	.354	.078	.073	--
	Samples.....number..	4	2	2	3	6	6	2	--
55	Lupine (Lupinus spp.).....grams..	--	.213	.313	.380	.280	.022	--	--
	Samples.....number..	--	1	1	1	1	1	--	--
56	Moth beans (Phaseolus aconitifolius).....grams..	.042	--	.280	.380	.308	.049	.028	.077
	Samples.....number..	1	--	1	1	1	1	1	--
57	Mung beans (Phaseolus aureus):								
	Average.....grams..	.046	.196	.346	.564	.427	.068	.039	.107
	Maximum.....do...	.066	.254	.392	.772	.578	.117	.074	--
	Minimum.....do...	.028	.118	.299	.478	.238	.039	.018	--
	Samples.....number..	6	6	2	5	8	7	5	--
58	Peanuts, flour, meal, peanut butter (Arachis hypogaea):								
	Average.....grams..	.069	.168	.257	.380	.223	.055	.094	.149
	Maximum.....do...	.125	.206	.315	.475	.283	.106	.120	--
	Minimum.....do...	.035	.100	.169	.281	.156	.032	.056	--
	Samples.....number..	26	21	22	23	26	24	8	--
59	Peas (Pisum sativum):								
	Average.....grams..	.066	.241	.352	.517	.458	.075	.081	.156
	Maximum.....do...	.124	.306	.446	.638	.554	.155	.125	--
	Minimum.....do...	.016	.175	.288	.425	.394	.019	.029	--
	Samples.....number..	14	10	7	7	10	55	30	--

TABLE 1.—AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											50
0.304	0.208	0.308	0.466	0.168	0.213	0.588	0.676	0.224	0.368	0.246	Average
.423	.306	.394	.562	.210	--	--	--	--	--	--	Maximum
.234	.125	.250	.267	.131	--	--	--	--	--	--	Minimum
7	4	5	8	5	1	1	1	1	1	1	Samples
											51
.327	.165	.353	.402	.189	--	--	--	--	--	--	Average
.364	--	.396	.472	.204	--	--	--	--	--	--	Maximum
.311	--	.327	.338	.162	--	--	--	--	--	--	Minimum
8	1	8	8	8	--	--	--	--	--	--	Samples
											52
.430	.162	.372	.356	.188	.276	.852	.946	.222	.406	.358	Average
.531	--	.406	.375	--	--	--	--	--	--	--	Maximum
.330	--	.338	.338	--	--	--	--	--	--	--	Minimum
2	1	2	2	1	1	1	1	1	1	1	Samples
											53
.276	.166	.340	.477	.137	.222	.730	.925	.325	.270	.285	Average
.332	.227	.403	.579	.160	--	--	--	--	--	--	Maximum
.234	.112	.301	.294	.106	--	--	--	--	--	--	Minimum
9	4	8	7	6	1	1	1	1	1	1	Samples
											54
.369	.164	.392	.397	.202	--	--	--	--	--	--	Average
.386	--	.485	.428	.214	--	--	--	--	--	--	Maximum
.352	--	.355	.368	.189	--	--	--	--	--	--	Minimum
2	1	4	5	2	--	--	--	--	--	--	Samples
.246	--	.257	.526	.157	--	.582	1.320	.224	--	--	55
1	--	1	1	1	--	1	1	1	--	--	Samples
.257	.319	.178	--	.185	--	--	--	--	--	--	56
1	1	1	--	1	--	--	--	--	--	--	Samples
											57
.299	.100	.370	.351	.139	.187	.536	.720	.115	.274	.188	Average
.389	.115	.400	.640	.168	--	--	.731	--	.329	.198	Maximum
.172	.086	.330	.160	.095	--	--	.708	--	.219	.178	Minimum
7	2	5	4	3	1	1	2	1	2	2	Samples
											58
.316	.224	.311	.669	.152	.222	.878	1.204	.347	.318	.412	Average
.388	.289	.469	.862	.198	.262	.944	1.360	.356	.325	--	Maximum
.269	.175	.231	.537	.112	.181	.811	1.090	.336	.310	--	Minimum
21	8	23	22	21	2	2	4	3	2	1	Samples
											59
.315	.252	.350	.552	.171	.252	.766	1.466	.362	.352	.243	Average
.401	.362	.488	.671	.288	.270	.990	1.990	.380	--	--	Maximum
.228	.166	.259	.375	.134	.235	.541	.941	.345	--	--	Minimum
10	6	8	8	10	2	2	2	2	1	1	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
LEGUMES (DRY SEED); COMMON NUTS; OTHER NUTS AND DRY SEEDS; THEIR PRODUCTS--Continued								
LEGUME SEEDS AND THEIR PRODUCTS--Continued								
60 Pigeon peas (Cajanus cajan):								
Average.....grams..	0.034	0.238	0.384	0.490	0.451	0.073	0.088	0.161
Maximum.....do...	.071	.369	.411	.542	.625	.135	.166	--
Minimum.....do...	.011	.144	.357	.437	.388	.021	.024	--
Samples.....number..	5	5	2	2	6	6	4	--
61 Soybeans and flour (Glycine max):								
Average.....grams..	.086	.246	.336	.482	.395	.084	.111	.195
Maximum.....do...	.138	.326	.462	.551	.512	.138	.146	--
Minimum.....do...	.044	.169	.250	.375	.230	.042	.062	--
Samples.....number..	74	54	65	59	90	95	24	--
62 Soybean curd.....grams..	--	--	--	--	--	.066	.074	.140
Samples.....number..	--	--	--	--	--	1	1	--
63 Soybean milk:								
Average.....grams..	.085	.296	.294	.512	.452	.090	.120	.210
Maximum.....do...	.109	.331	.312	.568	.611	.108	.145	--
Minimum.....do...	.071	.261	.275	.456	.319	.072	.070	--
Samples.....number..	3	2	2	2	3	4	3	--
64 Vetch (Vicia sativa):								
Average.....grams..	.044	.195	.477	.497	.412	.075	.073	.148
Maximum.....do...	.069	.295	.542	.582	.502	.136	.103	--
Minimum.....do...	.017	.143	.412	.412	.325	.025	.043	--
Samples.....number..	4	5	2	2	5	4	2	--
COMMON NUTS AND THEIR PRODUCTS:								
65 Almonds (Prunus amygdalus).....grams..	.049	.170	.243	.405	.162	.072	.105	.177
Samples.....number..	1	1	1	1	1	1	1	--
66 Brazil nuts (Bertholletia excelsa):								
Average.....grams..	.071	.160	.225	.428	.168	.357	.191	.548
Maximum.....do...	.082	.178	.235	.434	.202	.374	--	--
Minimum.....do...	.060	.142	.215	.421	.133	.340	--	--
Samples.....number..	2	2	2	2	2	2	1	--
67 Cashews (Anacardium occidentale).....grams..	.135	.211	.350	.436	.227	.101	.151	.252
Samples.....number..	1	1	1	1	1	1	1	--
68 Coconut and other palm family nuts and meals: 3/								
Average.....grams..	.052	.201	.281	.419	.237	.110	.097	.207
Maximum.....do...	.066	.236	.331	.494	.354	.150	.113	--
Minimum.....do...	.027	.182	.232	.378	.162	.078	.081	--
Samples.....number..	8	7	8	8	11	12	2	--
69 Filberts (Corylus spp.):								
Average.....grams..	.068	.173	.356	.392	.174	.058	.069	.127
Maximum.....do...	.124	.185	.434	.401	.196	.075	.154	--
Minimum.....do...	.068	.165	.282	.385	.151	.044	.027	--
Samples.....number..	3	3	3	3	3	3	3	--

Peanuts and peanut products. See Legume seeds.

3/ Including coconut (Cocos nucifera), babassu (Orbignya speciosa), palm cohune (Orbignya cohune), and palm nut (Elaeis guineensis).

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											60
0.535	0.207	0.329	0.425	0.176	0.244	0.767	1.128	0.213	0.332	0.372	Average
.568	--	.354	.476	.213	--	--	--	--	--	--	Maximum
.497	--	.312	.336	.140	--	--	--	--	--	--	Minimum
4	1	3	3	2	1	1	1	1	1	1	Samples
											61
.309	.199	.328	.452	.149	.257	.758	1.147	.261	.420	.408	Average
.344	.269	.400	.519	.188	.308	.796	1.330	.288	.462	.438	Maximum
.200	.118	.250	.330	.075	.206	.694	.965	.219	.378	.379	Minimum
57	11	58	68	57	2	3	37	4	2	2	Samples
--	--	--	--	--	--	--	--	--	--	--	62
--	--	--	--	--	--	--	--	--	--	--	Samples
											63
.328	.325	.312	.508	.203	.262	.719	.925	.288	--	.406	Average
.355	--	.355	.519	.231	--	--	--	--	--	--	Maximum
.300	--	.269	.497	.175	--	--	--	--	--	--	Minimum
2	1	2	2	2	1	1	1	1	--1	1	Samples
											64
.220	.080	.313	.488	.143	.150	.569	.589	.112	.319	.141	Average
.260	--	.438	--	.156	--	--	--	--	--	--	Maximum
.144	--	.208	--	.130	--	--	--	--	--	--	Minimum
3	1	3	1	2	1	1	1	1	1	1	Samples
.319	.172	.313	.760	.144	--	--	--	--	--	--	65
1	1	1	1	1	--	--	--	--	--	--	Samples
											66
.234	.183	.312	.852	.139	--	--	--	--	--	--	Average
.261	--	.321	.876	.142	--	--	--	--	--	--	Maximum
.206	--	.302	.827	.136	--	--	--	--	--	--	Minimum
2	1	2	2	2	--	--	--	--	--	--	Samples
.271	.204	.456	.601	.119	--	--	--	--	--	--	67
1	1	1	1	1	--	--	--	--	--	--	Samples
											68
.271	.158	.331	.757	.108	--	--	--	--	--	--	Average
.371	.250	.382	.912	.131	--	--	--	--	--	--	Maximum
.244	.118	.299	.656	.097	--	--	--	--	--	--	Minimum
9	4	8	9	9	--	--	--	--	--	--	Samples
											69
.224	.181	.390	.906	.120	--	.448	1.285	.593	.351	.604	Average
.234	--	.416	.973	.136	--	.459	1.310	.612	.358	.634	Maximum
.216	--	.370	.850	.111	--	.438	1.260	.574	.344	.574	Minimum
3	1	3	3	3	--	2	2	2	2	2	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

	ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METHIONINE	CYSTINE	TOTAL
	LEGUMES (DRY SEEDS); COMMON NUTS; OTHER NUTS AND DRY SEEDS; THEIR PRODUCTS--Continued								
	COMMON NUTS AND THEIR PRODUCTS--Continued								
70	Pecans (Carya illinoensis):								
	Average.....grams..	0.078	0.219	0.312	0.436	0.245	0.086	0.122	0.208
	Maximum.....do...	.089	.247	.315	.509	.338	.104	.128	--
	Minimum.....do...	.068	.193	.306	.388	.173	.075	.116	--
	Samples.....number..	2	3	3	3	3	3	2	--
71	Walnuts, English or Persian (Juglans regia).....grams..	.062	.208	.271	.434	.156	.108	.113	.221
	Samples.....number..	1	1	1	1	1	1	1	--
	OTHER NUTS AND SEEDS AND THEIR PRODUCTS:								
72	Acorns (Quercus spp.).....grams..	.064	.221	.286	.412	.324	.071	.094	.165
	Samples.....number..	1	1	1	1	1	1	1	--
73	Amaranth (Amaranthus paniculatus):								
	Average.....grams..	.054	.302	.320	.439	.390	.135	.189	.324
	Maximum.....do...	.056	.328	.431	.500	.512	.156	--	--
	Minimum.....do...	.051	.275	.210	.378	.269	.101	--	--
	Samples.....number..	2	2	2	2	2	3	1	--
74	Balsampear (Momordica charantia).....grams..	--	--	--	--	.160	--	.018	--
	Samples.....number..	--	--	--	--	1	--	--	--
75	Breadnuttree, Ramon (Brosimum alicastrum).....grams..	.144	.206	.300	.575	.231	.031	--	--
	Samples.....number..	1	1	1	1	1	1	--	--
76	Chinese tallow-tree nut flour (Sapium sebiferum).....grams..	.077	.200	.323	.400	.146	.085	.064	.149
	Samples.....number..	1	1	1	1	1	1	1	--
77	Chocolatetree, Nicaragua (Theobroma bicolor).....grams..	.081	.206	.288	.544	.306	.038	--	--
	Samples.....number..	1	1	1	1	1	1	--	--
78	Cottonseed flour and meal (Gossypium spp.):								
	Average.....grams..	.074	.221	.236	.369	.268	.086	.102	.188
	Maximum.....do...	.102	.271	.281	.403	.356	.131	.162	--
	Minimum.....do...	.025	.162	.203	.311	.154	.036	.041	--
	Samples.....number..	37	40	40	32	48	51	15	--
79	Earpodtree, Guanacaste (Enterolobium cyclocarpum).....grams..	.069	.161	.344	.712	.300	.056	--	--
	Samples.....number..	1	1	1	1	1	1	--	--
80	Leadtree (Leucaena spp.).....grams..	.042	.182	.363	.393	.256	.012	--	--
	Samples.....number..	1	1	1	1	1	1	--	--
81	Pumpkin seed (Cucurbita pepo):								
	Average.....grams..	.096	.160	.298	.418	.242	.099	--	--
	Maximum.....do...	.100	.175	.369	.500	.316	.129	--	--
	Minimum.....do...	.093	.146	.228	.336	.169	.069	--	--
	Samples.....number..	2	2	2	2	2	2	--	--
82	Safflower seed meal (Carthamus tinctorius):								
	Average.....grams..	.085	.184	.241	.345	.192	.092	--	--
	Maximum.....do...	.096	--	--	--	.244	.096	--	--
	Minimum.....do...	.074	--	--	--	.164	.087	--	--
	Samples.....number..	2	1	1	1	3	2	--	--

TABLE 1.—AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS—CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											70
0.318	0.178	0.296	0.668	0.154	—	—	—	—	—	—	Average
.329	.188	.336	.716	.186	—	—	—	—	—	—	Maximum
.297	.169	.221	.639	.131	—	—	—	—	—	—	Minimum
3	2	3	3	3	—	—	—	—	—	—	Samples
.271	.206	.344	.808	.143	—	—	—	—	—	—	71
1	1	1	1	1	—	—	—	—	—	—	Samples
.241	—	.366	.368	.128	—	—	—	—	—	—	72
1	—	1	1	1	—	—	—	—	—	—	Samples
											73
.414	—	.308	.634	.150	—	—	—	—	—	—	Average
.428	—	.381	.925	.181	—	—	—	—	—	—	Maximum
.400	—	.235	.344	.139	—	—	—	—	—	—	Minimum
2	—	2	2	2	—	—	—	—	—	—	Samples
.330	.078	—	.748	.116	—	—	—	—	—	—	74
1	1	—	1	—	—	—	—	—	—	—	Samples
.250	—	.512	.488	.081	—	—	—	—	—	—	75
1	—	1	1	1	—	—	—	—	—	—	Samples
.262	.185	.415	.923	.146	0.115	0.638	1.000	0.277	0.246	0.292	76
1	1	1	1	1	1	1	1	1	1	1	Samples
.362	—	.331	.581	.094	—	—	—	—	—	—	77
1	—	1	1	1	—	—	—	—	—	—	Samples
											78
.327	.171	.308	.702	.166	.270	.645	1.143	.291	.234	.286	Average
.366	.200	.343	.825	.228	.280	.677	1.250	.331	.253	.293	Maximum
.260	.094	.232	.469	.105	.251	.620	1.060	.276	.224	.278	Minimum
39	7	40	41	41	3	3	6	4	3	3	Samples
.206	—	.244	.444	.156	—	—	—	—	—	—	79
1	—	1	1	1	—	—	—	—	—	—	Samples
.188	—	.190	.530	.124	—	—	—	—	—	—	80
1	—	1	1	1	—	—	—	—	—	—	Samples
											81
.300	—	.288	.825	.122	—	—	—	—	—	—	Average
.331	—	.312	.962	.131	—	—	—	—	—	—	Maximum
.268	—	.263	.688	.112	—	—	—	—	—	—	Minimum
2	—	2	2	2	—	—	—	—	—	—	Samples
											82
.328	—	.308	.582	.124	—	—	—	.314	—	—	Average
—	—	—	.679	—	—	—	—	—	—	—	Maximum
—	—	—	.486	—	—	—	—	—	—	—	Minimum
1	—	1	2	1	—	—	—	1	—	—	Samples

TABLE 1.—AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
LEGUMES (DRY SEED); COMMON NUTS; OTHER NUTS AND DRY SEEDS; THEIR PRODUCTS--Continued								
OTHER NUTS AND SEEDS AND THEIR PRODUCTS--Continued								
83 Sesame seed and seed meal (Sesamum indicum):								
Average.....grams..	0.091	0.194	0.261	0.461	0.160	0.175	0.136	0.311
Maximum.....do...	.125	.225	.300	.531	.250	.225	.211	--
Minimum.....do...	.068	.138	.222	.382	.092	.111	.081	--
Samples.....number..	8	3	2	3	7	9	4	--
84 Sunflower seed meal (Helianthus annuus):								
Average.....grams..	.079	.210	.294	.400	.200	.102	.107	.209
Maximum.....do...	.119	.250	.388	.481	.238	.137	.115	--
Minimum.....do...	.061	.169	.244	.368	.169	.081	.100	--
Samples.....number..	8	8	7	8	8	6	3	--
GRAINS AND THEIR PRODUCTS:								
85 Barley (Hordeum vulgare):								
Average.....grams..	.073	.197	.248	.405	.197	.084	.117	.201
Maximum.....do...	.099	.251	.300	.444	.220	.131	.157	--
Minimum.....do...	.050	.138	.203	.345	.138	.061	.056	--
Samples.....number..	9	29	28	28	29	30	6	--
Bread:								
86 White made with refined wheat flour and 4 percent nonfat dry milk, flour basis:								
Average.....grams..	.061	.189	.288	.448	.151	.095	.134	.229
Maximum.....do...	--	.194	.304	.468	.158	.118	.136	--
Minimum.....do...	--	.185	.271	.429	.147	.080	.132	--
Samples.....number..	1	3	3	3	3	3	2	--
87 Other breads: 4/								
Average.....grams..	.061	.185	.237	.252	.149	.148	.116	.264
Maximum.....do...	.094	.193	.286	.427	.175	.188	.138	--
Minimum.....do...	.043	.175	.188	.076	.112	.089	.104	--
Samples.....number..	9	3	2	2	3	5	8	--
88 Buckwheat flour (Fagopyrum esculentum):								
Average.....grams..	.088	.246	.235	.365	.367	.110	.122	.232
Maximum.....do...	.105	.264	.252	.389	.384	.116	.168	--
Minimum.....do...	.066	.220	.216	.327	.348	.099	.077	--
Samples.....number..	4	3	3	3	3	3	2	--
89 Cañihua (Chenopodium pallidicaule).....grams..	.050	.300	.425	.362	.375	.112	.069	.181
Samples.....number..	1	1	1	1	1	1	1	--
Cereal combinations:								
90 Corn and soy grits.....grams..	.056	.275	.292	.575	.268	.094	.108	.202
Samples.....number..	1	1	1	1	1	1	1	--
91 Infant food, precooked mixed cereals with nonfat dry milk and yeast.....grams..	.036	--	--	--	.088	.100	.044	.144
Samples.....number..	1	--	--	--	1	1	1	--
92 Oat-corn-rye mixture, puffed.....grams..	.069	.219	.338	.550	.138	.156	.094	.250
Samples.....number..	1	1	1	1	1	1	1	--

4/ Based on miscellaneous samples including other white bread, hard bread with and without oatmeal, and pumpernickel.

TABLE 1.—AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											83
0.400	0.261	0.244	0.547	0.121	0.219	--	--	0.581	--	--	Average
.519	.294	.319	.628	.148	--	--	--	--	--	--	Maximum
.280	.219	.112	.469	.094	--	--	--	--	--	--	Minimum
3	3	3	3	2	1	--	--	1	--	--	Samples
											84
.281	.149	.312	.546	.135	.200	--	--	--	--	--	Average
.356	.162	.350	.706	.185	--	--	--	--	--	--	Maximum
.200	.136	.275	.369	.106	--	--	--	--	--	--	Minimum
8	2	8	8	7	1	--	--	--	--	--	Samples
											85
.301	.212	.293	.300	.109	.268	0.324	1.303	.265	0.526	0.271	Average
.356	.275	.326	.356	.150	.281	.400	1.440	.372	.638	.400	Maximum
.241	.175	.238	.172	.085	.256	.156	1.100	.181	.300	.172	Minimum
29	7	29	29	29	3	4	5	5	4	5	Samples
											86
.312	.163	.292	.228	.129	.180	.286	1.980	.202	.675	--	Average
.328	.211	.292	.236	.135	--	--	--	--	--	--	Maximum
.300	.137	.291	.221	.119	--	--	--	--	--	--	Minimum
3	3	3	3	3	1	1	1	1	1	--	Samples
											87
.322	.226	.238	.236	.129	--	--	--	--	--	--	Average
.344	.306	.288	.257	.144	--	--	--	--	--	--	Maximum
.300	.156	.188	.219	.112	--	--	--	--	--	--	Minimum
4	8	2	3	3	--	--	--	--	--	--	Samples
											88
.236	.128	.324	.497	.137	--	--	--	--	--	--	Average
.263	.129	.347	.596	.143	--	--	--	--	--	--	Maximum
.209	.126	.295	.356	.132	--	--	--	--	--	--	Minimum
3	2	3	4	3	--	--	--	--	--	--	Samples
.225	.125	.288	.494	.156	--	--	--	--	--	--	89
1	1	1	1	1	--	--	--	--	--	--	Samples
.289	.195	.366	.341	.164	--	--	--	--	--	--	90
1	1	1	1	1	--	--	--	--	--	--	Samples
.175	.144	--	.144	.075	--	--	--	--	--	--	91
1	1	--	1	1	--	--	--	--	--	--	Samples
.375	.250	.362	.312	.131	--	--	--	--	--	--	92
1	1	1	1	1	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
GRAINS AND THEIR PRODUCTS--Continued								
93 Corn, cornmeal, grits (Zea mays):								
Average.....grams..	0.038	0.249	0.289	0.810	0.160	0.116	0.081	0.197
Maximum.....do...	.074	.316	.400	1.344	.322	.248	.190	--
Minimum.....do...	.008	.194	.221	.582	.028	.059	.050	--
Samples.....number..	267	25	22	25	98	96	145	--
Corn products:								
94 Flakes:								
Average.....grams..	.040	.212	.236	.808	.119	.104	.117	.221
Maximum.....do...	.061	.231	.248	.832	.275	.138	--	--
Minimum.....do...	.018	.198	.225	.784	.027	.047	--	--
Samples.....number..	2	3	2	2	3	3	1	--
95 Germ:								
Average.....grams..	.062	.268	.249	.444	.341	.100	.056	.156
Maximum.....do...	.064	.281	.262	.481	.367	.106	--	--
Minimum.....do...	.061	.248	.238	.422	.325	.094	--	--
Samples.....number..	2	4	4	4	3	2	1	--
96 Gluten:								
Average.....grams..	.037	.215	.277	.977	.112	.176	.088	.264
Maximum.....do...	.054	.256	.321	1.544	.199	.312	.094	--
Minimum.....do...	.025	.169	.125	.680	.069	.079	.075	--
Samples.....number..	7	8	6	6	6	6	3	--
97 Hominy:								
Average.....grams..	.060	.227	.251	.582	.257	.071	--	--
Maximum.....do...	.071	.254	.273	.692	.272	.118	--	--
Minimum.....do...	.048	.198	.225	.519	.241	.042	--	--
Samples.....number..	3	3	3	3	3	3	--	--
98 Masa:								
Average.....grams..	.022	--	--	--	.230	.242	.066	.308
Maximum.....do...	.022	--	--	--	.244	.283	.067	--
Minimum.....do...	.022	--	--	--	.217	.200	.065	--
Samples.....number..	2	--	--	--	2	2	2	--
99 Pozol.....grams..	.044	.356	.322	.626	.248	.092	--	--
Samples.....number..	1	1	1	1	1	1	--	--
100 Tortilla:								
Average.....grams..	.033	.253	.372	1.012	.155	.120	--	--
Maximum.....do...	.041	.287	--	1.181	.174	.134	--	--
Minimum.....do...	.025	.219	--	.843	.137	.106	--	--
Samples.....number..	2	2	1	2	2	2	--	--
101 Zein:								
Average.....grams..	.004	.192	.319	1.236	.000	.109	.063	.172
Maximum.....do...	.006	.206	--	--	--	.126	.088	--
Minimum.....do...	.002	.179	--	--	--	.094	.038	--
Samples.....number..	4	2	1	1	5	6	2	--
102 Job's tears (Coix lacryma-jobi).....grams..	.028	.262	.450	1.481	.153	.194	.112	.306
Samples.....number..	1	1	1	1	1	1	1	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											93
0.284	0.382	0.319	0.220	0.129	0.622	0.778	1.103	0.212	0.522	0.353	Average
.333	.459	.467	.377	.200	.661	.803	1.400	.259	.601	.533	Maximum
.202	.132	.268	.131	.090	.582	.749	.943	.180	.443	.246	Minimum
17	118	23	124	121	2	2	3	3	2	3	Samples
											94
.273	.218	.296	.178	.174	--	--	--	--	--	--	Average
.338	.309	.316	.226	.200	--	--	--	--	--	--	Maximum
.234	.126	.279	.108	.153	--	--	--	--	--	--	Minimum
3	2	2	3	3	--	--	--	--	--	--	Samples
											95
.208	.148	.340	.459	.200	--	--	--	--	--	--	Average
.241	--	.369	.631	.225	--	--	--	--	--	--	Maximum
.177	--	.321	.350	.186	--	--	--	--	--	--	Minimum
3	1	3	3	3	--	--	--	--	--	--	Samples
											96
.349	.364	.320	.201	.125	--	.121	1.555	.252	--	--	Average
.417	.444	.356	.220	.140	--	--	1.610	.269	--	--	Maximum
.254	.231	.282	.188	.106	--	--	1.530	.234	--	--	Minimum
5	6	6	6	6	--	1	4	2	--	--	Samples
											97
.239	.238	.286	.319	.146	--	--	--	--	--	--	Average
.284	.251	.355	.361	.172	--	--	--	--	--	--	Maximum
.209	.225	.246	.262	.123	--	--	--	--	--	--	Minimum
3	2	3	3	3	--	--	--	--	--	--	Samples
											98
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
.269	--	.283	.209	.129	--	--	--	--	--	--	99
1	--	1	1	1	--	--	--	--	--	--	Samples
											100
.272	--	.328	.240	.138	--	--	--	--	--	--	Average
.275	--	.338	.275	.144	--	--	--	--	--	--	Maximum
.269	--	.318	.206	.133	--	--	--	--	--	--	Minimum
2	--	2	2	2	--	--	--	--	--	--	Samples
											101
.646	.381	.254	.111	.084	.687	.312	1.854	.088	.656	.402	Average
.850	.412	.258	.126	.096	.769	.338	2.250	--	.662	.435	Maximum
.441	.350	.250	.101	.077	.644	.285	1.516	--	.649	.369	Minimum
2	2	2	4	4	3	2	4	1	2	2	Samples
.297	--	--	.219	.134	--	--	--	--	--	--	102
1	--	--	1	1	--	--	--	--	--	--	Samples

TABLE 1--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
GRAINS AND THEIR PRODUCTS--Continued								
Millets:								
103 Foxtail millet (Setaria italica).....grams..	0.062	0.194	0.475	1.044	0.131	0.175	--	--
Samples.....number..	1	1	1	1	1	1	--	--
104 Little millet (Panicum miliare).....grams..	.038	.212	.419	.681	.112	.144	--	--
Samples.....number..	1	1	1	1	1	1	--	--
105 Pearlmillet (Pennisetum glaucum):								
Average.....grams..	.127	.233	.325	.893	.196	.138	0.078	0.216
Maximum.....do...	.153	.344	.441	1.150	.264	.175	.084	--
Minimum.....do...	.100	.150	.188	.583	.153	.100	.072	--
Samples.....number..	7	6	8	8	8	8	4	--
106 Ragimillet (Eleusine coracana):								
Average.....grams..	.080	.254	.374	.583	.190	.254	.176	.430
Maximum.....do...	.103	.260	.419	.654	.236	.368	.202	--
Minimum.....do...	.043	.248	.290	.534	.106	.162	.151	--
Samples.....number..	6	2	4	4	6	9	2	--
107 Oats, oatmeal, rolled oats (Avena sativa):								
Average.....grams..	.075	.193	.301	.437	.214	.086	.127	.213
Maximum.....do...	.097	.225	.369	.556	.320	.144	.188	--
Minimum.....do...	.037	.131	.259	.331	.119	.065	.083	--
Samples.....number..	46	27	24	52	55	53	10	--
108 Quinoa (Chenopodium quinoa):								
Average.....grams..	.068	.297	.410	.444	.414	.158	.061	.219
Maximum.....do...	.082	.337	.437	--	.460	.202	--	--
Minimum.....do...	.052	.272	.350	--	.369	.123	--	--
Samples.....number..	6	6	6	6	6	6	6	--
Rice (Oryza sativa):								
109 Brown, converted, white:								
Average.....grams..	.064	.233	.279	.513	.235	.107	.081	.188
Maximum.....do...	.096	.457	.421	.692	.333	.230	.154	--
Minimum.....do...	.022	.138	.192	.369	.076	.034	.050	--
Samples.....number..	49	67	55	59	82	62	33	--
110 Germ.....grams..	.113	.912	.264	.351	.715	.176	.071	.247
Samples.....number..	1	1	1	1	1	1	1	--
111 Puffed rice:								
Average.....grams..	.046	--	--	--	.056	--	.044	--
Maximum.....do...	.062	--	--	--	--	--	--	--
Minimum.....do...	.031	--	--	--	--	--	--	--
Samples.....number..	2	--	--	--	1	--	1	--
112 Rye (Secale cereale), whole grain, and flours of different extractions:								
Average.....grams..	.066	.216	.248	.392	.236	.092	.116	.206
Maximum.....do...	.125	.262	.275	.443	.262	.138	.176	--
Minimum.....do...	.034	.138	.219	.345	.207	.034	.037	--
Samples.....number..	9	7	7	7	7	6	6	--
113 Sorghum (Sorghum vulgare):								
Average.....grams..	.070	.224	.340	1.004	.170	.108	.104	.212
Maximum.....do...	.084	.312	.437	1.644	.244	.194	.147	--
Minimum.....do...	.053	.156	.256	.575	.094	.029	.077	--
Samples.....number..	15	12	17	17	17	17	6	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
0.419	--	0.431	0.225	0.131	--	--	--	--	--	--	103
1	--	1	1	1	--	--	--	--	--	--	Samples
.300	--	.381	.294	.119	--	--	--	--	--	--	104
1	--	1	1	1	--	--	--	--	--	--	Samples
											105
.259	--	.349	.268	.123	--	--	--	--	--	--	Average
.316	--	.399	.328	.147	--	--	--	--	--	--	Maximum
.216	--	.250	.219	.100	--	--	--	--	--	--	Minimum
8	--	8	5	7	--	--	--	--	--	--	Samples
											106
.247	--	.445	.094	.074	--	--	--	--	--	--	Average
.325	--	.527	--	.094	--	--	--	--	--	--	Maximum
.098	--	.361	--	.020	--	--	--	--	--	--	Minimum
5	--	4	1	4	--	--	--	--	--	--	Samples
											107
.311	.215	.347	.384	.107	0.356	0.241	1.174	0.265	0.332	0.233	Average
.450	.281	.438	.469	.148	.444	.281	1.280	.326	.406	.306	Maximum
.261	.094	.238	.259	.076	.256	.188	1.070	.194	.256	.148	Minimum
25	12	24	28	26	4	4	5	5	4	5	Samples
											108
.224	.144	.254	.466	.169	--	--	--	--	--	--	Average
.257	--	.308	.580	.215	--	--	--	--	--	--	Maximum
.186	--	.190	.420	.145	--	--	--	--	--	--	Minimum
6	1	6	6	6	--	--	--	--	--	--	Samples
											109
.299	.272	.416	.343	.100	--	.281	.815	.407	.288	.302	Average
.419	.365	.558	.714	.219	--	.289	1.510	.440	.314	.345	Maximum
.234	.165	.294	.145	.037	--	.259	.649	.362	.255	.271	Minimum
56	10	56	39	44	--	5	6	5	5	5	Samples
.314	.389	.393	.653	.190	--	.548	.523	.431	.364	.389	110
1	1	1	1	1	--	1	1	1	1	1	Samples
											111
.288	.125	--	.138	.138	--	--	--	--	--	--	Average
--	.131	--	--	--	--	--	--	--	--	--	Maximum
--	.119	--	--	--	--	--	--	--	--	--	Minimum
1	2	--	1	1	--	--	--	--	--	--	Samples
											112
.275	.188	.304	.285	.133	--	--	1.240	--	--	.241	Average
.350	.300	.337	.358	.142	--	--	--	--	--	--	Maximum
.240	.081	.241	.207	.106	--	--	--	--	--	--	Minimum
7	6	7	7	7	--	--	1	--	--	1	Samples
											113
.311	.172	.357	.237	.120	--	--	1.370	--	--	.316	Average
.401	.260	.504	.278	.158	--	--	--	--	--	--	Maximum
.244	.105	.275	.200	.088	--	--	--	--	--	--	Minimum
17	3	17	10	16	--	--	1	--	--	1	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
GRAINS AND THEIR PRODUCTS--Continued								
114 Teosinte (Euchlaena mexicana):								
Average.....grams..	0.014	--	--	--	0.099	0.141	--	--
Maximum.....do...	.016	--	--	--	.125	.158	--	--
Minimum.....do...	.009	--	--	--	.074	.114	--	--
Samples.....number..	4	--	--	--	4	4	--	--
Wheat (Triticum aestivum):								
115 Whole grain and whole grain flour:								
Average.....grams..	.072	0.168	0.253	0.391	.160	.089	0.128	0.217
Maximum.....do...	.085	.206	.298	.426	.215	.175	.169	--
Minimum.....do...	.052	.106	.189	.346	.134	.042	.053	--
Samples.....number..	19	63	57	57	83	85	36	--
116 Flour of intermediate extraction.....grams..	--	.186	.294	.439	.169	.094	.152	.246
Samples.....number..	--	1	1	1	1	1	1	--
117 White flour:								
Average.....grams..	.070	.164	.262	.439	.130	.075	.114	.189
Maximum.....do...	.088	.184	.292	.469	.156	.107	.147	--
Minimum.....do...	.039	.131	.231	.416	.077	.044	.088	--
Samples.....number..	10	6	3	3	8	7	4	--
Wheat products:								
118 Bran:								
Average.....grams..	.103	.180	.255	.377	.258	.076	.142	.218
Maximum.....do...	.137	.216	.337	.491	.331	.097	.174	--
Minimum.....do...	.062	.133	.212	.325	.200	.039	.114	--
Samples.....number..	5	6	6	6	7	6	3	--
119 Burghul.....grams..	.033	--	--	--	.202	.141	.150	.291
Samples.....number..	1	--	--	--	1	1	1	--
120 Farina:								
Average.....grams..	.065	--	--	--	.104	.075	.096	.171
Maximum.....do...	.088	--	--	--	.125	--	.106	--
Minimum.....do...	.050	--	--	--	.081	--	.088	--
Samples.....number..	4	--	--	--	4	1	3	--
121 Flaked wheat:								
Average.....grams..	.064	.188	.262	.470	.190	.067	.101	.168
Maximum.....do...	.076	.214	.312	.569	.253	.076	--	--
Minimum.....do...	.051	.162	.192	.390	.126	.057	--	--
Samples.....number..	2	2	4	4	2	2	1	--
122 Germ:								
Average.....grams..	.061	.309	.271	.393	.353	.093	.066	.159
Maximum.....do...	.078	.460	.384	.535	.418	.125	.100	--
Minimum.....do...	.046	.212	.224	.327	.269	.062	.038	--
Samples.....number..	5	5	5	6	8	6	3	--
123 Gluten:								
Average.....grams..	.061	.151	.262	.427	.109	.099	.123	.222
Maximum.....do...	.081	.175	.297	.455	.131	.120	.169	--
Minimum.....do...	.034	.131	.225	.350	.09[illegible]	.076	.097	--
Samples.....number..	33	23	23	23	29	31	28	--
124 Macaroni or spaghetti:								
Average.....grams..	.067	.222	.286	.378	.184	.086	.108	.194
Maximum.....do...	.081	--	--	--	--	--	--	--
Minimum.....do...	.053	--	--	--	--	--	--	--
Samples.....number..	2	1	1	1	1	1	1	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											114
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
											115
0.288	0.218	0.270	0.279	0.119	0.204	0.318	1.822	0.356	0.609	0.269	Average
.356	.300	.300	.344	.162	--	--	1.930	.471	--	--	Maximum
.232	.164	.224	.173	.081	--	--	1.500	.242	--	--	Minimum
59	8	62	69	64	1	1	25	2	1	1	Samples
.348	.159	.277	.261	.136	--	--	--	--	--	--	116
1	1	1	1	1	--	--	--	--	--	--	Samples
											117
.313	.195	.246	.253	.114	.172	.247	1.983	.207	.681	.294	Average
.350	.250	.279	.459	.154	--	.252	2.110	.212	.706	.294	Maximum
.280	.108	.181	.194	.081	--	.238	1.830	.196	.644	.294	Minimum
6	7	6	8	7	1	3	3	3	3	2	Samples
											118
.228	.136	.290	.390	.147	--	--	--	--	--	.333	Average
.280	.182	.319	.451	.170	--	--	--	--	--	--	Maximum
.196	.067	.242	.300	.125	--	--	--	--	--	--	Minimum
6	4	6	6	5	--	--	--	--	--	1	Samples
--	--	--	--	--	--	--	--	--	--	--	119
--	--	--	--	--	--	--	--	--	--	--	Samples
											120
.303	.234	--	.222	.140	--	--	--	--	--	--	Average
.350	.281	--	.325	.194	--	--	--	--	--	--	Maximum
.256	.212	--	.169	.100	--	--	--	--	--	--	Minimum
4	6	--	4	4	--	--	--	--	--	--	Samples
											121
.252	.164	.302	.295	.122	--	--	--	--	--	--	Average
.271	.215	.321	.332	.124	--	--	--	--	--	--	Maximum
.234	.113	.281	.258	.119	--	--	--	--	--	--	Minimum
2	2	4	2	2	--	--	--	--	--	--	Samples
											122
.209	.203	.314	.420	.158	--	--	--	--	--	--	Average
.262	.238	.394	.600	.268	--	--	--	--	--	--	Maximum
.156	.163	.275	.338	.128	--	--	--	--	--	--	Minimum
7	3	5	7	7	--	--	--	--	--	--	Samples
											123
.310	.185	.270	.248	.130	.128	.206	.209	.299	.726	.270	Average
.346	.322	.334	.296	.137	.169	.236	.241	.450	.883	.285	Maximum
.275	.134	.219	.111	.119	.106	.137	.179	.175	.667	.251	Minimum
23	24	23	27	23	8	24	27	21	17	6	Samples
											124
.298	.188	.324	.259	.135	--	--	--	--	--	--	Average
--	.219	--	--	--	--	--	--	--	--	--	Maximum
--	.157	--	--	--	--	--	--	--	--	--	Minimum
1	2	1	1	1	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
GRAINS AND THEIR PRODUCTS--Continued								
Wheat (Triticum aestivum)--Continued								
Wheat products--Continued								
125 Noodles (contain egg solids).....grams..	0.060	0.241	0.291	0.377	0.166	0.096	0.111	0.207
Samples.....number..	1	1	1	1	1	1	1	--
126 Shredded wheat.....grams..	.049	.234	.259	.395	.191	.080	.118	.198
Samples.....number..	1	1	1	1	1	1	1	--
127 Whole wheat with added wheat germ:								
Average.....grams..	.062	--	--	--	.212	--	.112	--
Maximum.....do...	.075	--	--	--	--	--	--	--
Minimum.....do...	.050	--	--	--	--	--	--	--
Samples.....number..	2	--	--	--	1	--	1	--
FRUITS:								
128 Abiu (Pouteria campechiana).....grams..	.104	--	--	--	.311	.048	--	--
Samples.....number..	1	--	--	--	1	1	--	--
129 Avocados (Persea americana):								
Average.....grams..	.068	--	--	--	.357	.058	--	--
Maximum.....do...	.088	--	--	--	.400	.067	--	--
Minimum.....do...	.046	--	--	--	.242	.046	--	--
Samples.....number..	9	--	--	--	9	9	--	--
Bananas, ripe:								
130 Common (Musa paradisiaca var. sapientum):								
Average.....grams..	.095	--	--	--	.289	.055	--	--
Maximum.....do...	.115	--	--	--	.319	.077	--	--
Minimum.....do...	.076	--	--	--	.241	.035	--	--
Samples.....number..	4	--	--	--	4	4	--	--
131 Dwarf (Musa nana).....grams..	.063	--	--	--	.253	.023	--	--
Samples.....number..	1	--	--	--	1	1	--	--
132 Dates (Phoenix dactylifera).....grams..	.173	.173	.211	.219	.184	.076	--	--
Samples.....number..	1	1	1	1	1	1	--	--
133 Grapefruit, juice and pulp (Citrus paradisi).....grams..	.012	--	--	--	.075	.000	--	--
Samples.....number..	1	--	--	--	1	1	--	--
134 Guavas, common (Psidium guajava):								
Average.....grams..	.060	--	--	--	.188	.060	--	--
Maximum.....do...	.094	--	--	--	.244	.078	--	--
Minimum.....do...	.026	--	--	--	.132	.042	--	--
Samples.....number..	2	--	--	--	2	2	--	--
135 Limes (Citrus spp.).....grams..	.025	--	--	--	.117	.017	--	--
Samples.....number..	1	--	--	--	1	1	--	--
136 Mamey (Mammea americana).....grams..	.071	--	--	--	.500	.086	--	--
Samples.....number..	1	--	--	--	1	1	--	--
137 Mangos (Mangifera indica):								
Average.....grams..	.125	--	--	--	.831	.071	--	--
Maximum.....do...	.225	--	--	--	1.550	.100	--	--
Minimum.....do...	.062	--	--	--	.525	.050	--	--
Samples.....number..	5	--	--	--	5	5	--	--
138 Muskmelons (Cucumis melo).....grams..	.011	--	--	--	.156	.022	--	--
Samples.....number..	1	--	--	--	1	1	--	--

TABLE 1.—AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
0.276	0.141	0.337	0.281	0.136	--	--	--	--	--	--	125
1	1	1	1	1	--	--	--	--	--	--	Samples
.278	.136	.333	.302	.136	--	--	--	--	--	--	126
1	1	1	1	1	--	--	--	--	--	--	Samples
											127
.344	.219	--	.338	.169	--	--	--	--	--	--	Average
--	.219	--	--	--	--	--	--	--	--	--	Maximum
--	.219	--	--	--	--	--	--	--	--	--	Minimum
1	2	--	1	1	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	128
--	--	--	--	--	--	--	--	--	--	--	Samples
											129
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
											130
--	.162	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	1	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	131
--	--	--	--	--	--	--	--	--	--	--	Samples
.180	--	.267	.139	.139	--	--	--	--	--	--	132
1	--	1	1	1	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	133
--	--	--	--	--	--	--	--	--	--	--	Samples
											134
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	135
--	--	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	136
--	--	--	--	--	--	--	--	--	--	--	Samples
											137
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	138
--	--	--	--	--	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
FRUITS--Continued								
139 Oranges, juice only (Citrus sinensis):								
Average.....grams..	0.021	--	--	--	0.165	0.018	--	--
Maximum.....do...	.042	--	--	--	.211	.029	--	--
Minimum.....do...	.008	--	--	--	.115	.008	--	--
Samples.....number..	4	--	--	--	4	4	--	--
140 Oranges, mandarin, segments (Citrus reticulata):								
Average.....grams..	.039	--	--	--	.221	.033	--	--
Maximum.....do...	.064	--	--	--	.245	.045	--	--
Minimum.....do...	.027	--	--	--	.191	.025	--	--
Samples.....number..	3	--	--	--	3	3	--	--
141 Papayas (Carica papaya).....grams..	.125	--	--	--	.400	.025	--	--
Samples.....number..	1	--	--	--	1	1	--	--
142 Pineapple (Ananas comosus).....grams..	.078	--	--	--	.144	.022	--	--
Samples.....number..	1	--	--	--	1	1	--	--
143 Plantain, green and ripe (Musa paradisiaca):								
Average.....grams..	.055	0.152	0.319	0.338	.262	.030	0.091	0.121
Maximum.....do...	.086	.169	--	--	.429	.050	--	--
Minimum.....do...	.039	.136	--	--	.154	.012	--	--
Samples.....number..	5	2	1	1	5	6	1	--
144 Soursop (Annona muricata).....grams..	.069	--	--	--	.375	.044	--	--
Samples.....number..	1	--	--	--	1	1	--	--
145 Sugarapple (Annona squamosa).....grams..	.032	--	--	--	.246	.029	--	--
Samples.....number..	1	--	--	--	1	1	--	--
VEGETABLES:								
IMMATURE SEEDS:								
146 Corn (Zea mays):								
Average.....grams..	.039	.255	.232	.687	.231	.122	.104	.226
Maximum.....do...	.053	.268	.275	.743	.262	.165	--	--
Minimum.....do...	.031	.231	.172	.638	.195	.088	--	--
Samples.....number..	7	5	5	5	7	7	1	--
147 Cowpeas (Vigna spp.):								
Average.....grams..	.066	.235	.309	.434	.410	.087	--	--
Maximum.....do...	.077	.256	.363	.482	.432	.092	--	--
Minimum.....do...	.054	.226	.282	.369	.371	.078	--	--
Samples.....number..	4	4	4	4	4	4	--	--
148 Lima beans, large- and small-seeded varieties (Phaseolus lunatus including var. macrocarpus):								
Average.....grams..	.081	.282	.383	.504	.395	.067	.069	.136
Maximum.....do...	.098	.324	.417	.541	.431	.082	--	--
Minimum.....do...	.069	.247	.324	.473	.358	.057	--	--
Samples.....number..	5	4	4	4	5	5	1	--
149 Peas, raw or canned (Pisum sativum):								
Average.....grams..	.052	.229	.287	.390	.295	.050	.068	.118
Maximum.....do...	.077	.298	.361	.546	.436	.093	.096	--
Minimum.....do...	.025	.173	.230	.293	.089	.017	.039	--
Samples.....number..	10	10	7	9	10	10	2	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											139
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
											140
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	141
--	--	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	142
--	--	--	--	--	--	--	--	--	--	--	Samples
											143
0.281	--	0.371	0.256	--	--	0.682	1.091	--	--	--	Average
--	--	.473	--	--	--	--	--	--	--	--	Maximum
--	--	.269	--	--	--	--	--	--	--	--	Minimum
1	--	2	1	--	--	1	1	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	144
--	--	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	145
--	--	--	--	--	--	--	--	--	--	--	Samples
											146
.350	0.209	.390	.294	0.161	--	--	--	--	--	--	Average
.469	--	.450	.388	.169	--	--	--	--	--	--	Maximum
.266	--	.347	.250	.150	--	--	--	--	--	--	Minimum
5	1	5	5	5	--	--	--	--	--	--	Samples
											147
.348	--	.341	.409	.206	--	--	--	--	--	--	Average
.359	--	.402	.494	.214	--	--	--	--	--	--	Maximum
.340	--	.310	.358	.199	--	--	--	--	--	--	Minimum
4	--	4	4	4	--	--	--	--	--	--	Samples
											148
.324	.216	.404	.378	.206	--	--	--	--	--	--	Average
.351	--	.421	.434	.216	--	--	--	--	--	--	Maximum
.266	--	.385	.274	.189	--	--	--	--	--	--	Minimum
4	1	4	4	4	--	--	--	--	--	--	Samples
											149
.240	.152	.256	.555	.102	0.183	.596	.442	0.202	--	--	Average
.313	.258	.328	.761	.198	--	--	--	--	--	--	Maximum
.151	.100	.194	.426	.061	--	--	--	--	--	--	Minimum
10	4	9	8	9	1	1	1	1	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
VEGETABLES--Continued								
LEAFY VEGETABLES:								
150 Amaranth (Amaranthus gangeticus):								
Average.....grams..	0.068	0.100	0.293	0.368	0.251	0.044	0.042	0.086
Maximum.....do...	--	.138	--	--	--	.066	--	--
Minimum.....do...	--	.062	--	--	--	.023	--	--
Samples.....number..	1	2	1	1	1	2	1	--
151 Beet greens (Beta vulgaris):								
Average.....grams..	.075	.239	.263	.404	.338	.106	--	--
Maximum.....do....	.075	.240	.264	.409	.338	.106	--	--
Minimum.....do....	.075	.238	.262	.400	.337	.106	--	--
Samples.....number..	2	2	2	2	2	2	--	--
152 Brussels sprouts (Brassica oleracea var. gemmifera):								
Average.....grams..	.063	.218	.264	.276	.280	.066	--	--
Maximum.....do...	.076	.236	.286	.278	.288	.070	--	--
Minimum.....do...	.050	.199	.242	.274	.272	.062	--	--
Samples.....number..	2	2	2	2	2	2	--	--
153 Cabbage (Brassica oleracea var. capitata):								
Average.....grams..	.050	.175	.180	.255	.295	.058	.125	.183
Maximum.....do...	.052	.188	.196	.279	.438	.084	.150	--
Minimum.....do...	.048	.163	.163	.231	.205	.032	.100	--
Samples.....number..	2	3	2	2	3	2	2	--
154 Chard (Beta vulgaris var. cicla):								
Average.....grams..	.064	.258	.267	.340	.246	.019	--	--
Maximum.....do...	.068	--	--	--	.316	.038	--	--
Minimum.....do...	.059	--	--	--	.177	.000	--	--
Samples.....number..	2	1	1	1	2	2	--	--
155 Chicory (Cichorium intybus).....grams..	.094	--	--	--	.202	.061	.024	.085
Samples.....number..	1	--	--	--	1	1	1	--
156 Collards (Brassica oleracea var. acephala):								
Average.....grams..	.088	.193	.194	.350	.324	.073	.094	.167
Maximum.....do...	.104	.275	.302	.465	.354	.077	--	--
Minimum.....do...	.072	.091	.087	.234	.294	.069	--	--
Samples.....number..	2	2	2	2	2	2	1	--
157 Kale (Brassica oleracea var. acephala):								
Average.....grams..	.068	.222	.213	.404	.194	.056	.058	.114
Maximum.....do...	.069	.226	.214	.406	.194	.056	--	--
Minimum.....do...	.068	.219	.212	.402	.193	.055	--	--
Samples.....number..	2	2	2	2	2	2	1	--
158 Lettuce (Lactuca sativa).....grams..	.061	--	--	--	.367	.022	--	--
Samples.....number..	1	--	--	--	1	1	--	--
159 Mustard greens (Brassica spp.).....grams..	.100	.164	.205	.168	.302	.064	.094	.158
Samples.....number..	1	1	1	1	1	1	1	--
160 Parsley, curly (garden (Petroselinum crispum).....grams..	.125	--	--	--	.400	.030	--	--
Samples.....number..	1	--	--	--	1	1	--	--
161 Spinach (Spinacia oleracea):								
Average.....grams..	.101	.276	.290	.478	.386	.106	.124	.230
Maximum.....do...	.140	.414	.400	.598	.560	.144	--	--
Minimum.....do...	.068	.140	.152	.271	.192	.041	--	--
Samples.....number..	8	9	8	8	9	8	1	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN SOLUBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											150
0.172	0.188	0.242	0.239	0.124	0.500	0.450	0.575	--	--	--	Average
--	--	.284	--	--	--	--	--	--	--	--	Maximum
--	--	.200	--	--	--	--	--	--	--	--	Minimum
1	1	2	1	1	1	1	1	--	--	--	Samples
											151
.362	--	.316	.260	.082	--	--	--	--	--	--	Average
.362	--	.319	.264	.082	--	--	--	--	--	--	Maximum
.361	--	.312	.256	.081	--	--	--	--	--	--	Minimum
2	--	2	2	2	--	--	--	--	--	--	Samples
											152
.210	--	.274	.396	.150	--	--	--	--	--	--	Average
.243	--	.286	.405	.151	--	--	--	--	--	--	Maximum
.176	--	.263	.388	.148	--	--	--	--	--	--	Minimum
2	--	2	2	2	--	--	--	--	--	--	Samples
											153
.136	.132	.193	.470	.112	.316	.675	1.188	--	--	0.188	Average
.200	--	.248	.484	.129	.444	--	--	--	--	--	Maximum
.102	--	.156	.455	.094	.188	--	--	--	--	--	Minimum
4	1	3	2	4	2	1	1	--	--	1	Samples
											154
.205	--	.246	.158	.080	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
1	--	1	1	1	--	--	--	--	--	--	Samples
--	.158	--	--	.093	--	--	--	--	--	--	155
--	1	--	--	1	--	--	--	--	--	--	Samples
											156
.198	.242	.312	.414	.140	--	--	--	--	--	--	Average
.254	--	.392	.461	.146	--	--	--	--	--	--	Maximum
.142	--	.231	.367	.134	--	--	--	--	--	--	Minimum
2	1	2	2	2	--	--	--	--	--	--	Samples
											157
.253	--	.295	.323	.100	.102	--	--	--	--	--	Average
.276	--	.302	.327	.101	--	--	--	--	--	--	Maximum
.208	--	.288	.319	.100	--	--	--	--	--	--	Minimum
3	--	2	2	2	1	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	158
--	--	--	--	--	--	--	--	--	--	--	Samples
.200	.329	.294	.454	.111	--	--	--	--	--	--	159
1	1	1	1	1	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	160
--	--	--	--	--	--	--	--	--	--	--	Samples
											161
.269	.198	.343	.316	.132	.198	--	.784	--	--	--	Average
.378	.270	.517	.448	.200	.297	--	--	--	--	--	Maximum
.108	.127	.187	.168	.069	.100	--	--	--	--	--	Minimum
10	2	9	8	6	2	--	1	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
VEGETABLES--Continued								
LEAFY VEGETABLES--Continued								
162 Turnip greens (Brassica rapa):								
Average.....grams..	0.098	0.270	0.230	0.447	0.278	0.113	0.096	0.209
Maximum.....do...	.116	.299	.278	.582	.479	.159	--	--
Minimum.....do...	.081	.250	.159	.351	.187	.016	--	--
Samples.....number..	4	4	4	4	4	4	1	--
163 Watercress (Roripoa nasturtium-aquaticum):								
Average.....grams..	.103	.310	.280	.483	.336	.037	--	--
Maximum.....do...	.130	.333	--	--	.348	.068	--	--
Minimum.....do...	.076	.287	--	--	.326	.020	--	--
Samples.....number..	2	2	1	1	3	3	--	--
STARCHY ROOTS AND TUBERS:								
164 Apio arracacia (Arracacia xanthorrhiza)..grams..	.042	--	--	--	.217	.017	--	--
Samples.....number..	1	--	--	--	1	1	--	--
165 Cassava, root and flour (Manihot esculenta):								
Average.....grams..	.081	.172	.174	.257	.258	.039	.069	.108
Maximum.....do...	.329	.238	.331	.350	.500	.089	.162	--
Minimum.....do...	.004	.118	.115	.184	.065	.000	.033	--
Samples.....number..	13	7	7	7	12	13	13	--
166 Potatoes (Solanum tuberosum):								
Average.....grams..	.067	.246	.274	.311	.333	.078	.060	.138
Maximum.....do...	.111	.292	.331	.352	.419	.104	.106	--
Minimum.....do...	.044	.215	.226	.268	.292	.038	.036	--
Samples.....number..	6	7	5	5	8	8	3	--
167 Sweetpotatoes (Ipomaea batatas):								
Average.....grams..	.109	.294	.301	.358	.295	.116	.100	.216
Maximum.....do...	.171	.369	.388	.425	.373	.170	--	--
Minimum.....do...	.050	.211	.225	.303	.183	.050	--	--
Samples.....number..	10	7	6	6	10	10	1	--
168 Taro (Colocasia spp.):								
Average.....grams..	.115	.294	.325	.556	.363	.070	--	--
Maximum.....do...	.142	--	--	--	.388	.079	--	--
Minimum.....do...	.088	--	--	--	.338	.062	--	--
Samples.....number..	2	1	1	1	2	2	--	--
169 Yam (Dioscorea spp.):								
Average.....grams..	.103	--	--	--	.326	.100	--	--
Maximum.....do...	.112	--	--	--	.393	.207	--	--
Minimum.....do...	.093	--	--	--	.262	.038	--	--
Samples.....number..	3	--	--	--	3	3	--	--
170 Yautia malange (Xanthosoma sagittaefolium)grams.	.085	--	--	--	.246	.058	--	--
Samples.....	1	--	--	--	1	1	--	--
OTHER VEGETABLES:								
171 Asparagus (Asparagus officinalis):								
Average.....grams..	.076	.188	.228	.272	.294	.090	--	--
Maximum.....do...	.099	.190	.231	.336	.315	.101	--	--
Minimum.....do...	.054	.185	.224	.208	.274	.080	--	--
Samples.....number..	2	2	2	2	2	2	--	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											162
0.315	0.226	0.321	0.360	0.110	--	--	--	--	--	--	Average
.335	.230	.402	.482	.157	--	--	--	--	--	--	Maximum
.291	.223	.270	.281	.088	--	--	--	--	--	--	Minimum
4	2	4	4	4	--	--	--	--	--	--	Samples
											163
.227	.133	.309	.194	.126	0.222	0.889	0.600	--	--	--	Average
.232	--	.351	--	--	--	--	--	--	--	--	Maximum
.222	--	.267	--	--	--	--	--	--	--	--	Minimum
2	1	2	1	1	1	1	1	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	164
--	--	--	--	--	--	--	--	--	--	--	Samples
											165
.175	.117	.190	.623	.096	.316	.331	.763	0.177	0.139	0.164	Average
.223	.216	.281	1.359	.219	.422	.433	1.174	.232	.185	.211	Maximum
.133	.081	.126	.076	.061	.252	.244	.509	.134	.093	.126	Minimum
7	8	7	24	8	6	6	6	6	6	6	Samples
											166
.276	.112	.334	.308	.090	.292	--	.625	--	.208	.250	Average
.338	.125	.378	.359	.106	--	--	--	--	--	--	Maximum
.193	.099	.296	.270	.075	--	--	--	--	--	--	Minimum
7	2	7	6	6	1	--	1	--	1	1	Samples
											167
.348	.281	.468	.326	.124	--	--	1.158	--	--	.158	Average
.423	--	.611	.579	.164	--	--	--	--	--	--	Maximum
.268	--	.263	.183	.085	--	--	--	--	--	--	Minimum
6	1	7	7	6	--	--	1	--	--	1	Samples
											168
.325	--	.375	.388	.106	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
1	--	1	1	1	--	--	--	--	--	--	Samples
											169
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	170
--	--	--	--	--	--	--	--	--	--	--	Samples
											171
.196	--	.301	.349	.103	--	--	--	--	--	--	Average
.210	--	.315	.357	.118	--	--	--	--	--	--	Maximum
.182	--	.287	.341	.088	--	--	--	--	--	--	Minimum
2	--	2	2	2	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
VEGETABLES--Continued								
OTHER VEGETABLES--Continued								
172 Beans, snap (Phaseolus vulgaris):								
Average.....grams..	0.086	0.236	0.282	0.362	0.328	0.090	0.063	0.153
Maximum.....do...	.121	.239	.283	.386	.360	.147	--	--
Minimum.....do...	.067	.233	.282	.337	.279	.031	--	--
Samples.....number..	3	2	2	2	3	9	1	--
173 Beets (Beta vulgaris):								
Average.....grams..	.054	.133	.201	.214	.336	.024	--	--
Maximum.....do...	--	.151	--	--	.462	.031	--	--
Minimum.....do...	--	.115	--	--	.210	.017	--	--
Samples.....number..	1	2	1	1	2	2	--	--
174 Broccoli (Brassica oleracea var. botrytis):								
Average.....grams..	.071	.231	.239	.308	.278	.094	--	--
Maximum.....do...	.082	.268	.294	.394	.424	.156	--	--
Minimum.....do...	.054	.194	.208	.237	.153	.070	--	--
Samples.....number..	9	9	9	9	9	9	--	--
175 Carrots (Daucus carota):								
Average.....grams..	.050	.223	.240	.339	.270	.054	.153	.207
Maximum.....do...	.074	.281	.300	.400	.356	.079	--	--
Minimum.....do...	.023	.169	.177	.288	.144	.019	--	--
Samples.....number..	7	6	6	5	6	6	1	--
176 Cauliflower (Brassica oleracea var. botrytis):								
Average.....grams..	.087	.265	.271	.421	.350	.123	--	--
Maximum.....do...	.112	.319	.288	.462	.375	.175	--	--
Minimum.....do...	.056	.179	.256	.327	.327	.032	--	--
Samples.....number..	4	5	4	4	5	5	--	--
177 Celery (Apium graveolens var. dulce).....grams..	.060	--	--	--	.102	.074	.028	.102
Samples.....number..	1	--	--	--	1	1	1	--
178 Chayote (Sechium edule).....grams..	.082	--	--	--	.400	.009	--	--
Samples.....number..	1	--	--	--	1	1	--	--
179 Cowpeas, yardlong (Vigna sesquipedalis):								
Average.....grams..	.062	--	--	--	.374	.038	--	--
Maximum.....do...	.065	--	--	--	.444	.038	--	--
Minimum.....do...	.060	--	--	--	.304	.037	--	--
Samples.....number..	2	--	--	--	2	2	--	--
180 Cucumbers (Cucumus sativus):								
Average.....grams..	.048	.167	.196	.265	.273	.059	--	--
Maximum.....do...	.055	--	--	--	.294	.098	--	--
Minimum.....do...	.040	--	--	--	.253	.033	--	--
Samples.....number..	3	1	1	1	3	3	--	--
181 Cushaw (Cucurbita moschata).....grams..	.058	--	--	--	.183	.033	--	--
Samples.....number..	1	--	--	--	1	1	--	--
182 Eggplant (Solanum melongena):								
Average.....grams..	.059	.214	.318	.384	.171	.033	--	--
Maximum.....do...	.067	.231	--	.393	.340	.053	--	--
Minimum.....do...	.051	.197	--	.374	.073	.013	--	--
Samples.....number..	3	2	1	2	3	2	--	--
183 Mallow (Malva sp.).....grams..	.244	.262	--	.438	.262	.050	--	--
Samples.....number..	1	1	--	1	1	1	--	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											172
0.149	0.131	0.300	0.263	0.118	0.253	--	--	--	--	--	Average
.219	.194	.308	.272	.134	--	--	--	--	--	--	Maximum
.079	.068	.291	.254	.108	--	--	--	--	--	--	Minimum
3	2	2	2	3	1	--	--	--	--	--	Samples
											173
.104	--	.192	.111	.085	--	0.615	1.000	--	0.115	--	Average
.115	--	--	--	--	--	--	--	--	--	--	Maximum
.094	--	--	--	--	--	--	--	--	--	--	Minimum
2	--	1	1	1	--	1	1	--	1	--	Samples
											174
.226	--	.322	.364	.119	--	--	--	--	--	--	Average
.272	--	.396	.422	.143	--	--	--	--	--	--	Maximum
.174	--	.206	.305	.100	--	--	--	--	--	--	Minimum
9	--	9	9	8	--	--	--	--	--	--	Samples
											175
.219	.103	.291	.216	.086	.302	.467	1.133	--	--	0.200	Average
.319	.133	.425	.344	.119	--	--	--	--	--	--	Maximum
.163	.073	.208	.088	.046	--	--	--	--	--	--	Minimum
7	2	6	4	6	1	1	1	--	--	1	Samples
											176
.196	.089	.374	.286	.126	.500	.516	.482	0.429	--	--	Average
.231	--	.456	.312	.138	--	--	--	--	--	--	Maximum
.107	--	.327	.234	.109	--	--	--	--	--	--	Minimum
5	1	5	4	4	1	1	1	1	--	--	Samples
--	.077	--	--	--	--	--	--	--	--	--	177
--	1	--	--	--	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	178
--	--	--	--	--	--	--	--	--	--	--	Samples
											179
--	--	--	--	--	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
--	--	--	--	--	--	--	--	--	--	--	Samples
											180
.147	--	.216	.471	.088	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
1	--	1	1	1	--	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	181
--	--	--	--	--	--	--	--	--	--	--	Samples
											182
.271	--	.368	.211	.107	--	--	--	--	--	--	Average
--	--	--	--	--	--	--	--	--	--	--	Maximum
--	--	--	--	--	--	--	--	--	--	--	Minimum
1	--	1	1	1	--	--	--	--	--	--	Samples
.281	--	.306	.319	.106	--	--	--	--	--	--	183
1	--	1	1	1	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOT
VEGETABLES--Continued								
OTHER VEGETABLES--Continued								
Mushrooms:								
184 (Agaricus campestris).....grams..	0.010	--	0.918	0.484	--	0.288	--	--
Samples.....number..	1	--	1	1	--	1	--	--
185 (Lactarius spp.).....grams..	.008	0.226	.292	.202	.127	.030	--	--
Samples.....number..	1	1	1	1	1	1	--	--
186 Okra (Hibiscus esculentus):								
Average.....grams..	.064	.228	.241	.351	0.265	.078	0.058	0.136
Maximum.....do...	.084	.239	.262	.384	.308	.108	.066	--
Minimum.....do...	.044	.222	.220	.314	.156	.054	.051	--
Samples.....number..	5	3	3	3	5	5	2	--
187 Onions (Allium cepa):								
Average.....grams..	.092	.098	.092	.165	.284	.060	--	--
Maximum.....do...	--	.103	--	--	--	.073	--	--
Minimum.....do...	--	.092	--	--	--	.046	--	--
Samples.....number..	1	2	1	1	1	2	--	--
188 Peppers (Capsicum frutescens):								
Average.....grams..	.045	.261	.237	.239	.266	.081	--	--
Maximum.....do...	.062	.278	.255	.262	.394	.225	--	--
Minimum.....do...	.037	.250	.226	.225	.184	.020	--	--
Samples.....number.	4	3	3	3	5	5	--	--
189 Pricklypears, Nopales (Opuntia spp.).....grams..	.051	.301	.250	.326	.250	.046	--	--
Samples.....number..	1	1	1	1	1	1	--	--
190 Pumpkin (Cucurbita pepo):								
Average.....grams..	.082	.148	.231	.328	.304	.056	--	--
Maximum.....do...	--	.172	--	--	.358	.075	--	--
Minimum.....do...	--	.125	--	--	.250	.038	--	--
Samples.....number..	1	2	1	1	2	2	--	--
191 Radishes (Raphanus sativus).....grams..	.024	.308	--	--	.176	.009	--	--
Samples.....number..	1	1	--	--	1	1	--	--
192 Seapweed (Dondia spp.).....grams..	.066	.214	.272	.366	.214	.032	--	--
Samples.....number..	1	1	1	1	1	1	--	--
193 Soybean sprouts (Glycine max).....grams..	--	.160	.227	.267	.213	.045	--	--
Samples.....number..	--	1	1	1	1	1	--	--
194 Squash, summer (Cucurbita pepo).....grams..	.056	.143	.198	.278	.238	.079	--	--
Samples.....number..	1	1	1	1	1	1	--	--
195 Tomatoes and cherry tomatoes (Lycopersicon esculentum and L. e. cerasiforme):								
Average.....grams..	.054	.207	.182	.255	.260	.042	--	--
Maximum.....do...	.064	.231	--	--	.310	.058	--	--
Minimum.....do...	.029	.182	--	--	.154	.012	--	--
Samples.....number..	4	3	1	1	6	5	--	--
196 Turnips (Brassica rapa).....grams..	--	--	.116	--	.324	.066	--	--
Samples.....number..	--	--	1	--	1	1	--	--
197 Waxgourd, Chinese (Benincasa hispida)....grams..	.038	--	--	--	.138	.050	--	--
Samples.....number..	1	--	--	--	1	1	--	--

TABLE 1.—AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
--	--	0.652	0.406	--	--	--	--	--	--	--	184
--	--	1	1	--	--	--	--	--	--	--	Samples
0.026	--	.168	.030	0.039	--	--	--	--	--	--	185
1	--	1	1	1	--	--	--	--	--	--	Samples
											186
.226	0.275	.315	.322	.103	--	--	--	--	--	--	Average
.247	--	.368	.411	.130	--	--	--	--	--	--	Maximum
.208	--	.286	.158	.066	--	--	--	--	--	--	Minimum
3	1	3	3	3	--	--	--	--	--	--	Samples
											187
.174	.206	.138	.802	.064	--	--	0.928	--	--	--	Average
--	--	--	1.193	--	--	--	--	--	--	--	Maximum
--	--	--	.412	--	--	--	--	--	--	--	Minimum
1	1	1	2	1	--	--	1	--	--	--	Samples
											188
.286	--	.173	.126	.071	--	--	--	--	--	--	Average
.302	--	.175	.127	.074	--	--	--	--	--	--	Maximum
.262	--	.170	.125	.070	--	--	--	--	--	--	Minimum
3	--	3	3	3	--	--	--	--	--	--	Samples
.337	--	.234	.179	.092	--	--	--	--	--	--	189
1	--	1	1	1	--	--	--	--	--	--	Samples
											190
.167	.083	.233	.224	.097	0.333	--	.750	--	--	0.167	Average
.209	--	.299	--	--	--	--	--	--	--	--	Maximum
.125	--	.167	--	--	--	--	--	--	--	--	Minimum
2	1	2	1	1	1	--	1	--	--	1	Samples
--	--	.154	--	--	--	0.538	1.308	--	--	.308	191
--	--	1	--	--	--	1	1	--	--	1	Samples
.280	--	.219	.148	.087	--	--	--	--	--	--	192
1	--	1	1	1	--	--	--	--	--	--	Samples
.187	--	.227	.227	.134	--	--	--	--	--	--	193
1	--	1	1	1	--	--	--	--	--	--	Samples
.166	--	.230	.277	.095	--	--	--	--	--	--	194
1	--	1	1	1	--	--	--	--	--	--	Samples
											195
.178	.090	.174	.182	.094	.316	.678	1.488	0.207	--	.207	Average
.207	.103	.209	.209	.118	.483	.769	1.769	--	--	--	Maximum
.154	.077	.138	.154	.069	.154	.586	1.207	--	--	--	Minimum
3	2	2	2	2	2	2	2	1	--	1	Samples
.111	.164	--	--	--	.376	--	--	--	--	--	196
1	1	--	--	--	1	--	--	--	--	--	Samples
--	--	--	--	--	--	--	--	--	--	--	197
--	--	--	--	--	--	--	--	--	--	--	Samples

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

ITEM NUMBER AND DESCRIPTION	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOT.
MISCELLANEOUS FOOD ITEMS:								
198 Vegetable patty or steak (principally wheat protein), canned, drained:								
Average.....grams..	0.054	0.156	0.336	0.410	0.122	0.096	--	--
Maximum.....do...	.054	.168	.339	.419	.125	.098	--	--
Minimum.....do...	.053	.144	.332	.400	.119	.095	--	--
Samples.....number..	2	2	2	2	2	2	--	--
Yeast:								
199 Baker's:								
Average.....grams..	.058	.312	.312	.548	.435	.118	0.057	0.175
Maximum.....do...	.075	.342	.356	.888	.482	.188	.107	--
Minimum.....do...	.031	.296	.262	.415	.400	.088	.040	--
Samples.....number..	3	4	4	4	5	4	6	--
200 Brewer's, dried:								
Average.....grams..	.096	.318	.324	.436	.446	.113	.074	.187
Maximum.....do...	.169	.406	.394	.488	.569	.188	.138	--
Minimum.....do...	.050	.194	.192	.359	.369	.062	.006	--
Samples.....number..	20	12	15	15	17	21	16	--
Primary, dried:								
201 (Saccharomyces cerevisiae):								
Average.....grams..	.086	.318	.366	.446	.451	.115	.060	.175
Maximum.....do...	.099	.375	.404	.531	.506	.175	.069	--
Minimum.....do...	.075	.188	.206	.300	.397	.059	.056	--
Samples.....number..	14	16	16	16	16	25	7	--
202 (Torulopsis utilis):								
Average.....grams..	.086	.315	.449	.501	.493	.096	.057	.153
Maximum.....do...	.119	.340	.536	.548	.545	.164	.118	--
Minimum.....do...	.035	.262	.344	.449	.425	.029	.025	--
Samples.....number..	10	7	6	6	6	8	11	--

TABLE 1.--AMINO ACIDS PER GRAM OF TOTAL NITROGEN IN EDIBLE PORTION OF FOODS--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
											198
0.308	--	0.268	0.227	0.122	--	--	--	--	--	--	Average
.314	--	.268	.228	.123	--	--	--	--	--	--	Maximum
.303	--	.268	.226	.121	--	--	--	--	--	--	Minimum
2	--	2	2	2	--	--	--	--	--	--	Samples
											199
.289	0.276	.400	.255	.168	0.572	0.760	1.158	0.375	0.247	0.300	Average
.353	.388	.585	.296	.258	--	--	--	--	--	--	Maximum
.244	.217	.312	.155	.119	--	--	--	--	--	--	Minimum
5	6	4	5	5	1	1	1	1	1	1	Samples
											200
.257	.257	.368	.304	.169	.467	.707	.856	.328	.268	.359	Average
.356	.400	.444	.406	.258	.572	.769	1.263	.381	.319	.469	Maximum
.118	.170	.294	.162	.112	.312	.665	.688	.232	.238	.150	Minimum
18	19	13	16	17	6	3	6	7	3	6	Samples
											201
.245	.334	.345	.261	.149	.181	.338	.776	.230	--	.288	Average
.291	.531	.416	.438	.202	--	--	1.010	.272	--	--	Maximum
.165	.156	.250	.191	.094	--	--	.444	.069	--	--	Minimum
16	15	16	16	16	1	1	8	8	--	1	Samples
											202
.319	.333	.392	.451	.169	--	--	.859	.425	--	--	Average
.397	.384	.411	.538	.177	--	--	.909	.492	--	--	Maximum
.244	.261	.369	.366	.161	--	--	.834	.290	--	--	Minimum
6	6	6	5	5	--	--	3	3	--	--	Samples

DISCUSSION OF DATA IN TABLE 2

Average amino acid contents of foods per 100 grams of the edible portion are shown in table 2. These values were calculated from the average amino acid content per gram of nitrogen shown in table 1 and the nitrogen content equivalent to the protein listed for the foods in the first column of table 2. Table 2 is to be used when an average protein content may be assumed. When the specific nitrogen (or protein) content of a food is known, use of data from table 1 would be preferable.

The protein content shown for an item was usually taken either from previous publications on food composition from this Department (7, 18, 27) or from the international tables of the Food and Agriculture Organization (5, 6). (These publications also give data for moisture, ash, and other proximate constituents corresponding to the protein specified.)

Only a limited number of items is included in table 2. For example, no items that required calculation from recipes were included. The drained solids of cooked and canned foods have been omitted because there may well be loss of the more soluble protein when the liquid is poured off, with greater loss of some amino acids than of others.

Procedure for calculating protein in foods. Since the procedure for obtaining figures on the protein content of foods is not generally understood, a brief explanation is included here.

Most of the nitrogen that occurs in foods and feeds is in the form of protein or its related products, peptides and amino acids. As compared with determinations of total nitrogen, protein analyses are not very satisfactory. However as all proteins contain nitrogen, it has long been customary to determine total nitrogen chemically and from this analysis to calculate protein.

Some of the first proteins analyzed were found to contain close to 16 percent nitrogen. The protein content of foods containing these proteins could be calculated by multiplying the nitrogen content found from analysis by the factor 6.25 [$100 \div 16 = 6.25$]. The results have sometimes been labeled "crude protein" to call attention to the fact that for the purpose of calculation, all nitrogen present was counted as protein nitrogen. From the first this assumption was known to introduce some error. Small amounts of other nitrogenous compounds such as choline, nitrates, nitrites, purine bases, and more recently several nitrogen-containing vitamins have been found in foods.

A food usually has more than one kind of protein and these proteins may differ in nitrogen content. Jones, in 1931, reported an extensive study of data available from his own research and from the literature on the composition of proteins in different foods and feeds (15). In the report he showed that the nitrogen content of protein in some of the well-known items of food varies considerably--from a high of 19.3 percent for amandin in almonds to a low of 13.4 percent for an alcohol-alkali soluble protein in avocado. Taking into consideration the various nitrogenous compounds and especially the nitrogen content of the chief protein or proteins present, he derived special factors for converting nitrogen to protein in foods for which he considered there was sufficient information.

The figures for protein in tables of food composition issued by the U. S. Department of Agriculture have been calculated by use of the factors published by Jones and a few additional factors recommended by him later in personal communication. These factors are as follows:

Food	Factor
Animal origin	
Eggs	6.25
Gelatin	5.55
Meat	6.25
Milk	6.38
Plant origin	
Grains and cereals:	
Barley	5.83
Corn (maize)	6.25
Millets	5.83
Oats	5.83
Rice	5.95
Rye	5.83
Sorghums	6.25
Wheat:	
Whole kernel	5.83
Bran	6.31
Embryo	5.80
Endosperm	5.70
Legumes:	
Beans:	
Adzuki	6.25
Castor	5.30
Jack	6.25
Lima	6.25
Mung	6.25
Navy	6.25

Food	Factor
Plant origin	
Legumes--Continued	
Beans--Continued	
Soybeans	5.71
Velvetbeans	6.25
Peanuts	5.46
Nuts:	
Almonds	5.18
Brazil	5.46
Butternuts	5.30
Cashew	5.30
Chestnuts	5.30
Coconuts	5.30
Hazelnuts	5.30
Hickory	5.30
Pecans	5.30
Pine nuts	5.30
Pistachio	5.30
Walnuts	5.30
Seeds:	
Cantaloup	5.30
Cottonseed	5.30
Flaxseed	5.30
Hempseed	5.30
Pumpkin	5.30
Sesame	5.30
Sunflower	5.30

The use of the factors listed above to obtain figures for protein content involves two assumptions: That all the nitrogen present is protein nitrogen, and that the protein or mixture of proteins on which the particular factor for a food was based has the same nitrogen content as other proteins that may be present in the food. Neither of these assumptions is entirely valid.

For items in table 2 that are not listed above, the nitrogen factor predominating for other items in the food group was assumed. For example, for oilseeds and nuts not listed, the factor 5.3 was used; for additional legumes and for all fruits and vegetables, 6.25. The nitrogen conversion factors actually used in making the calculations for each food have been indicated in the table.

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION

	ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
	MILK; MILK PRODUCTS:								
	MILK (Protein, N x 6.38):								
	Cow:								
1	Fluid, whole and nonfat (3.5% protein).....	0.049	0.161	0.223	0.344	0.272	0.086	0.031	0.117
	Canned:								
2	Evaporated, unsweetened (7.0% protein).....	.099	.323	.447	.688	.545	.171	.063	.234
3	Condensed, sweetened (8.1% protein).....	.114	.374	.518	.796	.631	.198	.072	.271
	Dried:								
4	Whole (25.8% protein).....	.364	1.191	1.648	2.535	2.009	.632	.231	.863
5	Nonfat (35.6% protein).....	.502	1.641	2.271	3.493	2.768	.870	.318	1.188
6	Goat (3.3% protein).....	.039	.217	.087	.278	.312	.065	--	--
7	Human (1.4% protein).....	.023	.062	.075	.124	.090	.028	.027	.055
8	Indian buffalo (4.2% protein).....	.059	.212	.204	.420	.331	.112	.058	.170
	MILK PRODUCTS:								
9	Buttermilk (3.5% protein, N x 6.38).....	.038	.165	.219	.348	.291	.082	.032	.114
10	Casein (100.0% protein, N x 6.29).....	1.335	4.277	6.550	10.048	8.013	3.084	.382	3.466
	Cheese (protein, N x 6.38):								
11	Blue mold (21.5% protein).....	.293	.799	1.449	2.096	1.577	.559	.121	.680
12	Camembert (17.5% protein).....	.239	.650	1.179	1.706	1.284	.455	.099	.554
13	Cheddar (25.0% protein).....	.341	.929	1.685	2.437	1.834	.650	.141	.791
14	Cheddar processed (23.2% protein).....	.316	.862	1.563	2.262	1.702	.604	.131	.735
15	Cheese foods, cheddar (20.5% protein).....	.280	.761	1.382	1.998	1.504	.533	.116	.649
16	Cottage (17.0% protein).....	.179	.794	.989	1.826	1.428	.469	.147	.616
17	Cream cheese (9.0% protein).....	.080	.408	.519	.923	.721	.229	.085	.314
18	Limburger (21.2% protein).....	.289	.788	1.429	2.067	1.555	.552	.120	.672
19	Parmesan(36.0% protein).....	.491	1.337	2.426	3.510	2.641	.937	.203	1.14[illegible]
20	Swiss (27.5% protein).....	.375	1.021	1.853	2.681	2.017	.715	.155	.87[illegible]
21	Swiss processed (26.4% protein).....	.360	.981	1.779	2.574	1.937	.687	.149	.83[illegible]
22	Lactalbumin (100.0% protein, N x 6.49).....	2.203	5.239	6.209	12.342	9.060	2.250	3.405	5.65[illegible]
	Whey (Protein, N x 6.49)								
23	Fluid (0.9% protein).....	.010	.048	.052	.074	.055	.013	.018	.031
24	Dried (12.7% protein).....	.147	.677	.734	1.043	.769	.188	.250	.43[illegible]
	EGGS, CHICKEN (Protein, N x 6.25):								
	Fresh or stored:								
25	Whole (12.8% protein).....	.211	.637	.850	1.126	.819	.401	.299	.70[illegible]
26	Whites (10.8% protein).....	.164	.477	.698	.950	.648	.420	.263	.68[illegible]
27	Yolks (16.3% protein).....	.235	.827	.996	1.372	1.074	.417	.274	.69[illegible]
	Dried:								
28	Whole (46.8% protein).....	.771	2.329	3.108	4.118	2.995	1.468	1.093	2.56[illegible]
29	Whites (85.9% protein).....	1.306	3.793	5.553	7.559	5.154	3.340	2.089	5.43[illegible]
30	Yolks (31.2% protein).....	.449	1.582	1.907	2.626	2.057	.799	.524	1.32[illegible]
	MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS:								
	MEAT (Protein, N x 6.25):								
	Beef carcass or side:								
31	Thin (18.8% protein).....	.220	.830	.984	1.540	1.642	.466	.238	.7[illegible]
32	Medium fat (17.5% protein).....	.204	.773	.916	1.434	1.529	.434	.221	.6[illegible]
33	Fat (16.3% protein).....	.190	.720	.853	1.335	1.424	.404	.206	.61
34	Very fat (13.7% protein).....	.160	.605	.717	1.122	1.197	.340	.173	.51
35	Medium fat, trimmed to retail basis (18.2% protein).....	.213	.804	.952	1.491	1.590	.451	.230	.6[illegible]
	Beef cuts, medium fat:								
36	Chuck (18.6% protein).....	.217	.821	.973	1.524	1.625	.461	.235	.6[illegible]
37	Flank (19.9% protein).....	.232	.879	1.041	1.630	1.738	.494	.252	.7[illegible]
38	Hamburger (16.0% protein).....	.187	.707	.837	1.311	1.398	.397	.202	.5[illegible]
39	Porterhouse (16.4% protein).....	.192	.724	.858	1.343	1.433	.407	.207	.61

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0.170	0.178	0.240	0.128	0.092	0.121	0.255	0.819	0.069	0.389	0.206	1
.340	.357	.481	.256	.185	.242	.511	1.639	.138	.779	.413	2
.393	.413	.557	.296	.214	.280	.591	1.897	.160	.902	.478	3
1.251	1.316	1.774	.944	.680	.891	1.883	6.039	.510	2.871	1.523	4
1.724	1.814	2.444	1.300	.937	1.228	2.595	8.320	.703	3.956	2.098	5
.121	--	.139	.174	.068	--	--	--	--	--	--	6
.060	.071	.086	.055	.030	.048	.109	.229	.030	.104	.056	7
.177	--	.255	.136	.086	--	--	--	--	--	--	8
.186	.137	.262	.168	.099	--	--	.620	--	--	.133	9
5.389	5.819	7.393	4.070	3.021	3.354	7.393	23.052	1.987	11.749	6.645	10
1.153	1.028	1.543	.785	.701	.603	1.254	5.881	.330	2.463	1.294	11
.938	.837	1.256	.639	.571	1.020	1.020	4.787	.269	2.005	1.053	12
1.340	1.195	1.794	.913	.815	.701	1.457	6.837	.384	2.864	1.505	13
1.244	1.109	1.665	.847	.756	.651	1.353	6.345	.356	2.658	1.396	14
1.099	.980	1.472	.749	.668	.575	1.195	5.607	.315	2.349	1.234	15
.917	.917	.978	.802	.549	--	--	--	--	--	--	16
.547	.408	.538	.313	.278	--	--	--	--	--	--	17
1.136	1.014	1.522	.774	.691	.595	1.236	5.799	.326	2.429	1.276	18
1.930	1.721	2.584	1.315	1.174	1.010	2.099	9.847	.553	4.125	2.167	19
1.474	1.315	1.974	1.004	.896	.771	1.603	7.521	.422	3.151	1.655	20
1.415	1.262	1.895	.964	.861	.741	1.539	7.221	.406	3.025	1.589	21
4.360	3.806	5.686	3.498	1.911	6.348	9.907	16.086	--	4.129	4.129	22
.023	.009	.045	.017	.011	--	--	.097	--	--	.027	23
.323	.131	.640	.235	.159	--	--	1.360	--	--	.374	24
.739	.551	.950	.840	.307	--	.897	1.583	.453	.543	1.075	25
.689	.449	.842	.634	.233	.816	.850	1.521	.404	.408	.848	26
.717	.756	1.121	1.132	.368	--	.897	1.951	.571	.722	1.314	27
2.703	2.014	3.474	3.070	1.123	--	3.280	5.788	1.655	1.984	3.931	28
5.484	3.573	6.693	5.044	1.855	6.487	6.762	12.095	3.216	3.244	6.748	29
1.373	1.448	2.147	2.167	.704	--	1.717	3.734	1.093	1.383	2.516	30
.773	.638	1.044	1.212	.653	1.086	1.754	2.846	1.164	.926	.788	31
.720	.594	.972	1.128	.608	1.011	1.632	2.649	1.084	.862	.734	32
.670	.553	.905	1.051	.566	.941	1.520	2.467	1.009	.803	.683	33
.563	.465	.761	.883	.476	.791	1.278	2.074	.848	.675	.574	34
.748	.617	1.010	1.174	.632	1.051	1.698	2.755	1.127	.897	.763	35
.765	.631	1.033	1.199	.646	1.074	1.735	2.815	1.152	.917	.780	36
.818	.675	1.105	1.283	.691	1.149	1.856	3.012	1.232	.981	.834	37
.658	.543	.888	1.032	.556	.924	1.492	2.422	.991	.788	.671	38
.674	.556	.911	1.057	.569	.947	1.530	2.482	1.015	.808	.687	39

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

	ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
							METH-IONINE	CYSTINE	TOTAL
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
	MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS--Continued								
	MEAT (Protein, N x 6.25)--Continued								
	Beef cuts, medium fat--Continued								
40	Rib roast (17.4% protein)	0.203	0.768	0.910	1.425	1.520	0.432	0.220	0.65[illegible]
41	Round (19.5% protein)	.228	.861	1.020	1.597	1.704	.484	.246	.73[illegible]
42	Rump (16.2% protein)	.189	.715	.848	1.327	1.415	.402	.205	.607
43	Sirloin (17.3% protein)	.202	.764	.905	1.417	1.511	.429	.219	.64[illegible]
44	Beef, canned (25.0% protein)	.292	1.104	1.308	2.048	2.184	.620	.316	.93[illegible]
45	Beef, dried or chipped (34.3% protein)	.401	1.515	1.795	2.810	2.996	.851	.434	1.28[illegible]
	Lamb carcass or side:								
46	Thin (17.1% protein)	.222	.782	.886	1.324	1.384	.410	.224	.[illegible]34
47	Medium fat (15.7% protein)	.203	.718	.814	1.216	1.271	.377	.206	.[illegible]
48	Fat (13.0% protein)	.168	.595	.674	1.007	1.052	.312	.171	.4[illegible]
	Lamb cuts, medium fat:								
49	Leg (18.0% protein)	.233	.824	.933	1.394	1.457	.432	.236	.65[illegible]
50	Rib (14.9% protein)	.193	.682	.772	1.154	1.206	.358	.195	.553
51	Shoulder (15.6% protein)	.202	.714	.809	1.208	1.263	.374	.205	.57[illegible]
	Pork, packer's carcass or side:								
52	Thin (14.1% protein)	.183	.654	.724	1.038	1.157	.352	.165	.517
53	Medium fat (11.9% protein)	.154	.552	.611	.876	.977	.297	.139	.436
54	Fat (9.8% protein)	.127	.455	.503	.721	.804	.245	.114	.[illegible]59
	Pork cuts, medium fat, fresh:								
55	Ham (15.2% protein)	.197	.705	.781	1.119	1.248	.379	.178	.557
56	Loin (16.4% protein)	.213	.761	.842	1.207	1.346	.409	.192	.601
57	Miscellaneous lean cuts (14.5% protein)	.188	.673	.745	1.067	1.190	.362	.169	.531
	Pork, cured:								
58	Bacon, medium fat (9.1% protein)	.095	.306	.399	.728	.587	.141	.106	.247
59	Fat back or salt pork (3.9% protein)	.006	.141	.110	.367	.317	.055	.043	.09[illegible]
60	Ham (16.9% protein)	.162	.692	.841	1.306	1.420	.411	.273	.664
	Luncheon meat:								
61	Boiled ham (22.8% protein)	.219	.934	1.135	1.762	1.915	.554	.368	.923
62	Canned, spiced (14.9% protein)	.143	.610	.741	1.151	1.252	.362	.241	.603
63	Rabbit, domesticated, flesh only (21.0% protein)	—	1.021	1.082	1.636	1.818	.541	--	--
	Veal, carcass or side:								
64	Thin (19.7% protein)	.258	.854	1.040	1.444	1.645	.451	.233	.684
65	Medium fat (19.1% protein)	.251	.828	1.008	1.400	1.595	.437	.226	.663
66	Fat (18.5% protein)	.243	.802	.977	1.356	1.545	.423	.219	.642
	Veal cuts, medium fat:								
67	Round (19.5% protein)	.256	.846	1.030	1.429	1.629	.446	.231	.677
68	Shoulder (19.4% protein)	.255	.841	1.024	1.422	1.620	.444	.230	.674
69	Stew meat (18.3% protein)	.240	.793	.966	1.341	1.528	.419	.217	.636
	POULTRY (Protein, N x 6.25):								
	Chicken, flesh only:								
70	Broilers or fryers (20.6% protein)	.250	.877	1.088	1.490	1.810	.537	.277	.814
71	Hens (21.3% protein)	.259	.907	1.125	1.540	1.871	.556	.286	.842
72	Ducks, domesticated, flesh only (21.4% protein)	--	.935	1.109	1.657	1.842	.531	--	--
73	Turkey, flesh only (24.0% protein)	--	1.014	1.260	1.836	2.173	.664	.330	.994
	FISH AND SHELLFISH (Protein, N x 6.25):								
74	Blue fish (20.5% protein)	.203	.889	1.040	1.548	1.797	.597	.276	.873
	Cod:								
75	Fresh (16.5% protein)	.164	.715	.837	1.246	1.447	.480	.222	.702
76	Dried (81.8% protein)	.811	3.547	4.149	6.178	7.172	2.382	1.099	3.481
77	Croaker (17.8% protein)	.177	.772	.903	1.344	1.561	.518	.2[illegible]	.757
78	Eel (18.6% protein)	.185	.806	.943	1.405	1.631	.542	.[illegible]	.792
79	Flounder (14.9% protein)	.148	.646	.756	1.125	1.306	.434	.[illegible]	.634
80	Haddock (18.2% protein)	.181	.789	.923	1.374	1.596	.530	.2[illegible]	.775

TABLE 2.—AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0.715	0.590	0.966	1.122	0.604	1.005	1.623	2.634	1.077	0.857	0.729	40
.802	.661	1.083	1.257	.677	1.126	1.819	2.952	1.207	.961	.817	41
.666	.550	.899	1.045	.562	.936	1.511	2.452	1.003	.798	.679	42
.711	.587	.960	1.116	.601	.999	1.614	2.619	1.071	.853	.725	43
1.028	.848	1.388	1.612	.868	1.444	2.332	3.784	1.548	1.232	1.048	44
1.410	1.163	1.904	2.212	1.191	1.981	3.200	5.192	2.124	1.690	1.438	45
.695	.594	.843	1.114	.476	.955	1.576	2.594	.999	.791	.684	46
.638	.545	.774	1.022	.437	.877	1.447	2.381	.917	.726	.628	47
.528	.451	.641	.847	.362	.726	1.198	1.972	.759	.601	.520	48
.732	.625	.887	1.172	.501	1.005	1.659	2.730	1.051	.832	.720	49
.606	.517	.734	.970	.415	.832	1.373	2.260	.870	.689	.596	50
.634	.542	.769	1.016	.434	.871	1.438	2.366	.911	.721	.624	51
.555	.503	.733	.864	.487	.654	1.336	2.157	.699	.623	.573	52
.468	.425	.619	.729	.411	.552	1.127	1.820	.590	.526	.484	53
.386	.350	.510	.601	.339	.455	.928	1.499	.486	.433	.398	54
.598	.542	.790	.931	.525	.705	1.440	2.325	.754	.671	.618	55
.646	.585	.853	1.005	.567	.761	1.553	2.509	.813	.724	.666	56
.571	.517	.754	.889	.501	.673	1.373	2.218	.719	.640	.589	57
.434	.234	.434	.622	.246	--	.858	1.022	.858	.482	.352	58
.157	.052	.168	.379	.035	--	--	--	--	--	--	59
.646	.652	.879	1.068	.544	--	1.517	2.390	1.114	.846	.695	60
.872	.879	1.186	1.441	.733	--	2.047	3.225	1.503	1.142	.938	61
.570	.575	.775	.942	.479	--	1.337	2.107	.982	.746	.613	62
.793	--	1.021	1.176	.474	--	2.127	3.629	.958	--	--	63
.801	.709	1.018	1.283	.634	1.169	1.942	3.073	.942	.794	.867	64
.776	.688	.987	1.244	.614	1.134	1.882	2.980	.914	.770	.840	65
.752	.666	.956	1.205	.595	1.098	1.823	2.886	.885	.746	.814	66
.792	.702	1.008	1.270	.627	1.158	1.922	3.042	.933	.786	.858	67
.788	.698	1.003	1.263	.624	1.152	1.912	3.026	.928	.782	.854	68
.744	.659	.946	1.192	.589	1.086	1.804	2.855	.875	.738	.805	69
.811	.725	1.012	1.302	.593	--	2.024	3.309	1.378	--	--	70
.838	.750	1.046	1.346	.613	--	2.093	3.422	1.425	--	--	71
.842	--	1.027	1.301	.486	--	2.253	3.636	1.079	--	--	72
.960	--	1.187	1.513	.649	--	2.427	4.270	1.298	--	--	73
.761	.554	1.092	1.155	--	--	1.807	2.611	1.132	1.250	.633	74
.612	.446	.879	.929	--	--	1.455	2.101	.911	1.006	.510	75
3.036	2.212	4.358	4.607	--	--	7.211	10.418	4.515	4.987	2.526	76
.661	.481	.948	1.002	--	--	1.569	2.267	.983	1.085	.550	77
.690	.503	.991	1.048	--	--	1.640	2.369	1.027	1.134	.574	78
.553	.403	.794	.839	--	--	1.314	1.898	.822	.908	.460	79
.676	.492	.970	1.025	--	--	1.605	2.316	1.005	1.109	.562	80

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

	ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
							METH-IONINE	CYSTINE	TOTAL
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
	MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS--Continued								
	FISH AND SHELLFISH (Protein, N x 6.25)--Continued								
81	Halibut (18.6% protein)	0.185	0.806	0.943	1.405	1.631	0.542	0.250	0.7[illegible]
	Herring:								
82	Atlantic (18.3% protein)	.182	.793	.928	1.382	1.605	.533	.246	.77[illegible]
83	Lake (18.5% protein)	.184	.802	.938	1.397	1.622	.539	.249	.7[illegible]
84	Pacific (16.6% protein)	.165	.720	.842	1.254	1.455	.483	.223	.70[illegible]
	Mackerel:								
85	Raw, common Atlantic (18.7% protein)	.186	.811	.948	1.412	1.640	.545	.251	.79[illegible]
	Canned, solids and liquid:								
86	Atlantic (19.3% protein)	.191	.837	.979	1.458	1.692	.562	.259	.82[illegible]
87	Pacific (21.1% protein)	.209	.915	1.070	1.593	1.850	.614	.284	.89[illegible]
	Salmon:								
88	Raw, Pacific (Chinook or King) (17.4% protein)	.173	.754	.883	1.314	1.526	.507	.234	.741
89	Canned, solids and liquid (Sockeye or red) (20.2% protein)	.200	.876	1.025	1.526	1.771	.588	.271	.85[illegible]
	Sardines, canned, solids and liquid:								
90	Atlantic type (21.1% protein)	.209	.915	1.070	1.593	1.850	.614	.284	.89[illegible]
91	Pacific type (17.7% protein)	.176	.767	.898	1.337	1.552	.515	.238	.75[illegible]
92	Shrimp, canned, solids and liquid (18.7% protein)	.186	.811	.948	1.412	1.640	.545	.251	.79[illegible]
	PRODUCTS FROM MEAT, POULTRY, AND FISH (Protein, N x 6.25):								
93	Brains (10.4% protein)	.138	.494	.504	.845	.760	.220	.145	.36[illegible]
94	Chitterlings (8.6% protein)	.094	.398	.308	.457	.670	.193	.109	.30[illegible]
95	Fish flour (76.0% protein)	.754	4.378	4.232	6.189	7.381	2.019	--	--
96	Gelatin (85.6% protein, N x 5.55)	.006	1.912	1.357	2.930	4.226	.787	.077	.86[illegible]
97	Gizzard, chicken (23.1% protein)	.207	1.072	1.094	1.689	1.567	.554	.218	.77[illegible]
	Heart:								
98	Beef or pork (16.9% protein)	.219	.776	.857	1.509	1.387	.403	.168	.57[illegible]
99	Chicken (20.5% protein)	.266	.941	1.040	1.830	1.683	.489	.203	.69[illegible]
	Kidney:								
100	Beef (15.0% protein)	.221	.665	.730	1.301	1.087	.307	.182	.48[illegible]
101	Pork (16.3% protein)	.240	.722	.793	1.414	1.181	.334	.198	.53[illegible]
102	Sheep (16.6% protein)	.244	.736	.807	1.440	1.203	.340	.202	.54[illegible]
	Liver:								
103	Beef or pork (19.7% protein)	.296	.936	1.031	1.819	1.475	.463	.243	.70[illegible]
104	Calf (19.0% protein)	.286	.903	.994	1.754	1.423	.447	.234	.66[illegible]
105	Chicken (22.1% protein)	.332	1.050	1.156	2.040	1.655	.520	.272	.79[illegible]
106	Sheep or lamb (21.0% protein)	.316	.998	1.099	1.939	1.572	.494	.259	.75[illegible]
	Pancreas:								
107	Beef (13.5% protein)	.175	.626	.683	1.054	.996	.244	--	--
108	Pork (14.5% protein)	.188	.673	.733	1.132	1.070	.262	--	--
109	Pork and beef, canned (14.3% protein)	.151	.618	.730	1.190	1.345	.327	.261	.59[illegible]
110	Potted meat (16.1% protein)	.149	.662	.641	1.203	1.061	.361	--	--
	Sausage:								
111	Bologna (14.8% protein)	.126	.606	.716	1.061	1.191	.313	.185	.49[illegible]
112	Braunschweiger (15.4% protein)	.172	.668	.754	1.291	1.200	.320	.187	.507
113	Frankfurters (14.2% protein)	.120	.582	.688	1.018	1.143	.300	.177	.477
114	Head cheese (15.0% protein)	.079	.418	.509	.946	.907	.250	.209	.45[illegible]
115	Liverwurst (16.7% protein)	.187	.724	.818	1.400	1.301	.347	.203	.55[illegible]
116	Pork, links or bulk, raw (10.8% protein)	.092	.442	.524	.774	.869	.228	.135	.36[illegible]
117	Pork, bulk, canned (15.4% protein)	.131	.631	.747	1.104	1.239	.325	.192	.517
118	Salami (23.9% protein)	.203	.979	1.159	1.712	1.923	.505	.298	.80[illegible]
119	Vienna sausage, canned (15.8% protein)	.134	.647	.766	1.133	1.272	.334	.197	.531

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0.690	0.503	0.991	1.048	--	--	1.640	2.369	1.027	1.134	0.574	81
.679	.495	.975	1.031	--	--	1.613	2.331	1.010	1.116	.565	82
.687	.500	.986	1.042	--	--	1.631	2.356	1.021	1.128	.571	83
.616	.449	.884	.935	--	--	1.463	2.114	.916	1.012	.513	84
.694	.506	.996	1.053	--	--	1.649	2.382	1.032	1.140	.577	85
.716	.522	1.028	1.087	--	--	1.701	2.458	1.065	1.177	.596	86
.783	.571	1.124	1.188	--	--	1.860	2.687	1.165	1.286	.652	87
.646	.470	.927	.980	--	--	1.534	2.216	.960	1.061	.537	88
.750	.546	1.076	1.138	--	--	1.781	2.573	1.115	1.231	.624	89
.783	.571	1.124	1.188	--	--	1.860	2.687	1.165	1.286	.652	90
.657	.479	.943	.997	--	--	1.560	2.254	.977	1.079	.547	91
.694	.506	.996	1.053	--	--	1.649	2.382	1.032	1.140	.577	92
.506	.433	.536	.614	0.278	0.599	1.135	1.456	.419	.478	.632	93
.359	.228	.462	1.406	.169	--	--	--	--	--	--	94
2.845	--	3.916	5.204	1.289	--	--	--	--	--	--	95
2.036	.401	2.421	7.866	.771	8.483	5.737	9.470	22.795	13.773	3.301	96
.968	.680	1.116	1.741	.480	--	--	--	--	--	--	97
.765	.627	.973	1.068	.433	1.114	1.782	2.515	.930	.814	.768	98
.928	.761	1.181	1.296	.525	1.351	2.162	3.050	1.128	.987	.932	99
.706	.557	.876	.934	.377	.912	1.538	1.958	.898	.775	.847	100
.767	.605	.952	1.015	.409	.991	1.672	2.128	.975	.842	.921	101
.781	.616	.969	1.033	.417	1.009	1.702	2.167	.993	.858	.938	102
.993	.738	1.239	1.201	.523	1.261	2.030	2.679	1.198	1.012	1.031	103
.958	.711	1.195	1.158	.505	1.216	1.958	2.584	1.155	.976	.994	104
1.114	.827	1.390	1.347	.587	1.414	2.277	3.006	1.344	1.135	1.156	105
1.058	.786	1.320	1.280	.558	1.344	2.164	2.856	1.277	1.079	1.099	106
.562	.590	.724	.771	.266	--	1.296	1.132	.823	--	--	107
.603	.633	.777	.828	.285	--	1.392	1.216	.884	--	--	108
.579	.570	.810	1.050	.460	--	1.489	2.009	1.119	.828	.599	109
.641	--	.943	1.002	.322	--	1.324	1.744	1.695	1.151	--	110
.540	.481	.744	1.028	.398	--	1.395	2.022	1.241	.843	.606	111
.700	.471	.956	.954	.456	--	1.491	1.762	1.380	.973	.752	112
.518	.461	.713	.986	.382	--	1.338	1.940	1.191	.809	.582	113
.569	.569	.617	1.075	.278	--	1.334	1.584	1.802	1.214	.588	114
.759	.510	1.037	1.034	.497	--	1.617	1.910	1.496	1.055	.815	115
.394	.351	.543	.750	.290	--	1.018	1.476	.905	.615	.442	116
.562	.500	.774	1.069	.414	--	1.451	2.104	1.291	.877	.631	117
.872	.776	1.201	1.660	.642	--	2.252	3.266	2.004	1.361	.979	118
.576	.513	.794	1.097	.425	--	1.489	2.159	1.325	.900	.647	119

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

	ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTOPHAN	THREONINE	ISOLEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METHIONINE	SULFUR CONTAINING CYSTINE	SULFUR CONTAINING TOTAL
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
	MEAT; POULTRY; FISH AND SHELLFISH; THEIR PRODUCTS--Continued								
	PRODUCTS FROM MEAT, POULTRY, AND FISH (Protein, N x 6.25)--Continued								
	Tongue:								
120	Beef (16.4% protein)	0.197	0.708	0.792	1.286	1.364	0.357	0.207	0.564
121	Pork (16.8% protein)	.202	.726	.812	1.317	1.398	.366	.212	.578
122	Veal and pork loaf, canned (17.2% protein)	.198	.627	.859	1.236	1.258	.418	.209	.627
	LEGUMES(DRY SEED); COMMON NUTS; OTHER NUTS AND DRY SEEDS; THEIR PRODUCTS:								
	LEGUME SEEDS AND THEIR PRODUCTS:								
	Beans (Phaseolus vulgaris) (N x 6.25):								
123	Pinto and red Mexican (23.0% protein)	.213	.997	1.306	1.976	1.708	.232	.228	.460
	Red kidney:								
124	Raw (23.1% protein)	.214	1.002	1.312	1.985	1.715	.233	.229	.462
125	Canned, solids and liquid (5.7% protein)	.053	.247	.324	.490	.423	.057	.057	.114
	Other common beans including navy, peabean, white marrow:								
126	Raw (21.4% protein)	.199	.928	1.216	1.839	1.589	.216	.212	.428
127	Baked with pork, canned (5.8% protein)	.057	.274	.291	.486	.354	.059	.018	.077
128	Black gram, raw (23.6% protein, N x 6.25)	.242	.801	1.390	2.062	1.510	.332	.287	.619
129	Broadbeans, raw (25.4% protein, N x 6.25)	.236	.829	1.593	2.211	1.426	.106	.179	.285
130	Chickpeas (20.8% protein, N x 6.25)	.170	.739	1.195	1.538	1.434	.276	.296	.572
131	Cowpeas (22.9% protein, N x 6.25)	.220	.901	1.110	1.715	1.491	.352	.297	.649
132	Dolichos, twinflower (21.6% protein, N x 6.25)	.221	.836	1.448	1.707	1.700	.294	.480	.774
133	Lentils, whole (25.0% protein, N x 6.25)	.216	.896	1.316	1.760	1.528	.180	.204	.384
134	Lima beans (20.7% protein, N x 6.25)	.195	.980	1.199	1.722	1.378	.331	.311	.642
135	Lupine (32.3% protein, N x 6.25)	--	1.101	1.618	1.964	1.447	.114	--	--
136	Moth beans (24.4% protein, N x 6.25)	.164	--	1.093	1.484	1.202	.191	.109	.300
137	Mung beans (24.4% protein, N x 6.25)	.180	.765	1.351	2.202	1.667	.265	.152	.417
138	Peanuts (26.9% protein, N x 5.46)	.340	.828	1.266	1.872	1.099	.271	.463	.734
139	Peanut flour (51.2% protein, N x 5.46)	.647	1.575	2.410	3.563	2.091	.516	.881	1.397
140	Peanut butter (26.1% protein, N x 5.46)	.330	.803	1.228	1.816	1.066	.263	.449	.712
	Peas (Pisum sativum) (N x 6.25):								
141	Entire seeds (23.8% protein)	.251	.918	1.340	1.969	1.744	.286	.308	.594
142	Split (24.5% protein)	.259	.945	1.380	2.027	1.795	.294	.318	.612
143	Pigeonpeas, without seed coat (21.9% protein, N x 6.25)	.119	.834	1.346	1.717	1.580	.256	.308	.564
144	Soybeans, whole (34.9% protein, N x 5.71)	.526	1.504	2.054	2.946	2.414	.513	.678	1.191
	Soybean flour, flakes, and grits (protein, N x 5.71):								
145	Low fat (44.7% protein)	.673	1.926	2.630	3.773	3.092	.658	.869	1.527
146	Medium fat (42.5% protein)	.640	1.831	2.501	3.588	2.940	.625	.826	1.451
147	Full fat (35.9% protein)	.541	1.547	2.112	3.030	2.483	.528	.698	1.226
148	Soybean curd (7.0% protein, N x 5.71)	--	--	--	--	--	.081	.091	.172
149	Soybean milk (3.4% protein, N x 5.71)	.051	.176	.175	.305	.269	.054	.071	.125
150	Vetch (28.8% protein, N x 6.25)	.203	.899	2.198	2.290	1.898	.346	.336	.682
	COMMON NUTS AND THEIR PRODUCTS:								
151	Almonds (18.6 protein, N x 5.18)	.176	.610	.873	1.454	.582	.259	.377	.635
152	Brazil nuts (14.4% protein, N x 5.46)	.187	.422	.593	1.129	.443	.941	.504	1.445
153	Cashews (18.5% protein, N x 5.30)	.471	.737	1.222	1.522	.792	.353	.527	.880
154	Coconut (3.4% protein, N x 5.30)	.033	.129	.180	.269	.152	.071	.062	.133

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0.661	0.548	0.840	1.065	0.412	1.044	1.616	2.506	1.097	0.918	0.764	120
.677	.562	.860	1.091	.422	1.070	1.656	2.567	1.124	.941	.782	121
.619	.468	.958	.916	.388	--	1.395	2.034	1.475	1.018	.658	122
1.270	.887	1.395	1.384	.655	1.310	1.542	3.680	.390	.990	1.196	123
1.275	.891	1.401	1.390	.658	1.316	1.549	3.696	.392	.994	1.201	124
.315	.220	.346	.343	.162	.325	.382	.912	.097	.245	.296	125
1.181	.825	1.298	1.287	.609	1.219	1.435	3.424	.363	.921	1.113	126
.333	.165	.312	.251	.186	--	--	--	--	--	--	127
1.242	.551	1.450	1.552	.559	.895	2.201	3.315	.982	.955	.955	128
1.057	.687	1.276	1.780	.748	.671	1.646	3.475	.947	1.150	1.451	129
1.012	.692	1.025	1.551	.559	.709	1.957	2.250	.745	1.225	.819	130
1.198	.678	1.293	1.473	.692	--	--	--	--	--	--	131
1.486	.560	1.286	1.230	.650	.954	2.945	3.269	.767	1.403	1.237	132
1.104	.664	1.360	1.908	.548	.888	2.920	3.700	1.300	1.080	1.140	133
1.222	.543	1.298	1.315	.669	--	--	--	--	--	--	134
1.271	--	1.328	2.718	.811	--	3.008	6.822	1.158	--	--	135
1.003	1.245	.695	--	.722	--	--	--	--	--	--	136
1.167	.390	1.444	1.370	.543	.730	2.093	2.811	.449	1.070	.734	137
1.557	1.104	1.532	3.296	.749	1.094	4.326	5.932	1.710	1.567	2.030	138
2.963	2.100	2.916	6.273	1.425	2.082	8.233	11.290	3.254	2.982	3.863	139
1.510	1.071	1.487	3.198	.727	1.061	4.197	5.755	1.659	1.520	1.969	140
1.200	.960	1.333	2.102	.651	.960	2.917	5.583	1.378	1.340	.925	141
1.235	.988	1.372	2.164	.670	.988	3.003	5.747	1.419	1.380	.953	142
1.875	.725	1.153	1.489	.617	.855	2.688	3.953	.746	1.163	1.303	143
1.889	1.216	2.005	2.763	.911	1.571	4.633	7.010	1.595	2.567	2.494	144
2.419	1.558	2.568	3.538	1.166	2.012	5.934	8.979	2.043	3.288	3.194	145
2.300	1.481	2.441	3.364	1.109	1.913	5.642	8.537	1.943	3.126	3.037	146
1.943	1.251	2.062	2.842	.937	1.616	4.766	7.211	1.641	2.641	2.565	147
--	--	--	--	--	--	--	--	--	--	--	148
.195	.193	.186	.302	.121	.156	.428	.550	.171	--	.242	149
1.014	.369	1.442	2.249	.659	.691	2.622	2.714	.516	1.470	.650	150
1.146	.618	1.124	2.729	.517	--	--	--	--	--	--	151
.617	.483	.823	2.247	.367	--	--	--	--	--	--	152
.946	.712	1.592	2.098	.415	--	--	--	--	--	--	153
.174	.101	.212	.486	.069	--	--	--	--	--	--	154

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

	ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTOPHAN	THREONINE	ISOLEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METHIONINE	SULFUR CONTAINING CYSTINE	SULFUR CONTAINING TOTAL
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
	LEGUMES (DRY SEED); COMMON NUTS; OTHER NUTS AND DRY SEEDS; THEIR PRODUCTS--Continued								
	COMMON NUTS AND THEIR PRODUCTS--Continued								
155	Coconut meal (20.3% protein, N x 5.30)	0.199	0.770	1.076	1.605	0.908	0.421	0.372	0.793
156	Filberts (12.7% protein, N x 5.30)	.211	.415	.853	.939	.417	.139	.165	.304
	Peanuts. See Legumes.								
157	Pecans (9.4% protein, N x 5.30)	.138	.389	.553	.773	.435	.153	.216	.369
158	Walnuts (English or Persian) (15.0% protein, N x 5.30)	.175	.589	.767	1.228	.441	.306	.320	.626
	OTHER NUTS AND SEEDS AND THEIR PRODUCTS (Protein, N x 5.30):								
159	Acorns (10.4% protein)	.126	.434	.561	.808	.536	.139	.184	.323
160	Amaranth (14.6% protein)	.149	.832	.882	1.209	1.074	.372	.521	.893
161	Balsampear seed meal (41.9% protein)	--	--	--	--	1.265	--	.142	--
162	Breadnuttree, Ramón (9.6% protein)	.261	.373	.543	1.041	.418	.056	--	--
163	Chinese tallow tree-nut flour (57.6% protein)	.837	2.174	3.510	4.347	1.587	.924	.696	1.620
164	Chocolatetree, Nicaragua (38.5% protein)	.588	1.496	2.092	3.952	2.223	.276	--	--
165	Cottonseed flour and meal (42.3% protein)	.591	1.764	1.884	2.945	2.139	.686	.814	1.500
166	Earpodtree, Guanacaste (34.1% protein)	.444	1.165	2.213	4.581	1.930	.360	--	--
167	Leadtree (24.1% protein)	.191	.828	1.651	1.787	1.164	.055	--	--
168	Pumpkin seed (30.9% protein)	.560	.933	1.737	2.437	1.411	.577	--	--
169	Safflower seed meal (42.1% protein)	.675	1.462	1.914	2.740	1.525	.731	--	--
	Sesame:								
170	Seed (19.3% protein)	.331	.707	.951	1.679	.583	.637	.495	1.132
171	Meal (33.4% protein)	.573	1.223	1.645	2.905	1.008	1.103	.857	1.960
	Sunflower:								
172	Kernel (23.0% protein)	.343	.911	1.276	1.736	.868	.443	.464	.907
173	Meal (39.5% protein)	.589	1.565	2.191	2.981	1.491	.760	.797	1.557
	GRAINS AND THEIR PRODUCTS:								
174	Barley (12.8% protein, N x 5.83)	.160	.433	.545	.889	.433	.184	.257	.441
175	Bread, white (4% nonfat dry milk, flour basis) (8.5% protein, N x 5.70)	.091	.282	.429	.668	.225	.142	.200	.342
	Buckwheat flour:								
176	Dark (11.7% protein, N x 6.25)	.165	.461	.440	.683	.687	.206	.228	.434
177	Light (6.4% protein, N x 6.25)	.090	.252	.241	.374	.376	.113	.125	.238
178	Cañihua (14.7% protein, N x 6.25)	.118	.706	1.000	.851	.882	.263	.162	.425
	Cereal combinations:								
179	Corn and soy grits (18.0% protein, N x 6.25)	.161	.792	.841	1.656	.772	.271	.311	.582
180	Infant food, precooked, mixed cereals with nonfat dry milk and yeast (19.4% protein, N x 6.25)	.118	--	--	--	.273	.310	.137	.447
181	Oat-corn-rye mixture, puffed (14.5% protein, N x 5.83)	.172	.545	.841	1.368	.343	.368	.234	.622
182	Corn, field (10.0% protein, N x 6.25)	.061	.398	.462	1.296	.288	.186	.130	.316
183	Corn flour (7.8% protein, N x 6.25)	.047	.311	.361	1.011	.225	.145	.101	.246
184	Corn grits (8.7% protein, N x 6.25)	.053	.347	.402	1.128	.251	.161	.113	.274
	Cornmeal:								
185	Whole ground (9.2% protein, N x 6.25)	.056	.367	.425	1.192	.265	.171	.119	.290
186	Degermed (7.9% protein, N x 6.25)	.048	.315	.365	1.024	.228	.147	.102	.249
	Corn products:								
187	Flakes (8.1% protein, N x 6.25)	.052	.275	.306	1.047	.154	.135	.152	.157
188	Germ (14.5% protein, N x 6.25)	.144	.622	.578	1.030	.791	.232	.130	.362
189	Gluten (10.0% protein, N x 6.25)	.059	.344	.443	1.563	.179	.282	.141	.423
190	Hominy (8.7% protein, N x 6.25)	.084	.316	.349	.810	.358	.099	--	--

TABLE 2.—AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
1.038	0.605	1.268	2.899	0.414	--	--	--	--	--	--	155
.537	.434	.934	2.171	.288	--	1.073	3.079	1.421	0.841	1.447	156
.564	.316	.525	1.185	.273	--	--	--	--	--	--	157
.767	.583	.974	2.287	.405	--	--	--	--	--	--	158
.473	--	.718	.722	.251	--	--	--	--	--	--	159
1.141	--	.849	1.747	.441	--	--	--	--	--	--	160
2.609	.617	--	5.914	.917	--	--	--	--	--	--	161
.453	--	.927	.884	.147	--	--	--	--	--	--	162
2.847	2.011	4.510	10.031	1.587	1.250	6.934	10.868	3.010	2.674	3.173	163
2.630	--	2.404	4.220	.683	--	--	--	--	--	--	164
2.610	1.365	2.458	5.603	1.325	2.155	5.148	9.122	2.322	1.868	2.263	165
1.325	--	1.570	2.857	1.004	--	--	--	--	--	--	166
.855	--	.864	2.410	.564	--	--	--	--	--	--	167
1.749	--	1.679	4.810	.711	--	--	--	--	--	--	168
2.605	--	2.446	4.623	.985	--	--	--	2.494	--	--	169
1.457	.951	.885	1.992	.441	.798	--	--	2.116	--	--	170
2.521	1.645	1.531	3.447	.763	1.380	--	--	3.661	--	--	171
1.220	.647	1.354	2.370	.586	.868	--	--	--	--	--	172
2.094	1.110	2.325	4.069	1.006	1.491	--	--	--	--	--	173
.661	.466	.643	.659	.239	.589	.712	2.861	.582	1.155	.595	174
.465	.243	.435	.340	.192	.268	.426	2.952	.301	1.006	--	175
.442	.240	.607	.930	.256	--	--	--	--	--	--	176
.242	.131	.332	.509	.140	--	--	--	--	--	--	177
.529	.294	.677	1.162	.367	--	--	--	--	--	--	178
.832	.562	1.054	.982	.472	--	--	--	--	--	--	179
.543	.447	--	.447	.233	--	--	--	--	--	--	180
.933	.622	.900	.776	.326	--	--	--	--	--	--	181
.454	.611	.510	.352	.206	.995	1.242	1.765	.339	.835	.565	182
.354	.477	.398	.275	.161	.776	.968	1.377	.265	.651	.441	183
.395	.532	.444	.306	.180	.866	1.080	1.535	.295	.727	.491	184
.418	.562	.470	.324	.190	.916	1.142	1.624	.312	.768	.520	185
.359	.483	.403	.278	.163	.786	.961	1.394	.268	.660	.446	186
.354	.283	.386	.231	.226	--	--	--	--	--	--	187
.483	.343	.789	1.134	.464	--	--	--	--	--	--	188
.558	.582	.512	.322	.200	--	.194	2.488	.403	--	--	189
.333	.331	.398	.444	.203	--	--	--	--	--	--	190

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

	ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTOPHAN	THREONINE	ISOLEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METHIONINE	SULFUR CONTAINING CYSTINE	SULFUR CONTAINING TOTAL
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
	GRAINS AND THEIR PRODUCTS--Continued								
	Corn products--Continued								
191	Masa (2.8% protein, N x 6.25)	0.010	--	--	--	0.103	0.108	0.030	0.138
192	Pozol (5.9% protein, N x 6.25)	.042	0.336	0.304	0.591	.234	.087	--	--
193	Tortilla (5.8% protein, N x 6.25)	.031	.235	.345	.939	.145	.111	--	--
194	Zein (16.1% protein, N x 6.25)	.010	.495	.822	3.184	--	.281	.162	.443
195	Job's tears (13.8% protein, N x 5.83)	.066	.620	1.065	3.506	.362	.459	.265	.724
	Millets:								
196	Foxtail millet (9.7% protein, N x 5.83)	.103	.323	.790	1.737	.218	.291	--	--
197	Little millet (7.2% protein, N x 5.83)	.047	.262	.517	.841	.138	.178	--	--
198	Pearlmillet (11.4% protein, N x 5.83)	.248	.456	.635	1.746	.383	.270	.152	.422
199	Ragimillet (6.2% protein, N x 5.83)	.085	.270	.398	.620	.202	.270	.187	.457
200	Oatmeal and rolled oats (14.2% protein, N x 5.83)	.183	.470	.733	1.065	.521	.209	.309	.518
201	Quinoa (11.0% protein, N x 6.25)	.120	.523	.722	.781	.729	.278	.107	.385
	Rice:								
202	Brown (7.5% protein, N x 5.95)	.081	.294	.352	.646	.296	.135	.102	.237
203	White and converted (7.6% protein, N x 5.95)	.082	.298	.356	.655	.300	.137	.103	.240
	Rice products:								
204	Flakes or puffed (5.9% protein, N x 5.95)	.046	--	--	--	.056	--	.044	--
205	Germ (14.2% protein, N x 5.95)	.270	2.177	.630	.838	1.707	.420	.169	.589
206	Rye (12.1% protein, N x 5.83)	.137	.448	.515	.813	.494	.191	.241	.432
	Rye flour:								
207	Light (9.4% protein, N x 5.83)	.106	.348	.400	.632	.384	.148	.187	.335
208	Medium (11.4% protein, N x 5.83)	.129	.422	.485	.766	.465	.180	.227	.407
209	Sorghum (11.0% protein, N x 6.25)	.123	.394	.598	1.767	.299	.190	.183	.373
210	Teosinte (22.0% protein, N x 6.25)	.049	--	--	--	.348	.496	--	--
	Wheat, whole grain:								
211	Hard red spring (14.0% protein, N x 5.83)	.173	.403	.607	.939	.384	.214	.307	.521
212	Hard red winter (12.3% protein, N x 5.83)	.152	.354	.534	.825	.338	.188	.270	.458
213	Soft red winter (10.2% protein, N x 5.83)	.126	.294	.443	.684	.280	.156	.224	.380
214	White (9.4% protein, N x 5.83)	.116	.271	.408	.630	.258	.143	.206	.349
215	Durum (12.7% protein, N x 5.83)	.157	.366	.551	.852	.348	.194	.279	.473
	Wheat flour:								
216	Whole grain (13.3% protein, N x 5.83)	.164	.383	.577	.892	.365	.203	.292	.495
217	Intermediate extraction (12.0% protein, N x 5.70)	--	.392	.619	.924	.356	.198	.320	.518
218	White (10.5% protein, N x 5.70)	.129	.302	.483	.809	.239	.138	.210	.348
	Wheat products:								
219	Bran (12.0% protein, N x 6.31)	.196	.342	.485	.717	.491	.145	.270	.415
220	Burghul (12.4% protein, N x 5.83)	.070	--	--	--	.430	.300	.319	.619
221	Farina (10.9% protein, N x 5.70)	.124	--	--	--	.199	.143	.184	.327
222	Flakes (10.8% protein, N x 5.70)	.121	.356	.496	.891	.360	.127	.191	.318
223	Germ (25.2% protein, N x 5.80)	.265	1.343	1.177	1.708	1.534	.404	.287	.691
224	Gluten, commercial (80.0% protein, N x 5.70)	.856	2.119	3.677	5.993	1.530	1.389	1.726	3.115
225	Gluten flour (41.4% protein, N x 5.70)	.443	1.097	1.903	3.101	.792	.719	.893	1.612
226	Macaroni or spaghetti (12.8% protein, N x 5.70)	.150	.499	.642	.849	.413	.193	.243	.436
227	Noodles, contain egg solids (12.6% protein, N x 5.70)	.133	.533	.621	.834	.411	.212	.245	.457
228	Shredded wheat (10.1% protein, N x 5.83)	.085	.405	.449	.684	.331	.139	.204	.343
229	Whole wheat with added germ (12.8% protein, N x 5.83)	.136	--	--	--	.466	--	.246	--

TABLE 2--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
--	--	--	--	--	--	--	--	--	--	--	191
0.254	--	0.267	0.197	0.122	--	--	--	--	--	--	192
.252	--	.304	.223	.128	--	--	--	--	--	--	193
1.664	0.981	.654	.286	.216	1.770	0.804	4.776	0.227	1.690	1.036	194
.703	--	--	.518	.317	--	--	--	--	--	--	195
.697	--	.717	.374	.218	--	--	--	--	--	--	196
.370	--	.471	.363	.147	--	--	--	--	--	--	197
.506	--	.682	.524	.240	--	--	--	--	--	--	198
.263	--	.473	.100	.079	--	--	--	--	--	--	199
.758	.524	.845	.935	.261	.867	.587	2.860	.646	.809	.568	200
.394	.253	.447	.820	.297	--	--	--	--	--	--	201
.377	.343	.524	.432	.126	--	.354	1.027	.513	.363	.381	202
.382	.347	.531	.438	.128	--	.359	1.041	.520	.368	.386	203
.286	.124	--	.137	.137	--	--	--	--	--	--	204
.750	.929	.938	1.559	.430	--	1.308	1.248	1.029	.869	.929	205
.571	.390	.631	.591	.276	--	--	2.573	--	--	.500	206
.443	.303	.490	.459	.214	--	--	1.999	--	--	.388	207
.538	.368	.594	.557	.260	--	--	2.424	--	--	.471	208
.547	.303	.628	.417	.211	--	--	2.411	--	--	.556	209
--	--	--	--	--	--	--	--	--	--	--	210
.691	.523	.648	.670	.286	.490	.764	4.375	.855	1.462	.646	211
.608	.460	.570	.589	.251	.430	.671	3.844	.751	1.285	.568	212
.504	.382	.472	.488	.208	.357	.556	3.188	.623	1.066	.471	213
.464	.351	.435	.450	.192	.329	.513	2.937	.574	.982	.434	214
.627	.475	.588	.608	.259	.444	.693	3.968	.775	1.326	.586	215
.657	.497	.616	.636	.271	.465	.725	4.156	.812	1.389	.614	216
.732	.335	.583	.549	.286	--	--	--	--	--	--	217
.577	.359	.453	.466	.210	.317	.455	3.653	.381	1.254	.542	218
.434	.259	.552	.742	.280	--	--	--	--	--	.633	219
--	--	--	--	--	--	--	--	--	--	--	220
.579	.447	--	.424	.268	--	--	--	--	--	--	221
.478	.311	.572	.559	.231	--	--	--	--	--	--	222
.908	.882	1.364	1.825	.687	--	--	--	--	--	--	223
4.351	2.596	3.789	3.461	1.825	1.796	2.891	2.933	2.933	10.189	3.789	224
2.252	1.344	1.961	1.801	.944	.930	1.496	1.518	1.518	5.273	1.961	225
.659	.422	.728	.562	.303	--	--	--	--	--	--	226
.610	.312	.745	.621	.301	--	--	--	--	--	--	227
.481	.236	.577	.523	.236	--	--	--	--	--	--	228
.755	.481	--	.742	.371	--	--	--	--	--	--	229

TABLE 2.—AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
FRUITS (Protein, N x 6.25):								
230 Abiu (1.7% protein)	0.028	--	--	--	0.085	0.013	--	--
231 Avocados (1.3% protein)	.014	--	--	--	.074	.012	--	--
Bananas, ripe:								
232 Common (1.2% protein)	.018	--	--	--	.055	.011	--	--
233 Dwarf (1.2% protein)	.012	--	--	--	.049	.004	--	--
234 Dates (2.2% protein)	.061	0.061	0.074	0.077	.065	.027	--	--
235 Grapefruit (0.5% protein)	.001	--	--	--	.006	.000	--	--
236 Guavas, common (1.0% protein)	.010	--	--	--	.030	.010	--	--
237 Limes (0.8% protein)	.003	--	--	--	.015	.002	--	--
238 Mamey (0.5% protein)	.006	--	--	--	.040	.007	--	--
239 Mangos (0.7% protein)	.014	--	--	--	.093	.008	--	--
240 Muskmelons (0.6% protein)	.001	--	--	--	.015	.002	--	--
241 Oranges, sweet (0.9% protein)	.003	--	--	--	.024	.003	--	--
242 Orange juice (0.8% protein)	.003	--	--	--	.021	.002	--	--
243 Oranges, mandarin including tangerines (0.8% protein)	.005	--	--	--	.028	.004	--	--
244 Papayas (0.6% protein)	.012	--	--	--	.038	.002	--	--
245 Pineapple (0.4% protein)	.005	--	--	--	.009	.001	--	--
246 Plantain or baking banana (1.1% protein)	.010	.027	.056	.059	.050	.005	0.016	0.021
247 Soursop (1.0% protein)	.011	--	--	--	.060	.007	--	--
248 Sugarapple (1.8% protein)	.009	--	--	--	.071	.008	--	--
VEGETABLES:								
IMMATURE SEEDS (Protein, N x 6.25):								
Corn, sweet, white or yellow:								
249 Raw (3.7% protein)	.023	.151	.137	.407	.137	.072	.062	.134
250 Canned, solids and liquid (2.0% protein)	.012	.082	.074	.220	.074	.039	.033	.072
251 Cowpeas (9.4% protein)	.099	.353	.465	.653	.617	.131	--	--
Lima beans:								
252 Raw (7.5% protein)	.097	.338	.460	.605	.474	.080	.083	.163
253 Canned, solids and liquid (3.8% protein)	.049	.171	.233	.306	.240	.041	.042	.083
Peas:								
254 Raw (6.7% protein)	.056	.245	.308	.418	.316	.054	.073	.127
255 Canned, solids and liquid (3.4% protein)	.028	.125	.156	.212	.160	.027	.037	.064
LEAFY VEGETABLES, RAW (Protein, N x 6.25):								
256 Amaranth (3.5% protein)	.038	.056	.164	.206	.141	.025	.024	.049
257 Beet greens (2.0% protein)	.024	.076	.084	.129	.108	.034	--	--
258 Brussels sprouts (4.4% protein)	.044	.153	.186	.194	.197	.046	--	--
259 Cabbage (1.4% protein)	.011	.039	.040	.057	.066	.013	.028	.041
260 Chard (1.4% protein)	.014	.058	.060	.076	.055	.004	--	--
261 Chicory (1.6% protein)	.024	--	--	--	.052	.016	.006	.022
262 Collards (3.9% protein)	.055	.114	.121	.218	.202	.046	.059	.105
263 Kale (3.9% protein)	.042	.139	.133	.252	.121	.035	.036	.071
264 Lettuce (1.2% protein)	.012	--	--	--	.070	.004	--	--
265 Mustard greens (2.3% protein)	.037	.060	.075	.062	.111	.024	.035	.059
266 Parsley, curly garden (2.5% protein)	.050	--	--	--	.160	.012	--	--
267 Spinach (2.3% protein)	.037	.102	.107	.176	.142	.039	.046	.085
268 Turnip greens (2.9% protein)	.045	.125	.107	.207	.129	.052	.045	.097
269 Watercress (1.7% protein)	.028	.084	.076	.131	.091	.010	--	--

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
--	--	--	--	--	--	--	--	--	--	--	230
--	--	--	--	--	--	--	--	--	--	--	231
--	0.031	--	--	--	--	--	--	--	--	--	232
--	--	--	--	--	--	--	--	--	--	--	233
0.063	--	0.094	0.049	0.049	--	--	--	--	--	--	234
--	--	--	--	--	--	--	--	--	--	--	235
--	--	--	--	--	--	--	--	--	--	--	236
--	--	--	--	--	--	--	--	--	--	--	237
--	--	--	--	--	--	--	--	--	--	--	238
--	--	--	--	--	--	--	--	--	--	--	239
--	--	--	--	--	--	--	--	--	--	--	240
--	--	--	--	--	--	--	--	--	--	--	241
--	--	--	--	--	--	--	--	--	--	--	242
--	--	--	--	--	--	--	--	--	--	--	243
--	--	--	--	--	--	--	--	--	--	--	244
--	--	--	--	--	--	--	--	--	--	--	245
.049	--	.065	.045	--	--	0.120	0.192	--	--	--	246
--	--	--	--	--	--	--	--	--	--	--	247
--	--	--	--	--	--	--	--	--	--	--	248
.207	.124	.231	.174	.095	--	--	--	--	--	--	249
.112	.067	.125	.094	.052	--	--	--	--	--	--	250
.523	--	.513	.615	.310	--	--	--	--	--	--	251
.389	.259	.485	.454	.247	--	--	--	--	--	--	252
.197	.131	.246	.230	.125	--	--	--	--	--	--	253
.257	.163	.274	.595	.109	0.196	.639	.474	0.217	--	--	254
.131	.083	.139	.302	.055	.100	.324	.240	.110	--	--	255
.096	.105	.136	.134	.069	.280	.252	.322	--	--	--	256
.116	--	.101	.083	.026	--	--	--	--	--	--	257
.148	--	.193	.279	.106	--	--	--	--	--	--	258
.030	.030	.043	.105	.025	.071	.196	.266	--	--	0.042	259
.046	--	.055	.035	.018	--	--	--	--	--	--	260
--	.040	--	--	.024	--	--	--	--	--	--	261
.124	.151	.195	.258	.087	--	--	--	--	--	--	262
.158	--	.184	.202	.062	.064	--	--	--	--	--	263
--	--	--	--	--	--	--	--	--	--	--	264
.074	.121	.108	.167	.041	--	--	--	--	--	--	265
--	--	--	--	--	--	--	--	--	--	--	266
.099	.073	.126	.116	.049	.073	--	.289	--	--	--	267
.146	.105	.149	.167	.051	--	--	--	--	--	--	268
.062	.036	.084	.053	.034	.060	.242	.163	--	--	--	269

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

	ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING METH-IONINE	CYSTINE	TOTAL
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
	VEGETABLES--Continued								
	STARCHY ROOTS AND TUBERS (Protein, N x 6.25):								
270	Apio arracacia (1.2% protein)	0.008	--	--	--	0.042	0.003	--	--
	Cassava:								
271	Flour (1.6% protein)	.021	0.044	0.045	0.066	.066	.010	0.018	0.028
272	Root (1.1% protein)	.014	.030	.031	.045	.045	.007	.012	.019
	Potatoes:								
273	Raw (2.0% protein)	.021	.079	.088	.100	.107	.025	.019	.044
274	Canned, solids and liquid (1.7% protein)	.018	.067	.075	.085	.091	.021	.016	.037
275	Flour (7.1% protein)	.076	.279	.311	.353	.378	.089	.068	.157
	Sweetpotatoes (Ipomaea batatas):								
276	Raw (1.8% protein)	.031	.085	.087	.103	.085	.033	.029	.062
277	Dehydrated (5.0% protein)	.087	.235	.241	.286	.236	.093	.080	.173
278	Taro (1.9% protein)	.035	.089	.099	.169	.110	.021	--	--
279	Yam (Dioscorea spp.) (2.1% protein)	.035	--	--	--	.110	.034	--	--
280	Yautia malanga (1.7% protein)	.023	--	--	--	.067	.016	--	--
	OTHER VEGETABLES (Protein, N x 6.25):								
	Asparagus:								
281	Raw (2.2% protein)	.027	.066	.080	.096	.103	.032	--	--
282	Canned, solids and liquid (1.9% protein)	.023	.057	.069	.083	.089	.027	--	--
	Beans, snap:								
283	Raw (2.4% protein)	.033	.091	.109	.139	.126	.035	.024	.059
284	Canned, solids and liquid (1.0% protein)	.014	.038	.045	.058	.052	.014	.010	.024
	Beets:								
285	Raw (1.6% protein)	.014	.034	.051	.055	.086	.006	--	--
286	Canned, solids and liquid (0.9% protein)	.008	.019	.029	.031	.048	.003	--	--
287	Broccoli (3.3% protein)	.037	.122	.126	.163	.147	.050	--	--
	Carrots:								
288	Raw (1.2% protein)	.010	.043	.046	.065	.052	.010	.029	.039
289	Canned, solids and liquid (0.5% protein)	.004	.018	.019	.027	.022	.004	.012	.016
290	Cauliflower (2.4% protein)	.033	.102	.104	.162	.134	.047	--	--
291	Celery (1.3% protein)	.012	--	--	--	.021	.015	.006	.021
292	Chayote (0.6% protein)	.008	--	--	--	.038	.001	--	--
293	Cowpeas, yardlong, immature pod (3.4% protein)	.034	--	--	--	.203	.021	--	--
294	Cucumbers (0.7% protein)	.005	.019	.022	.030	.031	.007	--	--
295	Cushaw (1.5% protein)	.014	--	--	--	.044	.008	--	--
296	Eggplant (1.1% protein)	.010	.038	.056	.068	.030	.006	--	--
297	Mallow (3.7% protein)	.144	.155	--	.259	.155	.030	--	--
	Mushrooms:								
298	(Agaricus campestris) 1/	.006	--	.532	.281	--	.167	--	--
299	(Lactarius spp.) 2/	.006	.156	.201	.139	.088	.021	--	--
300	Okra (1.8% protein)	.018	.066	.069	.101	.076	.022	.017	.039
301	Onions, mature (1.4% protein)	.021	.022	.021	.037	.064	.013	--	--
302	Peppers (1.2% protein)	.009	.050	.046	.046	.051	.016	--	--
303	Pricklypears (1.1% protein)	.009	.053	.044	.057	.044	.008	--	--
304	Pumpkin (1.2% protein)	.016	.028	.044	.063	.058	.011	--	--
305	Radishes (1.2% protein)	.005	.059	--	--	.034	.002	--	--

1/ Total nitrogen is 0.58%. This is equivalent to 2.4% protein on the basis that 2/3 of the nitrogen is protein nitrogen. If total nitrogen is used for the calculation, the protein content is 3.6%.

2/ Total nitrogen is 0.69%. This is equivalent to 2.9% protein on the basis that 2/3 of the nitrogen is protein nitrogen. If total nitrogen is used for the calculation, the protein content is 4.3%.

TABLE 2.—AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
--	--	--	--	--	--	--	--	--	--	--	270
0.045	0.030	0.049	0.159	0.025	0.081	0.085	0.195	0.045	0.036	0.042	271
.031	.021	.033	.110	.017	.056	.058	.134	.031	.024	.029	272
.088	.036	.107	.099	.029	.093	--	.200	--	.067	.080	273
.075	.030	.091	.084	.024	.079	--	.170	--	.057	.068	274
.314	.127	.379	.350	.102	.332	--	.710	--	.236	.284	275
.100	.081	.135	.094	.036	--	--	.334	--	--	.046	276
.278	.225	.374	.261	.099	--	--	.926	--	--	.126	277
.099	--	.114	.118	.032	--	--	--	--	--	--	278
--	--	--	--	--	--	--	--	--	--	--	279
--	--	--	--	--	--	--	--	--	--	--	280
.069	--	.106	.123	.036	--	--	--	--	--	--	281
.060	--	.092	.106	.031	--	--	--	--	--	--	282
.057	.050	.115	.101	.045	.097	--	--	--	--	--	283
.024	.021	.048	.042	.019	.040	--	--	--	--	--	284
.027	--	.049	.028	.022	--	.157	.256	--	.029	--	285
.015	--	.028	.016	.012	--	.089	.144	--	.017	--	286
.119	--	.170	.192	.063	--	--	--	--	--	--	287
.042	.020	.056	.041	.017	.058	.090	.218	--	--	.038	288
.018	.008	.023	.017	.007	.024	.037	.091	--	--	.016	289
.075	.034	.144	.110	.048	.192	.199	.185	.165	--	--	290
--	.016	--	--	--	--	--	--	--	--	--	291
--	--	--	--	--	--	--	--	--	--	--	292
--	--	--	--	--	--	--	--	--	--	--	293
.016	--	.024	.053	.001	--	--	--	--	--	--	294
--	--	--	--	--	--	--	--	--	--	--	295
.048	--	.065	.037	.019	--	--	--	--	--	--	296
.166	--	.181	.189	.063	--	--	--	--	--	--	297
--	--	.378	.235	--	--	--	--	--	--	--	298
.018	--	.116	.021	.027	--	--	--	--	--	--	299
.065	.079	.091	.093	.030	--	--	--	--	--	--	300
.039	.046	.031	.180	.014	--	--	.208	--	--	--	301
.055	--	.033	.024	.014	--	--	--	--	--	--	302
.059	--	.041	.032	.016	--	--	--	--	--	--	303
.032	.016	.045	.043	.019	.064	--	.144	--	--	.032	304
--	--	.030	--	--	--	.103	.251	--	--	.059	305

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

ITEM, PROTEIN CONTENT, AND NITROGEN CONVERSION FACTOR	TRYPTO-PHAN	THREO-NINE	ISO-LEUCINE	LEUCINE	LYSINE	SULFUR CONTAINING		
						METH-IONINE	CYSTINE	TOTAL
	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
VEGETABLES--Continued								
OTHER VEGETABLES (Protein, N x 6.25)--Continued								
306 Seepweed (2.6% protein)	0.027	0.089	0.113	0.152	0.089	0.013	--	--
307 Soybean sprouts (6.2% protein)	--	.159	.225	.265	.211	.045	--	--
308 Squash, summer (0.6% protein)	.005	.014	.019	.027	.023	.008	--	--
309 Tomatoes and cherry tomatoes (1.0% protein)	.009	.033	.029	.041	.042	.007	--	--
310 Turnips (1.1% protein)	--	--	.020	--	.057	.012	--	--
311 Waxgourd, Chinese (0.4% protein)	.002	--	--	--	.009	.003	--	--
MISCELLANEOUS FOOD ITEMS:								
312 Vegetable patty or steak (principally wheat protein) (15% protein, N x 5.70)	.142	.411	.884	1.079	.321	.253	--	--
Yeast:								
313 Baker's, compressed (3/, N x 6.25)	.122	.655	.655	1.151	.914	.248	0.120	0.368
314 Brewer's, dried (4/, N x 6.25)	.710	2.353	2.398	3.226	3.300	.836	.548	1.384
Primary, dried:								
315 (Saccharomyces cerevisiae) (4/, N x 6.25)	.636	2.353	2.708	3.300	3.337	.851	.444	1.295
316 (Torulopsis utilis) (4/, N x 6.25)	.636	2.331	3.323	3.707	3.648	.710	.422	1.132

3/ Total nitrogen is 2.1%. This is equivalent to 10.6% protein on the basis that 4/5 of the nitrogen is protein nitrogen. If total nitrogen is used for the calculation, the protein content is 13.1%.

4/ Total nitrogen is 7.4%. This is equivalent to 36.9% protein on the basis that 4/5 of the nitrogen is protein nitrogen. If total nitrogen is used for the calculation, the protein content is 46.1%.

TABLE 2.--AMINO ACID CONTENT OF FOODS, 100 GRAMS, EDIBLE PORTION--CONTINUED

PHENYL-ALANINE	TYROSINE	VALINE	ARGININE	HISTIDINE	ALANINE	ASPARTIC ACID	GLUTAMIC ACID	GLYCINE	PROLINE	SERINE	ITEM NUMBER
Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	
0.116	--	0.091	0.062	0.036	--	--	--	--	--	--	306
.186	--	.225	.225	.133	--	--	--	--	--	--	307
.016	--	.022	.027	.009	--	--	--	--	--	--	308
.028	0.014	.028	.029	.015	0.051	0.108	0.238	0.033	--	0.033	309
.020	.029	--	--	--	.066	--	--	--	--	--	310
--	--	--	--	--	--	--	--	--	--	--	311
.811	--	.705	.597	.321	--	--	--	--	--	--	312
.607	.580	.840	.536	.353	1.201	1.596	2.432	.788	0.519	.630	313
1.902	1.902	2.723	2.250	1.251	3.456	5.232	6.334	2.427	1.983	2.657	314
1.813	2.472	2.553	1.931	1.103	1.339	2.501	5.742	1.702	--	2.131	315
2.361	2.464	2.901	3.337	1.251	--	--	6.357	3.145	--	--	316

PUBLISHED SOURCES OF DATA USED IN AMINO ACID TABLES

Adriaens, E. L.
1955. Recherches sur la composition en acides aminés des proteines d'aliments végétaux du Congo Belge et du Ruanda-Urundi. Memoire couronne au concours annuel de 1954. 3(3):1-103.

Adriaens, E. L., and Bigwood, E. J.
1954. Contribution a l'étude de la composition en acides aminés de la matière protéique de la graine de coton. Soc. de Chim. Biol. Bul. 36: 579-583, illus.

Adrian, Jean
1955. Nouvelle technique pour le dosage microbiologique simultané des acides aminés indispensables et conditions de leur extraction en presence de glucides. Soc. de Chim. Biol. Bul. 37(1):107-121.

Ågren, G.
1949. Microbiological determinations of amino acids in foodstuffs. I. Acta Chem. Scand. 3: [931]-938.

Ågren, G.
1951. Microbiological determinations of amino acids in foodstuffs. II. Acta Chem. Scand. 5: [766]-773, illus.

Ågren, G.
1956. The amino acid composition of plant protein. Svenska Inst. f. Konserveringsforskning Pub. 115: 66-78.

Aguirre, F., Bressani, R., and Scrimshaw, N. S.
1953. The nutritive value of Central American corns. III. Tryptophane, niacin, thiamine, and riboflavin content of twenty-three varieties in Guatemala. Food Res. 18: 273-279.

Aguirre, F., Robles, C. E., and Scrimshaw, N. S.
1953. El valor nutritivo de las variedades de maiz cultivadas en Centro America. II. Contenido de lisina y metionina en veintitres variedades de Guatemala. Pan Amer. Union Bol. de la Ofic. Sanit., Sup. 1, pp. 89-95.

Aguirre, F., Robles, C. E., and Scrimshaw, N. S.
1953. The nutritive value of Central American corns. II. Lysine and methionine content of twenty-three varieties in Guatemala. Food Res. 18: 268-272.

Airan, J. W., and Desai, R. M.
1954. Chromatographic estimation of amino acids in Momordica charantia seed cakes. Bombay Univ. Jour. 22, Pt. 5, pp. [28]-31, illus.

Airan, J. W., Ghatge, N. D., and Kalyankar, G. D.
1950. Studies in Kolhapur fresh-water fishes. Part 2. Indian Jour. Med. Res. 38: 259-262.

Airan, J. W., and Joshi, J. V.
1952. Kolhapur fresh-water fishes: Part V. Bombay Univ. Jour. 21, Sect. A, Pt. 3, pp. 69-71.

Airan, J. W., and Kalyankar, G. D.
1952. Chemical analysis of the country hen egg. Bombay Univ. Jour. 20, Sect. A, Pt. 5 (Sci. No. 31), pp. 25-30.

Airan, J. W., and Thomas, A.
1953. Amino-acid contents of Bombay prawns. Bombay Univ. Jour. 22, Pt. 3, pp. 61-63, illus.

Airan, J. W., and Thomas, A.
1954. Amino acids in prawn from Bombay. Bombay Univ. Jour. 22, Pt. 5, pp. 48-49, illus.

Albanese, A. A.
1944. Colorimetric estimation of phenylalanine in some biological products. Jour. Biol. Chem. 155: 291-298, illus.

Albanese, A. A., Frankston, J. E., and Irby, V.
1944. The estimation of methionine in protein hydrolysates and human urine. Jour. Biol. Chem. 156: 293-302, illus.

Albanese, A. A., and Irby, V.
1948. The colorimetric determination of isoleucine in biological products. Arch. Biochem. 17: 21-30, illus.

Alderks, O. H.
1949. The study of 20 varieties of soybeans with respect to quantity and quality of oil, isolated protein, and nutritional value of the meal. Amer. Oil Chem. Soc. Jour. 26: 126-132.

Alexander, J. C., Beckner, C. W., and Elvehjem, C. A.
1953. The alanine, cystine, glycine and serine content of meat. Jour. Nutr. 51: 319-328, illus.

Arevalo Padilla, R.
1952. Estudio quimico bromatológico de la carne del "Acanthistius pictus" o Cherlo. An. Fac. Farm. Bioquim., Univ. Nac. Mayor San Marcos [Lima, Peru] 3: [160]- 163. (Abstract in Nutr. Abs. and Rev. 24: 520. 1954.)

Armbruster, G., and Murray, H. C.
1951. The effect of canning procedures on the nutritive value of the protein in peas. Jour. Nutr. 44: 205-216.

Armstrong, R. H.
1951. Amino-acids in the proteins of herbage. Jour. Sci. Food and Agr. [England] 2(4): 166-170.

Aurand, L. W.
1949. The influence of heredity on the nutritive content of corn. 163 pp., illus. [Doctor of Philosophy thesis, unpublished. Copy on file School of Agriculture, Penn. State Col.]

Bachstez, M., and Bustamante, M. S.
1955. Beiträge zur kenntnis Mexikanischer drogen, pflanzen und nahrungsmittel. XVI. Mitteilung. Verteilung der aminosäuren im essbaren anteil der krabbe (Penaus setiferus L.) Ztschr. f. Lebensmittel-Untersuch. u. -Forsch. 100(4): 266-269 (Abstracted in Nutr. Abs. and Rev. 25: 911. 1955.)

Bagchi, S. P., Ganguli, N. C., and Roy, S. C.
1955. Amino acid composition of some Indian pulses: Quantitative determination by paper chromatography. Ann. Biochem. and Expt. Med. 15(3): 149-153.

Balasubramanian, S. C., and Ramachandran, M.
1952. Essential amino acid composition and biological value of some cereal proteins. Soc. Biol. Chem. India, Proc. 9: 23-24.

Balasubramanian, S. C., Ramachandran, M., Viswanatha, T., and De, S. S.
1952. Amino-acid composition of Indian foodstuffs. Part I. Tryptophane, leucine, isoleucine and valine content of some cereals. Indian Jour. Med. Res. 40: 73-87, illus.

Balasubramanian, S. C., Ramachandran, M., Viswanatha, T., and De, S. S.
1952. Amino-acid composition of Indian foodstuffs. Part II. Lysine, methionine, phenylalanine and histidine content of some cereals. Indian Jour. Med. Res. 40: 219-234, illus.

Baldovinos, G., and Echegaray, A.
1951. Contenido de fenilalanina, tirosina y triptófano en proteinas de variedades y líneas seleccionadas de frijol. Chapingo 5: 692-699, illus.

Baptist, N. G.
1950. Amino-acid analysis of cereal protein--the nitrogen and threonine contents of Ceylon cereals. Trop. Agr. [Ceylon] 106: 3-6.

Baptist, N. G.
1951. Amino acids in finger seed (Ragi kurakkan) Eleusine coracana. Current Sci. [India] 20: 268.

Baptist, N. G.
1952. Amino-acids in legumes. Nature [London] 170: 76-77.

Baptist, N. G.
1954. Determination of essential amino-acids in some Ceylon vegetables. Brit. Jour. Nutr. 8: 205-217.

Baptist, N. G.
1954. Essential amino-acids of some common tropical legumes and cereals. Brit. Jour. Nutr. 8: 218-222.

Barton-Wright, E. C., and Moran, T.
1946. The microbiological assay of amino acids. II. The distribution of amino acids in the wheat grain. Analyst 71: 278-280.

Baumgarten, W., Mather, A. N., and Stone, L.
1945. Glutamic acid content of feed materials. Cereal Chem. 22: 514-521, illus.

Baumgarten, W., Mather, A. N., and Stone, L.
1946. Essential amino acid composition of feed materials. Cereal Chem. 23: 135-155, illus.

Beach, E. F., Bernstein, S. S., Hoffman, O. D., and others.
1941. Distribution of nitrogen and protein amino acids in human and in cow's milk. Jour. Biol. Chem. 139: 57-63.

Beach, E. F., Munks, B., and Robinson, A.
1943. The amino acid composition of animal tissue protein. Jour. Biol. Chem. 148: 431-439, illus.

Belfort Bethlem, M. L., Ferreira, H. N., Malouk, F., and Taviera, M.
1954. Teor de amino-ácidos em algumas variedades de feijões existentes no Brasil. Arq. de Bromatol. 2 (1/2): [3]-17.

Belton, W. E., and Hoover, C. A.
1948. Investigations on the mung bean (Phaseolus aureus Rox.). I. The determination of eighteen amino acids in the mung bean hydrolysate by chemical and microbiological methods. Jour. Biol. Chem. 175: 377-383, illus.

Benischke, H.
1948. Cystinbestimmung in pilanzensubstanzen. Ztschr. f. Pflanzenernähr., Dungung u. Bodenk. 41 (1): 42-53.

Bergh, H.
1952. Determination of the amino acids, cystine, methionine, tyrosine, and tryptophane in animal and vegetable materials. Acta Agr. Scand. 2: [158]-172.

Beuk, J. F., Chornock, F. W., and Rice, E. E.
1948. The effect of severe heat treatment upon the amino acids of fresh and cured pork. Jour. Biol. Chem. 175: 291-298.

Beveridge, J. M. R.
1947. Sulphur distribution in fish flesh proteins. Fish. Res. Bd. Canada Jour. 7: 51-54.

Bhagvat, K., and Sreeramamurthy, V. V.
1944. Liberation of tyrosine, tryptophane, cystine, and arginine from proteins. Indian Jour. Med. Res. 32: 145-148.

Bigwood, E. J.
1953. Free and combined amino-acids in foodstuffs. In Verzár, F., ed. Present Problems in Nutrition Research, Expt. Sup. I, 1952, pp. 88-99.

Bigwood, E. J.
1954. Quel parti nouveau peut-on tirer de la chromatographie sur colonces d'échangeurs d'ions selon la methode de Moore et Stein dans l'étude de la composition en acides aminés des produits alimentaires, des milieux biologiques ou de leurs constituants? Expt. Med. and Surg. 12: [23]-56, illus.

Bigwood, E. J., Crokaert, R., and Bilinski, E.
1953. Acides aminés du tissu musculaire (boeuf et veau). L'Acad. Roy. de Med. Belg. Bul. 18: [82]-110, illu
Block, R. J.
1951. Some amino acids, peptides and amines in milk, concentrated milks and cheese. Jour. Dairy Sci. 34(1):1-10.
Block, R. J., and Bolling, D.
1943. The amino acid yield from various animal and plant proteins after hydrolysis of the fat free tissue. Arch. Biochem. 3: 217-226.
Block, R. J., and Bolling, D.
1945. A note on the amino acids yielded by yeast, sunflower seed meal, and sesame seed after hydrolysis of th fat free tissue. Arch. Biochem. 6: 277-279.
Block, R. J., and Bolling, D.
1945. The amino acids yielded by various yeasts after hydrolysis of the fat-free material; a comparative investigation. Arch. Biochem. 7: 313-320.
Block, R. J., and Bolling, D.
1946. The amino acid composition of cow and human milk proteins. Arch. Biochem. 10: 359-363.
Block, R. J., and Bolling, D.
1951. The amino acid composition of proteins and foods. Ed. 2, 576 pp., illus. Springfield, Ill.
Block, R. J., Cannon, P. R., Wissler, R. W., and others.
1946. The effects of baking and toasting on the nutritional value of proteins. Arch. Biochem. 10: 295-301.
Booth, R. R., Hare, J. H., and VanLandingham, A. H.
1951. The glycine content of poultry ration components. W. Va. Acad. Sci. Proc. 23: 69-75, illus.
Boyd, M. J., and Logan, M. A.
1942. Colorimetric determination of serine. Jour. Biol. Chem. 146: 279-287, illus.
Bressani, R., Marcucci, E., Robles, C. E., and Scrimshaw, N. S.
1954. Nutritive value of Central American beans. I. Food Res. 19: 263-286.
Brüggemann, J., Drepper, K., and Drepper, G.
1952. Beitrag zur bausteinanalyse von proteinen in futtermitteln. I. Tryptophanbestimmung in futtermitteln. Hoppe-Seylers Ztschr. f. Physiol. Chem. 289: 211-217, illus.
Camien, M. N., Dunn, M. S., Malin, R. B., and others.
1949. Percentages of twelve amino acids in swine organs. Calif. Univ., Pubs., Physiol. 8(20):327-332.
Camien, M. N., Salle, A. J., and Dunn, M. S.
1945. Investigations of amino acids, peptides and proteins. XXII. Percentages of some amino acids in lactobacilli. Arch. Biochem. 8: 67-78.
Carpenter, K. J., and Ellinger, G. M.
1951. The effect of processing methods on the availability of lysine in groundnut meals. Biochem. Jour. 48: liii-liv.
Castillo la Rosa Sanchez, I.
1952. Estudio bromatológico de la carne de "Anisotremus scapularis" o "Chita." An. Fac. Farm y. Bioquim. Univ. Nac. Mayor San Marcos (Lima, Peru) 3: [136]-140. (Abstract in Nutr. Abs. and Rev. 24: 520-1954.)
Chang, I. C. L., and Murray, H. C.
1949. Biological value of the protein and the mineral, vitamin, and amino acid content of soymilk and curd. Cereal Chem. 26: 297-306.
Charkey, L. W., Dyar, E., and Wilgus, H. S., Jr.
1947. The nutrient content of high and low quality fresh eggs. I. Total solids, total nitrogen, and l(-)-tryptophane. Poultry Sci. 26: 626-631.
Chatterjee, K. P., Ray, A., and Banerjee, S.
1956. Essential amino acids composition of pulses and rice. Food Res. 21(5):569-570.
Cheung, M. W., Pratt, E. L., and Fowler, D. I.
1953. Total amino acid composition in mature human milk. Pediatrics 12(4):353-357.
Chiao, J. S., and Peterson, W. H.
1953. Yeasts. Methionine and cystine contents. Agr. and Food Chem. Jour. 1: 1005-1008.
Chibnall, A. C.
1946. The contribution of the analytical chemist to the problem of protein structure. Internatl. Soc. Leather Trades Chem. Jour. 30(1):1-20.
Chitre, R. G., and Keni, A. B.
1946. On the estimation of methionine by colorimetric procedure. In Letters to the Editor. Current Sci. [India] 15: 130-131.
Chitre, R. G., Williams, J. N., Jr., and Elvehjem, C. A.
1950. Nutritive value of canned foods. XLII. A study of the protein value of young and mature peas (Pisum sativum). Jour. Nutr. 42: 207-219, illus.
Cisneros Almontes, V.
1953. Preparación y estidio bromatológico de la carne ahumada del Mustelus dorsalis o "tollo blanco." An. Fac. Farm y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 75-80. (Abstract in Chem. Abs. 49: 13544. 1955.)
Clandinin, D. R., and Robblee, A. R.
1952. The effect of processing on the enzymatic liberation of lysine and arginine from soybean oil meal. Jour. Nutr. 46: 525-530, illus.

Clark, H. E., Hooper, A. S., and McCord, M. L.
1955. The nutritive value of the proteins of beef extracted with different solvents, and of egg, milk and wheat germ for the growing rat. Jour. Nutr. 55: 63-79, illus.

Close, J., Adriaens, E. L., Moore, S., and Bigwood, E. J.
1953. Composition en acides aminés d'hydrolysats de farine de manioc roui, variété amère. Soc. de Chim. Biol. Bul. 35: 985-992, illus.

Cook, B. B., Fraenkel-Conrat, J., Singer, B., and Morgan, A. F.
1951. The effect of heat treatment on the nutritive value of milk proteins. III. The effect of heat on casein, lactalbumin, and their lactose-induced derivatives, with special reference to digestibility and rate of release of lysine, methionine and tryptophan. Jour. Nutr. 44: 217-235, illus.

Córdova Urteaga, A.
1953. Estudio bromatológico de la carne de Cheilodactylus variegatus o "Pintadilla." An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 81-84. (Abstract in Chem. Abs. 49: 13544. 1955.)

Costa Saenz, F.
1952. Estudio bromatológico del "Ethmidium chilcae" o "Machete." An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 3: [150]-154. (Abstract in Nutr. Abs. and Rev. 24: 520. 1954.)

Cravioto, R. O., Cravioto, O. Y., Massieu H., G., and Guzman G., J.
1955. "El Pozol" forma indigena de consumir el maiz en el sureste de Mexico y su aporte de nutrientes a la dieta. Ciencia 15: 27-30, illus.

Cravioto, O. Y., Figueroa, F. de M., Cravioto, R. O., and Massieu H., G.
1953. Estudios sobre proteinas y aminoacidos en dietas mexicanas. I. Ciencia 13: 65-70.

Cravioto, R. O., Guzmán G. J., Cravioto, O. Y., and others.
1955. La Harina de pescado como suplemento proteinico. Ciencia 15(4-5): 83-88.

Csonka, F. A.
1941. Amino acids in oats and oat milling products, including rolled oats. Cereal Chem. 18: 523-529, illus.

Csonka, F. A., and Denton, C. A.
1946. Methionine determination in proteins and foods. Jour. Biol. Chem. 163: 329-338, illus.

Csonka, F. A., Lichtenstein, H., and Denton, C. A.
1944. Cystine determination in proteins and foods. Jour. Biol. Chem. 156: 571-576, illus.

Curtin, L. V., Abraham, J., Williams, H. H., and others.
1952. Estimation of amino acid requirements of swine from tissue analyses. (Abstract.) Fed. Amer. Soc. Expt. Biol. Proc. 11: 439.

Damanski, A. F., and Radej, N. V.
1954. L-threonine in wheat, white and yellow maize, beans, and peas. Acta Pharm. Jugoslav. 4: 109-114.

De, S. S.
1951. The amino-acid content of proteins of Indian foodstuffs and the biological availability of these amino-acids. Indian Counc. Med. Res. Tech. Rpt. Sci. Adv. Bd. 1951, pp. 61-64.

De, S. S.
1952. The essential amino-acid content of Indian foodstuffs. Indian Counc. Med. Res. Tech. Rpt. Sci. Adv. Bd. 1951, pp. 61-64.

Deas, C. P., and Tarr, H. L. A.
1949. Amino acid composition of fishery products. Fish. Res. Bd. Canada Jour. 7: 513-521.

De La Torre, A., M. C.
1952. Valuación de metionina, cistina y cisteina en algunos crustáceous consumidos en el Perú. An. Fac. Farm. y. Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 3: [141]-149.

Deshpande, P. D., and Radhakrishna Rao, M. V.
1954. Nitrogen complex and amino-acid composition of (i) amaranth (Amaranthus gangeticus) and (ii) aconite bean (Phaseolus aconitifolius). Indian Jour. Med. Res. 42: 77-83.

Desikachar, H. S. R., and De, S. S.
1947. The cystine and methionine contents of common Indian foolstuffs. Current Sci. [India] 16: 284.

Doermann, A. H.
1945. A bioassay for lysine by use of a mutant of neurospora. Jour. Biol. Chem. 160: 95-103, illus.

Doty, D. M., Bergdoll, M. S., Nash, H. A., and Brunson, A. M.
1946. Amino acids in corn grain from several single cross hybrids. Cereal Chem. 23: 199-209.

Duncan, C. W., Watson, G. I., Dunn, K. M., and Ely, R. E.
1952. Nutritive values of crops and cows' milk as affected by soil fertility. II. The essential amino acids in colostrum and milk proteins. Jour. Dairy Sci. 35: 128-139.

Dunn, M. S.
1947. Amino acids in food and analytical methods for their determination. Food Technol. 1: 269-286, illus.

Dunn, M. S., Camien, M. N., Eiduson, S., and Malin, R. B.
1949. The nutritive value of canned foods. I. Amino acid content of fish and meat products. Jour. Nutr. 39: 177-185.

Dunn, M. S., Camien, M. N., Malin, R. B., and others.
1949. Percentages of twelve amino acids in blood, carcass, heart, kidney, liver, muscle, and skin of eight animals. Calif. Univ., Pubs., Physiol. 8: 293-325.

Dunn, M. S., Camien, M. N., Rockland, L. B., and others.
1944. Investigations of amino acids, peptides, and proteins. XVII. The determination of glutamic acid in protein hydrolysates by a microbiological method. Jour. Biol. Chem. 155: 591-603, illus.

Dunn, M. S., Camien, M. N., Shankman, S., and others.
1944. Investigations of amino acids, peptides, and proteins. XIX. The determination of lysine in protein hydrolysates by a microbiological method. Jour. Biol. Chem. 156: 715-724.

Dunn, M. S., Camien, M. N., Shankman, S., and Block, H.
1946. Investigations of amino acids, peptides, and proteins. XXVI. The determination of methionine in protein hydrolysates with Lactobacillus fermenti 36. Jour. Biol. Chem. 163: 577-587.

Dunn, M. S., Camien, M. N., Shankman, S., and Block, H.
1947. The determination of d(-)- and l(+)- glutamic acid in protein hydrolysates. Jour. Biol. Chem. 168: 43-49.

Dunn, M. S., Camien, M. N., Shankman, S., and Block, H.
1948. Amino acids in lupine and soybean seeds and sprouts. Arch. Biochem. 18: 195-200.

Dunn, M. S., McClure, L. E., and Merrifield, R. B.
1949. The determination of proline in protein hydrolysates with Lactobacillus brevis. Jour. Biol. Chem. 179: 11-18.

Dunn, M. S., and Merrifield, B.
1945. The determination of amino acids in yeast protein by microbiological procedures. Nat. Res. Council, Comm. Food Comp. Rpt. 2, 21 pp. [Processed.]

Dunn, M. S., Shankman, S., and Camien, M. N.
1945. Investigations of amino acids, peptides, and proteins. XXIII. The determination of phenylalanine in protein hydrolysates with Leuconostoc mesenteroides P-60 and Lactobacillus casei. Jour. Biol. Chem. 161: 643-655.

Dunn, M. S., Shankman, S., and Camien, M. N.
1945. Investigations of amino acids, peptides, and proteins. XXV. The determination of histidine in protein hydrolysates with Lactobacillus fermenti 36. Jour. Biol. Chem. 161: 669-678, illus.

Dunn, M. S., Shankman, S., Camien, M. N., and Block, H.
1946. Investigations of amino acids, peptides, and proteins. XXVII. The determination of threonine in protein hydrolysates with Lactobacillus fermenti 36. Jour. Biol. Chem. 163: 589-598.

Dustin, J. P., Schram, E., Moore, S., and Bigwood, E. J.
1953. Dosage chromatographique des acides aminés d'une graine de céréale (orge), d'un foin, et d'un tourteau de lin. Soc. de Chim. Biol. Bul. 35: 1137-1147, illus.

Eastoe, J. E.
1955. The amino acid composition of mammalian collagen and gelatin. Biochem. Jour. 61(4):589-600.

Edwards, C. H., Carter, L. P., and Outland, C. E.
1955. Cystine, tyrosine, and essential amino acid contents of selected foods. Agr. and Food Chem. Jour. 3: 952-957.

Edwards, L. E., Sealock, R. R., O'Donnell, W. W., and others.
1946. Biological value of proteins in relation to the essential amino acids which they contain. IV. The analysis of fifteen protein foods for the ten essentials. Jour. Nutr. 32: 597-612.

Elvehjem, C. A.
1951. Amino acid content of meats. Natl. Livestock and Meat Bd. Ann. Rpt. (1950-51) 28: 52.

Elvehjem, C. A.
1952. Amino acid content of meats. Natl. Livestock and Meat Bd. Ann. Rpt. (1951-52) 29: 23-24.

Elvehjem, C. A.
1953. Amino acid content of meat. Natl. Livestock and Meat Bd. Ann. Rpt. (1952-53) 30: 27-28.

Esh, G. C., and Som, J. M.
1953. Nutritional survey of available food materials. Part IV: Availability of methionine, cystine and tryptophan in pulses. Indian Jour. Physiol. and Allied Sci. 7: [158]-162.

Esh, G. C., and Som, J. M.
1954. Nutritional survey on available food materials. V. Lysine, threonine, and phenylalanine contents of pulses. Inst. Chem. Jour. and Proc. [India] 26: 147-152.

Evans, R. J.
1945. Determination of the cystine and methionine content of plant and animal material by a differential oxidation procedure. Arch. Biochem. 7: 439-445.

Evans, R. J., Bandemer, S. L., Davidson, J. A., and others.
1954. Cottonseed rations' effect on eggs. Transfer of protein in stored shell eggs produced by hens fed crude cottonseed oil. Agr. and Food Chem. Jour. 2: 1077-1080.

Evans, R. J., Butts, H. A., Davidson, J. A., and Bandemer, S. L.
1949. The amino acid content of fresh and stored shell eggs. I. Leucine, isoleucine, valine, glycine, serine, threonine, aspartic acid, and glutamic acid. Poultry Sci. 28: 691-696.

Evans, R. J., Davidson, J. A., Bandemer, S. L., and Butts, H. A.
1949. The amino acid content of fresh and stored shell eggs. II. Arginine, histidine, lysine, methionine, cystine, tyrosine, tryptophan, phenylalanine, and proline. Poultry Sci. 28: 697-702.

Evans, R. J., Davidson, J. A., and Bauer, D. H.
1954. Changes in composition of crude egg protein preparations when kept under refrigeration for several years. Poultry Sci. 33: 208-209.

Evans, R. J., Davidson, J. A., and Butts, H. A.
1950. The amino acid content of fresh and stored shell eggs. III. Methionine, cystine, and lysine contents of eggs from hens fed diets differing in percentage of these amino acids. Poultry Sci. 29: 104-108.
Evans, R. J., and St. John, J. L.
1948. Influence of autoclaving dry peas on some properties of the proteins. Cereal Chem. 25: 377-385, illus.
Evans, R. J., St. John, J. L., Craven, P. M., and others.
1947. Some factors influencing the protein, cystine, and methionine content of dry peas. Cereal Chem. 24: 150-156.
Fang, S. C., Bullis, D. E., and Butts, J. S.
1953. Investigation of barcelona and du chilly filbert nuts. III. Amino acid and B-vitamin contents of oil-free filbert nut meals. Food Res. 18: 555-559.
Fernández Roig, G.
1950. Contribucion al estudio de las proteinas de origen vegetal en la ración del soldado Peruano. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 1: [54]-71.
Ferreyra Risso, L.
1953. Estudio bromatológico de la carne del Lóligo gahi "Jibia". An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 85-89. (Abstract in Chem. Abs. 49: 13545. 1955.)
Fitzpatrick, W. H., Esselen, W. B., Jr., and Weir, E.
1946. Composition and nutritive value of mushroom protein. Amer. Dietet. Assoc. Jour. 22: 318-323.
Flores Castañon, C.
1953. Valoración de los amino-acidos azufrados, metionina; cistina y cisteina en algunos moluscos. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 90-98. (Abstract in Nutr. Abs. and Rev. 26:45. 1956.)
Flynn, L. M., Zuber, M. S., Lewcke, D. H., and others.
1954. Relation between protein content of corn and concentration of amino acids and nicotinic acid. Cereal Chem. 31: 217-228.
Fredholm, H.
1954-55. Threonine in foods of animal origin. Acta Agr. Scand. 5: 11-22.
Frey, K. J.
1949. The inheritance of protein and certain of its components in maize. Amer. Soc. Agron. Jour. 41: 113-117.
Frey, K. J.
1951. The interrelationships of proteins and amino acids in corn. Cereal Chem. 28: 123-132, illus.
Frey, K. J.
1952. Variations in the protein and amino acid contents of different oat varieties. Cereal Chem. 29: 77-81.
Frost, D. V., Heinsen, J., and Olsen, R. T.
1946. Partial acid hydrolysates of proteins. IV. Intravenous use of high levels of fibrin and casein hydrolysates in hypoproteinemic dogs. Arch. Biochem. 10: 215-226.
Frost, D. V., and Sandy, H. R.
1949. Assay of dry proteins by rat repletion method. Nutritive value and amino acid composition of six reference proteins. Jour. Nutr. 39: 427-439, illus.
Gallegos Villa, A.
1953. Contribución al estidio bromatológico de la carne ahumada del Ethmidium chilcae o "Machete". An. Fac. Farm. y Bioquim, Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 103-108. (Abstract in Chem. Abs. 49: 13544. 1955.)
Garcia Terry, O. E.
1952. Valoracion de los amino-acidos azufrados: cistina, cisteína y metionina en peces. An. Fac. Farm. y Bioquim, Univ. Nac. Mayor San Marcos (Lima, Peru) 3: 99-115. (Abstract in Nutr. Abs. and Rev. 24: 520. 1954.)
Gerry, R. W., Carrick, C. W., and Hauge, S. M.
1948. Methionine and choline in a simplified chick ration. Poultry Sci. 27: 161-168.
Gerry, R. W., Carrick, C. W., and Hauge, S. M.
1948. Untoasted soybean oil meal in a simplified chick ration. Poultry Sci. 27: 621-626.
Ginger, I. D., Wachter, J. P., Doty, D. M., and others.
1954. Effect of aging and cooking on the distribution of certain amino acids and nitrogen in beef muscle. Food Res. 19: 410-416.
Giral, J., and Anza, S.
1951. Algunas investigaciones bioquimicas sobre el paiche Amazonico (Arapaima gigas Cuv.). Ciencia 11: 75-77.
Giri, K. C., Krishnamurthy, K., and Venkatasubramanian, T. A.
1952. Quantitative determination of amino acids separated by circular paper chromatography. Current Sci. [India] 21(2): 44-45.
Goncalves Ferreira, F. A.
1952. Dosagem microbiológica dos 10 ácidos aminados essenciais em quatro espécies de peixe. [In Portugese] Clinica, Higiene e Hidrologia [Lisbon] 18: 45-51.
Govindarajan, V. S., and Ramachandran, B. V.
1952. L-lysine content of some oilseed cakes. Jour. Sci. and Indus. Res. [India] 11B: 477-479.

Goyco, J. A., and Asenjo, C. F.
1949. New protein and growth-promoting values of three different types of yeast prepared under identical conditions. Jour. Nutr. 38(4): 517-525.
Graham, C. E., Smith, E. P., Hier, S. W., and Klein, D.
1947. An improved method for the determination of tryptophane with p-dimethylaminobenzaldehyde. Jour. Biol. Chem. 168: 711-716.
Grau, C. R.
1947. The phenylalanine and tyrosine contents of chicks and eggs. Jour. Biol. Chem. 168: 485-491, illus.
Grau, C. R., and Almquist, H. J.
1944. Sesame protein in chick diets. Soc. Expt. Biol. and Med. Proc. 57: 187-189.
Grau, C. R., and Almquist, H. J.
1945. The methionine content of feedstuff proteins. Arch. Biochem. 6: 287-294.
Greene, R. D., and Black, A.
1944. The microbiological assay of tryptophane in proteins and foods. Jour. Biol. Chem. 155: 1-8, illus.
Greenhut, I. T., Potter, R. L., and Elvehjem, C. A.
1947. The phenylalanine and tryptophan content of meats. Arch. Biochem. 15: 459-464.
Greenhut, I. T., Schweigert, B. S., and Elvehjem, C. A.
1946. The amino acid requirements of Streptococcus faecalis and the use of this organism for the determination of threonine in natural products. Jour. Biol. Chem. 162: 69-76, illus.
Greenhut, I. T., Schweigert, B. S., and Elvehjem, C. A.
1946. Hydrolysis procedures for the determination of tryptophane in proteins and foodstuffs by the microbiological procedure. Jour. Biol. Chem. 165: 325-332.
Greenhut, I. T., Sirny, R. J., and Elvehjem, C. A.
1948. The lysine, methionine and threonine content of meats. Jour. Nutr. 35: 689-701.
Greenwood, D. A., Kraybill, H. R., and Schweigert, B. S.
1951. Amino acid composition of fresh and cooked beef cuts. Jour. Biol. Chem. 193: 23-28.
Gunness, M., Dwyer, I. M., and Stokes, J. L.
1946. Microbiological methods for the determination of amino acids. III. Extension of the uniform assay method for the ten essential amino acids to include tyrosine. Jour. Biol. Chem. 163: 159-168, illus.
Guthneck, B. T., Bennett, B. A., and Schweigert, B. S.
1953. Utilization of amino acids from foods by the rat. II. Lysine. Jour. Nutr. 49: 289-294.
Hafner, F.
1942. The nutrition value of soy flour. Bakers Digest 16: 247-248; 251.
Hall, L. A.
1948. Protein hydrolysate--source of glutamate flavor. Food Indus. 20(8): 1158-1160.
Hankes, L. V., Riesen, W. H., Henderson, L. M., and Elvehjem, C. A.
1948. Liberation of amino acids from raw and heated casein by acid and enzyme hydrolysis. Jour. Biol. Chem. 176: 467-476, illus.
Hatakoshi, Y.
1953. The contents of methionine and threonine in meat proteins of various fishes. Agr. Chem. Soc. Japan, Jour. 27: 795-797.
Hatano, T., Kondo, F., and Itoh, H.
1954. Estimation of lysine in feeds by the use of l(+)-lysine decarboxylase. Tohoku Jour. Agr. Res. [Japan] 4: 161-166.
Hauschildt, J. D., Isaacs, T. L., and Wallace, W. B.
1947. On the determination of tryptophane. Jour. Biol. Chem. 167: 331-337.
Heathcote, J. G.
1950. The protein quality of oats. Brit. Jour. Nutr. 4: 145-154.
Heller, G. L., and Kirch, E. R.
1947. The microbiological determination of proline, cystine, and isoleucine. Amer. Pharm. Assoc. Jour. 36: 345-349, illus.
Henderson, L. M., Brickson, W. L., and Snell, E. E.
1948. A micromethod for the microbiological determination of amino acids. Jour. Biol. Chem. 172: 31-38, illus.
Henderson, L. M., and Snell, E. E.
1948. A uniform medium for determination of amino acids with various microorganisms. Jour. Biol. Chem. 172: 15-29, illus.
Hess, W. C., Kramke, E. H., Fritz, J. C., and Howard, H. W.
1948. A comparison of the nutritive value of egg proteins and their amino acid content. Soc. Expt. Biol. and Med. Proc. 67: 552-556.
Hier, S. W., Graham, C. E., Freides, R., and Klein, D.
1945. The microbiological determination of amino acids in animal proteins. Jour. Biol. Chem. 161: 705-716.
Hill, D. C., Slinger, S. J., and Marcellus, F. N.
1945. Tolerance of chicks for diets high in tyrosine. Poultry Sci. 24: 234-236.
Hirsch, J. S., Niles, A. D., and Kemmerer, A. R.
1952. The essential amino acid content of several vegetables. Food Res. 17: 442-447.

Hodson, A. Z.
1950. Amino acid content of evaporated milk on prolonged storage. Indus. and Engin. Chem. 42: 694-695.
Hodson, A. Z., and Krueger, G. M.
1946. Essential amino acid content of casein and fresh and processed cow's milk as determined microbiologically on hydrolyzates. Arch. Biochem. 10: 55-64.
Hodson, A. Z., and Krueger, G. M.
1947. Changes in the essential amino acid content of the proteins of dry skim milk on prolonged storage. Arch. Biochem. 12: 51-55.
Hodson, A. Z., and Krueger, G. M.
1947. Direct estimation of leucine application to milk and certain other foodstuffs. Arch. Biochem. 12: 435-440.
Holland, B. R., and Meinke, W. W.
1948. Chinese tallow nut protein. 1. Isolation, amino acid, and vitamin analysis. Amer. Oil Chem. Soc. Jour. 25: 418-419.
Holmes, P.
1953. The amino-acid composition of certain seed proteins. Austral. Jour. Expt. Biol. and Med. Sci. 31: [595]-602.
Hoover, A. A.
1951. Rice--an appraisal of its nutritive value. Trop. Agr. [Ceylon] 107: 3-8.
Horn, M. J., and Blum, A. E.
1956. A microbiological method for determination of cystine in foods. Cereal Chem. 33(1): 18-28.
Horn, M. J., Blum, A. E., Gersdorff, C. E. F., and others.
1953. Sources of error in microbiological determinations of amino acids on acid hydrolysates. I. Effect of humin on amino acid values. Jour. Biol. Chem. 203: 907-913.
Horn, M. J., Blum, A. E., Gersdorff, C. E. F., and Warren, H. W.
1955. Sources of error in microbiological determinations of amino acids on acid hydrolysates. II. Apparent loss of amino acids on storage. Cereal Chem. 32: 64-70.
Horn, M. J., Blum, A. E., Womack, N., and Gersdorff, C. E. F.
1952. Nutritional evaluation of food proteins by measuring availability of amino acids to micro-organisms. I. Cottonseed proteins. Jour. Nutr. 48: 231-241.
Horn, M. J., Fifield, C. C., Blum, A. E., Warren, H. W.
The distribution of amino acids in wheat and certain wheat products. Cereal Chem. [In press]
Hortencia Oliveros, E.
1951. Hidrólisis y valoración de amino-acidos indispensables en la proteína de los ríos peruanos. (Histidina, metionina, tirosina y triptofano.) An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 2: [239]-247. (Abstracted in Nutr. Abs. and Rev. 24: 48. 1954.)
Hou, H. C., Riesen, W. H., and Elvehjem, C. A.
1949. Influence of heating on the liberation of certain amino acids from whole soybeans. Soc. Expt. Biol. and Med. Proc. 70: 416-419.
Ingalls, R. L., Klocke, J. F., Plummer, J., and others.
1950. Nutritive value of fish from Michigan waters. Mich. Agr. Expt. Sta. Tech. Bul. 219, 24 pp., illus.
Ingram, G. R., Cravens, W. W., and Elvehjem, C. A.
1950. Evaluating cottonseed meal protein for chick growth by enzymatic release of amino acids. Poultry Sci. 29: 590-594.
Ingram, G. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G.
1950. Relation of tryptophan and lysine to egg production, hatchability and composition of the protein of hens' eggs. Poultry Sci. 29: 793-803.
Ingram, G. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G.
1951. The methionine requirement of the laying hen. Poultry Sci. 30: 431-434, illus.
Ingram, G. R., Riesen, W. W., Cravens, W. W., and Elvehjem, C. A.
1949. Evaluating soybean oil meal protein for chick growth by enzymatic release of amino acids. Poultry Sci. 28: 898-902.
Inskeep, G. C., Wiley, A. J., Holderby, J. M., and Hughes, L. P.
1951. Food yeast from sulfite liquor. Indus. and Engin. Chem. 43(8): 1702-1711.
Jaquot, R., Raulin, J., Adrian, J., and Rerat, A.
1955. Composición y valor alimenticio de las lágrimas de Job (Lagrimas de San Pedro o trigo Adlay) (Coix lacryma-Jobi). Arch. Venezolanos de Nutr. 6(1): 3-21.
Jelliffe, D. B., Arroyave, G., Aguirre, F., and others.
1956. The amino-acid composition of certain tropical pulses and cereals. Jour. Trop. Med. 59(9): 216-217.
Johanson, R.
1948. The cystine plus cysteine and methionine contents of the seed proteins, and sulphur distributions of seeds of Vicia spp. (vetches) with notes on their cyanogenesis. Austral. Jour. Expt. Biol. and Med. Sci. 26(4): 259-270.
Johanson, R., and Lugg, J. W. H.
1946. Sulphur distributions, with special reference to the cystine plus cysteine and methionine contents, of the seeds and seed proteins of two varieties of soya beans. Austral. Jour. Expt. Biol. and Med. Sci. 24: [321]-325.
Joshi, N. V., and Raj, H.
1954. Microbiological assay of essential amino acids in the milk of Indian buffalo. Indian Jour. Dairy Sci. 7: 139-146.

Kelley, E. G., and Baum, R. R.
1953. Protein amino acids. Contents of vegetable leaf proteins. Agr. and Food Chem. Jour. 1: 680-683.
Kemble, A. R., and Macpherson, H. T.
1954. Determination of monoamino monocarboxylic acids by quantitative paper chromatography. Biochem. Jour. 56: 548-555.
Kemmerer, A. R., and Acosta, R.
1949. The essential amino acid content of several vegetables. Jour. Nutr. 38: 527-533.
Kik, M. C.
1941. The nutritive value of the proteins of rice and its byproducts. III. Amino acid content. Cereal Chem. 18: 349-354.
Kik, M. C.
1942. Nutritive studies of rice and its byproducts. Ark. Agr. Expt. Sta. Bul. 416, 28 pp., illus.
Kik, M. C.
1954. Nutritive value of rice germ. Agr. and Food Chem. Jour. 2: 1179-1181.
Kik, M. C.
1955. Influence of processing on nutritive value of milled rice. Agr. and Food Chem. Jour. 3: 600-603.
Kik, M. C.
1956. Nutritional improvement of rice. Amer. Dietet. Assoc. Jour. 32(7): 647-650.
Kirimura, J., and Tamura, G.
1952. Microbiological determination of amino acids in human milk, cow milk, and amino-acid injections. Agr. Chem. Soc. Japan, Jour. 26: 603-605.
Klimenko, V. G.
1950. Azotsoderzhashchie veshchestva nekotorykh predstavitelei rodov semeistva bobovikh. Biokhimiya 15: 408-413. (In Russian. Abstract in Nutr. Abs. and Revs. 21: 288. 1951.)
Klose, A. A., and Fevold, H. L.
1945. Nutritional value of yeast protein to the rat and the chick. Jour. Nutr. 29(6): 421-430.
Klug, H. L., Harshfield, R. D., Pengra, R. M., and Moxon, A. L.
1952. Methionine and selenium toxicity. Jour. Nutr. 48: 409-420, illus.
Klungsøyr, M., Sirny, R. J., and Elvehjem, C. A.
1951. Effect of incomplete hydrolysis on microbiological determination of amino acids. Jour. Biol. Chem. 189: 557-569, illus.
Kratzer, F. H.
1944. The tryptophane content of feedstuff proteins. Jour. Biol. Chem. 156: 507-509.
Kratzer, F. H., and Williams, D. E.
1951. Safflower oil meal in rations for chicks. Poultry Sci. 30: 417-421, illus.
Kraut, H., Kofranyi, E. Telschow, H., and Weber, M.
1955. Aminosaurebestimmung in nahrungsmitteln durch chromatographie an starkesaulen. Hoppe-Seylers Ztschr. f. Physiol. Chem. 301(4-6): 165-184.
Kuiken, K. A.
1952. Availability of the essential amino acids in cottonseed meal. Jour. Nutr. 46: 13-25.
Kuiken, K. A., and Lyman, C. M.
1948. Availability of amino acids in some foods. Jour. Nutr. 36: 359-368.
Kuiken, K. A., and Lyman, C. M.
1949. Essential amino acid composition of soy bean meals prepared from twenty strains of soy beans. Jour. Biol. Chem. 177: 29-36.
Kuiken, K. A., Lyman, C. M., and Hale, F.
1947. Factors which influence the stability of tryptophan during the hydrolysis of proteins in alkaline solution. Jour. Biol. Chem. 171: 551-560, illus.
Kuiken, K. A., Lyman, C. M., and Hale, F.
1947. The tryptophan content of meat. Jour. Biol. Chem. 171: 561-564.
Kuiken, K. A., Norman, W. H., Lyman, C. M., and others.
1943. The microbiological determination of amino acids. I. Valine, leucine, and isoleucine. Jour. Biol. Chem. 151: 615-626, illus.
Kuiken, K. A., and Pearson, P. B.
1949. The essential amino acid (except tryptophan) content of colostrum and milk of the cow and ewe. Jour. Nutr. 39: 167-176.
Kurth, E. F., and Cheldelin, V. H.
1946. Feeding yeasts from wood sugar stillage. Indus. and Engin. Chem. 38(6): 617-619.
Kymal, P. K.
1955. Variation in amino-acid content of rice varieties. 62 pp. (Unpublished PhD. thesis. Copy on file Louisiana State Univ. Libr., Baton Rouge.)
Lal, S. B.
1950. Microbiological assay of amino-acids in gram and ragi. Indian Jour. Med. Res. 38: 131-137.
Langdraf, R. G., Jr.
1953. Technical note No. 27--Alaska pollock: proximate composition; amino acid, thiamine, and riboflavin content; use as mink feed. In U.S. Dept. Int. Com. Fisheries Rev. 15(7): 20-22, illus.
Lavine, T. F.
1943. An iodometric determination of methionine. Jour. Biol. Chem. 151: 281-297.
Lewis, J. C., Snell, N. S., Hirschmann, D. J., and Fraenkel-Conrat, H.
1950. Amino acid composition of egg proteins. Jour. Biol. Chem. 186: 23-35.

Lindan, O., and Work, E.
1951. The amino-acid composition of two yeasts used to produce massive dietetic liver necrosis in rats. Biochem. Jour. 48: 337-344, illus.

Lopez-Matas, A., and Fellers, C. R.
1948. Composition and nutritive value of fresh, cooked, and processed swordfish. Food Res. 13: 387-396, illus.

Lugg, J. W. H., and Weller, R. A.
1944. On the sulphur present in the seeds of certain legumes, with special reference to the cystine plus cysteine and methionine contents of the proteins. Austral. Jour. Expt. Biol. and Med. Sci. 22, Pt. 3, pp. [149]-155.

Luz Suarez, M. de la, Massieu, G., Cravioto, R. O., and Guzman Garcia, J.
1954. Nuevos datos sobre contenido en amino acidos indispensables en alimentos Mexicanos. Ciencia 14: 19-32.

Lyman, C. M., Butler, B., Moseley, O., and others.
1946. The methionine content of meat. Jour. Biol. Chem. 166: 173-176, illus.

Lyman, C. M., and Kuiken, K. A.
1949. The amino acid composition of meat and some other foods. 1. Arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Tex. Agr. Expt. Sta. Bul. 708, 31 pp.

Lyman, C. M., Kuiken, K. A., Blotter, L., and Hale, F.
1945. The microbiological determination of amino acids. II. Glutamic acid. Jour. Biol. Chem. 157: 395-405, illus.

Lyman, C. M., Kuiken, K., and Hale, F.
1947. The essential amino acid content of cottonseed, peanut and soybean products. Tex. Agr. Expt. Sta. Bul. 692, 12 pp.

Lyman, C. M., Kuiken, K. A., and Hale, F.
1947. The histidine content of meat. Jour. Biol. Chem. 171: 233-245, illus.

Lyman, C. M., Kuiken, K. A., and Hale, F.
1956. Essential amino acid content of farm feeds. Agr. and Food Chem. Jour. 4(12): 1008-1013.

Lyman, C. M., Moseley, O., Butler, B., and others.
1946. The microbiological determination of amino acids. III. Methionine. Jour. Biol. Chem. 166: 161-171, illus.

MacPherson, H. T.
1946. The basic amino-acid content of proteins. Biochem. Jour. 40: 470-481.

Mahadevan, V.
1950. Microbiological determination of cystine in foods and proteins. Jour. Sci. and Indus. Res. [India] 9B: 143-147, illus.

Mahadevan, V.
1951. Semi-micromethode microbiologique pour le dosage simultané de la cystine, de la méthionine et de la lysine. Soc. de Chim. Biol. Bul. 33: [200]-207, illus.

Mahon, J. H., and Common, R. H.
1950. The amino acid composition of the horsebean (Vicia faba L.) as related to its nutritive value for chicks. Sci. Agr. 30: 43-49.

Majumder, S. G., Dutta, R. N., and Ganguli, N. C.
1956. Amino acid composition of some Indian vegetables as determined by paper chromatography. Food Res. 21(4): 477-480.

Massieu, G. H., Guzmán, J., Cravioto, R. O., and Calvo, J.
1949. Determination of some essential amino acids in several uncooked and cooked Mexican foodstuffs. Jour. Nutr. 38: 293-304.

Massieu, G. H., Guzmán, J., Cravioto, R. O., and Calvo, J.
1950. Determinacion de algunos amino acidos esenciales en algunos alimentos Mexicanos crudos y cocinados. Pan Amer. Union Bol. de la Ofic. Sanit. 29: 614-622.

Massieu, G., Guzmán, J., Cravioto, R., and Torre, J. C. de la.
1950. Contenido en aminoacidos indispensables en algunas semillas Mexicanas. Ciencia 10: 142-144.

Master, F., and Magar, N. G.
1954. Studies in the nutritive value of Bombay fish. Part II. Amino-acid composition. Indian Jour. Med. Res. 42: [509]-513.

Mauron, J., Mottu, F., Bujard, E., and Egli, R. H.
1955. The availability of lysine, methionine and tryptophan in condensed milk and milk powder. In vitro digestion studies. Arch. Biochem. and Biophys. 59: 433-451, illus.

McCoy, T. A., Patterson, M. K., Jr., and Wender, S. H.
1953. An improved method of analysis for glycine using Streptococcus faecalis. Arch. Biochem. and Biophys. 43: 485-491.

McElroy, L. W., Clandinin, D. R., Lobay, W., and Pethybridge, S. I.
1949. Nine essential amino acids in pure varieties of wheat, barley and oats. Jour. Nutr. 37: 329-336.

Melhus, I. E., Aguirre, F., and Scrimshaw, N. S.
1953. Observaciones sobre el valor nutritivo del teosinte. Pan Amer. Union Bol. de la Ofic. Sanit., Sup. 1, pp. 76-78.

Melhus, I. E., Aguirre, F., and Scrimshaw, N. S.
1953. Observations on the nutritive value of teosinte. Science 117: 34-35.

Michel, R., and Schiller, J.
1945. Note technique sur le dosage colorimetrique du tryptophane. Soc. de Chim. Biol. Bul. 27: 456-458.
Millares, R., and Davis, S. G.
1949. Determination of tryptophan and lysine in microvolumes of protein hydrolyzates. Anal. Chem. 21: 414-416.
Millares, R., and Fellers, C. R.
1948. Amino acid content of chicken. Amer. Dietet. Assoc. Jour. 24: 1057-1061.
Millares, R., and Fellers, C. R.
1949. Vitamin and amino acid content of processed chicken meat products. Food Res. 14: 131-143.
Miller, B. S., Seiffe, J. Y., Shellenberger, J. A., and Miller, G. D.
1950. Amino acid content of various wheat varieties. I. Cystine, lysine, methionine, and glutamic acid. Cereal Chem. 27: 96-106, illus.
Miller, P. A., Hurst, T. L., and Brimhall, B.
1952. Relationships of lysine and niacin with the crude protein and certain protein components in corn grain. Agron. Jour. 44: 343-345.
Miller, R. C., Aurand, L. W., and Flach, W. R.
1950. Amino acids in high and low protein corn. Science 112: 57-58.
Miller, S., and Ruttinger, V.
1950. An improved method of hydrolysis for use in the microbiological determination of tryptophan in human milk. Arch. Biochem. 27: 185-190.
Miller, S., and Ruttinger, V.
1951. Essential amino acids in mature human milk. Soc. Expt. Biol. and Med. Proc. 77: 96-99.
Miller, S., Ruttinger, V. E., Kovach, E. S., and Macy, I. G.
1952. The amino acids in nine food proteins as determined by three methods. Pan Amer. Med. Woman's Jour. 59: 9-13, 17, 24.
Milligan, J. L., Machlin, L. J., Bird, H. R., and Heywang, B. W.
1951. Lysine and methionine requirement of chicks fed practical diets. Poultry Sci. 30: 578-586, illus.
Mitchell, H. H., and Block, R. J.
1946. Some relationships between the amino acid contents of proteins and their nutritive values for the rat. Jour. Biol. Chem. 163: 599-620, illus.
Mitchell, H. H., Hamilton, T. S., and Beadles, J. R.
1952. The relationship between the protein content of corn and the nutritional value of the protein. Jour. Nutr. 48: 461-476.
Mock Morales, E.
1953. Contribución al estudio bromatológico de la "Sciaena deliciosus" (Lorna). An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 118-122. (Abstract in Chem. Abs. 49: 13543. 1955.)
Mojonnier, M. L., Hedrick, L. R., and Porter, T.
1955. The microbiological assay of the amino acids of five genera of yeasts grown under controlled conditions. Jour. Nutr. 57: 579-591.
Morales Gallegos, L.
1953. Estudio bromatológico de la carne ahumada del "Solea vulgaris" o Lenguado. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 123-126. (Abstract in Chem. Abs. 49: 13544. 1955.)
Morries, P., and Stuckey, R. E.
1953. The chromatographic determination of glutamic acid in wheat gluten and gluten hydrolysates. Analyst 78: 636-640.
Munks, B., Robinson, A., Beach, E. F., and Williams, H. H.
1945. Amino acids in the productions of chicken egg and muscle. Poultry Sci. 24: 459-464.
Murphy, E. A., and Dunn, M. S.
1950. Nutritional value of peanut protein. Food Res. 15: 498-510, illus.
Murray, H. C., Kellenbarger, S., and Armbruster, G. A.
1952. Protein efficiency and methionine content of Alaska peas grown with the use of various fertilizers. Agron. Jour. 44: 252-253.
Nagahara, T., Sugimura, K., and Kawamura, T.
1954. Amino acid assay by microbiological method. Food Res. Inst. (Tokyo, Japan), Rpt. No. 9, pp. 7-11, illus.
Navia, J. M., Lopez, H., Cimadevilla, M., and others.
1955. Nutrient composition of Cuban foods. (I). Foods of vegetable origin. Food Res. 20: 97-113.
Neilands, J. B., Sirny, R. J., Sohljell, I., and others.
1949. The nutritive value of canned foods. II. Amino acid content of fish and meat products. Jour. Nutr. 39: 187-202.
Occhialini, N. di E.
1951. Variazioni del contenuto della tirosina nel latte. Chimica [Italy] 6: 258-259.
Okuyama, Bazan, V. Y.
1953. Contribución al estudio bromatológico [del] Thais chocolata o "caracol negro." An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 142-147. (Abstract in Chem. Abs. 49: 13544. 1955.)
Olcott, H. S.
1944. A method for the determination of glutamic acid in proteins. Jour. Biol. Chem. 153: 71-82.
Pallardel Peralta, T. H.
1951. Hidrólisis y valoración de amino-acidos indispensables, histidina y meteonina, en la proteina de peces de la costa Peruana. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 2: 248 -256. (Abstract in Chem. Abs. 48: 3575. 1954.)

Palomino G., J. A.
1953. Contribución al estudio bromatologico de la Sciaena starksi - Robalo. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 148-155. (Abstract in Nutr. Abs. and Rev. 26: 44. 1956.)
Patwardhan, M. V., and Vijayaraghavan, P. K.
1954. Nutritive value of duck egg white. Part I. Note on the essential amino-acid composition of duck egg white. Indian Jour. Med. Res. 42: [521]-523.
Patwardhan, V. N.
1954. Studies on the breast milk of Indian women. Indian Counc. Med. Res. Tech. Rpt. Sci. Adv. Bd. 1953, pp. 57-59.
Pence, J. W., Mecham, D. K., Elder, A. H., and others.
1950. Characterization of wheat gluten. II. Amino acid composition. Cereal Chem. 27: 335-341.
Portner, C., and Hogl, O.
1952. Dosage du tryptophane dans les proteines pures et dans quelques denrees alimentaire d'origine animale. Mitt. aus dem Geb. der Lebensmtl. Untersuch. u. Hyg. 43: 505-521, illus.
Pottinger, S. R., and Baldwin, W. H.
1946. The content of certain amino acids in seafoods. U.S. Dept. Int. Com. Fisheries Rev. 8: 5-8, illus.
Prescott, B. A., and Waelsch, H.
1946. A microdetermination of glutamic acid and its application to protein analysis. Jour. Biol. Chem. 164: 331-343, illus.
Price, S. A.
1950. The amino acid composition of whole wheat in relation to its protein content. (In Communications to the editor.) Cereal Chem. 27: 73-74.
Proctor, B. E., and Bhatia, D. S.
1950. Effect of high-voltage cathode rays on amino acids in fish muscle. Food Technol. 4: 357-361.
Raica, N., Heimann, J., and Kemmerer, A. R.
1956. Amino acid proportions in food proteins compared to proportions utilized in rat growth. Agr. and Food Chem. Jour. 4(8): 704-707.
Raj, H., and Joshi, N. V.
1955. Amino-acid composition of the milk of Indian buffaloes. I. Essential amino-acid composition of total proteins and protein fractions. Indian Jour. Med. Res. 43(4): 591-596.
Ramachandran, M., and Phansalkar, S. V.
1956. Essential amino-acid composition of certain vegetable foodstuffs. Indian Jour. Med. Res. 44(3): 501-509.
Ramachandran, L. K., and Sarma, P. S.
1952. The liberation of serine and threonine during proteolysis. Jour. Sci. and Indus. Res. [India] 11B: 379-382, illus.
Rao, D. Narayana, Sreeramamurthy, V. V., and Venkateswarlu, P.
1951. The supplementary effect of pulses and rice on tapioca diet. Indian Jour. Med. Res. 39(3): 329-342.
Rao, R. Venkateswara and Basu, K. P.
1954. Studies on essential amino acids in milk. Soc. Biol. Chem. India, Proc. 12: 19-20.
Rao, R. Venkateswara and Basu, K. P.
1954. The essential amino acid content of milk products. Soc. Biol. Chem. India, Proc. 12: 21-22.
Rao, R. Venkateswara and Basu, K. P.
1954. Effect of storage on the essential amino acid content of buffalo milk powder (spray dried), with and without the addition of glucose (5%). Soc. Biol. Chem. India, Proc. 12: 22-23.
Ray Sarkar, B. C., Luecke, R. W., Duncan, C. W., and others.
1949. The amino acid composition of bovine colostrum and milk. Jour. Dairy Sci. 32: 671-678.
Rees, M. W.
1946. The estimation of threonine and serine in proteins. Biochem. Jour. 40: 632-640, illus.
Renner, R., Bentley, C. F., and McElroy, L. W.
1953. Nine essential amino acids in the protein of wheat and barley grown on sulfur-deficient soil. Soil Sci. Soc. Amer. Proc. 17: 270-273.
Renner, R., Clandinin, D. R., Morrison, A. B., and Robblee, A. R.
1953. The effects of processing temperatures on the amino acid content of sunflower seed oil meal. Jour. Nutr. 50(4): 487-490.
Riccori, G.
1951. Il contenuto in arginina dei semi di graminacee e di leguminose. II. Ricerca quantitativa nei semi col metodo di Furth e Deutschberger modificato. Arch. di Sci. Biol., [Italy] 35: [185]-193.
Rice, A. C., and Ramstad, P. E.
1950. Amino acid compositions of wheat and carob glutens. Cereal Chem. 27: 238-243.
Riesen, W. H., Clandinin, D. R., Elvehjem, C. A., and Cravens, W. W.
1947. Liberation of essential amino acids from raw, properly heated, and overheated soy bean oil meal. Jour. Biol. Chem. 167: 143-150, illus.
Riesen, W. H., Schweigert, B. S., and Elvehjem, C. A.
1946. Microbiological determination of methionine in proteins and foodstuffs. Jour. Biol. Chem. 165: 347-358, illus.
Ringrose, R. C.
1949. Nutritive properties of torula yeast for poultry. Poultry Sci. 28(1): 75-83.
Robles, C. E.
1951. Determinacion microbiologica de lisina en diversas variedades de maiz geneticamente controladas. La Escuela de Farm. [Guatemala] 13: 15-27.

Roche, J., and Michel, R.
1946. Sur la valeur alimentaire des proteins des tourteaux. Oleagineux [France] 1: 205-207.

Rodriguez Velasco, J. A.
1950. Análisis quimico bromatológico de Pygidium rivulatum o suche. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 1: [149]-154. (Abstract in Chem. Abs. 48: 2944. 1954.)

Russell, W. C., Taylor, M. W., Mehrhof, T. G., and Hirsch, R. R.
1946. The nutritive value of the protein of varieties of legumes and the effect of methionine supplementation. Jour. Nutr. 32: 313-325.

Rutgers University. Bureau of Biological Research.
[1951.] Cooperative determinations of the amino acid content, and of the nutritive value of six selected protein food sources 1946-1950. 114 pp. Rutgers Univ., New Brunswick, N. J. [Processed.]

Sanchez Moreno, E.
1950. Valoracion de triptofano y tirosina en la proteina de peces de la costa Peruana. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 1: [135]-148. (Abstract in Chem. Abs. 48: 2944. 1954.)

Sandoval Colmenares, T.
1951. Análisis quimico - bromatológico del Scartichthys gigas o "borracho." An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 2: [271]-275. (Abstract in Chem. Abs. 48: 3587. 1954.)

Sauberlich, H. E., and Baumann, C. A.
1949. A microbiological determination of alanine. Jour. Biol. Chem. 177: 545-551, illus.

Sauberlich, H. E., Chang, W. Y., and Salmon, W. D.
1953. The amino acid and protein content of corn as related to variety and nitrogen fertilization. Jour. Nutr. 51: 241-250, illus.

Schormüller, J.
1949. Über den aminosauregehalt von proteinen und eweisshaltigen lebensmitteln vorwiegend pflanzlicher herkunft. Der gehalt an cystine und schwefel. Ernähr. u. Verpflegung 1: 93-104.

Schormüller, J.
1950. Der gehalt an gesamtem, gebundenem und freiem tryptophan in lebensmitteln. Ztschr. f. Lebensmtl.-Untersuch. u. Forsch. 90: [337]-344.

Schormüller, J.
1951. Über den tyrosin- und tryptophangehalt von proteinen und eweisshaltigen lebensmitteln vorwiegend pflanzlicher herkunft. Deut. Lebensmtl.-Rundschau 47: 175-180.

Schormüller, J.
1951. Über anderungen der aminosäurebilanz von lebensmitteln im gange der gewinnung und aufbereitung, studiert am tyrosin- und tryptophangehalt. Deut. Lebensmtl.-Rundschau 47: [191]-195.

Schormüller, J., and Ballschmieter, H.
1951. Der gehalt an methionin, cystin und schwefel in lebensmitteln pflanzlicher und tierischer herkunft. Zugleich ein beitrag zur aufstellung von schwefelbilanzen. Deut. Lebensmtl.-Rundschau 47: [1]-7.

Schormüller, J., and Ballschmieter, H.
1951. Über den aminosäuern- und kohlenhydratgehalt des gesamtproteins von trockenvollei im frischen, gelagerten und erhitzen zustand. Deut. Lebensmtl.-Rundschau 47: 119-128.

Schormüller, J., and Krempien, J.
1954. Über den einfluss der glucose auf den threonin-, serin- und stickstoffgehalt des caseins beim erhitzen. Ztschr. f. Lebensmtl.-Untersuch. u. Forsch. 98: 1-18, illus.

Schormüller, J., Störig, I., and Leichter, L.
1953. Über das verhalten einiger aminosäuren des kleberproteins bei der chemischen mehlbehandlung. Ztschr. f. Lebensmtl.-Untersuch. u. Forsch. 96: 1-24, illus.

Schormüller, J., and Walter, J.
1951. Der threonin- und seringehalt von volleiproteinen im frischen, gelagerten und erhitzen zustand. Deut. Lebensmtl.-Rundschau 47: 265-271, illus.

Schormüller, J., Winter, H., and Ballschmieter, H.
1950. Über den gehalt an methionin, cystin, tyrosin, tryptophan, histidin und nichteiweissartigen begleitsubstanzen in trockeneiweiss aus susswasserfischen. Biochem. Ztschr. 321: [180]-188.

Schram, E., Moore, S., and Bigwood, E. J.
1954. Chromatographic determination of cystine as cysteic acid. Biochem. Jour. 57: 33-37, illus.

Schweigert, B. S.
1947. Amino acid content of feeds. I. Leucine, valine, isoleucine and phenylalanine. Jour. Nutr. 33: 553-559.

Schweigert, B. S.
1948. The value of various feeds as sources of arginine, histidine, lysine and threonine for poultry. Poultry Sci. 27: 223-227.

Schweigert, B. S., Bennett, B. A., and Guthneck, B. T.
1951. Further studies on the amino acid composition of pork and lamb cuts. Jour. Biol. Chem. 190: 697-703.

Schweigert, B. S., Bennett, B. A., and Guthneck, B. T.
1954. Amino acid composition of organ meats. Food Res. 19: 219-223.

Schweigert, B. S., Bennett, B. A., McBride, B. H., and Guthneck, B. T.
1952. Amino acid content of processed meats. Amer. Dietet. Assoc. Jour. 28: 23-26.

Schweigert, B. S., and Guthneck, B. T.
1953. Utilization of amino acids from foods by the rat. I. Methods of testing for lysine. Jour. Nutr. 49: 277-287, illus.

Schweigert, B. S., and Guthneck, B. T.
1954. Utilization of amino acids from foods by the rat. Jour. Nutr. 54: 333-343, illus.
Schweigert, B. S., Guthneck, B. T., Kraybill, H. R., and Greenwood, D. A.
1949. The amino acid composition of pork and lamb cuts. Jour. Biol. Chem. 180: 1077-1083.
Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Strong, F. M.
1944. The direct determination of valine and leucine in fresh animal tissues. Jour. Biol. Chem. 155: 183-191, illus.
Schweigert, B. S., Tatman, I. E., and Elvehjem, C. A.
1945. The leucine, valine, and isoleucine content of meats. Arch. Biochem. 6: 177-184, illus.
Shankman, S., Camien, M. N., and Dunn, M. S.
1947. The determination of glycine in protein hydrolysates with Leuconostoc mesenteroides P-60. Jour. Biol. Chem. 168: 51-60.
Sibalic, S., and Radej, N.
1954. Un nouveau milieu de culture pour le dosage microbiologique du tryptophane. Inst. Pasteur. Ann. [Paris] 86: 793-797, illus.
Sirny, R. J., Cheng, L. T., Elvehjem, C. A.
1951. An arginine-proline interdependence in Leuconostoc mesenteroide P-60. Jour. Biol. Chem. 190: 547-556.
Sirny, R. J., Greenhut, I. T., and Elvehjem, C. A.
1950. The arginine and histidine content of meats. Jour. Nutr. 41: 383-398.
Slack, E. B.
1948. Nitrogenous constituents of the potato. Nature [London] 161: 211-212, illus.
Slinger, S. J., Hill, D. C., Gartley, K. M., and Branion, H. D.
1949. Soybean oil meal and sunflower seed oil meal in rations for broad-breasted bronze turkeys. Poultry Sci. 28: 534-540.
Soupart, P., Moore, S., and Bigwood, E. J.
1954. Amino acid composition of human milk. Jour. Biol. Chem. 206: 699-704, illus.
Spanyer, J. W., Jr., and Thomas, A. T.
1956. Determination and comparison of amino acid composition of yeast and distiller's solubles. Agr. and Food Chem. Jour. 4(7): 631-633.
Springer, R., and Quentin, K. E.
1951. Über die bindungsformen des protein-schwefels. 1. Biochem. Ztschr. 322: [180]-185.
Sreeramamurthy, V. V.
1945. Investigations on the nutritive value of tapioca. Indian Jour. Med. Res. 33(2): 229-247.
Srinivasan, P. R., and Ramanathan, M. K.
1954. Protein and sulphur amino acids in breast-milk of poor class Indian women in the Nilgiris. Indian Jour. Med. Res. 42: 51-54.
Srinivasan, P. R., and Vijayaraghavan, P. K.
1953. Threonine in bengal gram: A correction. Current Sci. [India] 22: 87.
Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C.
1945. Microbiological methods for the determination of amino acids. II. A uniform assay for the ten essential amino acids. Jour. Biol. Chem. 160: 35-49, illus.
Subramanian, N., and Srinivasan, M.
1952. Vegetable protein from a new source Amaranthus paniculatus Linn.: Its nutritive value. Soc. Biol. Chem. India, Proc. 10: 25-26.
Sugimura, K., Taira, H., Hoshino, N., and others.
1954. The amino acid content of fish muscle protein. Jap. Soc. Sci. Fish. Bul. 20: 520-524.
Sugimura, K., Taira, H., and Nagahara, T.
1954. Amino acid composition of rice protein. Jap. Soc. Food and Nutr. Jour. 7: 31-32.
Sure, B.
1953. Protein efficiency. Improvement in whole yellow corn with lysine, tryptophan, and threonine. Agr. and Food Chem. Jour. 1: 626-629.
Taranova, A. I.
1951. The amino-acid composition of wheat proteins as dependent on variety, locality of growth and milling. Biokhimiya 16: 239-245. (Abstract in Nutr. Abs. and Rev. 22: 331. 1952.)
Thompson, J. F.
1952. The analysis of the alcohol-insoluble nitrogen of plants by quantitative procedures based on paper chromatography. I. The analysis of certain pure proteins. Jour. Expt. Bot. 3: [170]-181.
Urco Najera, A.
1953. Contribución al estudio bromatológico y ensayo de conservación de la Paralabrax humeralis -cabrilla-. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 156-161. (Abstract in Nutr. Abs. and Rev. 26: 44. 1956.)
Vallejos Plantano, C.
1952. Análisis bromatológico de la carne de "Paralonchurus peruanus," "Coco" o "Suco." An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos, (Lima, Peru) 3: [155]-159. (Abstract in Nutr. Abs. and Rev. 24: 520. 1954.)
Vasquez Vassalo, A.
1951. Nutritional and chemical study of Rhinobatos planiceps (Guitarra) and of "corn chicha." An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 2: 207-218. (Abstract in Chem. Abs. 48: 3579. 1954.)

Vijayaraghavan, P. K., and Srinivasan, P. R.
1953. Essential amino acid composition of some common Indian pulses. Jour. Nutr. 51: 261-271.
Viñas Tello, E., Diaz, C., Roca, A., and others.
1953. El contenido en amino-acidos esenciales de la quinua. Salud y Bienestar Soc. No. 2, pp. 61-66. [Processed.]
Violante, A. M., Sirny, R. J., and Elvehjem, C. A.
1952. The aspartic acid, glutamic acid, proline and tyrosine content of meat. Jour. Nutr. 47: 307-315.
Wetzel, W.
1953. Methode zur bestimmung von glutaminsäure in proteinhydrolysaten durch chromatographie an anionotropem aluminiumoxyd. Ztschr. f. Lebensmtl.-Untersuch. u. Forsch. 96: [237]-244.
White, P. L., Alvistur, E., Dids, C., and others.
1955. Nutrient content and protein quality of quinua and cañihua, edible seed products of the Andes Mountains. Agr. and Food Chem. Jour. 3: 531-534.
Wilder, O. H. M., and Kraybill, H. R.
1947. Effect of cooking and curing on lysine content of pork luncheon meat. Jour. Nutr. 33: 235-242, illus.
Williams, Harold H.
1955. "Essential" amino acid content of animal feeds. N. Y. (Cornell) Agr. Expt. Sta. Mem. 337, pp. 3-31.
Williamson, M. B.
1944. The amino acid composition of human milk proteins. Jour. Biol. Chem. 156: 47-52.
Winter, H.
1954. Kritische überprüfung verschiedener methoden zur glykokollbestimmung. Ztschr. f. Lebensmtl.-Untersuch. u. Forsch. 99: 34-42.
Wooley, J. G., and Sebrell, W. H.
1945. Two microbiological methods for the determination of l(-)-tryptophane in proteins and other complex substances. Jour. Biol. Chem. 157: 141-151, illus.
Wu, C. H., and Fenton, F.
1953. Effect of sprouting and cooking of soybeans on palatability, lysine, tryptophane, thiamine, and ascorbic acid. Food Res. 18: 640-645.
Yaichnikov, I. S., Florenskaya, N. K., and Funikova, A. N.
1954. Determination of the most important amino acids in feeds. Zhur. Prikl. Khim. 27: 570-572. (Abstract in Chem. Abs. 48: 9584. 1954.)
Zambrano Reyes, A.
1953. Análisis bromatológico de la carne de la "Merluccius gayi" o Merluza seca (Peje Palo) An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 4: 178-182. (Abstract in Nutr. Abs. and Rev. 26: 45. 1956.)
Zanoni V., T.
1951. Análisis bromatológico de la carne de "Aetobatum peruvianus" o raya. An. Fac. Farm. y Bioquim., Univ. Nac. Mayor San Marcos (Lima, Peru) 2: [282]-286. (Abstract in Chem. Abs. 48: 3587. 1954.)

LITERATURE CITED

(1) Bird, F. H.
1956. Report on amino acids in feeds. Jour. Assoc. Off. Agr. Chem. 39: 123-124.

(2) Block, R. J., and Bolling, D.
1951. The amino acid composition of proteins and foods. Analytical methods and results. Ed. 2, 576 pp., illus. Springfield, Ill.

(3) _____ Durrum, E. L., and Zweig, G.
1955. A manual of paper chromatography and paper electrophoresus. 484 pp. New York.

(4) _____ and Weiss, K. W.
1956. Amino acid handbook, methods and results of protein analysis. 386 pp. Springfield, Ill.

(5) Chatfield, C.
1949. Food composition tables for international use. Food and Agr. Organ. Nutr. Studies 3, 56 pp.

(6) _____
1954. Food composition tables-minerals and vitamins for international use. Food and Agr. Organ. Nutr. Studies 11, 117 pp.

(7) _____ and Adams, G.
1940. Proximate composition of American food materials. U. S. Dept. Agr. Cir. 549, 91 pp.

(8) Colowick, S. P., and Kaplan, N. O., eds.
1957. Methods in enzymology. V. 3. Preparation and assay of substrates. 1154 pp., illus. New York.

(9) Csonka, F. A.
1937. Amino acids in staple foods. I. Wheat (Triticum vulgare). Jour. Biol. Chem. 118: 147-153.

(10) Doty, D. M., Bergdoll, M. S., Nash, H. A., and Brunson, A. M.
1946. Amino acids in corn grain from several single cross hybrids. Cereal Chem. 23: 199-209.

(11) Dunn, M. S.
1950. Determination of amino acids. In Analytical Methods in the Food Industry. Advances in Chemistry Series, No. 3, pp. 13-28.

(12) Gale, E. F.
1957. Determination of amino acids by use of bacterial amino acid decarboxylases. In Glick, D., ed., Methods of Biochemical Analysis, V. 4, pp. 285-306.

(13) Harvey, D.
1956. Tables of the amino acids in foods and feedingstuffs. Commonwealth Bureau of Animal Nutrition [Rowett Institute, Bucksburn, Aberdeenshire, Scotland]. Tech. Commun. No. 19, 52 pp.

(14) Horn, M. J., Fifield, C. C., Blum, A. E., and Warren, H. W.
The distribution of amino acids in wheat and certain wheat products. Cereal Chem. [In press.]

(15) Jones, D. B.
1941. Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. U. S. Dept. Agr. Cir. 183, 22 pp. (Slightly rev. ed.)

(16) Kelsey, H. P., and Dayton, W. A., eds.
1942. Standardized plant names. Ed. 2, 675 pp. Harrisburg, Pa.

(17) Kik, M. C.
1942. Nutritive studies of rice and its by-products. Ark. Agr. Expt. Sta. Bul. 416, 28 pp., illus.

(18) Leung, Woot-Tsuen Wu, Pecot, R. K., and Watt, B. K.
1952. Composition of foods used in far eastern countries. U. S. Dept. Agr. Handb. 34, 62 pp.

(19) National Academy of Sciences, National Research Council.
1956. Composition of concentrate by-product feeding stuffs. Pub. 449, 126 pp.

(20) Neilands, J. B., Sirny, R. J., Sohljell, I., and others.
1949. The nutritive value of canned foods. II. Amino acid content of fish and meat products. Jour. Nutr. 39: 187-202.

(21) Orr, M. L., and Watt, B. K.
1955. Amino acid content of foods. U. S. Dept. Agr., Agr. Res. Serv. FE 101, 41 pp. [Processed.]

(22) Rose, W. C., and Wixon, R. L.
1955. The amino acid requirements of man. XIII. The sparing effect of cystine on the methionine requirement. Jour. Biol. Chem. 216: 763-773.

(23) _____ and Wixon, R. L.
1955. The amino acid requirements of man. XIV. The sparing effect of tyrosine on the phenylalanine requirement. Jour. Biol. Chem. 217: 95-101.

(24) Rutgers University, Bureau of Biological Research.
[1951] Cooperative determinations of the amino acid content, and of the nutritive value of six selected protein food sources 1946-1950. 114 pp. Rutgers Univ. New Brunswick, N. J. [Processed.]

(25) Schweigert, B. S., and Snell, E. E.
1947. Microbiological methods for the estimation of amino-acids. Nutr. Abs. and Rev. 16: 497-510.

(26) Taranova, A. I.
1951. The amino-acid composition of wheat proteins as dependent on variety, locality of growth and milling. Biokhimiya 16: 239-245. (Abstract in Nutr. Abs. and Rev. 22: 331. 1952.)

(27) Watt, B. K., Merrill, A. L., Orr, M. L., and others.
1950. Composition of foods--raw, processed, prepared. U. S. Dept. Agr. Handb. 8, 147 pp.

☆ U. S. GOVERNMENT PRINTING OFFICE : 1957 O - 448975

Food Research, Vol. 20,, 310-314 (1955)

INFLUENCE OF MONOSODIUM GLUTAMATE ON TASTE PERCEPTION[a,b]

FRANCIS J. PILGRIM, HOWARD G. SCHUTZ, AND DAVID R. PERYAM
Quartermaster Food and Container Institute for the Armed Forces, Chicago 9, Illinois

(Manuscript received February 14, 1955)

The fact that monosodium glutamate (MSG) increases palatability of some foods, as demonstrated by consumer preference studies *(3)*, would place it among the seasonings and condiments. However, statements have been made *(1, 6, 7)* to the effect that MSG is a unique substance that does not impart a flavor of its own but serves only to enhance the natural flavors of foods by increasing the sensitivity of the taste receptors. This might be termed an "accentuation" or "sensitization" hypothesis.

Several investigators have tested this hypothesis. Lockhart and Gainer *(5)* used subliminal concentrations of MSG in supraliminal sugar and salt solutions along with control (no MSG) solutions in triangle tests. The MSG had no significant effect on the taste of sucrose or sodium chloride. In those cases where a change in taste was observed, the taste appeared to be that of glutamate rather than additional sweetness or saltiness. Mosel and Kantrowitz *(8)* determined the effect of exposure to a 5 times threshold concentration of MSG five minutes before measuring absolute thresholds for the four basic tastes. Compared to control thresholds, *sweet* and *salty* were unaffected, acuity to *sour* was increased somewhat and acuity to *bitter* was greatly increased. These authors also discuss other publications, pointing out that there has been confusion of palatability with intensity and that some results are inconsistent with the "sensitization" hypothesis. Another study *(10)* employing subliminal concentrations of MSG (0.75 times threshold) showed a lowering of thresholds for *sweet* and *salt* but not those for *bitter* and *sour*. The results of these three studies do not support the hypothesis that MSG is a general sensitizer for taste acuity. Others who have sponsored this hypothesis do not support it with data.

Our investigation was undertaken to obtain further evidence on the validity of this hypothesis. The experiments included three types of measurements to assess the effects of MSG on gustatory acuity and flavor perception: changes in the absolute threshold (RL); changes in the differential threshold (DL); and, changes in subjective intensity.

PROCEDURE

Materials. The sodium chloride, sucrose and hydrochloric acid were C.P. grade; the caffeine was U.S.P., and the MSG was commercial 99+% pure. Water for preparing

---

[a] This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned number 520 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

[b] Based on a paper presented at the 62nd Annual Meeting of the American Psychological Association, New York, N. Y., September 6, 1954.

solutions and for rinsing was distilled and then filtered through charcoal to remove all taste and odor. In all experiments the solutions were presented in 1 oz. glasses; the amount was 6 ml. for the RL's and DL's and about 25 ml. for subjective intensity. The time interval between samples was 30 seconds in all cases.

Subjects. Subjects for the threshold studies all had had considerable experience in laboratory sensory testing of foods and were familiar with the specific methods used. The latter are standard psychophysical techniques *(1)*. For the subjective intensity experiments subjects were drawn from a large group of people normally available for food preference studies.

Subjective intensity. The effect of MSG on weak, but supraliminal, concentrations of the primary taste qualities was determined by the method of paired comparisons. Each pair consisted of identical concentrations of one of the basic taste substances, with one sample of the pair containing MSG in addition to the basic taste substance. The question asked of each subject was: "Which is saltier?" or "Which is sweeter?," etc. The substances and the concentrations employed are shown in Table 1. The results were tested for significance against the expected chance distribution of 50-50.

TABLE 1

Subjective intensity[1] of basic taste solutions with and without added monosodium glutamate (0.2%)

Taste quality	No. of subjects	Substance and conc.		Samples with MSG judged stronger	p[2]
			%	%	
Sweet	35	Sucrose	2.0	34	.09
Sour	48	Hydrochloric acid	0.021	62	.11
Salty	30	Sodium chloride	0.2	80	.002
Bitter	48	Quinine sulfate	.00015	81	.001

[1] Method of paired comparisons. One pair of samples per subject (untrained consumer-observers).
[2] Probability that difference from expected distribution of 50-50 could have occurred by chance.

Absolute threshold. RL's for *sweet* and *sour* were determined by the method of constant stimuli, using 7 concentrations for each subject. The 7 concentrations were chosen from a larger series to encompass the range of each subject's RL. The 7 samples were presented in a different random order to each subject at a session and in a different order for a given subject at successive sessions. Control and experimental sessions were alternated for a total of 10 sessions. For the control sessions the subject rinsed his mouth with water for 5 seconds before each sample; for experimental sessions he rinsed for 5 seconds with 0.068% (0.001M) MSG. He rinsed with water after each sample in both experimental and control sessions. To each sample, the subject responded with a "Yes" or "No" indicating presence or absence of the taste. Another study on the sweet threshold, employing the method of limits, was made to assess the effect of method on the results. Only the ascending order was used, as is common practice in gustatory studies in order to avoid adaptation to the stimulus. In this study each subject rinsed with an MSG solution that was twice the concentration of his own threshold concentration.

Differential threshold. The DL for salt was measured by the method of a single stimuli using concentrations of 0.8, 0.9, 1.0 and 1.1% sodium chloride solutions. At each session each of 18 subjects received the 4 concentrations in ascending order, with knowledge, as a practice set, and then received the series twice in random order to be judged as to strength. The judgments were scored on the basis of proportion of times a sample was judged stronger than the hypothetical mid-point of 0.95% salt. For the control session he rinsed before and after each sample with water; for the experimental session he rinsed before with a 0.17% (.01M) solution of MSG and after with water. For the bitter DL the method of constant stimulus differences was used with .0020M (.039%) caffeine as the standard to be compared to concentrations of .0014, .0016, .0018, .0022, .0024, and .0026M solutions. The order of the pairs, and the position of the standard in the pairs, was randomized. The subject judged which sample in each pair was stronger. Seventeen subjects made a total of 72 judgments per point under both the experimental and control conditions. The rinsing procedures were the same as for salt.

Raw data were converted to proportion judged "Yes" in the case of RL's, and proportion judged stronger than the mid-point in the case of the DL's. Then these proportions were transformed to normal deviates, or sigma values, to convert the psychometric curves to straight lines *(1)*. Lines were fitted to the points by the least squares technique and the resulting equations were then solved for the required 50% and 75% points to obtain the RL's, DL's and PSE's (point of subjective equality).

RESULTS

That MSG has some effect on subjective intensity is evident from Table 1. *Salt* and *bitter* were intensified, but *sour* and *sweet* were not significantly affected. General flavor intensification was not apparent. It is questionable whether even the result for *salt* should be called intensification since a pure MSG solution will be called salty by many people. Hence, the increased saltiness may be only the result of increasing the concentration of salty-tasting solutes. The increase in bitterness cannot be explained as readily in the same way.

If MSG sensitizes the end organs of taste, then thresholds should be lower immediately after the mouth has been exposed to it. Table 2 shows the changes in thresholds for *sweet* and *sour*. Acuity to *sweet* and *sour* is actually decreased. This result for *sour* is the opposite of that obtained by Mosel and Kantrowitz *(8)*. This may be due to methodological differences.

The effect of an MSG rinse on the DL's for salty and bitter qualities is illustrated by data in Table 3. Neither result reaches the usually accepted levels of statistical significance.

DISCUSSION

No consistent pattern of the effect of MSG has emerged from these experiments. General intensification of taste is not evident. Intensity and acuity are reduced in some cases, increased in others, and sometimes there is no effect. These results substantiate those of other investigators. Selective intensification, such as was found in the experiments on subjective intensity, shown in Table 1, is congruent with traditional principles of flavor mixture and requires no special hypothesis.

Other studies from this laboratory *(2)* on preferences have shown that MSG improves hamburger that had developed rancidity due to exposure to ultra-violet light. According to the intensification hypothesis, these off-flavors should have been accentuated with a consequent loss in preference. Furthermore, preference studies have not shown MSG to be consistent in its effects on any one class of foods. For example, it improves some green vegetables and not others, using the criterion of consumer preference.

TABLE 2

Effect of a prior rinse with a monosodium glutamate solution on the RL's for two basic tastes

Taste quality	No. of subjects	Substance and conc.		Conc. of MSG solution	RL[1]	p[2]
			%	%	%	
Sweet[3]	5	Sucrose	0.05-0.7	0	.27	.05
				2 × thresh.	.32	
Sweet[4]	10	Sucrose	.25-.70	0	.34	.01
				.068	.43	
Sour[4]	8	Hydrochloric acid	0.0002-.002	0	.00128	.001
				.068	.00171	

[1] RL = absolute threshold.
[2] Probability that difference could have occurred by chance.
[3] Method of limits.
[4] Method of constant stimuli.

4. GUILFORD, J. P. *Psychometric Methods.* 2nd ed., 1954, McGraw-Hill, New York, N. Y.
5. LOCKHART, E. E., AND GAINES, J. M. Effect of monosodium glutamate on taste of pure sucrose and sodium chloride. *Food Research*, 15, 459-461 (1950).
6. LOGAN, P. Test of monosodium glutamate. *National Restaurant News Bulletin*, June 1949.
7. MELNICK, D. Monosodium glutamate: improver of natural food flavors. *Sci. Monthly*, 70, 199-201 (1950).
8. MOSEL, J. N., AND KAMROWITZ, G. The effect of monosodium glutamate on acuity to the primary tastes. *Am. J. Psychol.*, 65, 573-579 (1952).
9. QUARTERMASTER FOOD AND CONTAINER INSTITUTE PROGRESS REPORT, Project 7-84-15-007. Report No. 1: Nerve Responses to Organic Salts Applied to the Tongue (Florida State University), 1952.
10. VAN COTT, H., HAMILTON, C. E., AND LITTELL, A. The effects of sub-threshold concentrations of monosodium glutamate on absolute taste thresholds. (A paper presented at the 75th Annual Meeting of the Eastern Psychological Association at New York, April 9-10, 1954.)

Reprinted from Archives internationales de Pharmacodynamie et de Thérapie
Vol. 189 - No. 1 - January 1971

Neuromuscular Functions of Mature Mice Following Neonatal Monosodium Glutamate (1)

V. G. Prabhu and Y. T. Oester

The Department of Physiology and Pharmacology, Chicago College of Osteopathic Medicine Chicago, Illinois and Research and Education Service, Veterans Administration Hospital Hines, Illinois, U.S.A.

Abstract—One day old mice were injected, daily, with Monosodium Glutamate for the first ten days of their neonatal life. Control litter mates were under identical conditions and were injeeted with normal saline. At the end of the sixty day period of observation, the mean weight of the Monosodium Glutamate treated group became greater than that of the controls. A battery of behavior and pharmacological tests were applied, when these mice attained 20–28 grams body weight. There was no significant or observable differences in their responses to behavior tests. Similarily, no differences were noted in the responses of these two groups to selected drugs.

Introduction

Olney (1) reported that single subcutaneous injections of Monosodium Glutamate (MSG) in new born mice induced acute neuronal necrosis in several regions of the developing brain, including the hypothalamus. As adults the mice treated when newborn by subcutaneous single injection showed stunted skeletal growth, marked obesity and female sterility. Based on these results, Olney postulated that the adult syndrome presents a multi-faceted neuro-endocrine disturbance arising from the disruption of developing neural centers. MSG has also been known to cause degenerative lesions in the inner retina of infant mice (2, 3). MSG is used in many varieties of food products including baby foods. The cause of a characteristic malady commonly known as "Chinese Restaurant" syndrome has been traced to MSG in certain foods (4, 5). In view of the common use of MSG in human baby foods, it was considered

(1) This investigation was supported in part, by the Public Health Service Grant FR 5436-07 of National Institute of Health and was reported, in part, in Federation Proceeding 29, 620 (1970).

pertinent to examine the effects of MSG administration in infant mice. The purpose of this study was to investigate any subsequent neurological and pharmacological changes.

Methods

Infant litter mates of Swiss-Webster mice were divided into two groups. The experimental group received single daily subcutaneous injections of MSG for 10 consecutive days. The control litter mate group received injections of 0.9 % saline. All injections were of 0.02 ml volume. Each injection was made into the subcutaneous tissue along the dorsum of the body. The first MSG injection, started 24 hours after birth, contained 2 gm/kg of MSG. Subsequent daily doses were in ascending daily increments of 0.25 grams, 2.25, 2.5, 2.75 ... 4.25 gm/kg/day, through the tenth day. When the surviving MSG treated and control mice attained 20–28 grams body weight, they were subjected to a battery of behavioral and pharmacological tests as listed herein. In order to screen for any acute effect of MSG, a third group of mice, given intraperitoneal injection of 1 gm/kg of MSG, thirty minutes prior to the various tests, were also included as a part of this study.

(*a*) *Spontaneous Motor Activity.* The procedure of Bastian and Hill (6) was used in these tests. The total activity (mouse exploratory movements) was measured for a 15 minute period immediately after placing each mouse in the activity cage.

(*b*) *Test of Neurological Deficit.* A procedure described by Danham and Miya (7) was used. Mice were placed on a slowly rotating wooden rod (6–7 R.P.M.). Any mouse which could not maintain its balance on the rotating rod for a minimum of 2 minutes, was considered as having neurological deficit.

(*c*) *Grip-strength Endurance Test.* A mouse placed on a tight cord, immediately grabs the cord by its fore limbs and always brings in its hind limbs for additional support. Placed in this situation, the animal clings to the cord for awhile and then drops to a cage placed below, when it no longer can maintain this posture. The length of time required for the loss of grip from the cord, is an index of endurance.

(*d*) *Swimming Endurance Test.* The method of Wilber and Hunn (8) was employed. The temperature of the water was $20° \pm 1°$ C. The water in the tank was under constant agitation. Groups of 4 mice were tested at a time. The time required for the cessation of co-ordinated muscle movements, just prior to drowning, was the end point of swimming endurance.

(*e*) *Amphetamine Aggregation Toxicity.* The method was essentially the same as the general method of Chance (9) modified by Proctor *et al.* (10). Mice were aggregated in groups of 5 in such a manner as to yield 25 cm^2 floor space per

mouse. Unaggregated solitary mice were placed, one mouse per cage with a floor space of 500 cm^2 per mouse. Animals were either aggregated or placed in isolation cages immediately after the intraperitoneal injection of 10 mg/kg of *d*-amphetamine sulfate. The experiments were carried out in an air-conditioned room at $24° \pm 1°$ C. Animals were periodically checked for survival up to four hours.

(*f*) *Pentylenetetrazol (Metrazol®) Infusion Test.* The method of Orloff *et al.* (11) was employed. Metrazol solution (0.5 %) was infused into the tail veins of unanesthetized, lightly restrained mice. The infusion rate was 0.27 ml/minutes (constant speed syringe infusion pump, Sage Instruments Company). A #25 gauge needle connected to one end of a thin 16 inch piece of polyethylene tubing was inserted into the tail vein. The other end of the tubing was connected to a one ml tuberculin syringe mounted on the infusion pump. The quantity of infused metrazol necessary to produce each of three distinct signs, namely a first twitch, clonic convulsion, and finally tonic seizure was recorded.

(*g*) *Measurement of Sleeping and Paralysis Times.* Hexobarbital (100 mg/kg) and barbital (300 mg/kg) sleeping times and Zoxazolamine paralysis times (150 mg/kg dissolved in corn oil) were determined by measuring the time between the loss and recovery of righting reflex of mice given these agents by intraperitoneal injection. The utilization rate was determined by repeatedly injecting a predetermined dose of hexobarbital immediately after the mice regained their righting reflex from a previous dose. This process was repeated for a period of 4–5 hours. At the end, the sum total of hexobarbital (mg/kg) injected (for the entire sleeping period) divided by sleep time in hours represented the utilization rate (mg/kg/hr). The consistency of this technique had been previously checked (12).

Results

Clinical Observations of Infant Mice on MSG. Twenty-four hour old, infant mice (1.75–2.25 grams) were injected subcutaneously daily with MSG for ten days. The first dose was 2 gm/kg and the tenth dose was 4.25 gm/kg. During the same time, control litter mate mice received 0.9 % NaCl injections. Daily, all mice were weighed and observed for gross clinical changes. In pilot experiments, body damage and mutilation of injected mice were observed several hours after the injection. It was found that the mother attacked and killed the recently MSG treated offspring. This was avoided by keeping the mother away from the offspring for 1–2 hours after injection. In other preliminary experiments, a first day dose of 4.25 gm/kg was tried but found to be too toxic (20 % mortality). In general, all the MSG treated mice appeared to be in good health. The gain in body weight was comparable to that of control groups (Figures 1 and 2). It is apparent that at the end of the study period, 50 days, both males

and females were slightly heavier than controls. No untoward signs or symptoms, except a few injection marks were noted. The results of all of the behavioral and pharmacological tests were obtained when the mice weighed 20–28 grams. Barbiturate sleeping time, and Zoxazolamine paralysis time, were conducted on male mice only. This would avoid sex linked difference in drug metabolism. In a subsequent group of mice, with identical MSG treatment as group one, the body weight at approximately 180 days was from 75 to 80 grams, while the corresponding litter mate controls, weighed, 35–40 grams.

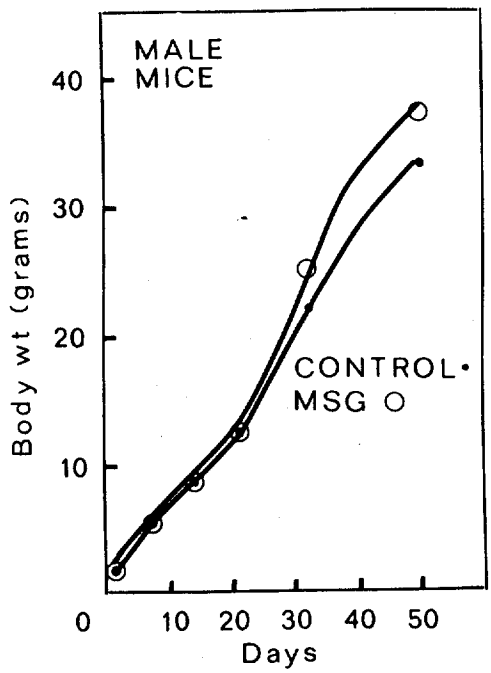


FIG. 1
Body weight of male mice given MSG in infancy

Neurological Tests

Spontaneous Motor Activity. In Table I are shown the mean motor activity counts of control, MSG treated (chronic) and MSG (acute) mice. The exploratory activity of control and MSG (chronic) groups were essentially of the same order. However, the spontaneous activity count of acute MSG mice (Gr III) was significantly greater.

Swimming and Grip Strength Endurance Tests. Results presented in Table I show that as far as endurance abilities are concerned, the MSG-treated (chronic)

mice and their control counter parts were in no way different from each other. Furthermore, actual presence of MSG in the body during testing (Gr III) did not alter the endurance abilities of the mice. In addition, Danham and Miya's test for neurological deficit, where the mice were made to balance on a rota-rod did not show any indication of neurological deficit.

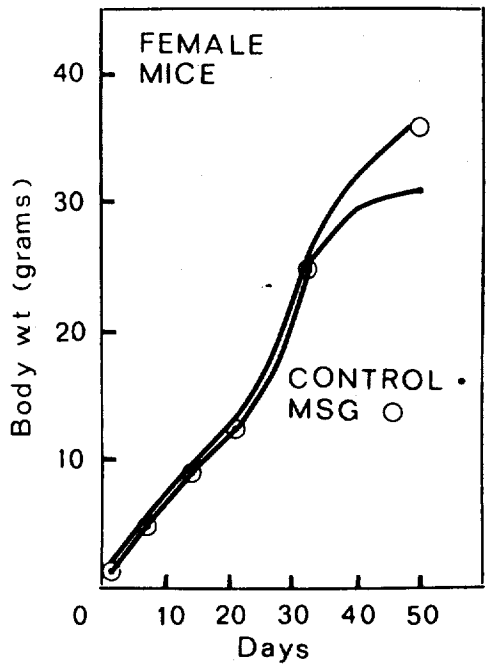


FIG. 2
Body weight of female mice given MSG in infancy

Zoxazolamine Paralysis and Amphetamine Aggregation Lethality. Results outlined in Table II show that chronic MSG treatment to infant mice did not influence the Zoxazolamine paralysis time or the *d*-amphetamine aggregation lethality when compared with their control litter mates. However, MSG, 1.0 gm/kg injected, 30 minutes prior to testing showed an antagonistic effect on Zoxazolamine paralysis time and enhancing effect on *d*-amphetamine aggregation mortality.

Effect of Barbiturates. The findings summarized in Table III show a trend similar to the results in Table II, namely the hexobarbital sleeping times of MSG (chronic) and control mice were not significantly different. Acute MSG treatment (GR III) appeared to decrease hexobarbital effect. In addition to sleeping time, Table III presents hexobarbital utilization data. The results

TABLE I

Neurological Tests on MSG Treated and Control Mice

Experimental Groups	Spontaneous Motor Activity		Swimming Endurance Test		Grip Strength Test	
	No. Mice	Counts 15 Min	No. Mice	Time for Exhaustion (min)	No. Mice	Time for Exhaustion (seconds)
I. Control	20	22.6 ± 7	15	9.4 ± 2	16	94.6 ± 13
II. MSG (Chronic)	20	25.3 ± 8 (1)	16	10.2 ± 2 (1)	15	95.9 ± 17 (1)
III. MSG (Acute)	12	49.8 ± 11 (2)	8	10.8 ± 3 (1)	10	89.4 ± 12 (1)

(1) Not significantly different from the controls. — (2) Significantly different from non-treated control.

TABLE II

Zoxazolamine Paralysis and d-Amphetamine Aggregation Effects of MSG Treated and Control Mice

Experimental Groups	Zoxazolamine (150 mg/kg) I.P.		*d*-Amphetamine (10 mg/kg) I.P. Aggregated	
	No. Mice	Paralysis Time	No. Mice	% Mortality
I. Control	16	74.5 ± 16	10	80 %
II. MSG (Chronic)	14	69.8 ± 12 (1)	10	80 %
III. MSG (Acute) 1.0 g/kg I.P. 30 min prior	10	38.6 ± 8 (2)	10	100 %

(1) Not significantly different from control group. — (2) Significantly different from control group.

TABLE III

Sleeping Time and Utilization Rate of Hexobarbital and Barbital in MSG-Treated and Control Mice

Experimental Groups	Hexobarbital		Barbital Sleep Time (minutes) 300 mg/kg I.P.
	Sleep Time Minutes 100 mg/kg I.P.	Utilization Rate mg/kg/hr	
I. Control	71.1 ± 8 (12)	39.8 ± 6 (8)	138 ± 35 (8)
II. MSG (Chronic) 2–4.25 g/kg 10 days	65.6 ± 12 (1) (14)	42.4 ± 5 (1) (10)	152 ± 42 (1) (8)
III. MSG (Acute) 1.0 g/kg	62 ± 9 (1) (8)	43.3 ± 7 (1) (6)	129 ± 28 (1) (8)

Numbers in parenthesis are number of mice used. — (1) Not significantly different from control.

TABLE IV

Constant Speed I.V. Infusion of Metrazol (0.5 % Solution): Infusion Rate—0.27 ml/min

Experimental Groups	No. Mice	Average Body Weight (gms)	ml of Metrazol Required for		
			1st Twitch	Clonic Convulsion	Tonic Seizure and Death
I. Control	16	22 ± 2	0.14 ± .04	0.20 ± .065	0.40 ± .15
II. MSG (Chronic) 2–4.25 g/kg/day 10 days	12	21 ± 3	0.13 ± .03 (1)	0.19 ± .049 (1)	0.38 ± .13 (1)
III. MSG (Acute) 1.0 g/kg I.P. 30 min Prior	10	21 ± 5	0.12 ± .06	0.15 ± .06	0.32 ± .07

(1) Not significantly different.

TABLE V

Organ Weight of MSG-Treated and Control Mice (1)

Experimental Groups	Body Weight Grams	Liver mg	Kidney mg	Spleen mg	Gonads mg	Heart mg	Lungs mg
I. Control							
Males	30.2 ± 7	2082.7 ± 5	419.6 ± 8	128.3 ± 6	258.5 ± 7	170.2 ± 7	192.4 ± 9
Females	23.3 ± 8	1318.6 ± 6	293.2 ± 7	122.2 ± 4		118.5 ± 4	172.6 ± 3
II. MSG (Chronic) 2–4.25 g/kg/day 10 days							
Males	34.2 ± 7	1845.5 ± 8	399.3 ± 8	115.5 ± 8	128.3 ± 3 (2)	184.6 ± 6	173.7 ± 5
Females	27.3 ± 8	1342.4 ± 9	291.8 ± 3	127.3 ± 6		115.4 ± 7	163.8 ± 7

(1) Organ weights are averages of 8 males and 6 females for each group. — (2) Significantly different from the control.

for MSG (chronic) and control group were not significantly different from each other. Barbital sleeping time in these two instances were almost similar (Table III).

Metrazol Infusion. Table IV outlines the data resulting from a constant speed metrazol infusion. Orloff *et al.* (11) had shown that a 0.5 % Metrazol infusion through a tail vein of the unanesthetized mouse produces three characteristic signs, namely a first twitch, secondly, a "pseudo clonic convulsion" and thirdly, a tonic seizure, leading to death. The quantity of metrazol required for each of these three steps is a reflection of the excitability of the CNS. Generally, hyperexcitability, pre-existing or induced, decreases the threshold for each of these steps, and hypoexcitability may increase the thresholds. Our metrazol infusion data shows that chronic-MSG or control mice did not show any significant difference in their responses to metrazol infusion. However, the acute MSG (1.0 gm/kg) demonstrated a decrease in the metrazol thresholds.

Organ Weights of MSG Treated and Control Mice. Table V summarizes the mean organ weights of mice from both groups. As can be seen from the data, the weights of liver, kidney, spleen, lungs and heart of both the groups were not significantly different. However, it is important to note that the testes of MSG treated mice were significantly smaller than those of control mice.

Discussion

The results of the experiments presented demonstrate that a short course (10 days) of high doses of MSG administered to newborn mice, does not appear to produce any grossly observable clinical signs in the early postnatal period or during the development to adulthood. As can be seen from the plots of body weights, the MSG treated and their control liter mates, had comparable weight gains during this period. This observation is comparable with the first part of Olney's findings (1). In order to ascertain whether or not any functional changes, not apparent by gross observation, might be revealed, a battery of tests were carried out. It was assumed that as adults, MSG-treated mice might exhibit some response, at least, different from those of their control litter mates. Thus, drugs like barbiturates, Zoxazolamine, amphetamine and metrazol were tested in an identical manner in both groups. Our results have not uncovered any differences between these animals. Olney (1) reported a marked neuronal necrosis and neuroendocrinological disturbance, with lesions in the developing brain centers of infant mice. It was logical to expect some degree of neurological deficit, or disturbances in spontaneous motor activity, or endurance to physical strain as a manifestation of such neurological damage. If a neurological test whose behavior effects were related to the appropriate brain center was available, this would be an ideal observation. However, the authors do not know of any such specific test. It was also logical to expect some difference

of CNS excitability, hepatic metabolism or renal elimination of drugs, caused by the same or related neurological lesions. Several investigators (13, 14), have used barbiturates and other test agents broadly in order to uncover possible involvements of the nervous, hepatic and renal systems of experimental animals. In our study aggregation toxicity, and metrazol infusion thresholds (Table II and IV) would seem to indicate that chronic MSG-treatment did not alter the overall excitiability of the CNS. This also agrees with another observation that the induction time for barbital action of MSG treated mice was not different from that of control mice. That the renal barbital eliminating mechanisms of MSG-treated mice, in all probability, were not affected is indicated by a lack of difference in barbital sleeping times. Barbital is not extensively metabolized and its effect is terminated primarily by its renal elimination. On the other hand, Zoxazolamine paralysis, hexobarbital sleeping time and utilization rate data (Tables II and III) rule out the unlikely effect of MSG on the hepatic microsomal enzyme systems of these mice. Such hepatic microsomal enzyme induction or inhibition produces appreciable change in hexobarbital activity (13).

The negative physiological and pharmacological finding in this study in contrast to the positive histological and obesity findings of Olney, may perhaps be related to a time factor as well as to the relatively localized site of neurological damage which Olney reported. It is important to realize that the marked obesity, according to Olney's findings, occurred much later in life 6–9 months. His positive histological findings, however, were made on 2–9 day old mice given MSG a few hours prior to sacrifice. The results of the present investigation were obtained from mice when they attained essentially adult weight, 20–28 gms or about two months of age. It would indeed be interesting to investigate whether or not detectable physiological changes might be present at an earlier or perhaps at a later date.

Summary

New born Swiss-Webster mice, 24 hours old, were injected daily, subcutaneously, with Monosodium Glutamate (MSG) for a period of 10 days. When the mice attained a body weight of 20–28 grams, they were subjected to a battery of behavioral and pharmacological tests to determine if MSG treatment in early life produced any demonstrable physiological disturbances. Neurological deficit, spontaneous motor activity, swimming and grip strength endurance tests and response to *d*-amphetamine, metrazol infusion, zoxazolamine and barbiturates were studied. Based on these tests, it was concluded that no obvious difference in the responses of MSG-treated and control mice were detected that could be attributed to MSG. Organ weights with the exception of testes were comparable.

References

1. Olney, J. W. *Science* **164**, 719 (1969).
2. Cohen, A. I. *Amer. J. Anat.* **120**, 319 (1967).
3. Lucas, D. R. and Newhouse, P. J. *Amer. med. Assoc. Arch. Opthalmol.* **58**, 193 (1957).
4. Schaumburg, H. H. and Byck, R. *N. Engl. J. Med.* **279**, 105 (1968).
5. Schaumburg, H. H., Byck, R., Cerstil, R. and Mashman, J. H. *Science* **163**, 826 (1969).
6. Bastian, H. W. and Hill, R. G. *J. Pharmacol. exp. Ther.* **119**, 826 (1969).
7. Danham, N. W. and Miya, T. S. *J. Amer. Pharm. Ass.* (Sc. Ed.) **46**, 208 (1957).
8. Wilber, C. G. and Hunn, B. *J. appl. Physiol.* **15**, 704 (1960).
9. Chance, M. R. A. *J. Pharmacol. exp. Ther.* **87**, 214 (1946).
10. Proctor, C. D., Ridlon, S. A., Fudema, J. J. and Prabhu, V. G. *Toxicol. appl. Pharmacol.* **6**, 1 (1964).
11. Orloff, M. G., William, H. L. and Pfeiffer, C. C. *Proc. Soc. exp. Biol.*, N.Y. **70**, 254 (1949).
12. Prabhu, V. G., Parikh, S. H. and Shah, G. *Indian J. med. Sci.* **21**, 524 (1967).
13. Conney, A. H. *Pharmacol. Rev.* **19**, 317 (1967).
14. Fuji, K., Jaffe, H. and Epstein, S. *Toxicol. appl. Pharmacol.* **13**, 431 (1968).

Received 13-7-1970.

ST. CATHERINE PRESS, TEMPELHOF 37, BRUGGE, BELGIUM

γ-AMINOBUTYRIC ACID IN BRAIN: ITS FORMATION FROM GLUTAMIC ACID*

BY EUGENE ROBERTS AND SAM FRANKEL

(*Department of Cancer Research, Washington University School of Medicine, St. Louis*)

(Received for publication, June 3, 1950)

Relatively large quantities of an unidentified ninhydrin-reactive material were found in numerous two-dimensional paper chromatograms of protein-free extracts of fresh mouse, rat, rabbit, guinea pig, human, and frog brains. At most, only traces of this material were found in a large number of extracts of many other normal and neoplastic tissues and in urine and blood. In the present experiments and in the following paper (1), the unknown compound in brain extract is conclusively identified as γ-aminobutyric acid. It is shown that this compound can be formed from glutamic acid by homogenates, washed residues, and acetone powders of brain. A preliminary report of some of these findings has been made (2).

EXPERIMENTAL

Chromatography.—One-dimensional and two-dimensional chromatograms were made by the descending method as outlined by Consden *et al.* (3) and extended by Dent (4, 5).

Separation of Unknown Material—Mice were killed by dislocation of the cervical vertebrae, and the brains were removed immediately and frozen in dry ice. A pooled sample weighing 7.8 gm. was homogenized in 100 ml. of 75 per cent alcohol. After centrifugation, the extract was evaporated almost to dryness, taken up in 2.6 ml. of water, and the aqueous extract was centrifuged to remove insoluble material. This extract was then employed for chromatography.

In Fig. 1 is shown a tracing of a chromatogram obtained from an aliquot of extract corresponding to 75 mg. of fresh mouse brain. Intense spots were given by aspartic acid, glutamic acid, glycine, glutamine, taurine, alanine, and the unknown material. Cystine, serine, and β-alanine were present in smaller amounts, and traces of valine, threonine, and glutathione were noted. Two substances which disappeared on acid hydrolysis were located to the left of aspartic and glutamic acids on the chromatograms. Among the substances which have been studied chromatographically (5), the unknown material corresponded most closely in its behavior to histi-

* Aided by grants from the Charles F. Kettering Foundation and the United States Public Health Service.

dine, methionine sulfoxide, and γ-aminobutyric acid. Its position on the chromatogram was especially favorable for separation from most of the other detectable constituents by one-dimensional chromatography with phenol, thus making possible a more adequate comparison of its properties with those of the aforementioned substances.

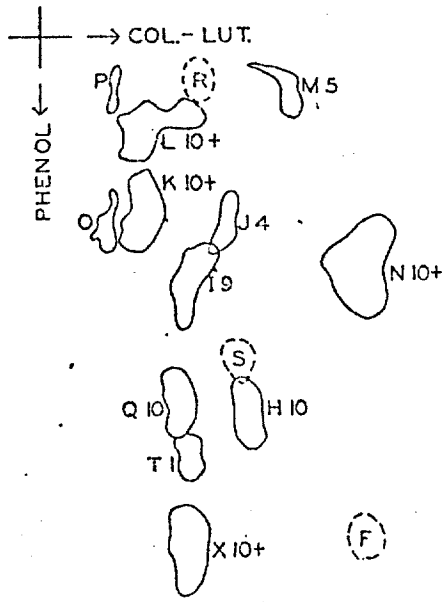


FIG. 1

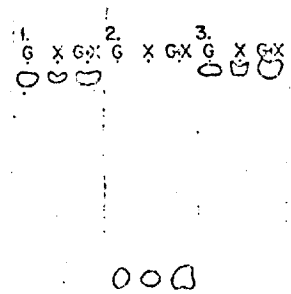


FIG. 2

FIG. 1. Tracing of a chromatogram of a peroxide-treated aliquot of brain extract corresponding to 75 mg. of fresh tissue. The letters identify the spots and the numbers represent visual estimates of relative intensities of color, a rating of 1 being taken as the color given by 1 γ of amino acid. The spots delineated by a broken line represent traces. *M*, cystine (cysteic acid); *L*, aspartic acid; *K*, glutamic acid; *J*, serine; *I*, glycine; *N*, taurine; *Q*, glutamine; *H*, alanine; *T*, β-alanine; *S*, threonine; *F*, valine; *X*, unknown substance; *R*, glutathione; *P* and *O*, unidentified materials which disappear on acid hydrolysis.

FIG. 2. Chromatography of unknown (*X*) and γ-aminobutyric acid (*G*) in (*1*) collidine-lutidine, (*2*) phenol, (*3*) butanol.

Ten spots of 10 μl., each corresponding to 30 mg. of fresh tissue, were placed along the narrow edge of each of four sheets of Whatman No. 1 filter paper and run in water-saturated phenol for 24 hours. The two end strips of each sheet were cut out and sprayed with a 0.1 per cent solution of ninhydrin in butanol. Six bands appeared in all instances. The unknown material was located in the band furthest from the starting point. The most likely contaminant would be the small quantity of valine shown in Fig. 1. With the two end strips as reference guides, the strips of paper

containing the unknown material were cut out of the four chromatograms. All ninhydrin-reactive material was removed from the strips by allowing water to flow down them into beakers. No color was given when these strips were sprayed subsequently with ninhydrin after drying. The eluate was evaporated to dryness and taken up in 2 ml. of distilled water. Subsequent chromatography of aliquots of the eluate before and after hydrolysis with 6 N HCl showed the presence of a large quantity of the unknown substance, which was stable to hydrolysis, and traces of valine and a peptide material.

Identification of Unknown Compound As γ-Aminobutyric Acid—Microbiological assay for histidine[1] and a comparison of the chemical and chromatographic properties with those of histidine and methionine sulfoxide showed that the unknown was neither of these compounds.

A known sample of γ-aminobutyric acid[2] (15 γ) and the unknown compound (52 μl.) were then chromatographed separately and in mixture in three different water-saturated solvent systems: *n*-butanol, phenol, and collidine-lutidine. The results are shown in Fig. 2. Although the R_F values (3) are widely different in the solvent systems employed, the main constituent of the unknown material and the γ-aminobutyric acid traveled at the same rate in each case and gave completely superimposable bands when mixed. It was therefore concluded that the unknown compound is γ-aminobutyric acid. This conclusion was confirmed by application of the isotope derivative method to the eluted material (1).

Quantitative Determination of γ-Aminobutyric Acid—Advantage was taken of the fact that this amino acid could be separated from all but traces of two of the ninhydrin-reactive materials found in brain by one-dimensional chromatography in phenol (see the previous discussion). By using smaller concentrations of brain extract the quantities of the interfering substances were reduced below a level at which positive reactions with ninhydrin were given. Under these conditions the color given by the γ-aminobutyric acid could be used for quantitative determination.

Spots corresponding to known amounts of γ-aminobutyric acid and accurately measured amounts of unknown samples were placed along the narrow edge of sheets of Whatman No. 1 filter paper and run in water-saturated phenol for 24 hours. After thorough removal of the phenol by directing a fan at the sheets for 18 to 24 hours, the sheets were sprayed on both sides with a 0.1 per cent solution of ninhydrin in butanol. Maximal color development took place in 24 to 36 hours at room temperature or in 30 minutes at 93°. The developed spots were cut out, care being taken

[1] The microbiological determinations were kindly performed by Dr. G. B. Ramasarma. The organism *Leuconostoc mesenteroides* P-60 was employed in the assay.

[2] γ-Aminobutyric acid was purchased from the Department of Chemistry, University of Illinois. It was recrystallized from alcohol.

to include the same total area of paper in all of the known and unknown samples in any set of determinations. Suitably chosen paper blanks were always included. The pieces of paper containing the spots were cut into small strips with minimal handling and were put into test-tubes. 5 ml. of water distilled in glass were then added and the tubes were shaken vigorously for a minute or two. Quantitative elutions of the color were achieved by this method. The paper fiber was then centrifuged and the color was read at 570 mμ in the Beckman spectrophotometer. The optical density was proportional to the concentration in the range studied (1 to 15 γ). The color was stable for at least 4 hours at room temperature when the tubes were kept out of strong light. Although the standard curves obtained at different times were in good agreement, a series of standards was run with each set of determinations in order to eliminate the influence of possible differences in paper and solvent on the development of the color. A similar procedure was employed in which the unknown and standards were run on two-dimensional chromatograms in phenol and collidine-lutidine. Good checks were obtained when brain extracts were analyzed by both the one-dimensional and two-dimensional methods. Three experimental samples of brain homogenates gave total values of 149, 202, and 278 γ of γ-aminobutyric acid by the one-dimensional procedure, while values of 156, 200, and 295 γ, respectively, were obtained for the same samples by two-dimensional chromatography. This indicates the adequacy of the one-dimensional method under the conditions of the experiment. Recoveries of γ-aminobutyric acid which had been added to brain homogenates ranged from 92 to 100 per cent. The presence of glutamic acid and aspartic acid in concentrations 200 times as great as those of the γ-aminobutyric acid did not affect the recovery.

The above methods have been applied successfully to the determination of several other amino acids in pure solutions and in protein hydrolysates.

γ-Aminobutyric Acid Present in Brain Chiefly in Free Form—The average value of a number of determinations of γ-aminobutyric acid in alcoholic extracts of mouse brain was 55 mg. per 100 gm. of fresh weight of tissue. Closely similar values were found in the supernatant solutions after heat coagulation of brain homogenates, in trichloroacetic acid extracts, and in acid hydrolysates of whole brain. It was also found that hydrolysis with 6 N HCl did not increase the content of γ-aminobutyric acid in the protein-free extracts. These results show that the γ-aminobutyric acid is present in brain chiefly in the free form.

Influence of Glutamic Acid on Content of γ-Aminobutyric Acid during Incubation of Brain Homogenates, Washed Residues, or Acetone Powders—Homogenates of freshly excised mouse brain containing 250 mg. of tissue per ml. were prepared in ice-cold 0.05 M phosphate buffer, pH 7.42. In

several experiments the homogenates were used directly. In others the homogenates were centrifuged in the cold at 3500 r.p.m., the supernatants discarded, and the residues washed with varying volumes of phosphate buffer one or more times. The residues were then suspended in a volume of buffer such that the final volume of the suspension was the same as that of the original homogenate. The acetone powders were prepared by suspending fresh brain in 100 to 500 volumes of acetone at −15° in a Waring blendor, filtering rapidly with suction, resuspending in the same volume of fresh acetone, filtering, and finally drying *in vacuo* over P_2O_5 and paraffin at 4°. Suitable amounts of the powders were then suspended in phosphate buffer with the aid of a ground glass homogenizer prior to use.

Immediately after preparation of the suspensions, 2 ml. aliquots were pipetted into tubes containing 8.45 ml. of 95 per cent alcohol and 0.67 ml. of distilled water to serve as blanks. 2 ml. portions of the preparations were also added to 0.67 ml. of water or to 0.67 ml. of a solution containing a suitable concentration of glutamic acid, previously adjusted to pH 7.4. These latter mixtures were then incubated for 2 hours at 38° in an atmosphere of O_2 in a Dubnoff incubator. The reactions were stopped by the addition of 8.45 ml. of 95 per cent alcohol and the mixtures stirred thoroughly and centrifuged. The clear supernatants were poured off and aliquots were used for analysis. Whenever aliquots greater than 0.3 ml. were used, the two-dimensional chromatographic procedure was employed.

The data for representative experiments are summarized in Table I. In all of the preparations employed, the increase of γ-aminobutyric acid was accelerated by the presence of added glutamic acid. Placing the preparations in boiling water for 1 minute destroyed the activity. In several instances, there were increases in the content of γ-aminobutyric acid in the absence of added glutamic acid. This occurred to the largest extent in the crude homogenates. Even in the most extensively washed preparations there were still easily detectable amounts of those amino acids which were present in the fresh tissue in the free form to the largest extent. From this it would be expected that constituents of brain, other than those which react with ninhydrin, would also adhere to the washed residues or acetone powders. For this reason it was not possible to conclude from these experiments that glutamic acid was a precursor of γ-aminobutyric acid. The possibility was not ruled out that glutamic acid was indirectly stimulating the formation of γ-aminobutyric acid from some other substance. The following experiments were undertaken to determine whether or not the carbon of glutamic acid would be found in γ-aminobutyric acid after incubation with a preparation which was actively forming the latter compound.

Formation of γ-Aminobutyric Acid from Glutamic Acid by Brain Acetone

Powder—Glutamic acid which was uniformly labeled with C^{14} was kindly furnished to us by Dr. Konrad Bloch. This amino acid had been isolated from a hydrolysate of algae grown in $C^{14}O_2$. The glutamic acid had a specific activity of 27,900 c.p.m. per mg. when counted as the amino acid with a windowless proportional counter connected to a scaling unit. The samples were deposited from dilute aqueous solution on cups 10.7 sq. cm. in area. A sample containing 1 mg. of the labeled amino acid to which

TABLE I

Influence of Glutamic Acid on Formation of γ-Aminobutyric Acid in Brain Preparations

Incubation conditions described in the text. In the case of the homogenates and washed residues an amount of tissue equivalent to 500 mg. of original fresh weight of tissue was employed. 50 mg. of acetone powder were used.

Type of preparation	Experiment No.	Final molarity of added glutamic acid	Change in content of γ-aminobutyric acid per tube
			γ
Homogenate	1	0	49
		0.1250	111
	2	0	50
		0.1250	100
Washed residue	1	0	−5
		0.0008	4
		0.0039	35
		0.0078	47
	2	0	22
		0.0078	69
		0.0313	85
		0.1250	179
		0.1250*	3
Acetone powder	1	0	8
		0.1250	369
		0.1250*	8
	2	0.0367	40
	3	0.1250	144

* Heat-inactivated.

5 γ of γ-aminobutyric acid had been added was subjected to two-dimensional chromatography. At the same time a chromatogram was run containing 5 γ of γ-aminobutyric acid, and one containing a series of samples of known concentrations was run to serve as a standard curve. There were no ninhydrin-reactive constituents other than glutamic and γ-aminobutyric acids on the paper containing the radioactive glutamic acid. On elution and estimation of the quantity of γ-aminobutyric acid, 5 γ were

recovered. The eluate contained no radioactivity whatsoever. This shows that the sample of glutamic acid employed contained no γ-aminobutyric acid or other ninhydrin-reactive impurities, that no γ-aminobutyric acid was formed from the glutamic acid during the chromatographic procedures, and that no radioactive contaminants were present which migrated to the same position as the γ-aminobutyric acid on the chromatogram. The recovery from the paper containing γ-aminobutyric acid alone was 4.7 γ, a value within the range of experimental error.

An experiment was then performed in which 40 mg. of fresh acetone powder from mouse brain were incubated with 146,000 counts of glutamic acid (5.25 mg.) in a final volume of 1 ml. under the conditions previously described; 3.16 ml. of 95 per cent alcohol were then added. Samples of

TABLE II

Radioactivity Found in Eluates of Ninhydrin-Positive Areas from Two-Dimensional Chromatograms after Incubation of C^{14}-Labeled Glutamic Acid with Acetone Powder of Mouse Brain

146,000 counts of glutamic acid were added originally to the incubation mixture.

Amino acid	Total counts in sample
	c.p.m.
Glutamic acid	127,800
γ-Aminobutyric acid	354
Glutamine	20
Glycine	0
Leucines	0
Valine	0
Alanine	0
Taurine	0

the clear supernatant (0.75 ml.) were subjected to two-dimensional chromatography. It was found that the total content of γ-aminobutyric acid after incubation exceeded by 40 γ that found in the zero time control.

The spots corresponding to glutamic acid and to all of the detectable constituents which were clearly separated from the large glutamic acid spot were eluted with suitable volumes of water, plated, and counted. In this manner it was possible to obtain results for glutamic acid, γ-aminobutyric acid, glutamine, glycine, the leucines, valine, alanine, and taurine. The counts are shown in Table II.

Of the constituents other than glutamic acid, by far the largest activity was found in the γ-aminobutyric acid. Glutamine was the only other constituent having a significant number of counts. The activity recovered in the glutamic acid spot (127,800 c.p.m.) was somewhat higher than would

have been expected if 1 mole of CO_2 had been liberated per mole of glutamic acid present (117,000 c.p.m.). The reaction with ninhydrin probably does not go to completion on paper when such large quantities of amino acid are present.

The above experiment shows conclusively that the carbon chain of glutamic acid can be converted to that of γ-aminobutyric acid. It is not possible at the present time to deal with the stoichiometry of the reaction because of the relatively low specific activity of the glutamic acid and the weak activity of the enzyme preparation employed. It appears highly probable, however, that the formation of γ-aminobutyric acid from glutamic acid in brain takes place by α decarboxylation.

Toxicity of γ-Aminobutyric Acid—Two mice, weighing approximately 20 gm., were given a single intraperitoneal injection of 100 mg. of γ-aminobutyric acid in 0.5 ml. of water, and two other mice were given 0.25 ml. of the same solution. There were no signs of toxicity and the mice appeared to be completely normal thereafter. This indicates that the acute toxicity of γ-aminobutyric acid is low.

DISCUSSION

An extensive survey of the free amino acids in many normal and malignant mouse tissues, a part of which has been reported (6, 7), revealed the presence of considerable quantities of a ninhydrin-reactive substance in extracts of brain which was present, at most, in traces in the other tissues examined. This substance was also detected in the brains of other species. The experiments reported in this paper and in the following one (1) established with certainty that this compound is γ-aminobutyric acid. Experiments with homogenates, washed residues, and acetone powders of mouse brain invariably showed increases in the content of γ-aminobutyric acid upon incubation with glutamic acid. With isotopically labeled glutamic acid, it was found that glutamic acid could serve as a precursor of γ-aminobutyric acid. Recent unpublished experiments in our laboratory with homogenates and sterile autolysates of liver, muscle, and tumors have shown increases of a substance which corresponds closely in its properties to γ-aminobutyric acid after incubation for varying periods of time. It may thus be that the formation of γ-aminobutyric is of wide occurrence in mammalian tissues. The uniquely high concentrations of this substance in brain may possibly be a result of a greater rate of formation than in other tissues, a slower rate of removal, or a combination of both. A study is being made of the metabolism of γ-aminobutyric acid.

γ-Aminobutyric acid has recently been reported to be present in bacteria (8), plants (9), human blood and urine (5), adult ox and embryo calf muscle (10), and yeast (11). In bacteria and plants, this compound can

arise as a result of the action of glutamic acid decarboxylase (12, 13). The presence of this enzyme has not yet been demonstrated in any of the other sources in which γ-aminobutyric acid has been reported. The experiments reported in this paper indicate that such an enzyme may be present in brain tissue.

SUMMARY

1. γ-Aminobutyric acid has been shown to be a constituent of brain in which it exists preponderantly in the free form.

2. Experiments with various crude enzyme preparations from mouse brain showed a net increase of γ-aminobutyric acid, which was accelerated by the addition of glutamic acid.

3. It was shown by the use of isotopically labeled glutamic acid that the latter amino acid can serve as a precursor for the γ-aminobutyric acid.

4. It is suggested that the formation of this compound in brain takes place by the α decarboxylation of glutamic acid.

BIBLIOGRAPHY

1. Udenfriend, S., *J. Biol. Chem.*, **187**, 65 (1950).
2. Roberts, E., and Frankel, S., *Federation Proc.*, **9**, 219 (1950).
3. Consden, R., Gordon, A. H., and Martin, A. J. P., *Biochem. J.*, **38**, 224 (1944).
4. Dent, C. E., *Biochem. J.*, **41**, 240 (1947).
5. Dent, C. E., *Biochem. J.*, **43**, 169 (1948).
6. Roberts, E., and Frankel, S., *Cancer Res.*, **9**, 645 (1949).
7. Roberts, E., Frankel, S., and Harman, P. J., *Proc. Soc. Exp. Biol. and Med.*, **74**, 383 (1950).
8. Work, E., *Biochim. et biophys. acta*, **3**, 400 (1949).
9. Steward, F. C., Thompson, J. F., and Dent, C. E., *Science*, **110**, 439 (1949).
10. Gordon, A. H., *Biochem. J.*, **100**, 99 (1949).
11. Reed, L. J., *J. Biol. Chem.*, **183**, 451 (1949).
12. Gale, E. F., *Advances in Enzymol.*, **6**, 1 (1946).
13. Schales, O., Mims, V., and Schales, S. S., *Arch. Biochem.*, **10**, 455 (1946).

GLUTAMIC ACID DECARBOXYLASE IN BRAIN*

By EUGENE ROBERTS and SAM FRANKEL

(From the Division of Cancer Research, Washington University School of Medicine, St. Louis, Missouri)

(Received for publication, October 9, 1950)

We have previously reported the presence of relatively large amounts of free γ-aminobutyric acid in brain (1, 2) and have shown the existence of an enzyme which can form this compound from glutamic acid (2). In the present paper are reported some manometric studies of the properties of the glutamic acid decarboxylase of mouse brain.

EXPERIMENTAL

Preparation of Tissues—Mice were killed by dislocation of the cervical vertebrae, and the brains were removed rapidly. The homogenates were prepared in ice-cold 0.05 M phosphate buffer, pH 5.9, with a ground glass homogenizer. The acetone powders were prepared by suspending fresh brain in a large excess of acetone at −15° in a Waring blendor and filtering rapidly with suction. All operations were performed in the cold room at 4° with glassware previously stored in a deep freeze. The powders were dried and kept *in vacuo* over silica gel and paraffin at 4°. Suitable amounts of the powders were suspended in buffer with the aid of a homogenizer prior to use.

Manometric Assay—All assays were carried out in Warburg flasks in an N_2 atmosphere with a final volume of 3.0 ml. at 38°. The pH of the incubation mixtures was between 6.2 and 6.3. The main compartments contained quantities of the enzyme preparations and buffer to make a final volume of 2.0 ml. One side arm contained 0.5 ml. of glutamic acid solution (pH 7.0 to 7.4) or distilled water. This was tipped in after equilibration. The other side arm contained 0.5 ml. of 1.2 N H_2SO_4. This was added to stop the reaction and to liberate bound CO_2. Suitable corrections for bound CO_2 were made.

Control flasks containing KOH papers were included in a number of experiments to determine whether the changes in gas pressure observed were attributable entirely to CO_2 formation. In no case was there a significant increase in pressure in the presence of KOH. It seems certain, therefore, that CO_2 was the only gas liberated. However, it was noted that both in the flasks containing homogenates and those with acetone powders there

* Aided by grants from the United States Public Health Service and the Charles F. Kettering Foundation.

was a significant gas consumption (probably O_2) when ordinary tank nitrogen was used. This difficulty was completely eliminated by flushing the apparatus for 15 minutes with specially purified N_2 (99.9 per cent, Linde).

The final concentrations of the added glutamic acid in the incubation mixtures were either 0.05 or 0.1 M. Under the conditions used, maximal activity was achieved with 0.05 M glutamic acid, the rates at this concen-

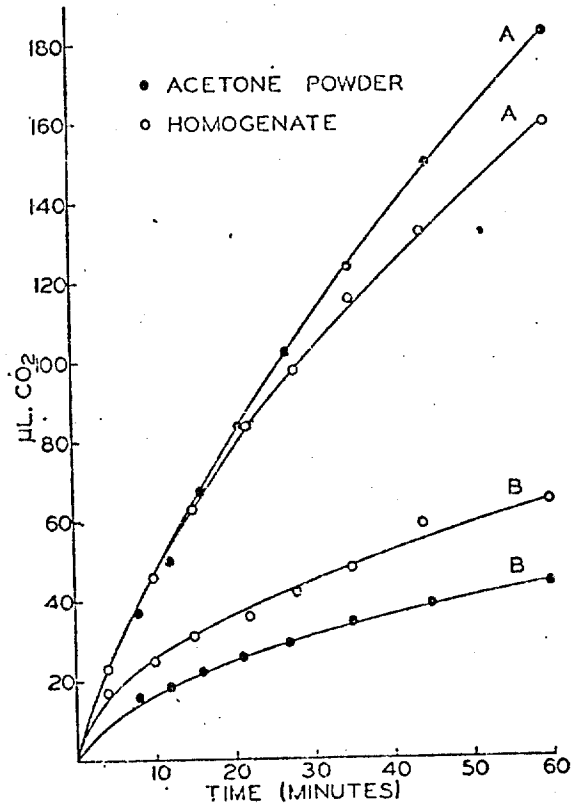


FIG. 1. Typical curves obtained with acetone powders and homogenates in presence (Curves A) and absence (Curves B) of added glutamic acid.

tration being identical with those found at the higher one. When the effects of hydroxylamine and semicarbazide were observed, these substances were added to the substrate; so that both substrate and inhibitor were added to the enzyme at the same time. In those experiments in which pyridoxal phosphate was studied, it was added directly to the main compartment prior to the addition of the tissue preparations.

Chromatographic Determination of γ-Aminobutyric Acid—γ-Aminobutyric acid was determined by the chromatographic method previously described (1).

Results

Rate of CO_2 Formation by Homogenates and Acetone Powders—In Fig. 1 are shown typical time-activity curves for acetone powders and homogenates of mouse brain in the presence and absence of added glutamic acid. The activity of 100 mg. of acetone powder was compared with that of 2 ml. of a homogenate containing 500 mg. of fresh weight of brain.

TABLE I

Equivalence of Formation of γ-Aminobutyric Acid and CO_2 by Brain Acetone Powder

Incubation time, 115 minutes. 52 mg. of acetone powder per flask. 0.5 ml. of 0.5 M glutamic acid added from side arm.

Flask No.	γ-Aminobutyric acid formed	CO_2 formed
	μM	μM
46*	2.3	2.3
18*	2.3	2.4
17*	2.3	2.3
62	6.8	5.3
29	6.0	5.3
38	5.2	5.3

*No added glutamate.

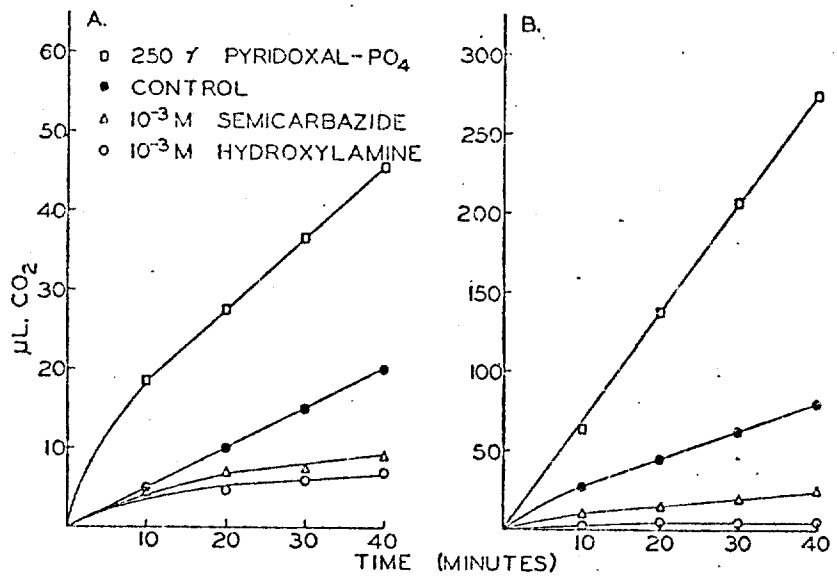


FIG. 2. Influence of pyridoxal phosphate, semicarbazide, and hydroxylamine on activity of acetone powder in absence (*A*) and presence (*B*) of added glutamic acid. 120 mg. of acetone powder per flask; 0.5 ml. of 0.25 M glutamate added.

This was done because the final dry weight of acetone powder is approximately 20 per cent of that of the starting material. In the presence of excess glutamic acid, the activities of both preparations were virtually identical for 20 minutes. Thereafter, the activity of the homogenate seemed to decline at a more rapid rate. In the absence of added glutamic acid, the CO_2 production of the homogenate was greater than that of the

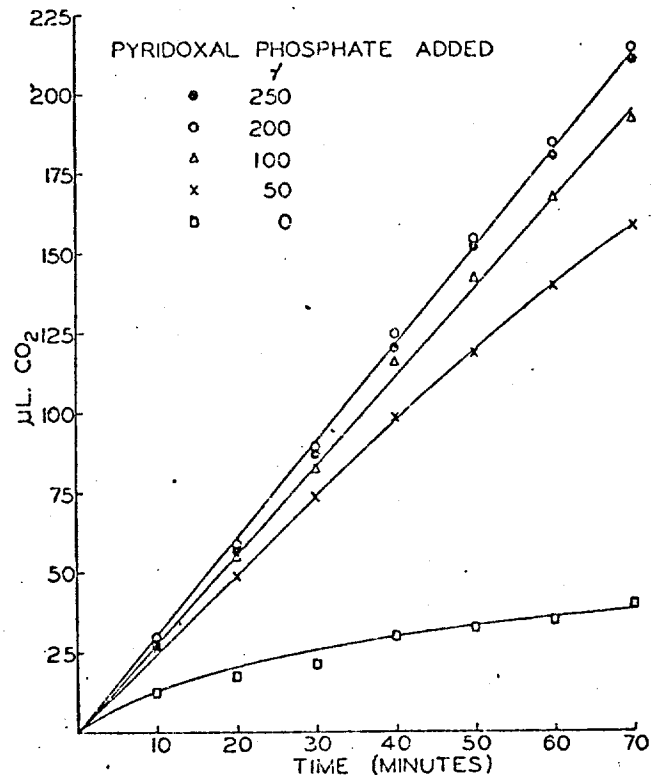


FIG. 3. Effects of different amounts of pyridoxal phosphate on the activity of acetone powder. 50 mg. of acetone powder per flask; 0.5 ml. of 0.5 M glutamate added to each flask.

acetone powder. Brain tissue contains large quantities of free glutamic acid and glutamine, from which glutamic acid is formed readily. Two-dimensional chromatograms of alcoholic extracts of the acetone powders showed the presence of appreciable amounts of free or loosely bound glutamic acid and glutamine. Presumably the CO_2 liberated in the absence of added glutamic acid could be formed from the glutamic acid already present in the preparations. Experiments to be cited on the stoichiometry

of the endogenous formation of CO_2 and the influence on CO_2 formation of inhibitors and pyridoxal phosphate support this view.

Formation of CO_2 and γ-Aminobutyric Acid by Brain Acetone Powder—An experiment was performed to determine whether the formation of CO_2 and γ-aminobutyric acid takes place in stoichiometric amounts under the conditions employed. The results are shown in Table I. It may be said

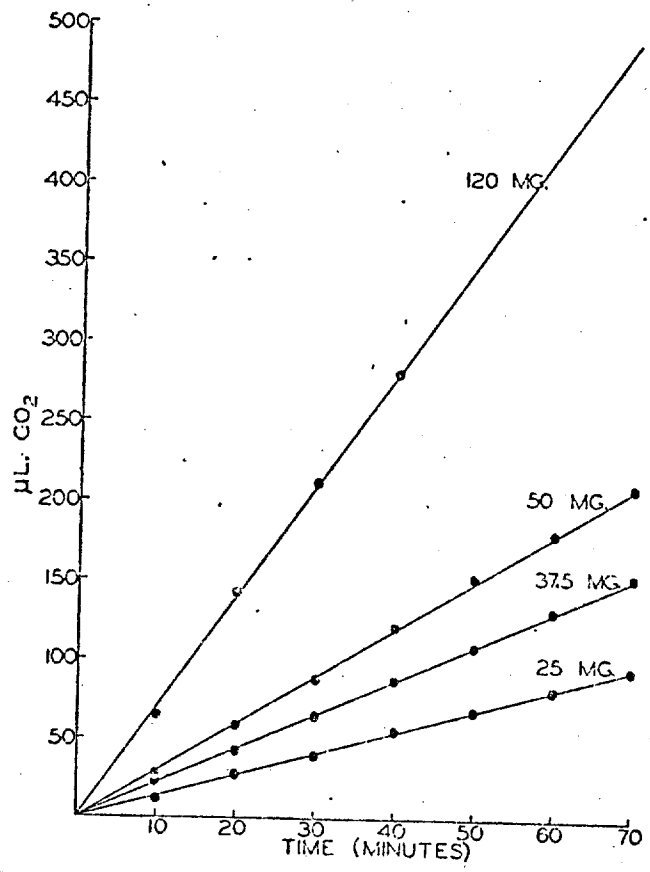


FIG. 4. Demonstration of zero order reaction with different quantities of acetone powder. All flasks contained 250 γ of pyridoxal phosphate.

that, within experimental error, there is a mole for mole formation of CO_2 and of γ-aminobutyric acid. The fact that this relationship holds even in the absence of added glutamic acid shows that the glutamic acid present in the acetone powder preparation is attacked readily. This experiment also shows that at most only very small amounts of CO_2 arise from substances other than glutamic acid.

Influence of Pyridoxal Phosphate, Hydroxylamine, and Semicarbazide on Decarboxylase Activity of Acetone Powder—Since decarboxylases are known to be sensitive to carbonyl-trapping agents and all of the decarboxylases that have been studied extensively have been shown to require pyridoxal phosphate as coenzyme (3, 4), it was of interest to study the effects of these substances in our system. It is seen from Fig. 2 that semicarbazide and hydroxylamine inhibit the decarboxylation of glutamic acid by the acetone powder in the absence (Fig. 2, *A*) and presence (Fig. 2, *B*) of

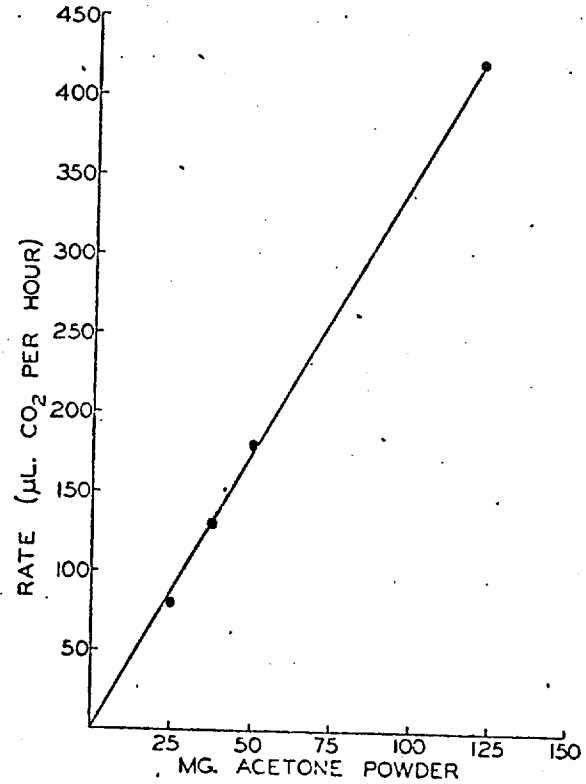


FIG. 5. Proportionality between the rate of reaction and enzyme concentration

added glutamic acid. Pyridoxal phosphate is a potent activator of the enzyme. It appears, therefore, that the glutamic acid decarboxylase of brain is similar to the glutamic decarboxylases of bacteria and plants.

Effect of Varying Concentrations of Pyridoxal Phosphate on Activity of Acetone Powder—Fig. 3 shows that increasing concentrations of added pyridoxal phosphate produced progressive increases in the activity of the enzyme. In the system employed, 200 γ produced complete activation, no further activation being noted when 250 γ were added. There

was only a small increase in activity when the quantity of pyridoxal phosphate was increased from 100 to 200 γ.

Assay for Decarboxylase Activity—In the presence of excess glutamic acid and pyridoxal phosphate, it was possible to obtain zero order reactions with different quantities of acetone powder (Fig. 4). The activities thus obtained were proportional to the quantity of enzyme employed (Fig. 5). With this method for assay, it will be possible to perform further studies of the properties of this enzyme, to follow its purification, and to determine the relative quantities of the apoenzyme in different parts of the nervous system. Preliminary chromatographic evidence has indicated that liver, muscle, and tumors can form γ-aminobutyric acid from glutamic acid. It is planned to extend the manometric studies to these tissues also.

DISCUSSION

Glutamic acid decarboxylase is now known to be present in plants, bacteria, and animals (2–4). γ-Aminobutyric acid, the end-product of glutamic acid decarboxylation, is also widely distributed (5–9). Obviously the rôle of the decarboxylase is to produce γ-aminobutyric acid. The key to the importance of the system probably lies in the knowledge of the transformations which the γ-aminobutyric acid undergoes. Experiments are now in progress in our laboratory dealing with this subject.

SUMMARY

1. The presence of glutamic acid decarboxylase in mouse brain is reported.

2. The activity was demonstrated in homogenates and acetone powders.

3. The enzyme behaved like a typical decarboxylase, requiring pyridoxal phosphate as coenzyme and being inhibited by semicarbazide and hydroxylamine.

4. Conditions for assay are described in which the reaction is of zero order and the activity is proportional to the quantity of enzyme employed.

BIBLIOGRAPHY

1. Roberts, E., Frankel, S., and Harman, P. J., *Proc. Soc. Exp. Biol. and Med.*, **74**, 383 (1950).
2. Roberts, E., and Frankel, S., *J. Biol. Chem.*, **187**, 55 (1950).
3. Gale, E. F., *Advances in Enzymol.*, **6**, 1 (1946).
4. Gunsalus, I. C., *Federation Proc.*, **9**, 556 (1950).
5. Work, E., *Biochim. et biophys. acta*, **3**, 400 (1949).
6. Steward, F. C., Thompson, J. F., and Dent, C. E., *Science*, **110**, 439 (1949).
7. Dent, C. E., *Biochem. J.*, **43**, 169 (1948).
8. Gordon, A. H., *Biochem. J.*, **45**, 99 (1949).
9. Reed, L. J., *J. Biol. Chem.*, **183**, 451 (1950).

[Reprinted from FOOD TECHNOLOGY, 1956, Vol. X, No. 7, Pages 299-302]

Incorporation and Distribution of Monosodium Glutamate in Chicken Meat

Charles J. Rogers, Paul A. Mills,[a] and George F. Stewart
University of California, Davis

(Manuscript received February 20, 1956)

THE DISTRIBUTION of monosodium glutamate (MSG) obtained by several methods of incorporation are reported in this paper. Previously, scant data *(7)* have been available to poultry meat processors who wish to incorporate MSG into eviscerated poultry. The determination of optimum quantities of MSG for flavor enhancement also has been studied. The results of these studies will be reported later.

Three methods—liquid injection, a dip procedure, and a dusting procedure—were considered for incorporation of MSG into chicken meat. The "Hypospray" unit injects graded amounts, of up to one ml. (interstitially in the form of a high velocity jet), which penetrates the skin through a very small hole. Bauer *et al.* *(1)* reported that rapid distribution of liquids is obtained using this method in human subjects as compared to using a hypodermic needle.

In the dip procedure, fryer carcasses were cut up and dipped for varying lengths of time in an MSG solution. For the dusting method, weighed quantities of finely powdered MSG were sprinkled evenly over specific areas inside the cavity of freshly drawn birds.

The initial analyses for MSG were done by the microbiological method of Hac *et al.* *(5)*. To facilitate conducting large numbers of analyses, a specific decarboxylation method, based on a report by Roberts *(3)*, was adapted.

MSG distribution in various tissues was also determined by tracer techniques, utilizing DL-glutamic acid-2-C^{14}.

All chickens used in these experiments were New Hampshire or Indian River fryers, 10 weeks old, and 2½ to 2¾ pounds in weight. All birds were slaughtered by the Kosher method, scalded 0.8 minutes at 140° F., and then plucked on an automatic picker.

ANALYTICAL METHODS

Decarboxylation technique. Ten grams of muscle was disintegrated in a Waring blender with approximately 35 ml. 0.1N acetate buffer, pH 4.5, and made up to 50 ml. with the buffer. The mixture was centrifuged and the liquor filtered through glass wool. Two ml. of the extract was placed in a Warburg flask which contained a previously prepared suspension of 3 mg. of L-glutamic acid decarboxylase in 0.5 ml. of the acetate buffer in the side arm. The flask was attached to a manometer and placed in the Warburg apparatus at 28° C. After temperature equilibration, a reading was recorded, the decarboxylase mixed with the sample, and the reaction allowed to proceed with readings being taken after 30 and 40 minutes reaction times. Trial runs were made with different quantities of decarboxylase and that quantity producing maximum CO_2 in 30 minutes from 2 mg. MSG was used. Three mg. of the enzyme produced 96-102% recoveries. The addition of cocarboxylase was not necessary.

Isotopic technique. To determine the actual distribution of MSG obtained by the "Hypospray" injection method, one ml. of 35% MSG containing 0.8 μc. of DL-glutamic acid-2-C^{14} was injected into the fleshy part of the chicken. The chicken was then quick-frozen immediately or after aging (to allow for distribution), cut cross-wise against the tissue fibers into slices approximately 0.7 cm. thick, and lyophilized. Each slice was then placed on a grid, and subjected to count analysis using a Geiger unit. This procedure enabled counts per minute per 0.40 square centimeter of area to be obtained, thus permitting construction of "isoactivity" diagrams with data expressed as counts per minute per square centimeter (corrected for background count).

Similar treatment was accorded whole chicken breasts and legs dipped into 35% MSG solution containing 0.8 μc. of DL-glutamic acid-2-C^{14} per ml.

Radioautographs were prepared to show the distribution obtained by the dusting technique. The cavity areas adjacent to the thigh were dusted with 2 g. and the breast area with 4 g. of MSG containing 1.67 μc. of DL-glutamic acid-2-C^{14} per g. After storage and freezing, followed by cutting the breast and leg perpendicularly to the treated surface and lyophilizing, the slices were placed in contact with high speed film for 7 days and then developed.

EXPERIMENTAL DATA

"Hypospray" injection. It was considered that the best distribution with the "Hypospray" method would be obtained by injecting the live bird, whereby normal fluid movement in the body would aid in transferring the MSG throughout the flesh. However, experiments with "Hypospray" inoculation with 1 ml. of 35% MSG solution showed that this is not feasible. Numerous small blood vessels were ruptured, leaving a large "black and blue" area at the site of injection, thus giving the dressed chicken a very unsightly appearance.

Other experiments were made on eviscerated birds. Since diffusion of MSG would conceivably be more rapid in a warm than a cold bird, six fryers were injected immediately after bleeding, then dressed and eviscerated. The fryers were then cut up, broiled and subjected to organoleptic examination. The sites of injections gave the sensation of high glutamate concentration, and were considered quite unpalatable. Samples from which the sites of injection were cut out, could not be differentiated from the controls. These results suggested that little or no diffusion of MSG occurred, even after cooking.

In another trial, 1 ml. portions of 35% MSG solution were injected into each breast, leg, and thigh of a young frying chicken, immediately after being slaughtered, dressed, and eviscerated. It was then stored for one week at 40° F. to allow for further diffusion of the MSG. A control bird was similarly treated (except for MSG injection). Microbiological analyses revealed that even in this case, the glutamate remained largely at the site of injection; analyses showed 0.22% MSG for the injected muscle versus 0.08% for muscles from control birds as well as for muscles adjacent to injected areas.

In another experiment two drumsticks and two breasts from freshly dressed and eviscerated chickens were injected with MSG containing DL-glutamic acid-2-C^{14}. One each of the drumsticks and breasts were immediately quick-frozen; the other pieces were stored for one week at 35° F. before freezing. These samples were then treated as given under the isotopic technique; the resulting "isoactivity" diagrams are shown in Figures 1, 2 and 3. Due to experimental difficulties, reliable data on the stored drumstick samples were not obtained and these have been omitted from the figures.

Dip procedure. Early studies made on dipping cut-up, freshly dressed, warm fryers, in a 35% MSG solution at 50° F. for 10 minutes showed a significant increase in glutamate absorption. Data obtained by microbiological analyses of the total muscle tissue are presented as part of Table 1. Pre-chilled chickens were used in subsequent dipping experiments since this seemed to be most feasible for possible commercial application. Chilled, cut-up fryers were taken from the packaging line in a commer-

[a] Present address, Food Division, Food and Drug Administration, Washington 25, D. C.

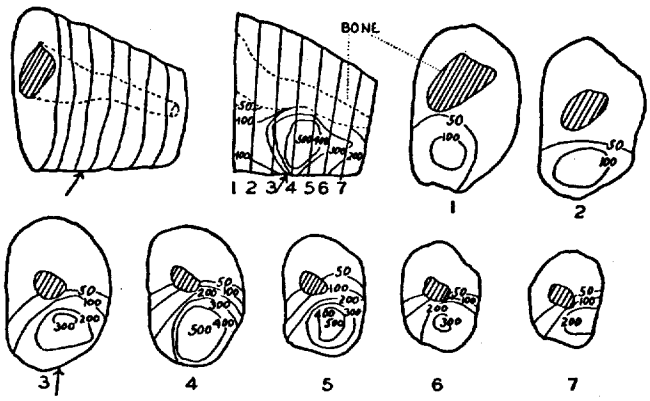


Figure 1. Distribution of MSG in chicken leg by "Hypospray" injection. (Analysis made immediately after injection.)
Illustration shows position of slices in injected leg.
Leg was injected with one ml. of 35% MSG solution containing 0.8 μc of dl-glutamic acid-2-C^{14}.
Data given in counts per min. per sq. cm. (0.4 times natural size).

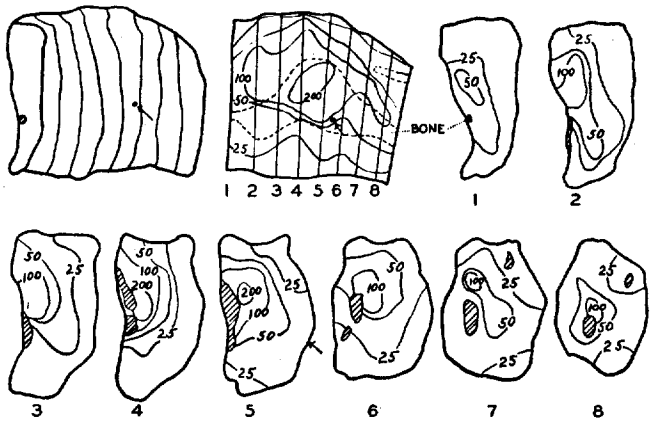


Figure 2. Distribution of MSG in chicken breast by "Hypospray" injection. (Analysis made immediately after injection.)
Injected with one ml. 35% MSG solution containing 0.8 μc of dl-glutamic acid-2-C^{14}.
Data given in counts per min. per sq. cm. (0.4 times natural size).

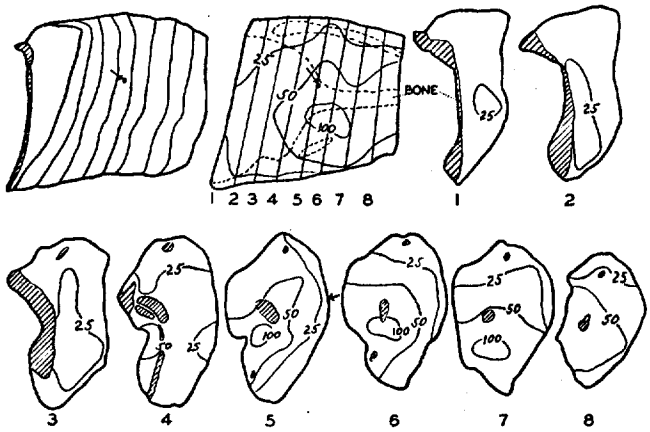


Figure 3. Effect of holding period on distribution of MSG after injection of chicken breast by "Hypospray." (Analysis made after holding sample one week at 35° F.)
Injected with one ml. 35% MSG solution containing 0.8 μc of dl-glutamic acid-2-C^{14}.
Data expressed as counts per min. per sq. cm. (0.4 times natural size).

cial plant and dipped for one minute in a 35% MSG solution at 65° F. These samples plus the controls were immediately frozen for subsequent treatment and analysis. The inner muscles

TABLE 1

Amount of MSG incorporated by dipping

Sample	Treatment[1]						
	A	B			C		
	Total edible portion (less skin)	Edible portion[2]			Edible portion[2]		
		Inner	Outer	Total	Inner	Outer	Total
	%	%	%	%	%	%	%
Breast	1.46	0.24	0.83	0.75	0.13	0.69	0.60
Thigh	2.29	0.26	0.75	0.63	0.13	0.42	0.32
Leg	0.95	0.04	0.37	0.23	0.10	0.25	0.19

[1] Treatments: Thigh and leg dipped as one piece.
A—Warm freshly dressed portions dipped in 35% MSG at 50° F. for 10 minutes.
B—Chilled freshly dressed portions dipped in 35% MSG at 65° F. for 1 minute.
C—Same as B, except samples cooked in deep fat before analysis.

[2] Edible portions	Approximate Percent of Total		
	Breast	Thigh	Leg
Inner	20	25	40
Outer	80	75	60

and outer muscle layers with the skin were analyzed separately by the decarboxylation method. Two of the chickens were cooked in deep fat at 375° F. until golden brown. The results are given in Table 1. In both the cooked and uncooked samples, the drumstick and thigh were dipped as one piece (i.e. they were not disjointed).

Radioactive glutamate was also used to determine the distribution of MSG using the dip procedure. In this case a freshly killed and plucked fryer was chilled to 35° F., then eviscerated and cut up into frying portions. Since the skin seemed to be an effective barrier to glutamate diffusion, the skin of the thigh was left on the drumstick and sealed so that none of the dipping solution came in direct contact with the cut end of the drumstick. Two drumsticks and two half breasts were dipped for 10 minutes in the C^{14} labeled glutamate solution at 35° F. One

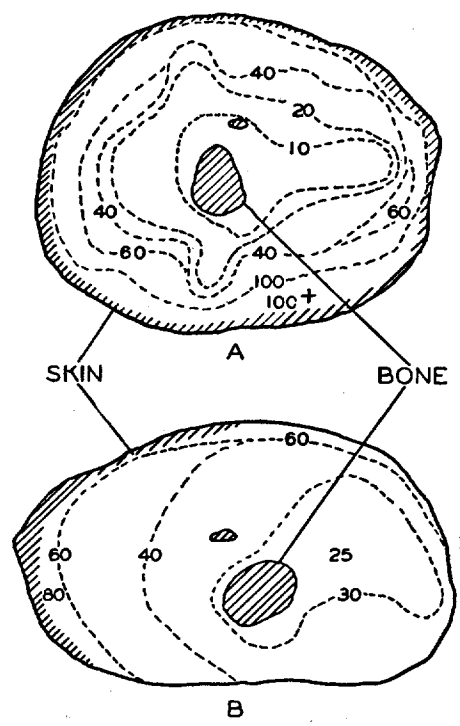


Figure 4. Distribution of MSG in chicken leg using dip procedure.
A. Cross-section from center of leg immediately after dipping.
B. Cross-section from center of leg stored one week at 35° F. after dipping.
Pieces dipped in 35% MSG solution containing 0.8 μc dl-glutamic acid-2-C^{14} per ml. Data expressed as counts per sq. cm. (Illustrations 0.7 times natural size.)

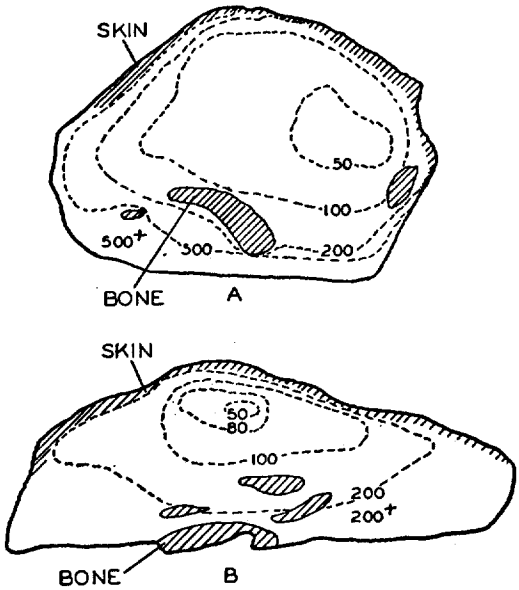


Figure 5. Distribution of MSG in chicken breast using dip procedure.

A. Cross-section from center of breast immediately after dipping.

B. Cross-section from center of breast stored one week at 35° F. after dipping.

Pieces dipped in 35% MSG solution containing 0.8 μc dl-glutamic acid-2-C^{14} per ml. Data expressed as counts per sq. cm. (Illustrations 0.7 times natural size.)

drumstick and half breast were then frozen immediately while the remainder was stored 7 days at 35° F. before freezing. The samples were then prepared and counted with results shown in Figures 4 and 5.

Dusting procedure. Most of the dusting experiments were conducted by sprinkling 2 g. of finely powdered MSG over each area of the cavity adjacent to the things and 4 g. on the breast cavity. [These quantities approximated addition of 0.75% MSG which was found to be the level for a significant effect on chicken flavor *(4, 8)*.]

All analyses were made by the decarboxylation method. The birds were hard frozen when sampled by use of an electric meat saw. The breast and thighs were divided in slices parallel to the dusted sections of the body cavity as shown in Figure 6. The thin layers adjacent to the cavity and carrying the unabsorbed MSG were discarded in order that only MSG actually diffused into the flesh was measured. Untreated chickens were included in all experiments as controls. The results are the averages of two birds with duplicate subsamples.

The effects of storage at 35° F. for various lengths of time, thawing, and various lengths of frozen storage on the diffusion of glutamate were studied. The type of treatment, storage conditions and results are tabulated in Table 2.

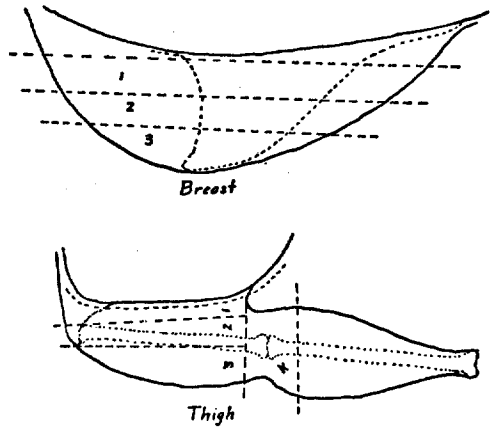


Figure 6. Location of analyzed samples in dusted chickens.

TABLE 2

Influence of sotrage conditions and thawing on distribution of MSG in dusted samples

Sample[3]		Storage conditions						Thawing conditions	
		Days						Hours	
		2	4	7	7[2]	30	104	24	36
		°F.						°F.	
		35°	35°	35°	35°	0°	0°	70°	45°
Breast	1	% 0.59	% 0.82	% 0.71	% 0.47	% 0.10	% 0.12	% 0.49	% 0.30
	2	0.28	0.56	0.67	0.44	0.09	0.02	0.29	0.19
	3	0.19	0.61	0.61	0.28	0.13	0.09	0.41	0.32
Thigh	1	0.97	0.58	1.03	0.50	0.16	0.30	0.97	0.89
	2	0.39	0.40	0.64	0.31	0.06	0.08	0.22	0.17
	3	0.24	0.22	0.40	0.18	0.07	0.07	0.32	0.17
Leg	4	0.17	0.11	0.15	0.11	0.04	0.10	0.20	0.09

[1] 4 g. MSG sprinkled on cavity area adjacent to breast.
2 g. MSG sprinkled on cavity area adjacent to thigh.
[2] ½ the above quantity of MSG used.
[3] Samples taken as shown in Figure 6.

Radioautographs were prepared from chickens dusted by the above technique with powdered MSG containing 3.33 μc. DL-glutamic acid-2-C^{14} per gram of MSG. One chicken was allowed to remain at 35° F. for 7 days, then frozen, sampled, lyophilized and its radioautograph obtained. Another was frozen immediately after dusting, held 7 days at 0° F., thawed 24 hours at 45° F. and refrozen for radioautograph preparation. The results obtained with the thigh are similar, though with slightly less diffusion, to those obtained with the breast, Figure 7.

RESULTS AND DISCUSSION

Although the "isoactivity" diagrams (Figures 1, 2, and 3) of "jet" injected leg and breast samples show some immediate and latent distribution of MSG, several factors must be investigated before this method can be considered for commercial application. Small panel tests on fried chicken *(2)*, chicken patties *(8)*, and canned chicken *(4)* have shown that from 0.2 to 0.75% MSG is required to produce a significant effect. If we consider 0.5% MSG as the minimum necessary concentration, 6.5 ml. of 35% MSG would be required per pound of chicken flesh. Since high concentrations in any particular location must be avoided (because of unpalatability), injections would have to be in small portions and at numerous sites. Proper design of injection equipment may prove this method to be feasible.

Our preliminary experiments on dipping warm, cut-up fryers in 35% MSG solution at 50° F. for 10 minutes, have shown considerable incorporation. Table 1. Further experiments using pre-chilled birds were carried out, since this would seem to be more practical in a processing plant. These experiments, in which pre-chilled cut-up fryers were dipped in 35% MSG at 65° F. for one minute showed (Table 1) an appreciable incorporation of MSG. Although the inner edible portions showed a low concentration, this pattern of concentration may be satisfactory for the effect desired. Unreported taste panel work in our laboratory and work in other laboratories *(6)* show that MSG has what may be considered a "lingering" taste effect. That is, once the mouth has been "sensitized" by glutamate, the effect can be carried over to samples that have no glutamate. Thus a relatively high concentration on the

surface would give a "lingering" effect which might last through consumption of the remainder of the pieces. Further studies on this aspect of MSG need to be carried out.

Comparison of "isoactivity" diagrams (Figures 4 and 5) of samples dipped in 35% MSG solution containing DL-glutamic acid-2-C^{14} show latent diffusion of MSG during storage for 7 days at 35° F. The skin is a considerable barrier to MSG penetration. This can be seen by the higher counts obtained on the breast samples compared to those on the leg and also by noting the lower activity adjacent to the skin layer on the breast samples.

The dipping procedure, due to dangers involved of a contaminated dipping solution and wastefulness of an expensive ingredient, is not recommended and was studied solely to demonstrate the amount of MSG absorbed by this method. On the other hand it is possible that a quantity of MSG solution might be sprayed into the cavity of each bird or over boxes of cut-up fryers before the over-wrap is applied. Although no data are available on this procedure, the information obtained in the dusting procedure may be applied.

Due to the low penetration of MSG through chicken skin, the dusting experiments were carried out by sprinkling definite quantities of MSG on specific areas inside the cavity of drawn birds. From Table 2, it can be seen that the amount of MSG absorbed increased with time on storage at 35° F. Very little absorption took place during frozen storage even when stored for 104 days at 0° F. Absorption of MSG during thawing of birds frozen immediately after dusting was fairly rapid. The applicability of freezing birds immediately after treatment would depend on whether the consumer would thaw the birds slowly and cook them without washing.

Considering the anatomy of the bird, it is only necessary to apply the powdered MSG to the breast and thigh areas of the carcass cavity. Whn the amount of MSG dusted in the various areas was reduced by half, the quantity absorbed into the tissues was proportionally reduced (Table 2).

Radioautograms (Figure 7) of breast samples, dusted with 4 g. of MSG containing 13.3 μc. DL-glutamic acid-2-C^{14}, demonstrate visually the greater distribution obtained in holding samples 7 days at 35° F. after dusting over the immediate freezing and 24 hours thawing at 45° F.

SUMMARY

Methods of incorporating MSG into eviscerated poultry have been considered and partially evaluated.

It is suggested from the results obtained that satisfactory incorporation may be obtained by improved multiple jet injection, by spraying the cavity of freshly drawn birds with a concentrated MSG solution, or by dusting the cavity with finely powdered MSG.

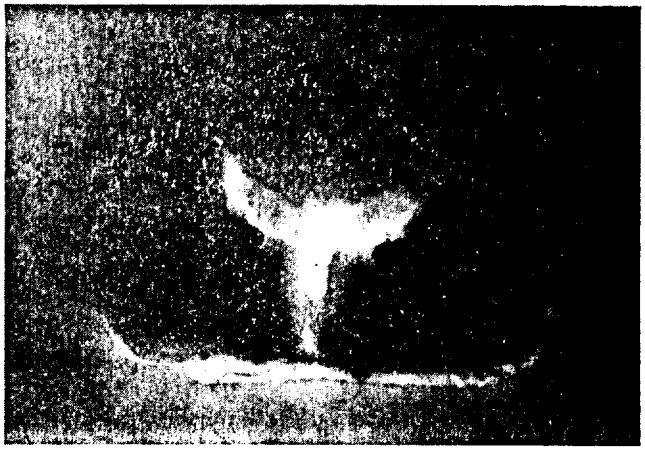
Acknowledgment

This work was financially supported by the International Minerals and Chemical Corporation of Chicago. Credit for technical suggestions is due to Dr. B. F. Buchanan of the International Minerals and Chemicals Corporation.

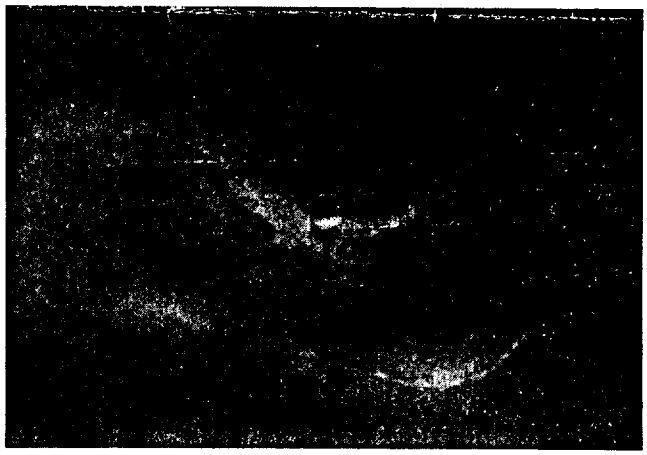
LITERATURE CITED

1. Bauer, F. K., Cassen, B., Youtcheff, E., Shoop, L. "Jet" Injection of Radioisotopes. Wadsworth General Hospital, Veterans Administration Center, Los Angeles, California.
2. Blish, M. J. An industry-wide research program. *Monosodium Glutamate*, second symposium, Sept., 1955. Research and Development Associates, Food and Container Institute, Inc., Chicago, Illinois.
3. Roberts, Eugene. Inhibition of bacterial glutamic decarboxylase by hydroxylamine. *J. Biol. Chem.*, **198**, 495 (1952).
4. Girardot, N. F., and Peryam, D. R. MSG's power to perk up foods. *Food Eng.*, **26**, 71-72, 182-185 (1954).
5. Hac, L. R., Long, M. L., and Blish, M. J. The occurrence of free L-glutamic acid in various foods. *Food Technol.*, **3**, 351-354 (1952).
6. Mosel, J. N., and Kantrowitz, G. The effect of monosodium glutamate on acuity to the primary tastes. *Am. J. Psychol.*, **65**, 573-579 (1952).
7. Norton, K. B., Tressler, D. K., and Farkas, L. D. The use of monosodium glutamate in frozen foods. *Food Technol.*, **6**, 405-411 (1952).
8. Stewart, G. F. (Unpublished data.)

Figure 7. Cross-section radioautographs of C^{14} dusted chicken breast.



A. Frozen immediately after dusting, held 7 days at 0° F., thawed 24 hrs. at 45° F.



B. Stored 7 days at 35° F. after dusting.

Dusted on cavity side only with 4 gm. MSG containing 13.3 μc dl-glutamic acid-2-C^{14}.

107. *An Evaluation of the Effects of Monosodium Glutamate in Man and Animals.*

I. ROSENBLUM, D. M. SERRONE, J. C. KILLEEN, JR., J. BRADLEY, J. H. WILLS, and F. COULSTON, Institute of Experimental Pathology and Toxicology, Albany Medical College, New York State Department of Health, Albany, New York, and Medical Department of Clinton Prison, Dannemora, New York.

Three aspects of the effects of monosodium glutamate (MSG) have been studied. The ability of MSG to sensitize or desensitize receptors to endogenous mediators was investigated in isolated tissues of several species. Results indicate that MSG sensitizes the frog rectus abdominis to potassium and acetylcholine but desensitizes the rabbit aorta strip to histamine.

Acute oral administration of 5 or 8 g of MSG to monkeys and intravenous infusions of lesser amounts to rabbits and monkeys did not affect the general behavior pattern, gross appearance, or electrocardiogram.

Three groups of rats were given 1, 2, or 4 gm/kg of MSG as a single daily oral dose, and one group received an iso-osmotic amount of NaCl. This experiment was carried out for 90 days. Body weight gain, hematologic values, blood chemical determinations, and urinalysis did not differ significantly between groups. A histologic appraisal of tissues taken at sacrifice showed no significant pathologic changes.

Ninety-eight male volunteers of varying age and background were fed a single 5-g dose of MSG dissolved in either tap water or diluted chicken soup stock. Each volunteer filled out a questionnaire consisting of 14 symptoms; they were also closely observed. Approximately one-half of the subjects affirmed one or more symptoms, but only one of these appeared to have "reacted" to MSG.

Appendix to "The Effect of Perorally Administered Monosodium Glutamate on the Reproductive Function and Somatic Growth in Rats"

— Histopathological Examinations —

Tsutomu Shimizu

Kageaki Aibara

(National Institute of Health, Japan)

Appendix to "The Effect of Perorally Administered Monosodium Glutamate on the Reproductive Function and Somatic Growth in Rats"

— Histopathological Examinations —

The animals explained in page 3 of the main volume, were killed with ether, and brain, hypophysis and eye were removed, fixed with 10% neutral buffered formalin and embeded in paraffin. Sections of 8μ in thickness were prepared and then stained with Hematoxylin-Eosin and Luxol fast blue-cresyl echt violet for histopathological examination. Arcuate nuclei and median eminence in hypothalamus and retina, (where olney observed acute neuronal necrosis due to the subcutaneous injection of MSG,) were observed microscopically, but no finding suggesting the difference between the control and MSG-loaded groups was observed.

(Ref. Photographs of hypothalamic area).

107. *An Evaluation of the Effects of Monosodium Glutamate in Man and Animals.*

I. ROSENBLUM, D. M. SERRONE, J. C. KILLEEN, JR., J. BRADLEY, J. H. WILLS, and F. COULSTON, Institute of Experimental Pathology and Toxicology, Albany Medical College, New York State Department of Health, Albany, New York, and Medical Department of Clinton Prison, Dannemora, New York.

Three aspects of the effects of monosodium glutamate (MSG) have been studied. The ability of MSG to sensitize or desensitize receptors to endogenous mediators was investigated in isolated tissues of several species. Results indicate that MSG sensitizes the frog rectus abdominis to potassium and acetylcholine but desensitizes the rabbit aorta strip to histamine.

Acute oral administration of 5 or 8 g of MSG to monkeys and intravenous infusions of lesser amounts to rabbits and monkeys did not affect the general behavior pattern, gross appearance, or electrocardiogram.

Three groups of rats were given 1, 2, or 4 gm/kg of MSG as a single daily oral dose, and one group received an iso-osmotic amount of NaCl. This experiment was carried out for 90 days. Body weight gain, hematologic values, blood chemical determinations, and urinalysis did not differ significantly between groups. A histologic appraisal of tissues taken at sacrifice showed no significant pathologic changes.

Ninety-eight male volunteers of varying age and background were fed a single 5-g dose of MSG dissolved in either tap water or diluted chicken soup stock. Each volunteer filled out a questionnaire consisting of 14 symptoms; they were also closely observed. Approximately one-half of the subjects affirmed one or more symptoms, but only one of these appeared to have "reacted" to MSG.

[Reprinted from Food Technology, 1948, Vol. 2, No. 4, Pages 317-321]

THE ROLE OF MONOSODIUM GLUTAMATE IN THE SEASONING OF CERTAIN VEGETABLES*

L. B. SJÖSTRÖM AND E. C. CROCKER
Arthur D. Little, Inc., Cambridge, Mass.

Most vegetables, raw or cooked, were found to be markedly improved in flavor by the addition of monosodium glutamate as a seasoning. The principal effects noted were balancing, blending, and reinforcement of the natural flavors and suppression of sourness, rawness, earthiness, and certain other undesirable notes. In the concentrations needed for this work, the presence of glutamate was scarcely noticeable.

Interest in glutamic acid, and especially in its monobasic salt, monosodium glutamate, has been long maintained in our laboratories. During the past two years we have made an intensive study of the effects of this remarkable substance in foods, particularly in vegetables, and our findings are reported herewith for their general interest.

The early forms of monosodium glutamate were crude and variable. With advances in food technology and the application of scientific methods to its production, monosodium glutamate has become an increasingly purer product, until today the commercial article is a chemically-pure, white, crystalline substance having practically no odor. It has a persistent taste, primarily sweet and salty, and imparts a mouth-filling sensation. When tasted in quantity, it produces a slight tingling on the tongue, especially near the tip. In water solution, the taste threshold is about one part in three thousand.

Monosodium glutamate is probably best classified as a seasoning agent; its unique action being more of the salt type than the condiment type *(1)*. Its compatilibility with meats is well known through extensive use in food products containing meats, but, by contrast, knowledge of its action in vegetables is meager. Consequently, our food study has been centered on the common vegetables to determine whether or not monosodium glutamate is complimentary to their flavor and to ascertain the specific effects of its use.

FLAVOR PANELS

Since all the information reported in this study was gained through the use of an organoleptic laboratory panel, it is perhaps fitting to refer briefly to panels. An organoleptic panel is a group of individuals who judge the quality of a product through use of their senses of feeling, smelling and tasting. The psychic aspects of food and flavor appreciation, no doubt, involve, all of the senses. There are three general types of panels: consumer, expert, and laboratory.

Consumer panels are used to obtain the public reaction to a food commodity, where tasting is usually done in the home. Results are obtained by analysis of the overall subjective impressions that the food creates due to flavor, texture, and many other factors.

Expert panels, such as the United States Board of Tea Experts, are composed of individuals or small groups highly skilled in evaluating one particular commodity.

Laboratory panels consist of trained individuals of tested sensitivity who work independently under favorable conditions. Their findings are considered objective when based on careful comparisons against standards. Usually, panel workers evaluate specific points of differences between a standard and the material under consideration. The usual means of obtaining familiarity with a new product is

* Presented before the Eighth Annual I. F. T. Convention, Philadelphia, Pa., June 9, 1948.

afforded the taster by "open panel" sessions. Such orientative sessions are informal meetings where the article to be tested is examined and discussed. The free discussion helps to develop a specific vocabulary applicable to the characteristics of the particular food and fosters exchange of ideas and a focusing of attention on the points of specific or critical interest. In a "closed panel," the members individually examine the article, without discussion, and report their findings.

TECHNIQUE OF TESTING

Raw and cooked fresh, canned, and frozen vegetables were examined. Not all varieties of each vegetable were studied, nor were most samples in prime condition, as many were obtained in the retail market. In every case, all variables besides the effect of glutamate were controlled as well as possible. For example, if broccoli was to be tested, several heads of fresh broccoli were cleaned, cut up and boiled in glass until nearly cooked. Then sub-samples were prepared in one-liter beakers by taking aliquots of the broccoli and cooking water. Glutamate, calculated on the weight of the broccoli, was added in various proportions to all but the control samples. Then cooking was completed, long enough to assure good blending. Samples were presented to the panel seated at a circular, freely-revolving table. Aromas in the beakers were observed by all members who individually wrote down their findings. Then each individual was served with portions from the various lots. The silver and china used had previously been cleaned and oven-baked to insure freedom from aroma. After tasting the samples, the panel members again recorded their findings. Then discussion followed.

In the orientative work with each vegetable, preliminary tests were made with one or two tasters to ascertain the minimal effective amount of glutamate; in other words, the smallest amount of glutamate that would just produce an effect in the food to which it was added. Then the food under consideration was prepared and served to the panel for evaluation and estimation of compatibility. No other seasoning was added during the orientative tests.

Following the orientative work, confirmatory tests were made by a larger panel on the same vegetables. In this instance four sub-samples were examined. The first was an unseasoned control, the next was the otherwise unseasoned sample treated with glutamate, the third was a fully seasoned control, and the fourth was fully seasoned plus glutamate. By this time, it was usual for the panel members to characterize the aroma and the taste of each unseasoned vegetable. For example, the aroma of one sample of canned peas was given the following description; for aroma: pea-like, fragrant, beany, burnt; and for flavor by mouth: sweet, eggy, and sour, with bitter aftertaste. The effect of adding seasoning to these peas could then be observed in detail, particularly the effect of glutamate on the individual characteristics noted of each vegetable, with and without other seasoning agents.

Further tests were made on a series of conventionally seasoned vegetables. Each vegetable was subdivided into several test samples: fully-seasoned control; fully-seasoned samples containing minimum effective quantity of monosodium glutamate; and several fully seasoned samples containing increasing amounts of glutamate. The latter were prepared in order to ascertain the maximum tolerable amounts or the greatest quantity of added glutamate which would just not superimpose its own character on that of the cooked vegetable under study.

Perhaps the least scientific but most practical method for the purpose was that of evaluating the compatibility of monosodium glutamate with raw vegetables. A few of the commonly used salad vegetables were cleaned and cut into thin slices. Each panel member was furnished with shakers, one filled with table salt and the

other with monosodium glutamate, for seasoning the raw vegetables. To further evaluate the effect on raw vegetables, tossed salads were prepared with mayonnaise containing monosodium glutamate.

FINDINGS

In every instance it was found that glutamate altered the character of cooked vegetables by blending the components of aroma and taste into a smoother flavor.

Table 1 lists the raw vegetables seasoned with glutamate. When used in conjunction with salt, glutamate was found compatible with most of the raw vegetable slices tested. In the tossed salads prepared with mayonnaise monosodium glutamate was thoroughly compatible and produced interesting results. Tables 2, 3 and 4 present the vegetables we examined cooked fresh, cooked canned, and cooked frozen. A range of proportions of glutamate used is given for each cooked vegetable. The lower figure represents the minimal effective amount of glutamate and the higher figure the tolerable amount or the most that could be added without disguising the identity of the vegetable.

By adding monosodium glutamate in gradually increasing amounts beyond the minimum effective quantity, an increase in the effect was perceived up to the point

TABLE 1

Monosodium Glutamate Used on Raw Vegetables

Vegetable	Proportion of Glutamate Used
	%
Cabbage	about 0.2
Carrots	about 0.2
Celery	about 0.2
Lettuce	about 0.2
Radishes	about 0.2*
Tomatoes	about 0.2

*Of questionable compatibility.

All tasted in conjunction with salt.

TABLE 2

Monosodium Glutamate Cooked With Fresh Vegetables

Vegetable	Range of Useful Proportions
	%
Asparagus	0.5 – 3.0
Beans, green	0.1 – 0.2
Beans, lima	0.3 – 0.4
Beets	0.1 – 0.5
Broccoli	0.5 – 1.0
Cabbage	0.2 – 0.3
Carrots	0.2 – 2.0
Cauliflower	0.5 – 1.0
Celery	0.1 – 0.2
Corn	0.1 – 1.0
Mushrooms	0.1 – 1.0
Peas	0.2 – 2.0
Potatoes, sweet	0.1 – 0.2
Potatoes, white	0.2 – 1.5
Spinach	0.2 – 3.0
Squash, summer	0.5 – 1.0
Squash, winter	0.2 – 1.0
Tomatoes	0.1 – 0.3
Turnips, yellow	0.2 – 0.3

TABLE 3

Monosodium Glutamate Cooked With Canned Vegetables

Vegetable	Range of Useful Proportions
	%
Asparagus	0.1 – 1.0
Beans, green	0.2 – 1.0
Beans, lima	0.1 – 02
Beansprouts	0.05 - 0.1
Beets	0.1 – 0.5
Carrots	0.2 – 1.0
Corn, whole kernel	0.2 – 0.75
Corn, cream style	0.1 – 0.25
Hominy	not compatible
Mushrooms	0.1 – 1.0
Peas	0.1 – 1.0
Potatoes, sweet	0.1 – 0.3
Potatoes, white	0.1 – 0.3
Sauerkraut	0.1 – 0.2
Squash, winter	0.1 – 0.3
Tomatoes	0.1 – 0.3
Tomatoes, juice (not cooked)	0.2 – 3.0

TABLE 4

Monosodium Glutamate With Cooked Frozen Vegetables

Vegetable	Range of Useful Proportions
	%
Asparagus	0.5 – 1.0
Beans, green	0.1 – 0.2
Beans, lima	0.1 – 0.2
Broccoli	0.5 – 1.0
Cauliflower	0.1 – 0.2
Corn, whole kernel	0.1 – 0.3
Peas	0.1 – 0.3

where the glutamate itself was noticeable. We noted that the blander the food, the narrower was the range where glutamate could be employed as a seasoning. A good example of this was found in sweet potatoes.

Monosodium glutamate has been found to have certain specific effects. The natural flavor characteristics of carrots and cauliflower, for instance, were intensified. It reduced the sharpness of highly acidic foods. It diminished the inherent bitterness of fresh spinach. It obviated the sharpness of onions and suppressed the bite of radishes, and aided materially in the reduction of peel taste and earthiness in white potatoes and certain other root vegetables. It lessened the fishy note that is sometimes present in lima beans. Glutamate added to shelf-aged canned tomato puree at 0.2 percent, notably cut the metallic taste. When added at the same level to canned tomato juice, shelf-aged and artificially aged at 45° C. for two months, monosodium glutamate was found definitely effective in reduction of the "tin-can" flavor. It was able to augment the intensity of flavor and aroma that had been overshadowed by the addition of butter to hot, fully seasoned vegetables such as green beans.

Recent work in another laboratory *(2)* has shown that a number of cooked vegetables, such as peas, green beans, mushrooms and asparagus contain from 0.03 to 0.2 percent of glutamate. (This is a private communication from Dr. Lucile Hac.) Perhaps it is the naturally occurring glutamate in such vegetables that contributes

to the "mouthfullness" and satisfaction of stews made from them. Additional glutamate still further enhances this action.

A new observation made in this study was that considerably larger amounts of glutamate than were required for minimum effect often produced unusually interesting results. While these tastes were quite desirable, the economics of the situation might limit the extent to which they can be used.

In practically every instance, we found monosodium glutamate compatible with vegetables, the minimum effective range being, for the most part, under 0.5 percent. In these low proportions, glutamate possessed the ability to merge the individual components of aroma and taste, without diminishing the intensity, of nearly every cooked vegetable studied and without itself being noticeable. It was found that glutamate intensified sweetnes or saltiness in a food when these characteristics were near their optimal value. It helped correct rawness of flavor. It gave relish to overcooked vegetables by imparting taste body and "mouthfullness" or satisfaction.

LITERATURE CITED

1. Cairncross, S. E. The effect of monosodium glutamate on food flavor. Presented at the Symposium of the Quartermaster Food Institute, Chicago, March, 1948.
2. Hac, Lucile R. Research Laboratories, International Minerals and Chemical Corporation. Personal communication, work to be published.

The Taste of Amino Acids, Peptides, and Proteins

Juerg Solms

The common pure amino acids have the following taste properties near neutrality: no taste at all or a barely perceptible taste (D-Ala, D- and L-Arg, D- and L-Asp, D-Glu, L-His, D- and L-Ile, D- and L-Lys, D- and L-Pro, D- and L-Ser, D- and L-Thr, D- and L-Val), sweet taste (in order of decreasing sweetness: D-Try, D-His, D-Phe, D-Tyr, D-Leu, L-Ala, Gly), bitter taste (in order of decreasing bitterness: L-Try, L-Phe, L-Tyr, L-Leu), and sulfurous taste (D- and L-CySH and D- and L-Met). L-Glutamic acid has a unique taste-potentiating property: a similar effect has been reported for tricholomic acid and ibotenic acid. Pure peptides have been investigated to a lesser extent. Examples are presented of peptides with bitter and other taste sensations. Proteins have been found to provide tactual and astringent effects. The contributions of these substances to the flavor of food products often exceed the taste properties of the pure compounds.

The many important biological functions that amino acids, peptides, and proteins fulfill has focused research in the past decades on the physiological role of these compounds. Recently, the less fundamental aspects such as taste and flavor have become of interest. The present review is concerned with the direct contributions of amino acids, peptides, and proteins to the flavor of foods; their importance as flavor precursors is not considered.

The taste qualities of the pure substances will be considered first. The literature on this subject is controversial (Berg, 1953; Crocker, 1948; Ferguson and Lawrence, 1958; Galvin, 1948; Lawrence and Ferguson, 1959; Meister, 1957; Stone, 1967). This is probably because pure D- and L-amino acids were not readily available in the past, that differences in taste between the optical isomers were often not duly considered, and that chemical impurities often conveyed agreeable "hydrolysate flavors" to the test substances. Moreover, many taste tests were conducted only on a qualitative basis and at varying concentrations.

In Table I, results are presented from statistical taste tests conducted with a selected panel of eight to 12 people, using pure L- and D-amino acids (Solms *et al.*, 1965). The amino acids with taste properties were compared with standard substances having the same taste quality and known taste intensities. All taste tests were conducted at the same fixed concentration of amino acid (0.3%), although it is known that taste quality may vary with concentration. Experimental details are reported elsewhere (Solms *et al.*, 1965).

Three groups of amino acids can be established. Group 1 consists of amino acids whose L- and D-enantiomorphs have no taste at all, or only a barely perceptible taste. Group 2 consists of amino acids with complex taste sensations, which are difficult to evaluate in the pure state. This group contains the unique L-glutamic acid, known for its flavor-enhancing properties in suitable mixtures (Neukom, 1956; Oeda, 1963; Solms, 1967a, 1967b), and the sulfur-containing amino acids. The latter probably form decomposition products, which are responsible for their sulfurous taste. Group 3 comprises amino acids with distinctive tastes, either bitter or sweet, which were compared quantitatively with caffeine and sucrose solutions, respectively. The taste intensities of the amino acids in this group, especially those of the aromatic amino acids, are noteworthy. L-Tryptophan is approximately half as bitter as caffeine; D-tryptophan is 35 times sweeter than sucrose. L- and D-phenylalanine and L- and D-tyrosine have similar but less intense taste qualities. Alanine and histidine form a special subgroup, with one isomer flat in taste, the other sweet. Alanine is the only amino acid tested with a truly sweet-tasting L-enantiomorph. Glycine, named for its sweet taste, has the lowest relative sweetness of the amino acids in this group. Free aromatic L-amino acids occur in relatively high concentrations, together with other amino acids, in oriental food preparations (Murata *et al.*, 1967; Yokotsuka, 1960), and in many cheese varieties, especially in ripe ones (Hintz *et al.*, 1956; Kosikowski and Dahlberg, 1954; Ritter *et al.*, 1966), and may directly influence the taste or off-taste of these foods.

Two amino acids, which have not been tested, are ibotenic acid and tricholomic acid (Solms, 1967b, Takemoto, 1966). They are reported to exert flavor enhancing properties similar to glutamic acid, with much higher intensity.

Finally, a compound derived from glutamic acid, pyrrolidone-carboxylic acid, is claimed to possess a disagreeable bitter "off-taste" (Mahdi *et al.*, 1961; Shallenberger and Moyer, 1958).

Much less is known about the taste of peptides, but there are probably no simple relations to the amino acids. The peptide L-glycyl-L-tryptophan, for example, is not bitter in taste, although it contains L-tryptophan (Solms, 1968b). It is a well-known observation that bitter tasting products are formed during enzymatic hydrolysis of proteins. This apparently is due to the formation of bitter tasting peptides, especially phosphopeptides—e.g., in cheese production (Harwalkar, 1967; Murray and Baker, 1952; Raadsveld, 1953; Stone and Naff, 1967). Although many peptides have been isolated from enzymatic protein hydrolyzates and from cheese, detailed studies of their structures combined with taste evaluations have not as yet been published. Thus far only one peptide with a bitter taste has been examined in detail, and it contains the following amino acids: leucine, glutamic acid, and valine (Carr *et al.*, 1956).

Many peptides have been isolated from plants and animals, but have not been tested for their taste properties. At least two peptides, γ-L-glutamyl-*S*-(prop-1-enyl)-L-

Institute of Agricultural Chemistry, Swiss Federal Institute of Technology, 8006 Zurich, Switzerland

Table I. Taste of Amino Acids in 0.3% Aqueous Solutions[a,b]

Amino Acid	Taste: L-Form	Taste: D-Form
Group 1. Amino Acids without Taste		
Arginine	Flat	Sl. sweet (D, L)
Aspartic acid	Flat	Flat
Isoleucine	Flat	Flat
Lysine	Flat	Flat
Proline	Flat, sl. sweet	Flat (D, L)
Serine	Flat	Flat
Threonine	Flat	Flat
Valine	Flat	Flat
Glutamic acid	...	Flat
Group 2. Amino Acids with Varying Tastes		
Cysteine	Sulfurous	Sulfurous (D, L)
Glutamic acid	Unique, "glutamate"	...
Methionine	Sulfurous, meaty Sl. sweet	Sulfurous, meaty Sl. sweet
Group 3. Amino Acids with Bitter or Sweet Tastes (as compared with solutions of caffeine or sucrose)		
Alanine	Sweet, 0.54% sucrose	Flat
Histidine	Flat	Sweet, 2.23% sucrose
Leucine	Bitter, 0.011% caffeine	Sweet, 1.30% sucrose
Phenylalanine	Bitter, 0.069% caffeine	Sweet, 2.20% sucrose
Tryptophan	Bitter, 0.133% caffeine	Sweet, 11.00% sucrose
Tyrosine[c]	Bitter, 0.017% caffeine	Sweet, 1.65% sucrose
Glycine	Sweet, 0.45% sucrose	

[a] The amino acids marked (D, L) were not available in pure D-isomer form; therefore, the racemates were used in the tests.
[b] pH adjusted to pH 6.0 with NaOH or HCl.
[c] Due to the low solubility of tyrosine, the taste tests were conducted at elevated temperatures.

cysteine isolated from chives, and γ-L-glutamyl-*S*-(prop-1-enyl)-L-cysteine sulfoxide isolated from onions, were evaluated for taste, and they exhibited a characteristic biting taste sensation (Virtanen, 1965, 1968). An agreeable taste has been described for anserine (Jones, 1961). Certain flavor-enhancing properties have been assigned to calcium pantothenate (Dixon, 1968) and to several copolypeptides containing glutamic acid, aspartic acid, lysine, and ornithine (Kyowa Hakko Kogyo Co., 1965, 1967a, 1967b). For many years it has been claimed that glutathione is responsible for meaty taste (Bouthilet, 1951), but this is probably not correct.

It is generally accepted that proteins exert no taste activity; however, they contribute to flavor indirectly, with tactile and similar effects. This is especially true for protein-rich foods like milk, milk products, and meat (Matz, 1962). Astringency should also be mentioned as a direct contribution of these substances to taste (Josephson *et al.*, 1967).

In food preparations the contribution of amino acids and peptides to the flavor of the product often exceeds the taste properties of the pure compounds themselves, because of the phenomena of taste interactions, like synergism and potentiation (Amerine *et al.*, 1965; Solms, 1967a, 1967b). The best known example is L-glutamic acid, which exerts a pronounced taste-improving effect in appropriate mixtures, or in foods, due to flavor potentiation. Other compounds are much less effective, and must be regarded as secondary substances, which provide a background for a flavor potentiator (Hashimoto, 1965).

To assess the taste contribution of these substances, they must be tested in their natural environment. The first systematic approach, in this regard, has been described by Mulder (1952) with the "components balance theory" of cheese flavor. The theory states that flavor is not related to single compounds, but to a mixture of volatile and nonvolatile compounds, including amino acids, which must occur in a certain specific balance. The theory has been examined and confirmed by Silverman and Kosikowski (1953) and by Kosikowski and Mocquot (1958). Hashimoto (1965) developed a similar approach for evaluating the flavor of meat and fish; Craske and Reuter (1965) applied a somewhat different technique for mushroom flavor. The difficulties of the method were recently discussed for cheese flavor (Day *et al.*, 1957). They are mainly due to the fact that the amino acids do not impart a typical flavor as such, but appear to function by providing a general background on which the typical flavor notes are superimposed. The character of the background depends on the qualitative and quantitative composition of the mixture (Dylanyan *et al.*, 1966).

The amino acid fraction of Swiss cheese is an interesting example. Only a combination of the amino acids L-proline (6000 p.p.m.), glycine (1600 p.p.m.), L-serine (1950 p.p.m.), L-threonine (1950 p.p.m.), L-aspartic acid (2500 p.p.m.), L-glutamic acid (3000 p.p.m.), L-cysteine (760 p.p.m.), L-tryptophan (2200 p.p.m.), L-histidine (3700 p.p.m.), and L-lysine (2200 p.p.m.), together with selected free fatty acids and selected volatiles, gave reconstituted Swiss cheese flavor (Langler *et al.*, 1967).

Other amino acid mixtures of specific qualitative and quantitative composition, which contribute to basic flavor notes, are reported for onions (Take and Otsuka, 1968), crabmeat (Take *et al.*, 1968), fish and fish extracts (Hashimoto, 1965), soy sauce and other seasonings (Kikkoman Shoyu Ltd., 1967, 1968), and chicken and meat broth (Ajinomoto Co., 1967; Kazeniac, 1961). In the latter, hydroxyproline seems to be of special interest (Kawano, 1962).

The importance of free amino acids in the flavor of potatoes has been investigated in some detail (Solms *et al.*, 1969). The nonvolatile extracts of boiled potatoes con-

Table II. Proposed Integrated Flavor Picture

Total flavor sensation	Volatile fraction	Low boiling compds. High boiling compds.
	Nonvolatile fraction	Flavor potentiators, synergists Compds. with taste and tactile effects

tain at least three interesting fractions—namely, (I) nucleotides (adenosine-5′-phosphate, cytidine-5′-phosphate, guanosine-5′-phosphate, guanosine-2′,3′-phosphate, uridine-5′-phosphate, uridine-2′,3′-phosphate), (II) L-glutamic acid and (III) other free amino acids (L-Ala, L-Arg, L-Asp, Gly, L-His, L-Ile, L-Leu, L-Lys, L-Met, L-Pro, L-Phe, L-Ser, L-Thr, L-Try, L-Tyr, L-Val). A stepwise recombination of these fractions with pure substances at the proper concentrations gives a distinct stepwise increase in taste quality, as judged by panel comparison tests. The final, total mixture has practically no odor but has a distinct basic potato taste. However, a fully-reconstituted potato flavor is not achieved, because other nonvolatiles and the whole volatile flavor fraction are missing.

Amino acids, peptides, and proteins seem to have a place in an integrated flavor picture. Possible flavor relations for various compounds were recently proposed for meat flavor (Solms, 1968a). Other flavors are composed in a similar fashion of four groups of substances, with close interrelationships, each of which should occur in a specific balance to give an over-all flavor sensation (Table II). Amino acids, peptides, and proteins are located at the base of this scheme, providing taste and tactile effects; certain compounds act as flavor potentiators and synergists, and act as a bridge between the nonvolatile and volatile fractions. The volatile fractions are located at the top of the scheme and contribute important odor effects described elsewhere in the flavor literature. All groups together then form the integrated flavor picture.

REFERENCES

Ajinomoto Co., Inc., French Patent **1,474,613** (Feb. 13, 1967).
Amerine, M. A., Pangborn, R. M., Roessler, E. B., "Principles of Sensory Evaluation of Food," Academic Press, New York, 1965.
Berg, C. P., *Physiol. Rev.* **33**, 145 (1953).
Bouthilet, R. J., *Food Res.* **16**, 201 (1951).
Carr, J. W., Loughheed, T. C., Baker, B. E., *J. Sci. Food Agr.* **7**, 629 (1956).
Craske, J. D., Reuter, F. H., *J. Sci. Food Agr.* **16**, 243 (1965).
Crocker, E. C., "Monosodium Glutamate," W. F. Dove, Ed., p. 25, Food and Container Institute, Chicago, 1948.
Day, E. A., "Symposium on Foods, The Chemistry and Physiology of Flavors," H. W. Schultz, E. A. Day, C. M. Libbey, Eds., p. 331, Avi Pub. Co., Inc., Westport, Conn., 1967.
Dixon, M. P., *Rev. Nutr. Food Sci.* **1968**, 6 (10).
Dylanyan, Z., Agababyan, A., Ioanissyan, J., Zakharyan, L., XVII. Intern. Dairy Congress, Sect. D, Cheese, p. 301, Munich, 1966.
Ferguson, L. N., Lawrence, L. W., *J. Chem. Educ.* **35**, 136 (1958).
Galvin, S. L., "Monosodium Glutamate," W. F. Dove, Ed., p. 39, Food and Container Institute, Chicago, 1948.
Harwalkar, V. R., *J. Dairy Sci.* **50**, 956 (1967).
Hashimoto, V., "The Technology of Fish Utilization," R. Kreuzer, Ed., p. 57, Fishing News Ltd., London, 1965.
Hintz, P. C., Slatter, W. L., Harper, W. J., *J. Dairy Sci.* **39**, 235 (1956).
Jones, N. R., "Flavor Chemistry Symposium," p. 61, Campbell Soup Co., Camden, N. J., 1961.
Josephson, R. V., Thomas, E. L., Morr, C. V., Coulter, T., *J. Dairy Sci.* **50**, 1376 (1967).
Kawano, Y., Japanese Patent Appl. **56,792/62** (Dec. 24, 1962).
Kazeniac, S. J., "Flavor Chemistry Symposium," p. 37, Campbell Soup Co., Camden, N. J., 1961.
Kikkoman Shoyu Ltd., French Patent **1,469,024** (Jan. 2, 1967).
Kikkoman Shoyu Co., *CA* **68**, 28635 (1968).
Kosikowski, F. V., Dahlberg, A. C., *J. Dairy Sci.* **37**, 167 (1954).
Kosikowski, F. C., Mocquot, G., "Progrès de la technologie du fromage," Etudes Agricoles, FAO, No. 38, p. 151, Rome, 1958.
Kyowa Hakko Kogyo Co., Ltd., French Patent **1,413,451** (Aug. 30, 1965).
Kyowa Hakko Kogyo Co., Ltd., British Patent **1,068,478** (Apr. 4, 1967a).
Kyowa Hakko Kogyo Co., Ltd., British Patent **1,083,189** (1967b).
Langler, J. E., Libbey, L. M., Day, E. A., J. AGR. FOOD CHEM. **15**, 386 (1967).
Lawrence, A. R., Ferguson, L. N., *Nature* **183**, 1469 (1959).
Mahdi, A. A., Rice, A. C., Weckel, K. G., J. AGR. FOOD CHEM. **9**, 143, (1961).
Matsuda, I., Shiga, A., Kanemitsu, A., U. S. Patent **3,340,069** (1968).
Matz, S. A., "Food Texture," Avi Pub. Co., Westport, Conn., 1962.
Meister, A., "Biochemistry of the Amino Acids," Academic Press, New York, 1957.
Mulder, H., *Neth. Milk Dairy J.* **6** (2), 157 (1952).
Murata, K., Ikehata, H., Miyamoto, T., *J. Food Sci.* **32**, 580 (1967).
Murray, T. K., Baker, B. E., *J. Sci. Food Agr.* **3**, 470 (1952).
Neukom, H., *Chimia* **10**, 203 (1956).
Oeda, H., "Monosodium Glutamate," in "Encyclopedia of Chemical Technology," 2nd ed., **2**, 198 (1963).
Raadsveld, C. W., *Proc. XIII Intern. Dairy Congr.* **2**, 676 (1953).
Ritter, W., Schilt, P., Lowe, A., *XVII Intern. Dairy Congr.*, Sect. D, Cheese, p. 269 (1966).
Shallenberger, R. S., Moyer, J. C., J. AGR. FOOD CHEM. **6**, 604 (1958).
Silverman, G. J., Kosikowski, F. V., *J. Dairy Sci.* **36**, 574 (1953).
Solms, J., "Aroma- und Geschmacksstoffe in Lebensmitteln," J. Solms, H. Neukom, Eds., p. 199, Forster Verlag, Zurich, 1967a.
Solms, J., *Chimia* **21**, 169 (1967b).
Solms, J., *Fleischwirtschaft* **48**, 287 (1968a).
Solms, J., unpublished observation, 1968b.
Solms, J., Signer, V., Buri, R., *Lebensm.-Wiss. Technol.*, to be published (1969).
Solms, J., Vuataz, L., Egli, R. H., *Experientia* **21**, 692 (1965).
Stone, H., "Olfaction and Taste II," T. Hayashi, Ed., p. 289, Pergamon Press, Oxford, 1967.
Stone, W. K., Naff, D. M., *J. Dairy Sci.* **50**, 1497 (1967).
Take, T., Otsuka, H., *CA* **68**, 28563 (1968).
Take, T., Yoshimura, Y., Otsuka, H., *CA* **68**, 28562 (1968).
Takemoto, T., *Japan. Med. Gaz.* **3** (5), 5 (1966).
Virtanen, A. I., *Phytochemistry* **4**, 207 (1965).
Virtanen, A. I., Biochemical Research Institute, Helsinki, Finland, personal communication, 1968.
Yokotsuka, T., *Advan. Food Res.* **10**, 75 (1960).

Received for review October 10, 1968. Accepted February 17, 1969. Presented at Symposium on Importance of Nonvolatile Compounds in Flavor, Division of Agricultural and Food Chemistry, 156th Meeting, ACS, Atlantic City, N. J., September 1968.

J. Biol. Chem. 179 (1949)

THE ENZYMATIC SYNTHESIS OF GLUTAMINE, A REACTION UTILIZING ADENOSINE TRIPHOSPHATE*

By JOHN F. SPECK

(From the Department of Biochemistry, University of Chicago, Chicago)

(Received for publication, November 3, 1948)

The preceding paper (2) describes experiments on the synthesis of glutamine in pigeon liver dispersions, which, together with the results of Krebs and his collaborators (3, 4) and of Bujard and Leuthardt (5), suggested that adenosine triphosphate may play an essential rôle in amide formation. Proof for this function of adenosine triphosphate has been obtained in studies with tissue extracts, which are the subject of the present paper. Partially fractionated extracts of acetone-dried pigeon liver have been found to catalyze a stoichiometric reaction between adenosine triphosphate, glutamate, and ammonia, in the presence of magnesium ions, to form glutamine, adenosine diphosphate, and inorganic phosphate.

Methods

Materials—Commercial preparations of most amino acids, amines, and carboxylic acids were used. Diphenylamidophosphate was prepared according to Audrieth and Toy (6) and hydrolyzed to potassium acid amidophosphate by the method of Stokes (7). Methionine sulfoxide was made from DL-methionine by the procedure of Toennies and Kolb (8) as modified by Waelsch *et al.* (9). Adenosine triphosphate was isolated from rabbit muscle by the method of LePage (10); the preparation was carried through two mercury precipitations and finally precipitated as the barium salt with $BaCl_2$. Dried *in vacuo* over $CaCl_2$, the product gave the following analysis: 965 mg. of dibarium adenosine triphosphate contained 5.00 milliatoms of total nitrogen, 1.03 mM of adenine (from measurement of light absorption at 260 mμ), 3.00 mM of total organic phosphate, 1.96 mM of easily hydrolyzable phosphate, and 0.03 mM of inorganic phosphate. Solutions were prepared by dissolving the dibarium salt in dilute HCl, adding a small excess of Na_2SO_4 solution, neutralizing with NaOH to brom thymol blue as an internal indicator, diluting to the desired volume, and centrifuging. Adenosine diphosphate was prepared from adenosine triphosphate by means of yeast hexokinase (11); it contained easily hydrolyzable phos-

* This work was aided by grants from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, and from The National Foundation for Infantile Paralysis. A note describing part of this work has appeared previously (1).

phate equal to 51 per cent of the total organic phosphate. Other materials used are described in the preceding paper (2).

Procedure for Enzyme Experiments—The reaction mixtures were made up in 12 ml. conical tipped centrifuge tubes. The final volume was generally 1.0 or 1.5 ml. for experiments with hydroxylamine and 2 ml. for experiments with ammonia. After all the components except adenosine triphosphate had been added, the samples were placed in a water bath at 30° for 5 minutes; then adenosine triphosphate was added to start the reaction. At the end of the incubation period, the reaction was stopped by addition of an equal volume of 10 per cent trichloroacetic acid, and analyses were performed on the supernatants after centrifugation. Samples identical in essential respects with the incubated samples were treated with trichloroacetic acid before addition of adenosine triphosphate and analyzed for initial values. The changes resulting from enzyme action were obtained by difference.

Analytical Methods—Procedures for the distillation and colorimetric determination of ammonia and for the hydrolysis of glutamine are presented in the preceding paper (2). For inorganic phosphate analyses the method of Gomori (12) was modified by substituting an acid-molybdate reagent containing 5.0 N H_2SO_4 for the original reagent containing 2.5 N H_2SO_4; this decreases the possibility of interference from traces of silicate. Protein was determined by the biuret procedure of Robinson and Hogden (13); a liver protein fraction, the nitrogen content of which had been established by micro-Kjeldahl analysis, was used as a standard.

Hydroxamic acid was determined by a method essentially identical with that of Lipmann and Tuttle (14). The total sample plus an equal volume of 10 per cent trichloroacetic acid or an aliquot of a trichloroacetic acid filtrate was brought to 3.0 ml. with water. 1.0 ml. of 2 M NH_2OH of pH 6.5 ($NH_2OH \cdot HCl$ neutralized with NaOH) and 2.0 ml. of 2.5 per cent $FeCl_3 \cdot 6H_2O$ in 1.5 N HCl were added. If the protein had not been removed previously, the samples were centrifuged, and the colors were read in an Evelyn photoelectric colorimeter with Filter 540, in 18 × 150 mm. tubes. A blank and standards of 1.25 to 3.75 μM of succinyl hydroxamic acid, all containing the same amount of trichloroacetic acid as the unknowns, were read at the same time. Values for hydroxamic acid are expressed as succinyl hydroxamic acid equivalents, since a suitable standard of glutamyl hydroxamic acid was not available.

Results

All the detailed experiments on glutamine synthesis were performed with enzyme preparations obtained from acetone-dried pigeon liver, but similar enzyme activity is found in other tissues and other species of ani-

mals. The pigeon liver preparations catalyze a reaction between adenosine triphosphate, glutamate, and ammonia to form glutamine, adenosine diphosphate, and inorganic phosphate. The reaction can be followed by measuring the disappearance of ammonia and formation of amide and inorganic phosphate. Ammonia may be replaced by certain simple derivatives, such as hydroxylamine, hydrazine, and methylamine. When hydroxylamine is substituted for ammonia, a hydroxamic acid is produced. Because of the convenience of analyzing for the hydroxamic acid, hydroxylamine was generally employed in routine enzyme experiments, such as establishment of optimal reaction conditions and assay of fractions during enzyme purification.

Purification and Properties of Pigeon Liver Enzyme—Acetone-dried pigeon liver served as the source of the enzyme preparations. This material was obtained by dispersing fresh pigeon livers in about 6 parts of ice-cold acetone in a Waring blendor, filtering on a Büchner funnel, resuspending the solid in cold acetone, filtering again, and drying on the funnel in a stream of air and then in a vacuum desiccator over $CaCl_2$. Acetone powders kept in a desiccator in the cold yielded active enzyme preparations after at least a year.

Enzymatic activity of acetone powder extracts or of protein fractions obtained from the extracts was assayed in a system of the following composition: 0.1 M tris(hydroxymethyl)aminomethane buffer of pH 7.0 (15), 0.01 M $MgSO_4$, 0.04 M HCN of pH 7 (NaCN freshly neutralized with HCl), 0.05 M sodium L-glutamate, 0.05 M NH_2OH of pH 7 ($NH_2OH \cdot HCl$ freshly neutralized with NaOH), and 0.006 M adenosine triphosphate. The temperature was 30° and the reaction period 10 minutes. About 0.2 ml. of the crude extracts and smaller volumes of the purified fractions were used in each 1 ml. final volume of reaction mixture. Formation of hydroxamic acid and inorganic phosphate was measured. The same protein fractions were incubated in a system containing only buffer, magnesium ions, cyanide, and adenosine triphosphate; liberation of inorganic phosphate in this system was used as a measure of hydrolysis of adenosine triphosphate.

A study of conditions for extraction of the enzyme activity from the acetone powder showed that alkaline salt solutions were most effective, and 0.2 M $NaHCO_3$ was selected as the best of the solutions tested. Generally the acetone powder was ground with 10 parts of ice-cold 0.2 M $NaHCO_3$, and the solid was removed by centrifugation after the mixture had stood in the ice box for a few hours. Reextraction of the solid with 5 parts of 0.2 M $NaHCO_3$ increased the yield of activity by about one-third.

Extracts of acetone-dried pigeon liver cause hydrolysis of adenosine triphosphate. This activity interferes with the study of glutamine syn-

thesis by obscuring the stoichiometry and diminishing the quantity of adenosine triphosphate available for the synthesis. Hydrolysis of adenosine triphosphate by pigeon liver extracts is strongly activated by magnesium ions; calcium ions are less effective. Different batches of acetone powder yield different levels of adenosinetriphosphatase activity. Extracts from one powder, which was used for the experiment described in a previous note (1), contained little adenosinetriphosphatase and gave fairly good stoichiometry in glutamine formation without purification. Extracts from another large batch of powder, which was used in all the experiments described in the present paper, brought about hydrolysis of adenosine

TABLE I

Enzyme Assays during Purification

The fractionation procedure and assay systems are described in the text. Hydroxamic acid and phosphatase activities are expressed as micromoles of hydroxamic acid or phosphate per ml. of enzyme solution per 10 minutes and protein as mg. of N per ml. of enzyme solution.

Fraction	Volume	Hydroxamic acid activity		Phosphatase activity	Phosphatase / Hydroxamic acid	Protein	Hydroxamic acid / Protein
		Concentration	Total				
	ml.	μM per ml.	μM	μM per ml.		mg. N per ml.	μM per mg. N
Extract	246	8.3	2040	5.2	0.63	4.78	1.74
Isoelectric ppt.	120	16.5	1980	2.4	0.15	2.80	5.00
Ppt. 22% ethanol	35	10.0	350	3.5	0.35	3.72	2.68
" 22–43% ethanol	35	20.2	710	0.0	0.00	1.09	18.5

triphosphate almost as rapidly as utilization for glutamine synthesis. The extracts did not show significant glutaminase activity.

Studies on the precipitation of enzyme activity were carried out so that a method for freeing the extracts from adenosinetriphosphatase might be devised. A purification procedure was adopted which consisted of isoelectric precipitation and ethanol fractionation. All operations were carried out with cold solutions in a room at a temperature of about 1°. One such preparation is described here, and the assay results are given in Table I. (*a*) 20 gm. of pigeon liver acetone powder were extracted twice, first with 180 ml. and then with 100 ml. of 0.2 M $NaHCO_3$. The combined solutions were dialyzed overnight against 0.15 M NaCl-0.005 M $NaHCO_3$ and assayed (extract). (*b*) 246 ml. of extract were mixed with 123 ml. of 0.2 M acetate of pH 4.2. The precipitate was removed by centrifugation and suspended in a mixture of 50 ml. of 0.2 M $NaHCO_3$ and 30 ml. of 0.15 M NaCl-0.005 M $NaHCO_3$. After an hour the solid was centrifuged and reextracted with

40 ml. of NaCl-$NaHCO_3$. The combined solutions were dialyzed overnight against NaCl-$NaHCO_3$ and assayed (isoelectric precipitate). (*c*) To 120 ml. of the solution of the isoelectric precipitate 34 ml. of ethanol were slowly added, with efficient stirring and cooling in an ice bath; the final ethanol concentration was 22 volumes per cent. After 15 minutes the precipitate was centrifuged and suspended in 35 ml. of NaCl-$NaHCO_3$, and the solution was clarified by centrifugation (precipitate, 22 per cent ethanol). (*d*) 136 ml. of supernatant from the first ethanol precipitation were treated in the same manner with a further 50 ml. of ethanol, to give a final ethanol concentration of 43 volumes per cent. The precipitate was centrifuged and dissolved in NaCl-$NaHCO_3$, and the slight turbidity was removed by centrifugation (precipitate, 22 to 43 per cent ethanol).

It can be seen from the data of Table I that 35 per cent of the original activity was obtained in the fraction precipitating between 22 and 43 volumes per cent of ethanol; this fraction has an activity per unit protein 10 times as great as that of the original extract and is free from adenosinetriphosphatase activity. This degree of purification is consistently achieved, and the yield of activity is usually greater than in this particular example (up to 50 per cent of the original activity). Different preparations of this fraction were used for the experiments with the pigeon liver enzyme which are reported in this paper. A repetition of the isoelectric precipitation and ethanol fractionation has given a preparation 20 times as active per unit protein as the original extract.

The enzyme activity in both the original dialyzed extracts and the purified fractions is quite stable. The preparations may be kept at 2° for several weeks without significant loss in activity. The activity of the original extracts is not diminished by incubation for 3 hours at 30°. The heat stability of the enzyme in the crude extracts was tested by heating for 5 minutes at various temperatures in the presence of 0.05 M L-glutamate, which was added to protect specifically the glutamine enzyme. No activity was lost at temperatures up to 50°, but complete inactivation occurred at 60°. No differential effect on glutamine synthesis and adenosinetriphosphatase activity was observed.

The enzyme is associated with a protein fraction having the solubility properties of a euglobulin. When crude extracts or purified fractions are thoroughly dialyzed against distilled water, a precipitate appears which contains all the enzyme activity and which can be redissolved in 0.15 M NaCl-0.005 M $NaHCO_3$. Nearly all the protein of the purified fraction is precipitated by water dialysis.

It is not yet clear whether the pigeon liver preparations contain one or more than one protein component essential for the over-all enzymatic reaction.

Stoichiometry of Reaction—The formation of amide by pigeon liver preparations requires the simultaneous presence of glutamate, amine, adenosine triphosphate, and magnesium ions. Omission of any one of these components leads to complete inactivity in the synthesis. The stoichiometry is most clearly seen in experiments with ammonia as the amine, when measurements of disappearance of ammonia, formation of amide, and liberation of inorganic phosphate can be made. The amide is determined as ammonia released by heating with 1 N H_2SO_4 for 11 minutes at 100°, after previous removal of free ammonia by vacuum distillation. Table II gives the results of an experiment showing parallel utilization of ammonia and formation of amide and inorganic orthophosphate during various reaction periods. A series of 52 individual samples, similar to those described in

TABLE II

Stoichiometry of Glutamine Synthesis

The samples contained 0.1 M tris(hydroxymethyl)aminomethane buffer of pH 7.55, 0.01 M $MgSO_4$, 0.04 M HCN of pH 7.4, 0.05 M L-glutamate, 0.01 M NH_4Cl, 0.006 M adenosine triphosphate, and 0.05 ml. of purified pigeon liver enzyme (31 γ of N) in 1.0 ml. Temperature 30°; times as indicated. The figures given are micromoles per ml. of reaction mixture.

Time	Ammonia used	Glutamine formed	Phosphate liberated
min.			
5	1.7	1.04	1.80
10	3.1	2.08	2.96
20	3.9	4.08	3.94
30	4.2	4.72	4.83

Table II but including variations in pH, in quantity of enzyme, and in concentration of adenosine triphosphate and ammonia, gave the following average ratios: 1.05 moles of ammonia used, 1.00 mole of amide formed, and 1.00 mole of inorganic phosphate liberated. In the individual samples amide and phosphate were always formed in proportions very close to 1:1, but ammonia utilization was more variable, probably because it was determined less accurately as the difference between large initial and final ammonia values.

These results indicate the formation of a monoamide of glutamic acid. This amide is very labile and is completely split by acid under the same conditions which give quantitative results with authentic samples of glutamine, the γ-amide of glutamic acid. The observations of Melville (16) show that the α-amide, isoglutamine, is much more stable and is comparable to asparagine, which requires 3 hours for complete hydrolysis by 1 N

H_2SO_4 at 100° (17). The formation of glutamine by pigeon liver is known from the work of Örström *et al.* (4), who isolated in crystalline form an amide produced by pigeon liver slices from pyruvate and ammonia and identified it as glutamine hydrochloride. It may be concluded that the amide formed in the present experiments is glutamine.

Two observations indicate that adenosine diphosphate is the end-product formed from adenosine triphosphate. First, when samples containing relatively large amounts of enzyme are incubated for long periods of time, the reaction stops when 1 mole of glutamine and 1 mole of inorganic phosphate have been formed for each mole of adenosine triphosphate added. Second, adenosine diphosphate itself cannot be utilized in the test system, unless the enzyme myokinase is added to convert it in part to adenosine triphosphate.

On the basis of these findings the reaction may be written as shown in Equation 1.

$$\text{Glutamate} + \text{ammonia} + \text{adenosine triphosphate} \rightarrow \text{glutamine} + \text{adenosine diphosphate} + \text{phosphate} \quad (1)$$

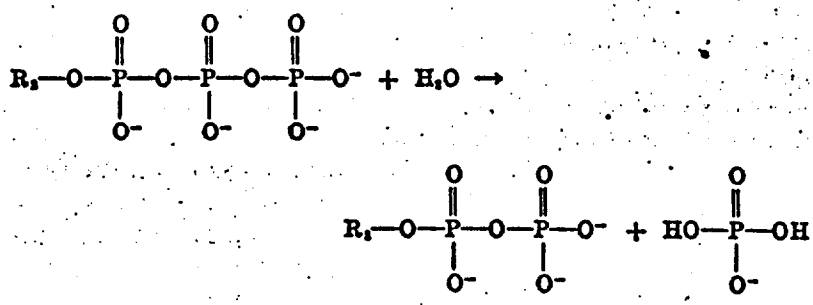
When hydroxylamine is substituted for ammonia, a hydroxamic acid is formed, which can be determined colorimetrically by reaction with $FeCl_3$, and at the same time phosphate is liberated from adenosine triphosphate. Data from several individual experiments are presented later, and only average values are given here. A series of 154 samples, similar in composition to the reaction system for enzyme assay but including variations of reaction time, enzyme level, pH, and concentration of each of the reactants, gave the following relation: for 1.00 mole of inorganic phosphate released, 1.12 moles of succinyl hydroxamic acid equivalent were formed. The proportion was not always constant, varying from 0.98 to 1.80 as extremes; this was due in part to variability in the hydroxamic acid determination. The changes were not related to alterations in the conditions of the enzyme reaction. The failure to observe a 1:1 relation between phosphate liberation and hydroxamic acid formation probably results from the use of a standard of succinyl hydroxamic acid, which may not give the same color per mole as the hydroxamic acid formed from glutamate.

Reaction between adenosine triphosphate, glutamate, and ammonia at neutral pH should result in the formation of H^+ ions. For purposes of calculation the over-all process may be divided into two steps: (1) combination of carboxylation ion and ammonium ion (Equation 2),[1]

$$R_1COO^- + NH_4^+ \rightarrow R_1CONH_2 + H_2O \quad (2)$$

[1] R_1 represents $COO^-CHNH_3^+CH_2CH_2^-$.

which involves no acid-base change, and (2) splitting of inorganic phosphate from adenosine triphosphate (Equation 3),[3]



(3)

which results in the formation of one acid group (inorganic phosphate) with a pK′ of 6.8. Therefore, at pH 7.4, for each mole of glutamine (or phosphate) formed, 0.80 mole of H^+ ion should be released. When hydroxylamine, with a pK′ of about 5.9, replaces ammonia, the first step becomes (Equation 4)

$$R_1COO^- + NH_2OH \rightarrow RCONHOH + OH^- \quad (4)$$

It can be calculated that 0.97 mole of OH^- ion should be released in this step; so that the over-all acid-base change in hydroxamic acid formation at pH 7.4 should be liberation of 0.17 mole of OH^- ion. In the same way it can be estimated that reaction with methylamine, pK 10.6, should release 0.80 mole of H^+ ion, as in the case of ammonia, and that reaction with hydrazine, pK 8.5, should release 0.73 mole of H^+ ion. Table III gives the results of an experiment with different amines in bicarbonate buffer. Acid-base changes were followed by measuring manometrically the CO_2 released or absorbed, and formation of inorganic phosphate in the same samples was used as a measure of amide synthesis. The observed evolution of CO_2 is in rather good agreement with that calculated by multiplying the phosphate by the factors described above.

Intermediates—Since it seemed probable that a process involving so many components as glutamine synthesis proceeds in steps, attempts were made to detect intermediate compounds.

Reaction of adenosine triphosphate with glutamate might produce such compounds as γ-glutamyl phosphate or γ-glutamyl adenosine diphosphate, which then could react with an amine to yield the amide. It might be expected that enzymatic catalysis would be necessary for the first step and perhaps also for the second. To test for the formation of an intermediate from glutamate, reaction mixtures containing buffer, adenosine triphosphate, glutamate, Mg^{++} ions, cyanide, and purified pigeon liver enzyme

[3] R_2OH represents adenosine.

were incubated for various times, and the enzyme was then inactivated by mild treatment so as to preserve labile compounds. Heating for 30 seconds in boiling water and immediately cooling and treating with ice-cold trichloroacetic acid and immediately neutralizing were two methods used; a substance like acetyl phosphate would suffer relatively little decomposition during such procedures. The samples were then analyzed for acyl phosphates by the hydroxamic acid method of Lipmann and Tuttle (14) (an acyl adenosine diphosphate might also react in this determination) and by differential phosphate determinations according to Lowry and Lopez (18) and Gomori (12). No intermediate was detected. It may be mentioned that if the enzyme was not inactivated before the addition of neu-

TABLE III

Acid Formation during Amide Synthesis

The samples were made up in Warburg vessels and contained 0.03 M $NaHCO_3$, 0.01 M $MgSO_4$, 0.04 M HCN of pH 7.4, 0.05 M L-glutamate, 0.02 M amine, 0.006 M adenosine triphosphate (tipped in from the side arm after temperature equilibration), and 0.10 ml. of purified pigeon liver enzyme (160 γ of N) in 2.0 ml. Gas phase 5 per cent CO_2-95 per cent N_2; temperature 30°; time 30 minutes. The figures given are micromoles per ml. of reaction mixture. Calculated CO_2 evolution was obtained by multiplying the phosphate formed by the factors given in the text.

Amine	Phosphate liberated	CO_2 evolution calculated	CO_2 evolution observed
None	0.05	0.04	0.2
Ammonium chloride	4.1	3.3	3.1
Hydroxylamine	3.4	−0.6	0.4
Hydrazine hydrochloride	3.2	2.3	2.3
Methylamine hydrochloride	2.3	1.8	2.0

tral hydroxylamine, as in the original method of Lipmann and Tuttle (14), hydroxamic acid was always found. Since the pigeon liver enzyme remains completely active in the presence of high concentrations of hydroxylamine, hydroxamic acid formation under these conditions cannot be regarded as evidence for the enzymatic synthesis and accumulation of an acyl phosphate which subsequently reacts non-enzymatically with hydroxylamine.

Adenosine triphosphate might yield phosphate or adenosine diphosphate derivatives of the amines, which then could react with glutamate to give the corresponding amides. To test this possibility, reaction mixtures containing buffer, adenosine triphosphate, hydroxylamine, Mg^{++} ions, cyanide, and enzyme were incubated for various times, and the enzyme was then inactivated. Differential phosphate analyses failed to show accumulation

of labile phosphate compounds. Addition of glutamate did not result in hydroxamic acid formation; this indicates that intermediates capable of reacting non-enzymatically with glutamate were not present. One intermediate which might be formed from adenosine triphosphate and ammonia, amidophosphate, was prepared synthetically and tested with the purified pigeon liver enzyme. Amidophosphate could not replace adenosine triphosphate plus ammonia in reacting with glutamate to form glutamine. It was slowly hydrolyzed by the enzyme preparations and could partially replace ammonia, provided adenosine triphosphate was also added. This

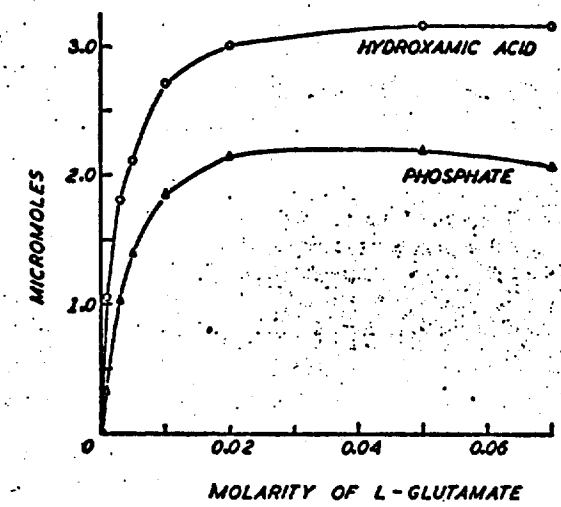


FIG. 1. Effect of glutamate concentration on the rate of amide synthesis and phosphate liberation. The samples contained 0.01 M $MgSO_4$, 0.04 M HCN of pH 7, 0.4 M NH_2OH of pH 7.0, L-glutamate in the concentrations shown, 0.006 M adenosine triphosphate, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Temperature 30°; time 10 minutes. The figures given are micromoles per ml. of reaction mixture.

lack of reactivity indicates that amidophosphate is probably not an intermediate in glutamine synthesis.

These negative results are not conclusive evidence for a reaction mechanism involving only one step or against a mechanism involving two or more steps. The experimental procedures available were not completely satisfactory for detecting the types of intermediates suggested above. If intermediates exist, they may be exceedingly labile or may not accumulate in the incomplete systems in sufficient quantities for analysis.

The following sections describe the effects of varying the conditions of reaction and the components of the system.

Glutamate—In Fig. 1 the rate of amide synthesis is plotted as a function

of the level of L-glutamate. A glutamate concentration of 0.05 M is required to give a maximum rate. The method of Lineweaver and Burk (19) was used in calculating the glutamate-enzyme dissociation constant from such data. Data from three experiments gave an average value for the dissociation constant of 0.0027 M (range 0.0011 to 0.0038 M).

A number of other carboxylic acids were tested for their ability to replace glutamate in forming a hydroxamic acid, but none were effective, either in the original dialyzed extracts or in the purified fraction. The substances tested included formate, acetate, butyrate, succinate, fumarate, *cis*-aconitate, lactate, malate, citrate, pyruvate, oxalacetate, α-ketoglutarate, glycine, alanine, valine, leucine, isoleucine, serine, threonine, hydroxy-

TABLE IV

Activity of Various Amines in Amide Synthesis

The samples contained 0.1 M tris(hydroxymethyl)aminomethane buffer of pH 7.0, 0.01 M $MgSO_4$, 0.04 M HCN of pH 7, 0.05 M L-glutamate, 0.02 M amine, 0.006 M adenosine triphosphate, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Temperature 30°; time 10 minutes. The figures given are micromoles per ml. of reaction mixture.

System	Phosphate liberated
No glutamate or amine	0.06
With glutamate, no amine	0.18
Glutamate + methylamine hydrochloride	2.02
" + ammonium chloride	2.23
" + hydroxylamine	2.28
" + hydrazine hydrochloride	2.60
" + L-cysteine	0.78

proline, cysteine, methionine, aspartate, phenylalanine, tyrosine, tryptophan, histidine, arginine, lysine, and *p*-aminobenzoate.

Amine—The ability of various amines to react with glutamate plus adenosine triphosphate was tested by taking advantage of the fact that in the absence of amines there occurs virtually no breakdown of adenosine triphosphate, while in the presence of a reactive amine inorganic phosphate is liberated. Ammonia, hydroxylamine, hydrazine, and methylamine were found to react at approximately the same rates (Table IV) and cysteine more slowly. The following amines and amino acids gave negative results by this testing procedure: dimethylamine, trimethylamine, *p*-aminobenzoate, glycine, alanine, valine, leucine, isoleucine, serine, threonine, hydroxyproline, methionine, aspartate, phenylalanine, tyrosine, tryptophan, histidine, arginine, and lysine.

It has previously been indicated that the reaction product formed with

ammonia is glutamine, the γ-amide of glutamic acid. Hydroxylamine yields a substance giving a violet color with $FeCl_3$, characteristic of a hydroxamic acid, an N-substituted amide. It is very probable that all the amines effective in the testing procedure described above react in the same

TABLE V

Competition of Amines in Amide Synthesis

The samples contained 0.1 M tris(hydroxymethyl)aminomethane buffer of pH 7.0, 0.01 M $MgSO_4$, 0.04 M HCN of pH 7, 0.05 M L-glutamate, 0.02 M amine, 0.006 M adenosine triphosphate, and 0.10 ml. of purified pigeon liver enzyme (150 γ of N) in 1.0 ml. Temperature 30°, time 10 minutes. The figures given are micromoles per ml. of reaction mixture.

Amine	Hydroxamic acid formed	Phosphate liberated
Hydroxylamine	3.20	2.78
Ammonia		3.28
Hydrazine		3.76
Methylamine		2.18
Hydroxylamine + ammonia	1.09	3.16
" + hydrazine	1.75	3.58
" + methylamine	2.67	2.89

TABLE VI

Effect of Ammonia Concentration on Glutamine Synthesis

The samples contained 0.1 M tris(hydroxymethyl)aminomethane buffer of pH 7.5, 0.01 M $MgSO_4$, 0.04 M HCN of pH 7.5, 0.05 M L-glutamate, NH_4Cl as indicated, 0.006 M adenosine triphosphate, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Temperature 30°; time 20 minutes. The figures given are micromoles per ml. of reaction mixture.

NH_4Cl added	Glutamine formed	Phosphate liberated
0.0	0.27	0.14
1.0	1.45	1.20
3.0	3.08	3.20
5.0	5.11	5.04
10.0	5.25	5.34
20.0	5.02	5.07

way, and this supposition is supported by the observation that the other amines are competitive with respect to hydroxylamine. When added along with hydroxylamine, they depress hydroxamic acid formation, while total amide synthesis as measured by phosphate liberation proceeds at a rate intermediate between those observed with the two amines separately (Table V).

The dissociation constants for reversible combination of ammonia or hydroxylamine with the enzyme are probably quite low. When the concentration of amine is decreased, the rate of reaction is not diminished until the absolute quantity of amine limits stoichiometrically the extent of reaction. Illustrative data for ammonia are given in Table VI.

Adenosine Triphosphate—Curves showing the relation between rate of amide synthesis and adenosine triphosphate concentration are given in Fig. 2. No amide is formed when adenosine triphosphate is omitted.

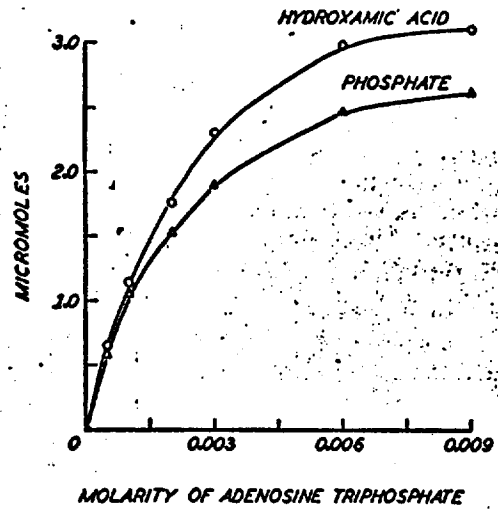


FIG. 2. Effect of adenosine triphosphate concentration on the rate of amide synthesis and phosphate liberation. The samples contained 0.01 M $MgSO_4$, 0.04 M HCN of pH 7, 0.05 M L-glutamate, 0.4 M NH_2OH of pH 7.0, adenosine triphosphate in the concentrations shown, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Temperature 30°, time 10 minutes. The figures given are micromoles per ml. of reaction mixture.

Application of the method of Lineweaver and Burk (19) to such data gave an average value for the adenosine triphosphate-enzyme dissociation constant of 0.0025 M (three experiments, range 0.0020 to 0.0028 M). It is not practical in routine experiments to add sufficient adenosine triphosphate to saturate the enzyme (a concentration of at least 0.02 M would be required); instead the initial concentration of adenosine triphosphate was kept uniform in samples which were to be compared.

Adenosine-5-monophosphate and adenosine diphosphate cannot replace adenosine triphosphate in the usual test system, but adenosine diphosphate is active if the enzyme myokinase is added to convert it to adenosine triphosphate plus adenosine monophosphate (Table VII). Both adenosine diphosphate and adenosine monophosphate inhibit amide synthesis when

they are present along with adenosine triphosphate. Since it seemed likely that this represented a competitive inhibition, rates of reaction were determined for samples containing various concentrations of adenosine triphosphate in the presence and absence of different concentrations of adenosine monophosphate and diphosphate, and the data were analyzed according to Lineweaver and Burk (19). The results agreed with the hypothesis of competitive inhibition, and the dissociation constants for both adenosine monophosphate and adenosine diphosphate and the enzyme were estimated to be about 0.01 M.

TABLE VII

Effect of Adenosine Monophosphate and Adenosine Diphosphate on Amide Synthesis

The samples contained 0.01 M $MgSO_4$, 0.04 M HCN of pH 7, 0.05 M L-glutamate, 0.4 M NH_2OH of pH 7.0, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Adenine nucleotides were added in a concentration of 0.006 M. Temperature 30°; time 10 minutes. The figures given are micromoles per ml. of reaction mixture. Myokinase was prepared according to Colowick and Kalckar (11) and lyophilized.

Addition	Hydroxamic acid formed	Phosphate liberated
No nucleotide	0.03	0.07
Adenosine-5-monophosphate	0.00	0.14
Adenosine diphosphate	0.27	0.13
" " + 7 mg. myokinase	1.84	1.52
" triphosphate	3.31	2.28
" " + adenosine monophosphate	2.74	2.16
" " + " diphosphate	2.54	1.51

Magnesium Ions—Addition of Mg^{++} ions is essential for amide synthesis. Variation of reaction rate with Mg^{++} concentration is shown in Fig. 3; a maximum rate is obtained with 0.01 M $MgSO_4$. The curve is of such a shape as to suggest combination of more than one Mg^{++} ion with each molecule of enzyme. Mn^{++} ions can replace Mg^{++} ions; the optimal concentration of Mn^{++} is 0.002 M and the maximum rate is only half that obtained with Mg^{++}.

Reducing Agents—Addition of cysteine, glutathione, or cyanide is necessary to achieve maximum rates of amide formation with crude or purified liver enzyme preparations (Table VIII). Probably free sulfhydryl groups on the enzyme protein are essential for activity. Cyanide gives the most rapid reaction rates, and a concentration of 0.01 M is sufficient to produce a maximum effect. When a reducing agent is present, the reaction rate is the same with either air or nitrogen in the gas phase. In perform-

ing all enzyme experiments, the enzyme and reducing agent were mixed in an incomplete reaction system which was incubated for 5 minutes before adenosine triphosphate was added to start amide synthesis.

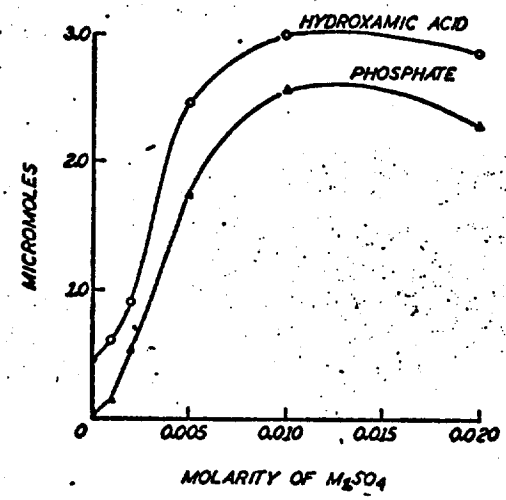


FIG. 3. Effect of Mg^{++} concentration on the rate of amide synthesis and phosphate liberation. The samples contained $MgSO_4$ in the concentrations shown, 0.04 M HCN of pH 7, 0.05 M L-glutamate, 0.4 M NH_2OH of pH 7.0, 0.006 M adenosine triphosphate, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Temperature 30°; time 10 minutes. The figures given are micromoles per ml. of reaction mixture.

TABLE VIII

Effect of Reducing Agents on Amide Synthesis

The samples contained 0.01 M $MgSO_4$, 0.05 M L-glutamate, 0.4 M NH_2OH of pH 7.0, 0.006 M adenosine triphosphate, reducing agents in a concentration of 0.04 M, and 0.13 ml. of purified pigeon liver enzyme (81 γ of N) in 1.0 ml. Gas phase air; temperature 30°; time 10 minutes. The figures given are micromoles per ml. of reaction mixture.

Reducing agent	Hydroxamic acid formed	Phosphate liberated
None	2.31	1.93
HCN	3.42	2.86
Cysteine	2.96	2.56
Glutathione	2.86	2.49

pH—If hydroxylamine is added as the amine component and hydroxamic acid formation is measured, the reaction occurs most rapidly when the pH of the buffer is 7.0 (Fig. 4). The rate falls off rapidly on the acid

side and more slowly on the alkaline side of this pH. When ammonia is the amine and glutamine synthesis is measured, the optimal pH lies between 7.5 and 8.0. This difference may be related to the difference be-

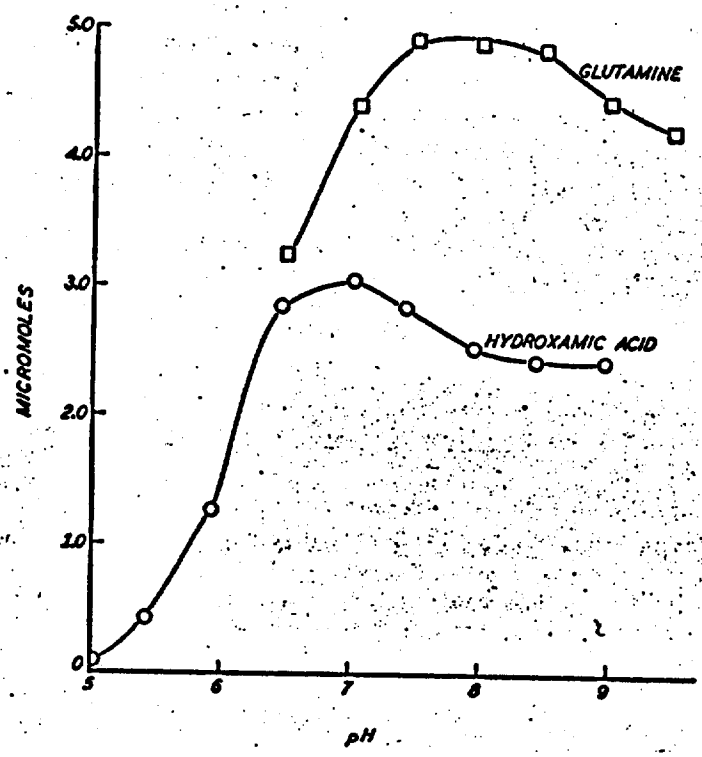


FIG. 4. Effect of pH on the rate of amide synthesis. The hydroxamic acid samples contained 0.01 M $MgSO_4$, 0.04 M HCN, 0.05 M L-glutamate, 0.4 M NH_2OH at the pH shown, 0.006 M adenosine triphosphate, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Temperature 30°; time 10 minutes. The glutamine samples contained 0.1 M buffer of the pH shown, 0.01 M $MgSO_4$, 0.04 M HCN, 0.05 M L-glutamate, 0.02 M NH_4Cl, 0.006 M adenosine triphosphate, and 0.10 ml. of purified pigeon liver enzyme in 1.0 ml. Temperature 30°; time 20 minutes. Tris(hydroxymethyl)aminomethane buffer was used up to pH 8 and 2-amino-2-methyl-1,3-propanediol buffer (15) from pH 8.5 to 9.5. The figures given are micromoles per ml. of reaction mixture.

tween the pK′ values of the two bases (5.9 for hydroxylamine and 9.4 for ammonia).

Other Components—It is unlikely that other substances readily dissociable from the enzyme protein are essential for glutamine synthesis by pigeon liver preparations. The enzyme may be dialyzed for long periods of time without inactivation, and during purification large losses of total activity

do not occur. In particular the cofactor containing pantothenic acid which is required for acetylation of sulfanilamide (20) and choline (21) is not involved in glutamine formation. Prolonged storage in the ice box or incubation for a few hours at room temperature does not reduce the activity of pigeon liver extracts in synthesizing glutamine, although such

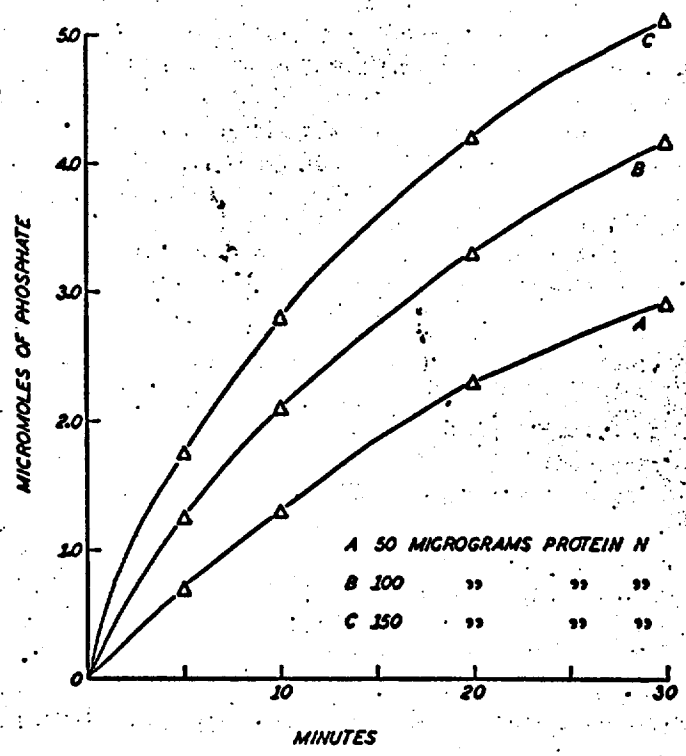


FIG. 5. Kinetics of phosphate liberation during amide synthesis. The samples contained 0.1 M tris(hydroxymethyl)aminomethane buffer of pH 7.0, 0.04 M HCN of pH 7, 0.01 M $MgSO_4$, 0.05 M L-glutamate, 0.05 M NH_2OH of pH 7, 0.006 M adenosine triphosphate, and the indicated quantities of purified pigeon liver enzyme in 1.0 ml. Temperature 30°; times as shown. The figures given are micromoles per ml. of reaction mixture.

treatment completely abolishes the capacity for acetylation of sulfanilamide by causing destruction of the coenzyme (22).[3]

Kinetics—The course of amide synthesis over a period of 30 minutes with different quantities of enzyme is represented in Fig. 5. Hydroxyl-

[3] In a personal communication Dr. Fritz Lipmann has stated that addition of a purified preparation of his coenzyme A to dialyzed pigeon liver extracts does not accelerate hydroxamic acid formation from glutamate.

amine was present as the amine, but only data for phosphate liberation from adenosine triphosphate are given. In this test system all the components except adenosine triphosphate are present in sufficient excess to saturate the enzyme protein for the whole reaction period. Calculations indicate that the reaction rate falls off too rapidly to be first order with respect to adenosine triphosphate. By use of the experimentally determined dissociation constants and the concentrations of adenosine diphosphate (equal to the inorganic phosphate liberated) and of adenosine triphosphate (from the initial concentration by difference), the concentration of the adenosine triphosphate-enzyme complex at each time interval can be calculated, but the observed reaction rates decline more rapidly than the concentration of

TABLE IX

Effect of Sodium Fluoride on Amide Synthesis

The samples contained 0.01 M $MgSO_4$, 0.04 M HCN of pH 7, 0.05 M L-glutamate, 0.2 M NH_2OH of pH 7.0, 0.006 M adenosine triphosphate, sodium fluoride as indicated, and 0.10 ml. of purified pigeon liver enzyme (62 γ of N) in 1.0 ml. Temperature 30°; time 10 minutes. The figures given are micromoles per ml. of reaction mixture.

NaF added	Hydroxamic acid formed	Phosphate liberated
M		
0.0	3.94	3.30
0.0001	3.55	2.73
0.0003	2.61	2.22
0.001	1.55	1.07
0.003	0.64	0.35
0.01	0.39	0.06
0.03	0.42	0.07

this complex. A simple formulation of the kinetics is not apparent at present.

The variation of reaction rate with temperature was studied over the range 10–40°, with phosphate liberation in a system containing hydroxylamine as a measure of rate. The following temperature coefficients (Q_{10}) were observed: 3.1 for the temperature interval 10–20°, 2.1 for the interval 20–30°, and 1.8 for the interval 30–40°.

Inhibitors—The formation of amide by pigeon liver enzyme preparations is strongly depressed by sodium fluoride in low concentrations. Illustrative data are given in Table IX. The inhibition is apparent with 0.0001 M fluoride and is nearly complete with 0.01 M fluoride. The inhibitory action of sodium fluoride on glutamine synthesis in fresh pigeon liver dispersions (2) is probably a result of this specific effect on the linking of glutamate and ammonia.

Methionine sulfoxide was found by Waelsch *et al.* (9) to inhibit the

growth of lactobacilli; the inhibition could be overcome by addition of large amounts of glutamate or small amounts of glutamine, and it was suggested that methionine sulfoxide acts as a competitive inhibitor for the formation of glutamine from glutamate. McIlwain *et al.* (23) have shown that methionine sulfoxide prevents formation of glutamine by streptococci. Recently Elliott and Gale (24) have demonstrated directly that methionine sulfoxide is a competitive inhibitor with respect to glutamate for the formation of glutamine from glutamate, ammonia, and adenosine triphosphate in extracts of staphylococci. DL-Methionine sulfoxide also depresses the synthesis of glutamine by purified pigeon liver preparations. Experiments with varying concentrations of glutamate and methionine sulfoxide were run, and analysis of the results by the method of Lineweaver and Burk (19) showed that methionine sulfoxide was competing with glutamate for combination with the enzyme. The effects are much smaller than those observed with the bacterial extracts; with pigeon liver enzyme 0.09 M DL-methionine sulfoxide in the presence of 0.01 M L-glutamate causes 50 per cent inhibition, while with staphylococcus extracts 0.011 M methionine sulfoxide in the presence of 0.033 M L-glutamate causes 72 per cent inhibition. This variation results from differences in the dissociation constants for the glutamate and methionine sulfoxide complexes of the two enzyme proteins. With purified pigeon liver enzyme the dissociation constant for L-glutamate is 0.0027 M and for DL-methionine sulfoxide about 0.03 M. From the graph given by Elliott and Gale (24) the dissociation constants for the staphylococcus enzyme can be estimated as 0.03 M for L-glutamate and 0.002 M for methionine sulfoxide. The lower dissociation constant for methionine sulfoxide and the higher constant for glutamate should make the bacterial enzyme much more susceptible to competitive inhibition than the pigeon liver enzyme.

Crystal violet has been reported to inhibit glutamate metabolism in Gram-positive cocci (25) and glutamine synthesis in extracts of *Staphylococcus aureus* (24), and it was found to depress glutamine formation in fresh pigeon liver dispersions (2). In concentrations up to 0.001 M this dye does not affect the rate of glutamine synthesis in pigeon liver extracts, although such concentrations completely inhibit amide formation in pigeon liver dispersions or bacterial extracts. Probably crystal violet depresses glutamine synthesis in pigeon liver dispersions by interfering with the supply of oxidative energy available through respiration, since oxygen uptake is also inhibited. The difference between extracts of pigeon liver and of bacteria in sensitivity to crystal violet presumably is due to differences in the enzyme proteins, since the reactions catalyzed by the two preparations are the same (adenosine triphosphate, glutamate, and ammonia, reacting in the presence of Mg^{++} ions and reducing agents to give glutamine).

Distribution of Enzyme Activity—Acetone powders of brain, liver, and kidney of various species were extracted with bicarbonate solutions and tested for activity by the hydroxamic acid procedure. Single batches of these powders, sometimes rather old, were used; so that for the most part quantitative comparisons of activity could not be made. Some activity was found in all the acetone powders tested; these included pigeon liver and brain, rat liver, kidney, and brain, guinea pig liver, kidney, and brain, rabbit liver, kidney, and brain, cat liver, kidney, and brain, beef liver, and pig liver. When comparable powders of different tissues from a single species were available, it was found that brain gave more active enzyme preparations than did liver or kidney (except for the pigeon). Pigeon liver powder was the best source of enzyme in terms of total activity per unit weight of powder or of activity per unit of extracted protein. The various acetone powder extracts also caused hydrolysis of adenosine triphosphate, but those preparations which formed hydroxamic acid readily also split inorganic phosphate from adenosine triphosphate more rapidly when the components of the amide-synthesizing system (glutamate plus hydroxylamine) were present than when they were omitted. This observation, together with the fact that no hydroxamic acid was formed when adenosine triphosphate was not added, indicates that the enzymes from other sources catalyze the same sort of reaction between adenosine triphosphate, glutamate, and amine that has been demonstrated for the pigeon liver enzyme.

DISCUSSION

These studies show that the synthesis of glutamine in enzyme preparations from pigeon liver involves a reaction between glutamate, ammonia, and adenosine triphosphate, in the presence of magnesium ions, to form the amide, adenosine diphosphate, and inorganic phosphate. Adenosine triphosphate supplies the energy necessary for the synthesis of the amide linkage by participating stoichiometrically in the reaction. Similar enzyme activity is found in brain, liver, and kidney of a number of vertebrates. Elliott (26) has described a similar reaction system for glutamine synthesis, using acetone-dried sheep brain as the source of enzyme, and Elliott and Gale (24) have obtained extracts of *Staphylococcus aureus* which bring about the same reaction. It is likely that an analogous mechanism is utilized in the synthesis of glutamine and asparagine in plants.

The enzymatic synthesis of glutamine shows interesting similarities to the synthesis of acetylcholine (27, 28) and of sulfanilamide (22, 29). In these reactions adenosine triphosphate is utilized as the source of energy for the formation of an amide or ester linkage. In all cases the enzymes are found in extracts of acetone powders, and reducing agents must be added for maximum activity. However, there are significant differences

among these systems. Mg^{++} ions are necessary for the synthesis of glutamine and of acetylcholine (30) but not of acetyl sulfanilamide (22). A cofactor containing pantothenic acid is required for the acetylation reactions (20, 21) but not for glutamine formation.

Utilization of the energy of adenosine triphosphate for the synthesis of amide linkages seems to be a reaction of general importance. In addition to glutamine and acetyl sulfanilamide, *p*-aminohippuric acid (31) and hippuric acid (32) are probably formed in this way. Such a mechanism is also a plausible one for the synthesis of true peptide bonds between amino acids.

SUMMARY

Purified extracts of acetone-dried pigeon liver catalyze a stoichiometric reaction between adenosine triphosphate, glutamate, and ammonia, in the presence of Mg^{++} ions, to form glutamine, adenosine diphosphate, and inorganic phosphate. The preparation and some of the properties of the enzyme are described. The stoichiometry of the reaction is shown by measurements of amide formation, ammonia utilization, inorganic phosphate liberation, and H^+ ion formation.

In this system other carboxylic acids than glutamic acid do not form amides. Hydroxylamine, hydrazine, and methylamine can be substituted for ammonia; a hydroxamic acid is formed when hydroxylamine is present. Adenosine monophosphate and adenosine diphosphate cannot replace adenosine triphosphate in amide synthesis. Mn^{++} ions can be substituted for Mg^{++} ions. Reducing agents such as cyanide or sulfhydryl compounds must be added to obtain maximum rates of reaction.

Synthesis of glutamine is strongly depressed by low concentrations of sodium fluoride. High concentrations of methionine sulfoxide inhibit competitively with respect to glutamate.

Similar enzyme activity is found in acetone powder extracts of brain, liver, and kidney of a number of vertebrates.

It is a pleasure to acknowledge the assistance of Mrs. Mary Bandurski and Mrs. Anna-May McCreedy in performing these experiments.

BIBLIOGRAPHY

1. Speck, J. F., *J. Biol. Chem.*, 168, 403 (1947).
2. Speck, J. F., *J. Biol. Chem.*, 179, 1387 (1949).
3. Krebs, H. A., *Biochem. J.*, 29, 1951 (1935).
4. Örström, A., Örström, M., Krebs, H. A., and Eggleston, L. V., *Biochem. J.*, 33, 995 (1939).
5. Bujard, E., and Leuthardt, F., *Helv. physiol. et pharmacol. acta*, 5, C39 (1947).
6. Audrieth, L. F., and Toy, A. D. F., *J. Am. Chem. Soc.*, 63, 2117 (1941).

7. Stokes, H. N., *Am. Chem. J.*, 15, 193 (1893).
8. Toennies, G., and Kolb, J. J., *J. Biol. Chem.*, 128, 399 (1939).
9. Waelsch, H., Owades, P., Miller, H. K., and Borek, E., *J. Biol. Chem.*, 166, 273 (1946).
10. LePage, G. A., in Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric techniques and related methods for the study of tissue metabolism, Minneapolis, 181 (1945).
11. Colowick, S. P., and Kalckar, H. M., *J. Biol. Chem.*, 148, 117 (1943).
12. Gomori, G., *J. Lab. and Clin. Med.*, 27, 955 (1942).
13. Robinson, H. W., and Hogden, C. G., *J. Biol. Chem.*, 135, 707 (1940).
14. Lipmann, F., and Tuttle, L. C., *J. Biol. Chem.*, 159, 21 (1945).
15. Gomori, G., *Proc. Soc. Exp. Biol. and Med.*, 62, 33 (1946).
16. Melville, J., *Biochem. J.*, 29, 179 (1935).
17. Vickery, H. B., Pucher, G. W., Clark, H. E., Chibnall, A. C., and Westall, R. G., *Biochem. J.*, 29, 2710 (1935).
18. Lowry, O. H., and Lopez, J. A., *J. Biol. Chem.*, 162, 421 (1946).
19. Lineweaver, H., and Burk, D., *J. Am. Chem. Soc.*, 56, 658 (1934).
20. Lipmann, F., and Kaplan, N. O., *Federation Proc.*, 5, 145 (1946).
21. Lipmann, F., and Kaplan, N. O., *J. Biol. Chem.*, 162, 743 (1946).
22. Kaplan, N. O., and Lipmann, F., *J. Biol. Chem.*, 174, 37 (1948).
23. McIlwain, H., Roper, J. A., and Hughes, D. E., *Biochem. J.*, 42, 492 (1948).
24. Elliott, W. H., and Gale, E. F., *Nature*, 161, 129 (1948).
25. Gale, E. F., and Mitchell, P. D., *J. Gen. Microbiol.*, 1, 299 (1947).
26. Elliott, W. H., *Nature*, 161, 128 (1948).
27. Nachmansohn, D., and Machado, A. L., *J. Neurophysiol.*, 6, 397 (1943).
28. Nachmansohn, D., and John, H. M., *J. Biol. Chem.*, 158, 157 (1945).
29. Lipmann, F., *J. Biol. Chem.*, 160, 173 (1945).
30. Nachmansohn, D., and Berman, M., *J. Biol. Chem.*, 165, 551 (1946).
31. Cohen, P. P., and McGilvery, R. W., *J. Biol. Chem.*, 171, 121 (1947).
32. Borsook, H., and Dubnoff, J. W., *J. Biol. Chem.*, 168, 397 (1947).

TOXICOLOGY AND APPLIED PHARMACOLOGY 23, 635–639 (1972)

Electroencephalographic Study of L-Glutamate Induced Seizures in Rats[1]

CHARLES N. STEWART, DAVID B. COURSIN AND HEMMIGE N. BHAGAVAN

Department of Psychology, Franklin and Marshall College, Lancaster, Pennsylvania 17604, and Research Institute, St. Joseph Hospital, Lancaster, Pennsylvania 17604

Received March 23, 1972

Electroencephalographic Study of L-Glutamate Induced Seizures in Rats. STEWART, CHARLES, N., COURSIN, DAVID B. and BHAGAVAN, HEMMIGE, N. (1972). *Toxicol. Appl. Pharmacol.* 23, 635–639. The ip administration of L-glutamate was found to induce clonic movements and tonic-clonic seizures in rats at a dose level of 2.0 mmole/100 g body weight. The susceptibility to the seizures was not affected by dietary pyridoxine deficiency. The seizure episodes were characterized by 3–5 cps high amplitude spikes ranging from 200 μV/cm to 1000 μV/cm. In the interseizure periods records showed 1 cps high voltage slow waves. The results are discussed in terms of possible biochemical mechanisms operative in the glutamate induced seizures.

The incidence of epileptiform seizures in rats following peripheral administration of L-glutamate has been described previously (Bhagavan *et al.*, 1971), and their similarity to those reported by Wiechert and Herbst (1966) and Wiechert and Gollnitz (1968) following intracisternal injection of this compound has been noted. While Olney (1969, 1971) and Arees and Mayer (1970) have found lesions in the hypothalamus of infant rats and mice treated with L-glutamate, Adamo and Ratner (1970) and Oser *et al.* (1971) have been unable to replicate these findings. In none of the investigations to date, however, has there been an attempt to monitor the effects of peripherally administered L-glutamate using techniques of electroencephalographic (EEG) recording. Such a study is reported here using both normal and pyridoxine-deficient animals which were included because of the important role pyridoxine plays in the metabolism of L-glutamate and γ-aminobutyric acid.

METHODS

Male Sprague-Dawley rats[2] were raised from weanling (21 days) on either pyridoxine deficient or control diets[3] (Bhagavan *et al.*, 1971) and after 7 wk were operated on for purposes of electrode implanatation. Electrode placement was done with the rat under Na pentobarbital (40 mg/kg, ip) and chlorprothixene (4.4 mg/kg, ip) with the tips being

[1] This investigation was supported by National Institutes of Health (NB 05789 and GRS RR 05657), U.S. Public Health Service.

[2] Charles River Breeding Laboratories, Wilmington, Massachusetts 01887.

[3] Prepared for us by General Biochemicals Inc., Chagrin Falls, Ohio 44022.

Copyright © 1972 by Academic Press, Inc.
All rights of reproduction in any form reserved.

placed over the visual and motor cortex on the surface of the dura. A third ground electrode was placed in the os nasale to reduce movement artifact during convulsions. One week was allowed for postoperative recovery before L-glutamate injection and EEG recording. The rats used in this study were randomly selected from larger groups of rats being maintained on diets for other experiments in process and ranged in weight from 86 to 100 g ($N = 6$; deficient) to 365 to 450 g ($N = 5$; control).

Recordings were taken on a Beckman type R Dynograph for 20 min prior to and 2 hr following the ip injection of L-glutamate (monosodium) at a dose level of 2.0 mmoles/100 g body weight (2.5 mmoles/ml in distilled water, pH 7.0). During recording the animal was restrained by binding it loosely with an elastic bandage to a plexiglass platform located in a shielded chamber. Recordings were taken at an amplification of 100 μV/cm and at a speed of 10 mm/sec except during severe seizures when the amplification had to be reduced to 200 μV and occasionally to 500 μV.

RESULTS

Of the 6 pyridoxine-deficient animals injected with L-glutamate 3 were observed to have tonic-clonic seizures which were detectable both behaviorally as well as on the EEG. Of the 5 control animals, 2 were observed to display tonic-clonic seizures, and 1 displayed only spastic tremors accompanied by clonic movements of the front paws. The dietary treatment was therefore not associated with any altered susceptibility to L-glutamate induced seizures in this small sample of animals. Other aspects of the seizure pattern, i.e., running seizures noted in a previous publication (Bhagavan *et al.*, 1971) could not be detected in this study because of the physical restraint imposed upon the animals for purposes of recording the EEG.

In both control and pyridoxine-deficient animals the baseline preinjection record consisted of 4–6 cps waves at 50–100 μV/cm in control and 100–300 μV/cm in the deficient animals. In both groups the above pattern was interspersed with 3–4 cps slow waves and occasional spindles of 10–13 cps along with periodic superimposed fast activity. The time of onset of seizure patterns in the EEG ranged from 21 min to 103 min for both control and deficient animals. There were no prodromal signs of seizures onset in the recordings, in that the preseizure periods were characterized by patterns identical to those found in the preinjection period. The seizure patterns themselves which occurred while the animals were observed to be having tonic-clonic seizures were characterized at the onset by 3–5 cps high amplitude peaks ranging from 200 μV/cm in some rats to 1000 μV/cm in others. The highest amplitude seizures were noted in the deficient rats, but the small sample size precluded any statistical analysis of this phenomenon. The duration of these seizures episode varied in different animals from 2- or 3- sec bursts to a continuous seizure pattern of 16 min duration noted in 1 animal. The immediate postseizure periods were characterized by a marked reduction in amplitude (50–100 μV/cm) and frequency (1–2 cps), followed by a return to baseline and in some instances by 1–2 cps slow waves. These characteristics wave form are shown in Fig. 1, which represents a record obtained from a control animal. The preinjection record is found in line I, the onset of a tonic-clonic seizure in line II (at 40 min postinjection) and the last seizure displayed by this rat at 80 min post-injection in line III. Comparable recordings

FIG. 1. EE
clonic seizure

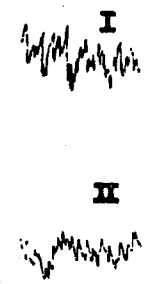


FIG. 2. EE
tonic-clonic se

FIG. 3. EE
control rat.

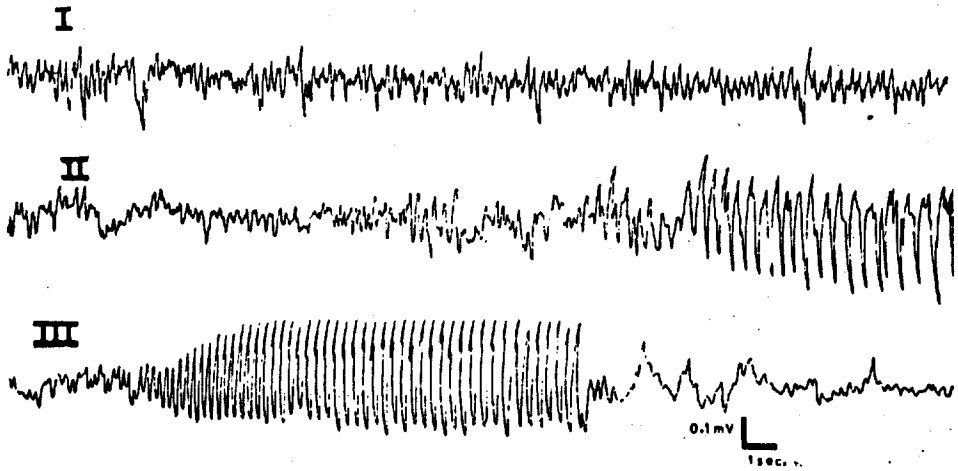


FIG. 1. EEG of L-glutamate injected control rat. (I) Preinjection record; (II) onset of first tonic-clonic seizure; (III) last seizure displayed by this rat.

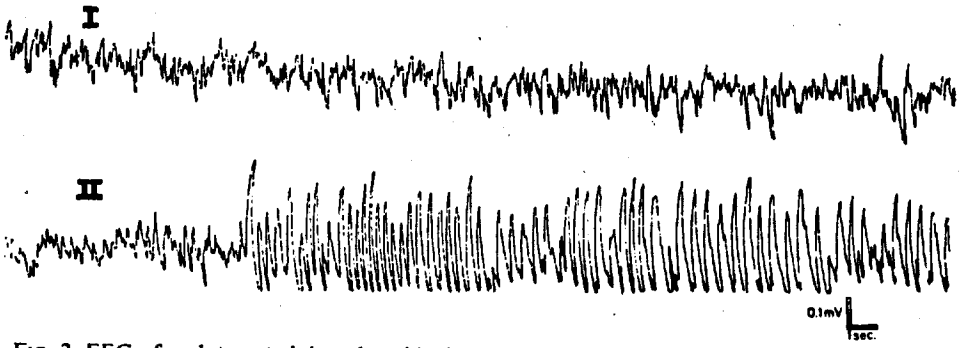


FIG. 2. EEG of L-glutamate injected pyridoxine-deficient rat. (I) Preinjection record; (II) onset of tonic-clonic seizure.

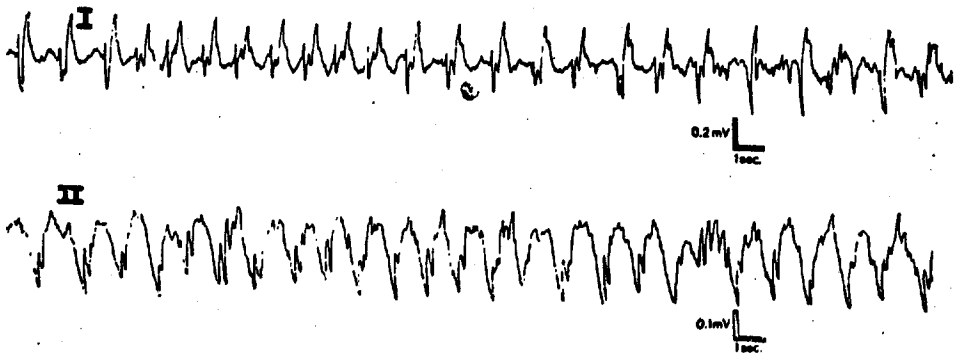


FIG. 3. EEG during period of inter-seizure slow wave activity. (I) Pyridoxine-deficient rat; (II) control rat.

from a pyridoxine-deficient rat during the preinjection and seizure are shown in Fig. 2 in lines I and II, respectively.

Figure 3 shows an inter-seizure recording from a deficient animal (line I, 200 μV/cm, 10 mm/sec) and a control animal (line II, 100 μV/cm, 10 mm/sec), which shows the characteristic 1 cps slow waves with occasional serrated domes in some animals between the tonic-clonic seizure episodes.

DISCUSSION

The seizures induced by the peripheral administration of L-glutamate are clearly reflected in the EEG tracings and are similar in form to the tonic seizures reported by Wiechert and Herbst (1966) following intracisternal administration in dogs. Although the pyridoxine-deficient animals exhibited consistently higher amplitudes of response than the controls, no difference was apparent with this sample size in the susceptibility and in the rate of onset of seizure between the deficient and the control rats. Using a lower dose of L-glutamate, Himwich *et al.* (1957) reported convulsions and dysrhythmia of the EEG in rats 10 days old but not in adults. The blood brain barrier (BBB) is not fully developed in the neonatal stage (Himwich and Himwich, 1955), so that the age discrepancy is not surprising if L-glutamate exerts its effect after its entry into the brain.

The induction of seizures by L-glutamate is consistent with the report of Prabhu and Oester (1971) that sc administration to mice (100 mg/100 g body weight) significantly lowered the threshold for metrazol-induced convulsions. This findings has been interpreted as a reflection of increased CNS excitability. This would be consistent with excitatory effects obtained upon direct microiontophoretic application of L-glutamate to the cerebral cortex (Curtis and Watkins, 1965; Steiner and Ruf, 1966; Crawford, 1970).

Since no neurochemical measures were taken in the present study, the EEG effects cannot be interpreted as due to direct effects of L-glutamate crossing the BBB to excite motor neurons in the cortex either directly or by altering the L-glutamate to GABA ratio, which previous workers (Elliot and van Gelder, 1958; Wiechert and Herbst, 1966) have considered to be critical in the control of neuronal excitability. Alternate and less direct effects of L-glutamate such as hypocalcemia due to chelation in the plasma bathing the brain must be given consideration, and a detailed study of the mechanism of induction of seizures by L-glutamate appears to be warranted.

ACKNOWLEDGMENTS

The authors wish to acknowledge the technical assistance of Robert Brackbill, John M. Koogler, Jr. and George F. Strutt.

REFERENCES

ADAMO, N. J. and RATNER, A. (1970). Monosodium glutamate: Lack of effect on brain and reproductive function in rats. *Science* **169**, 673–674.

ARIES, E. A. and MAYER, J. (1970). Monosodium glutamate-induced brain lesions: Electron-microscopic examination. *Science* **170**, 549–550.

BHAGAVAN, H. N., COURSIN, D. B. and STEWART, C. N. (1971). Monosodium glutamate induces convulsive disorders in rats. *Nature* **232**, 275–276.

CRAWFORD, J. I
acetylcholine.
CURTIS, D. R. an
aminobutyric
ELLIOT, K. A. a
acid by brain
HIMWICH, H. E.
glutamic acid
(H. Waelsch,
HIMWICH, W. A
function of ag
OLNEY, J. W. (
monosodium
OLNEY, J. W. (19
J. Neuropatho
OSER, B. L., CA
stration of m
411–413.
PRABHU, V. G. a
neonatal mon
STEINER, F. A.
activity in rat
Helv. Physiol.
WIECHERT, P. a
geschehens. I
ansgeloster K
WIECHERT, P. a
natural balan
59–64.

CRAWFORD, J. M. (1970). The sensitivity of cortical neurones to acidic amino acids and acetylcholine. *Brain Res.* **17**, 287–296.

CURTIS, D. R. and WATKINS, J. C. (1965). The pharmacology of amino acids related to gamma-aminobutyric acid. *Pharmacol. Rev.* **17**, 347–391.

ELLIOT, K. A. and VAN GELDER, N. M. (1958). Occlusion and metabolism of γ-aminobutyric acid by brain tissue. *J. Neurochem.* **3**, 28–40.

HIMWICH, H. E. and HIMWICH, W. A. (1955). The permeability of the blood-brain barrier to glutamic acid in the developing rat. In: *Biochemistry of the Developing Nervous System* (H. Waelsch, ed.), pp. 202–207. Academic Press, New York.

HIMWICH, W. A., PETERSEN, J. C. and ALLNE, M. L. (1957). Hematoencephalic exchange as a function of age. *Neurology* **7**, 705–710.

OLNEY, J. W. (1969). Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science* **164**, 719–721.

OLNEY, J. W. (1971). Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. *J. Neuropathol. Exp. Neurol.* **30**, 75–90.

OSER, B. L., CARSON, S., VOGIN, E. E. and COX, G. E. (1971). Oral and subcutaneous administration of monosodium glutamate to infant rodents and dogs. *Nature (London)* **229**, 411–413.

PRABHU, V. G. and OESTER, Y. T. (1971). Neuromuscluar functions of mature mice following neonatal monosodium glutamate. *Arch. Int. Pharmacodyn.* **189**, 59–71.

STEINER, F. A. and RUF, K. (1966). Excitatory effects of L-glutamic acid upon single unit activity in rat brain and their modification by thiosemicarbazide and pyridoxal-5′-phosphate. *Helv. Physiol. Acta* **24**, 181–192.

WIECHERT, P. and GOLLNITZ, G. (1968). Stoffwechseluntersuchungen des cerebralen Anfallsgeschehens. Die Aktivität der Glutamatdecarboxylase vor und während experimentell ansgeloster Krampfanfalle. *J. Neurochem.* **15**, 1265–1270.

WIECHERT, P. and HERBST, A. (1966). Provocation of cerebral seizures by derangement of the natural balance between glutamic acid and gamma-aminobutyric acid. *J. Neurochem.* **13**, 59–64.

(Ref.36)

The Effect of Perorally Administered Monosodium Glutamate on the Reproductive Function and Somatic Growth in Rats

- A Preliminary Report -

Yoshisuke Suzuki

Michio Takahashi

(Department of Veterinary Physiology,

Faculty of Agriculture, University of Tokyo)

The Effect of Perorally Administered Monosodium Glutamate on the Reproductive Function and Somatic Growth in Rats

- A Preliminary Report -

Introduction

Olney (1969) has reported that administration of MSG (0.5-4g/kg) induces obesity and female sterility in mice and rats due to hypothalamic degenerative lesion. To examine whether such symptoms could be induced by the continuous peroral administration of MSG, the present experiment was attempted.

Newborn mice and rats are nourished only by maternal milk until the day of eye opening (around 17 days after birth), but they start to feed themselves besides suckling after this age. From this point of view, we are in the opinion that, to examine the effects of peroral administration of MSG on the embryonic or neonatal animals, MSG should be given perorally to their mothers and the direct peroral administration should be started from around 17 days after birth.

Based upon such consideration, the present experiment was conducted on the experimental groups of animals as shown in Table 1, to see the effects of a long-term peroral MSG administration on the regularity in the recurrence of estrous cycles, reproductive functions and somatic growth. Since intake level of MSG could not be estimated exactly when test animals were fed ad libitum on MSG-containing diet, MSG corresponding to about 3 to 4g/kg body weight were given by a stomach tube everyday.

The experiment was carried out during a period between June 27, 1969 and February 21, 1970. Although histopathological examination of the brain and other organs is in progress at another laboratory and is not yet accomplished, the experimental results that have been obtained up to the present are summarized in this preliminary report.

Experimental Methods

The outline of the experimental design is shown in Table 1

Group M: Two adult female rats (M_1, M_2) of Wister strain (3 month old, 250-300mg body weight) showing regular estrous cycles of 4 days were selected from our own breeding colony and they were mated with an intact male. The day when sperms were first detected in the vaginal lavage was designated as the 1st day of pregnancy. From this day on, 2ml of aqueous solution containing 1g MSG was administered once everyday except for sundays and holidays by a stomach tube. The administration was continued also after delivery.

Group I - III: The number of litter from M_1 and M_2 was unified so that each litter consists of 4 females and 4 males on the 2nd day after birth. The offspring from M_1 and M_2 were designated as Group I and II, respectively, and they were nursed by their own mothers. Group III was composed of the remaining offspring from M_1 and M_2, 2 males and 6 females and they were nursed by an intact foster mother which had been delivered of the young 2 days before. Group I, the offspring from M_1, were given daily dose of MSG increasing with their age as follows: 0.25g/0.5ml after age 20 days, 0.5g/1.0ml after age 31 days and 1.0g/2.0ml after age 55 days on. The treatment was made everyday except for sundays and holidays throughout the experiment.

All the young were weaned 27 days after birth, and males and females were kept separately thereafter.

Group CM and C: In addition to the above mentioned groups, two intact mothers (CM_1 and CM_2) of the same age as Group M were mated and served as controls, and their young were also served as control Group C to be compared to Group I to III. These control groups were maintained in the same manner as the experimental groups, except that they were not treated with MSG.

All the animals were kept at 25°C under a light condition of 14L:10D (Light time; 5:00 - 19:00) and were given the standard diet of our laboratory and water ad libitum.

Items of observation:

In Groups M and CM were observed the courses of pregnancy, lactation, and recurrence of estrous cycles determined by daily examination of vaginal smear.

In Group I to III as well as Group C, the time of ear or eye opening, signs of sexual maturation in both sexes, course of recurrence of estrous cycles, and somatic growth were observed. In addition, the reproductive ability was examined around 100 days of age in Group I to III by sib-mating of one or two pairs from each group.

At the end of the experiment (February 21, 1970), mothers and their offspring were of ages 336 and 220 days, respectively, while the young of Group C were of age 214 days. Autopsy was executed in some animals of Group C and all of Groups I, II, III, M and CM were autopsied, although several animals subjected to mating tests or died of a false administration into the respiratory tract were excluded.

After general observation and weighing of various organs, some organs including brain were fixed with 10% neutralized formalin and were subjected to histopathological observations.

Experimental Results

1. Effects of perorally administered MSG on adult females

i) Effects on the pregnancy and lactation: In mothers which had received MSG perorally (M_1 and M_2), the placental sign appeared on the 13th to 14th day of pregnancy, and M_1 was delivered at the 22nd day of 6 females and 8 males, while M_2 of 10 females and 6 males. In control mothers (CM_1 and CM_2), the placental sign was observed on the 13th to 15th day, and CM_1 was delivered at the 22nd day of 7 females and 7 males while CM_2 of 5 females and 10 males. Body weights of these offspring at 2 days after birth are presented in Table 2. There was no appreciable difference between the MSG-treated groups and control one, in the course of pregnancy or delivery, littor size, sex proportion, and growth of the offspring. The period under the control of progesuten, in other words, the physiological persistent diestrous period after delivery, of M_1, M_2, CM_1 and CM_2 persisted for 22, 23, 22 and 23 days, respectively, as shown in Fig. 1. There was no significant difference between the groups. Later, one case of either groups (M_1 and CM_2) immediately recurred a regular estrous cycle of 4 days, but the remaining 2 cases (M_2 and CM_2) showed pseudopregnancy before recurrence of regular cycles, but this is not unusual. The growth curves of sucklings that would represent lactating ability of their mothers were not different between Group I or II and Group C (Fig. 3 and 4).

The results described above suggest that perorally administered MSG does not produce any noticeable effects on pregnancy and lactating function.

ii) Effects on the regularity in the recurrence of estrous cycles:

Group M received perorally 1g MSG everyday for more than 200 days including a period of pregnancy and lactation of 50 days. In this group, however, regular estrous cycles of 4 days recurred following lactation without any disturbances through the experiment as presented in Fig. 1. As M_2 had got into a habit of biting the stomach tube, anesthesia was necessary for peroral administration. Although pseudopregnancy was observed once in this case, it was hardly attributed to MSG treatment, but presumably due to anesthesia. This animal died of a false administration into the respiratory tract at the age of 318 days.

iii) Effects on the body weight: As shown in Fig. 2, changes in body weight during pregnancy and lactation did not differ significantly between the groups. In the further observation, abnormal weight gain suspected of obesity was never observed in the MSG-treated group. On the contrary, an intact mother (CM_1) showed unusual weight gain following lactation and weighed 450 g at autopsy. This is not considered to be obese, because such remarkable weight gain is sometimes encountered.

No appreciable changes were detected at autopsy in animals of Group M and CM.

2. Results in the offspring receiving continual peroral MSG load

The experimental results of the following groups were compared as stated below.

(a) offspring under the effect of maternally administered MSG through placenta (Group III); (b) those under the combined effects of MSG through placenta and milk (Group II); (c) those affected by MSG via placenta and milk and further treated perorally with about 3 to 4g MSG/kg everyday since the initiation of eating by themselves. (Goup I); (d) intact controls (Group C).

i) Effects on embryonic and neonatal animals and their sexual maturation:

As described already, there is no noticeable difference in the growth in embryonic and suckling stage between MSG-treated groups and control one (See Table 2 and Fig. 3 and 4).

As indices representing the somatic development, the day of ear or eye opening was compared between the groups. Descent of testis and changes of the pennis type from W into U in males and the day of viginal opening and the initiation of estrous cycles in females were also compared as indices for the signs of sexual maturation. As presented in Table 3 the results with all the MSG-treated groups were not different from those with controls.

ii) Effects on the growth: Fig. 3 and 4 show growth curves (changes in average body weights) of each group separated by sexes after birth. Weight gain was quite normal in MSG treated rats as well as controls until the time of autopsy at 220 days of age, except for one male of Group III (III-1) which weighed 600g at autopsy. This exceptional case was not considered to be obese due to MSG-treatment, because (i) such remarkable weight gain was never observed in the animals of Group I, Group II, both of which received severer MSG load, and in the remaining ones of this Group III, (ii) this tendency is sometimes encountered also in normal animals as observed in an intact mother (CM_1).

iii) Effects on the regularity in recurrence of estrous cycles:

Patterns of the recurrence of estrous cycles of each group are shown in Fig. 5 to 7. It is often experienced that the 4-day-cycle does not pass regularly in many normal females for some time after the appearance of the first cycle. In this experiments, some controls (Group C) as well as MSG-treated animals showed such irregularity. However, after the initiation of the regular estrous cycles at the age of 60 days, almost complete 4-day cycles were observed through the experiment in all the groups, except during the pregnancy and lactation in animals subjected to mating tests. Fifteen ova were detected in the oviduct of one case from Group I (I-5), which received the severest MSG load, by the autopsy on the day of estrous smear. In addition, as described later, all the females of Group I to III were dilivered normally after regular pregnancy by mating with males on the day of proestrous smear. These results confirm that vaginal smear cycles of MSG treated rats are perfect ones associated with normal ovulation.

iv) Effects on the fertility of females and males: Table 4 shows the results of sib-mating tests within each MSG treated Group (Group I to III). All the cases were delivered after normal period of pregnancy, and there was no abnormality in litter size and sex proportion of the young. Except for one offspring of Group III (III-9), all the young showed normal growth and were found to be fertile after maturation. III-9 was also delivered normally, but did not nurse the young. However, this is not unusual for the first delivery. Since the remaining one of the same group (III-10) was delivered and also nursed normally, there would be no disorder in fertility of this group.

In this experiment, persistence of regular recurrence of estrous cycle was considered to be rather important for detection of minor disturbances in the reproductive functions than mating tests. Consequently, mating test was made only with a part of each group.

The experimental results ii) and iii) described above, however, indicate that MSG treatment never causes sterility and any other disturbances in female reproductive ability. From the results of sib-mating tests, it would be conclusive that no disorders in males are induced by the MSG treatment.

v) Autopsy: There was no difference in macroscopic findings at autopsy between MSG treated animals and non-treated ones. In Group I which were killed 1 to 2 hours after administration of MSG (1g/2ml), the stomach and small intestine were filled with a large quantity of aqueous fluid, However, contents of the cecum and colon of this group were of normal appearance, and normal feces were contained in the rectum, and no diarrhea was observed in this Group through the experiment. Accordingly, it can be assumed that the exudative change in the stomach and small intestine was a transient phenomenon caused by administration of the hypertonic solution.

Body and organ weights of each group are presented in Table 5-a and 5-b. Arround 220 days of age, difference in the weights became considerable between males and females, suggesting a need to compare the values within each sex. However, in such way the number of cases was too small to be analyzed statistically, and the values of both sexes are presented together for each group in Table 8-a and 8-b. It can be concluded that no marked difference was shown between MSG-treated groups and non-treated ones.

Compared to controls, however, some minor changes were noticeable in Group I which received the severest MSG load,

changes having the following tendencies, for example: inhibition of thymus regression or increase in weight of the ovary and hypophysis in females, and increase in weight of the seminal vesicles and epididymis in males.

Discussion

It is widely accepted that genital glands of females and males regress resulting in sterility, if some lesions have been placed experimentally in basal hypothalamic area. By contrast, lesions located in anterior hypothalamic area causes only in females a persistent estrus due to the lack of periodical LH-Surge (LH liberation needed for ovulation) resulting in sterility. Also, it is well known that lesions involving hypothalamus including ventromedial nuclei cause an obesity associated with hyperphagia.

Olney reported that administration of MSG to rats and mice induced hypothalamic lesions followed by the appearance of sterility in females and obesity without hyperphagia. Although this should be amended partly in localization of the lesions and resulting clinical signs, it is strongly suggested that there occurred some functional disturbances in hypothalamus, as described above.

These morphological changes in hypothalamus are inevitably associated with a certain functional dissorders, but it can be readily assumed that disorder was not to be associated with appreciable morphological changes. For example, a single administration of androgen to newborn rats causes a persistent estrus in females after maturation resulting in sterility (This is called androgen sterility). And, when female rats showing normal estrous cycles are placed under continuous illumination, they exhibit persistent estrus and become sterile.

No morphological lesions are detectable in the brain of these cases. Consequently, specific functional changes seem to be more reliable to detect minor disturbances as compared to morphological ones.

From this point of view, we have studied disturbances of hypothalamus caused by a long-term peroral administration of MSG, attaching importance to characteristic symptoms indicated by Olney.

As described already, through this experiment, no functional disorders indicating hypothalamic lesions were observed in adult and young rats treated with MSG.

At autopsy, however, the thymus and hypophysis in females and sexual glands in males seem to be slightly larger in weight in animals that have received the most heavy load of MSG than in non-treated controls. As the number of cases was too small in the present experiment, further studies are needed to see whether such difference is significant or not.

Although histopathological examination of brain and other organs is now in progress, the non-existence of any functional abnormalities suggesting hypothalamic lesions in our experiments can be said to indicate the absence of serious adverse effects of MSG.

Table 1 Design of Experiment and Experimental Groups

Group	No. of rats Sex	Relation	MSG Load	
			Indirect (Maternal)	Direct Administered*
M	♀ 2 (M_1, M_2)	Mothers of I, II, III	no	Daily 1g. MSG perorally for 242 days including plegnancy & lactation
CM (Control)	♀ 2 (CM_1, CM_2)	Mothers of C	no	no
I	♂ 4 ♀ 4	Suckling of M_1	via placenta+milk	Daily MSG** perorally from 20 day old for 200 days
II	♂ 4 ♀ 4	Suckling of M_2	via placenta+milk	no
III	♂ 2 ♀ 6	Mixed young of M_1 M_2 nursed with foster mother	via placenta	no
C (Control)	C_1:♂4, ♀4 C_2:♂4, ♀4	Sucking of CM_1 Sucking of CM_2	no	no

* MSG agueous solution was given by stomach tube daily except Sunday and the National Holidays
** Daily doses of MSG: 0.25g/0.5ml for 20-30 day old, 0.5g/1.0ml for 31-54 day old and 1.0g/2ml for 55-200 day old.

Table 2 Body Weight and Sex Ratio of Offspring at 2nd Day After Birth

	Mother	No. of female offspring	Average Body weight (g)	No. of male offspring	Average Body weight (g)
MSG-treated	M_1	6	5.2	8	5.5
	M_2	10	5.5	6	5.7
Control	CM_1	7	5.6	7	5.8
	CM_2	5	5.3	10	5.6

Table 3 Appearing Age (in Day) of Maturity Indications

Group	Open of ears	Open of eyes	Descent of testes	Penile type change W to J	Open of Vaginal Orifice
I	3-4	15-16	24	43-45	34-35
II	3-4	15-16	24	43-45	34-42
III	3-4	15-16	24	42-43	34-39
C_1	3-4	15-16	23	43-46	35-36
C_2	3-4	15-16	23	43-47	33-36

Table 4 Result of Sib-mating Test

Group	Female No.	Male No.	Age of mating (day)	Term of gestation (day)	No. of pups and their sex	Remarks
I	8	3	95	23	♀ - 7 ♂ - 7	Eight pups of each group were nursed and matured normally. Some of each group were proved as fertile by sib-mating
II	6	4	113	23	♀ - 6 ♂ - 6	
III	10	1	90	22	♀ - 5 ♂ - 7	
	9	1	90	22	♀ - 4 ♂ - 6	Mother did not nurse pups

Table 5 - a Body Weight and Weight of Organs

Group	No.	Sex	Body Wt. g.		Heart g.		Kidney g.		Liver g.		Digestive tract g.		Spleen mg.		Thymus mg.		Hypophysis mg.		Adrenal mg.	
I	-1	♂	449	445	1.49	1.64	4.46	4.78	14.9	16.9	29.7	29.9	829	817	153	165	12.5	12.8	40.7	52.9
	-3	♂	440		1.79		5.10		18.9		30.0		804		176		13.1		65.1	
	-6	♀	305		1.05		2.81		13.1		31.5		816		274		13.4		68.5	
	-7	♀	346	322	1.31	1.12	2.47	2.77	13.6	13.5	31.2	33.4	738	746	300	291	16.1	15.4	59.6	63.5
	-8	♀	315		1.01		3.04		13.9		38.4		685		299		16.7		62.5	
II	-1	♂	499		1.60		4.33		17.3		32.1		931		182		14.6		48.6	
	-3	♂	472	490	1.53	1.65	4.25	4.36	16.8	17.4	26.6	30.3	970	921	166	171	13.6	13.6	48.6	48.5
	-4	♂	499		1.83		4.51		18.2		32.1		862		166		13.7		48.4	
	-5	♀	313		0.99		2.10		12.4		25.0		744		224		11.6		57.7	
	-6	♀	320	326	1.04	1.02	2.18	2.28	11.3	12.7	26.4	27.6	779	891	222	230	15.3	13.7	55.9	62.0
	-7	♀	345		1.03		2.56		14.4		31.4		1150		255		14.3		72.3	
III	-1	♂	600	549	1.94	1.84	5.86	5.44	31.1	24.2	50.1	39.2	1365	1251	254	218	18.1	17.2	69.4	65.3
	-2	♂	497		1.74		5.01		17.2		28.2		1136		178		16.2		61.2	
	-5	♀	345		1.00		2.29		11.8		27.4		685		237		14.3		53.4	
	-6	♀	320	332	1.02	1.03	2.39	2.39	14.9	12.9	31.1	28.1	750	770	222	224	11.2	13.1	62.2	57.8
	-7	♀	330		1.07		2.43		12.1		25.7		875		213		13.8		57.7	
C	C 1-1	♂	506		1.02		4.51		22.0		37.4		1002		195		13.2		51.0	
	C 1-4	♂	472	492	1.47	1.34	4.66	4.57	16.1	18.2	36.6	35.8	774	858	227	196	13.8	14.4	51.1	52.1
	C 2-1	♂	497		1.54		4.54		16.5		33.3		799		167		16.1		54.3	
	C 1-6	♀	345		0.99		2.31		13.7		33.7		741		235		12.3		48.0	
	C 2-6	♀	320	322	0.90	1.04	2.27	2.32	13.1	12.7	27.6	28.9	690	689	204	225	12.9	12.5	51.9	50.9
	C 2-7	♀	300		0.93		239		11.4		25.3		637		231		12.4		54.6	
M	M2	♀	370		1.10		2.70		13.4		38.8		822		121		15.4		55.3	
CM	-1	♀	465	415	1.37	1.27	3.15	3.01	20.5	17.0	56.8	42.3	1250	964	316	228	15.9	15.1	69.8	63.9
	-2	♀	365		1.14		2.86		13.5		27.7		678		133		14.8		58.0	

Table 5 - b Body Weight and Weight of Organs

Group	No.	Sex	Thyroid mg.		Ovary mg.		Uterus mg.		Testis g.		Epididymis g.		Seminal Vesicle g.		Ventral Prostate mg.		Vaginal smear	descent of T. or Penis Type	
I	-1	♂	39.4	42.2	•		•		2.99	3.02	1.21	1.20	2.39	2.56	488	507	•	+	U
	-3	♂	44.9		•		•		3.04		1.18		2.73		525		•	+	U
	-6	♀	32.2		87.3		499		•		•		•		•		E	•	
	-7	♀	22.6	29.5	90.1	90.4	411	437	•		•		•		•		D_1	•	
	-8	♀	33.8		93.7		400		•		•		•		•		E	•	
II	-1	♂	34.2		•		•		2.85		1.13		2.62		448		•	+	U
	-3	♂	39.4	39.0	•		•		2.74	2.82	1.19	1.13	2.61	2.65	705	555	•	+	U
	-4	♂	44.3		•		•		2.86		1.07		2.72		513		•	+	U
	-5	♀	25.6		76.2		684		•		•		•		•		E	•	
	-6	♀	32.2	25.5	91.6	81.0	432	566	•		•		•		•		E	•	
	-7	♀	18.7		75.2		583		•		•		•		•		D_1	•	
III	-1	♂	31.9	31.1	•		•		3.11	2.94	1.13	1.17	2.27	2.69	569	592	•	+	U
	-2	♂	30.2		•		•		2.77		1.21		3.11		615		•	+	U
	-5	♀	18.9		78.5		453		•		•		•		•		D_1	•	
	-6	♀	29.6	24.8	87.4	79.6	549	538	•		•		•		•		D_1	•	
	-7	♀	26.0		73.0		613		•		•		•		•		E	•	
C	C1-1	♂	35.2		•		•		2.95	2.79	1.25	1.15	2.62	2.45	570	670	•	+	U
	C1-4	♂	29.[illegible]	31.4	•		•		2.67		1.14		2.67		880		•	+	U
	C2-1	♂	29.2		•		•		2.76		1.07		2.06		561		•	+	U
	C1-6	♀	27.7		79.8		643		•		•		•		•		D_2	•	
	C2-6	♀	19.2	22.3	74.5	71.9	493	536	•		•		•		•		D_1	•	
	C2-7	♀	20.1		61.3		468		•		•		•		•		E	•	
M	M_2	♀			69.5		605		•		•		•		•		D_1	•	
CM	-1	♀	•		86.2	90.6	489	516	•		•		•		•		D_1	•	
	-2	♀	26.8		94.9		541		•		•		•		•		E	•	

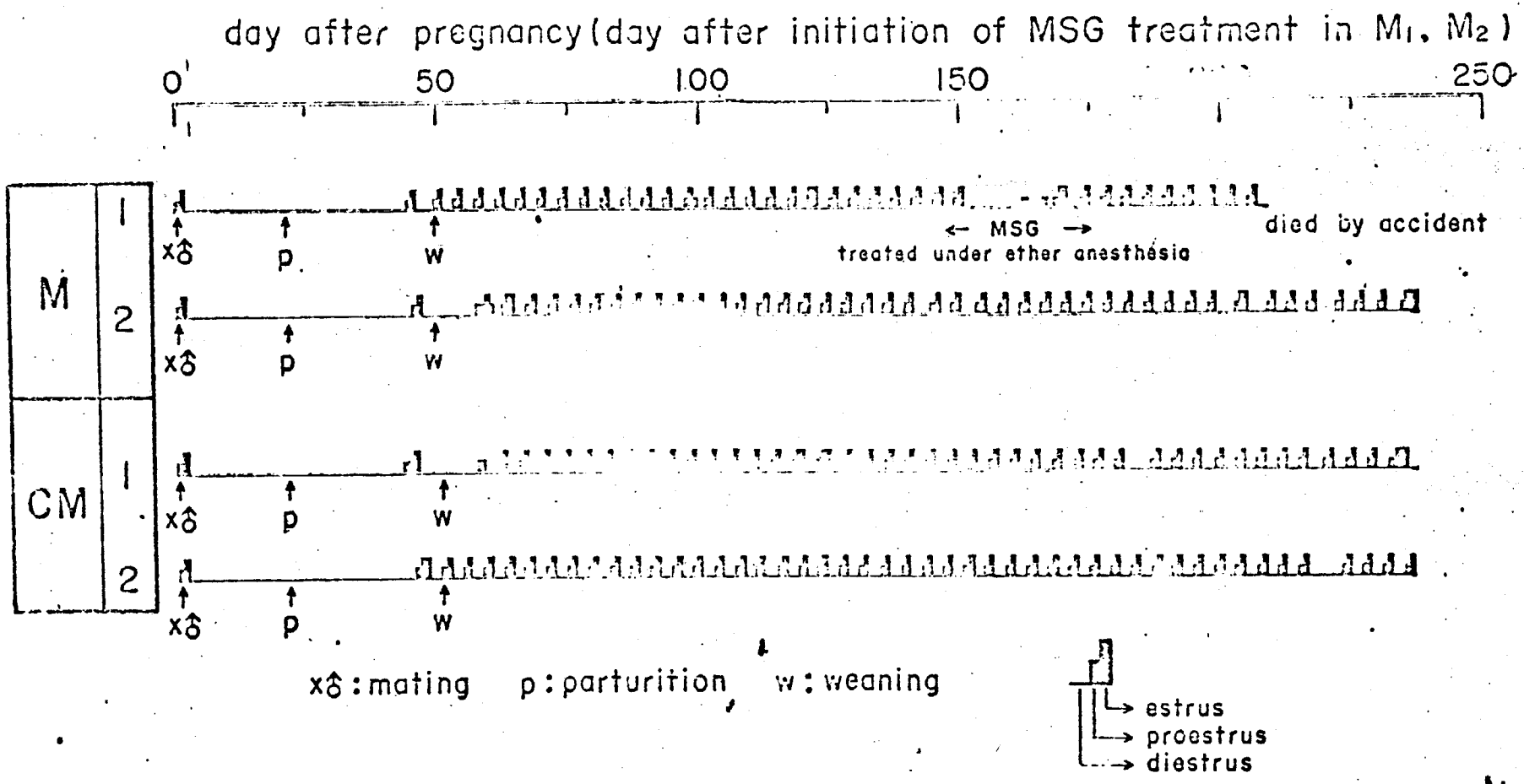


Fig 1 Recurence of Estrous Cycle in Mothers

M : MSG (1g/2ml) perorally, daily throughout experimental period

CM : Intact control

Figure 2 Progressive Change of Mothers' Body Weight

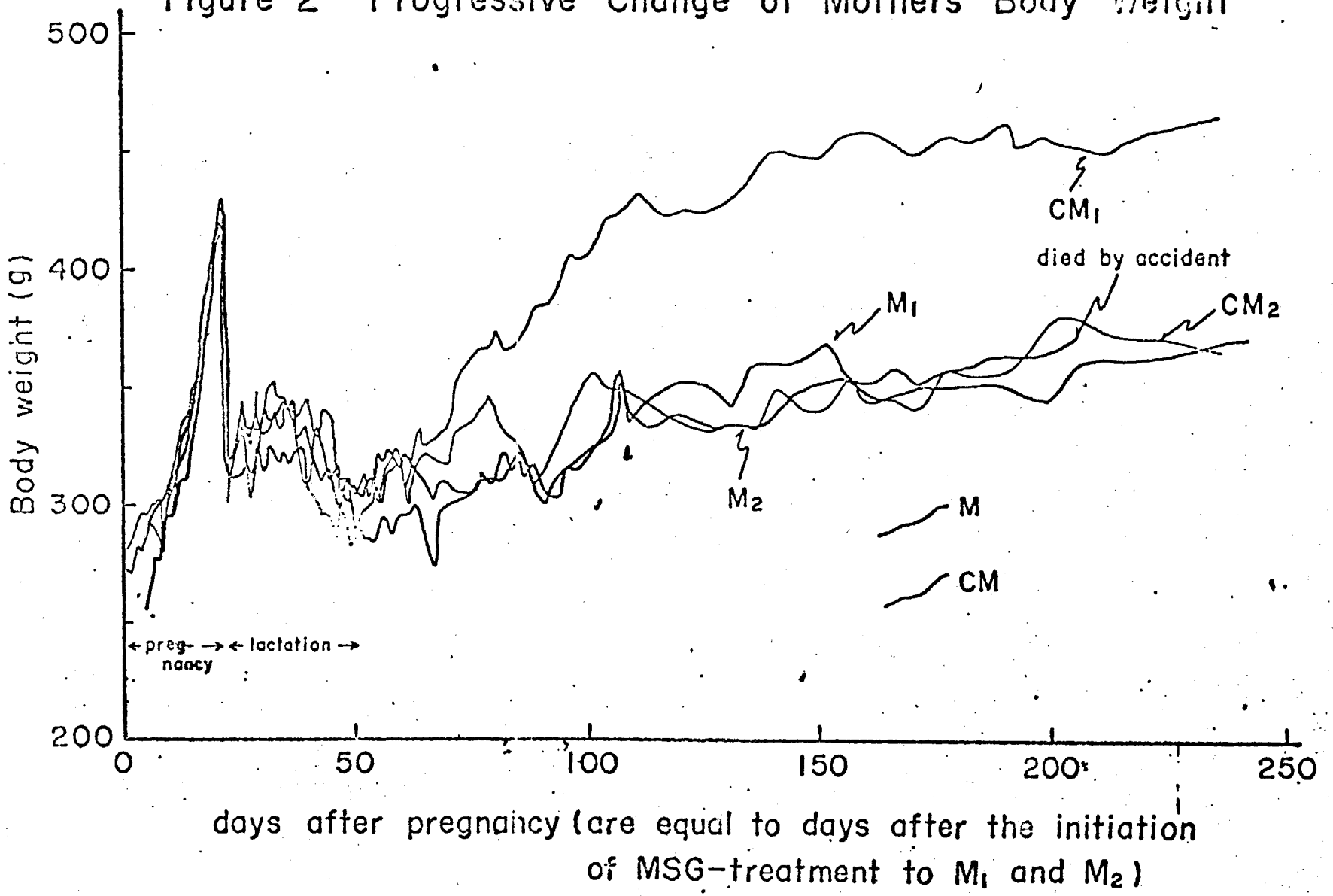


Figure 3 Average Growth Curve of Female Offspring*

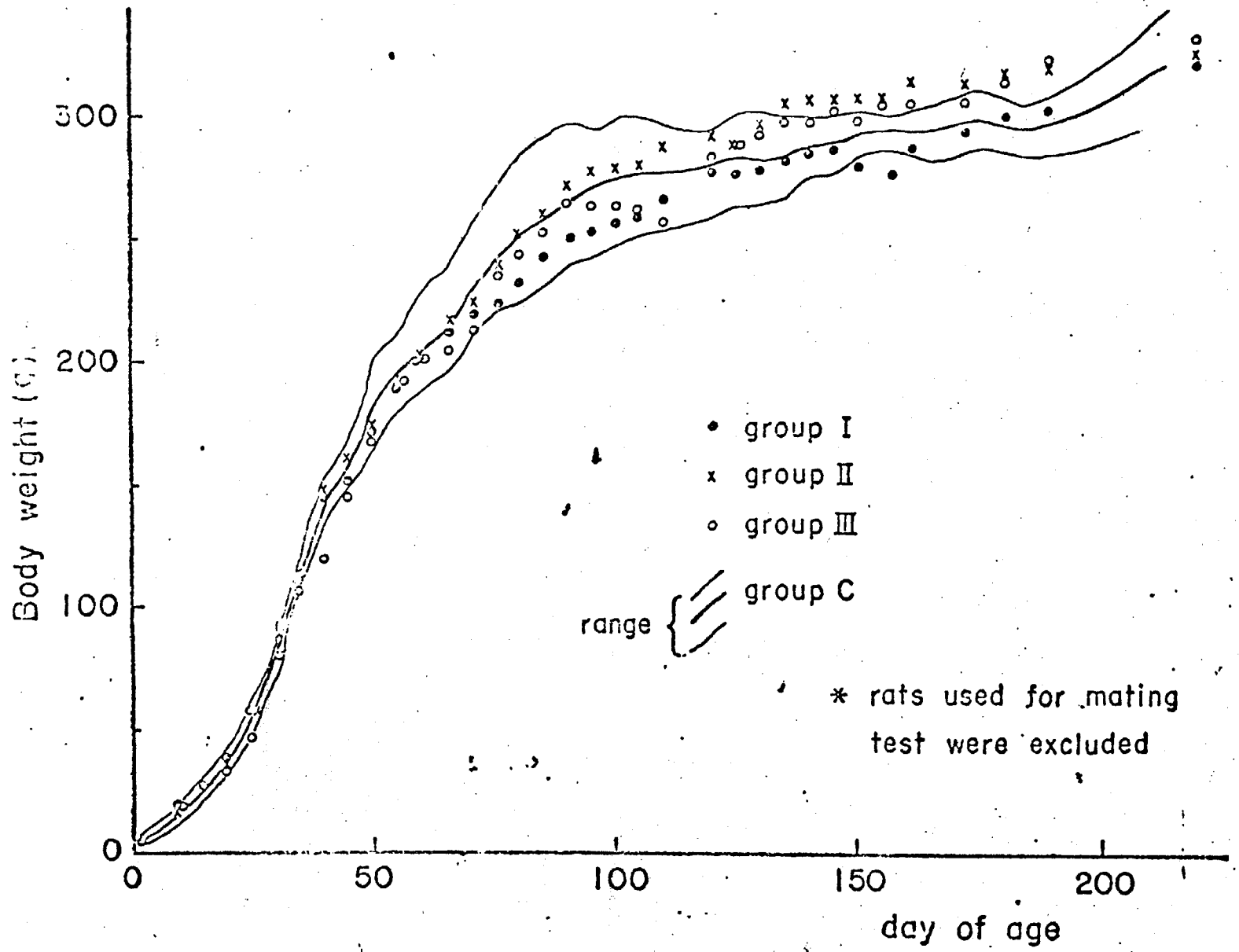
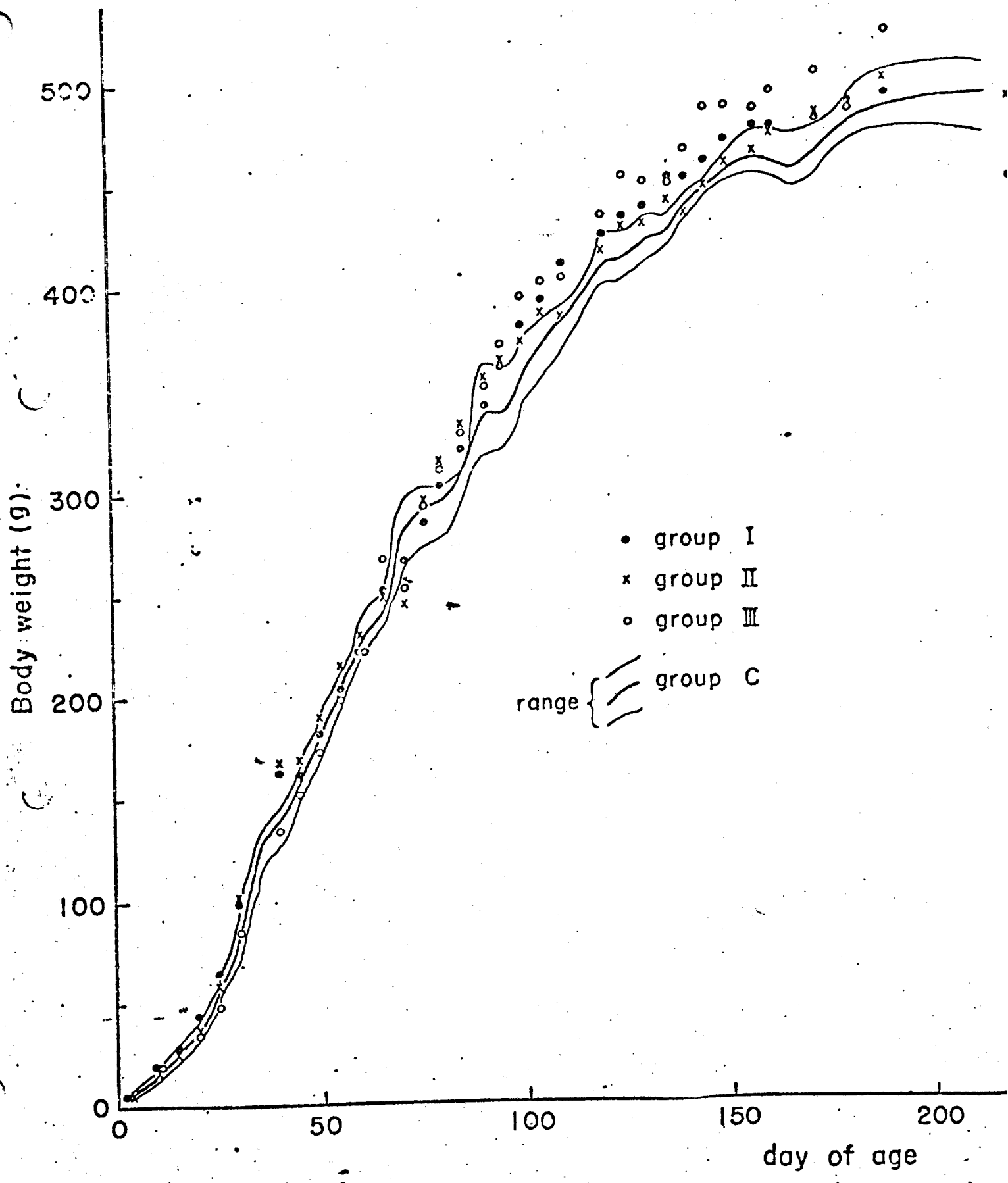


Figure 4 Average Growth Curve of Male Offspring



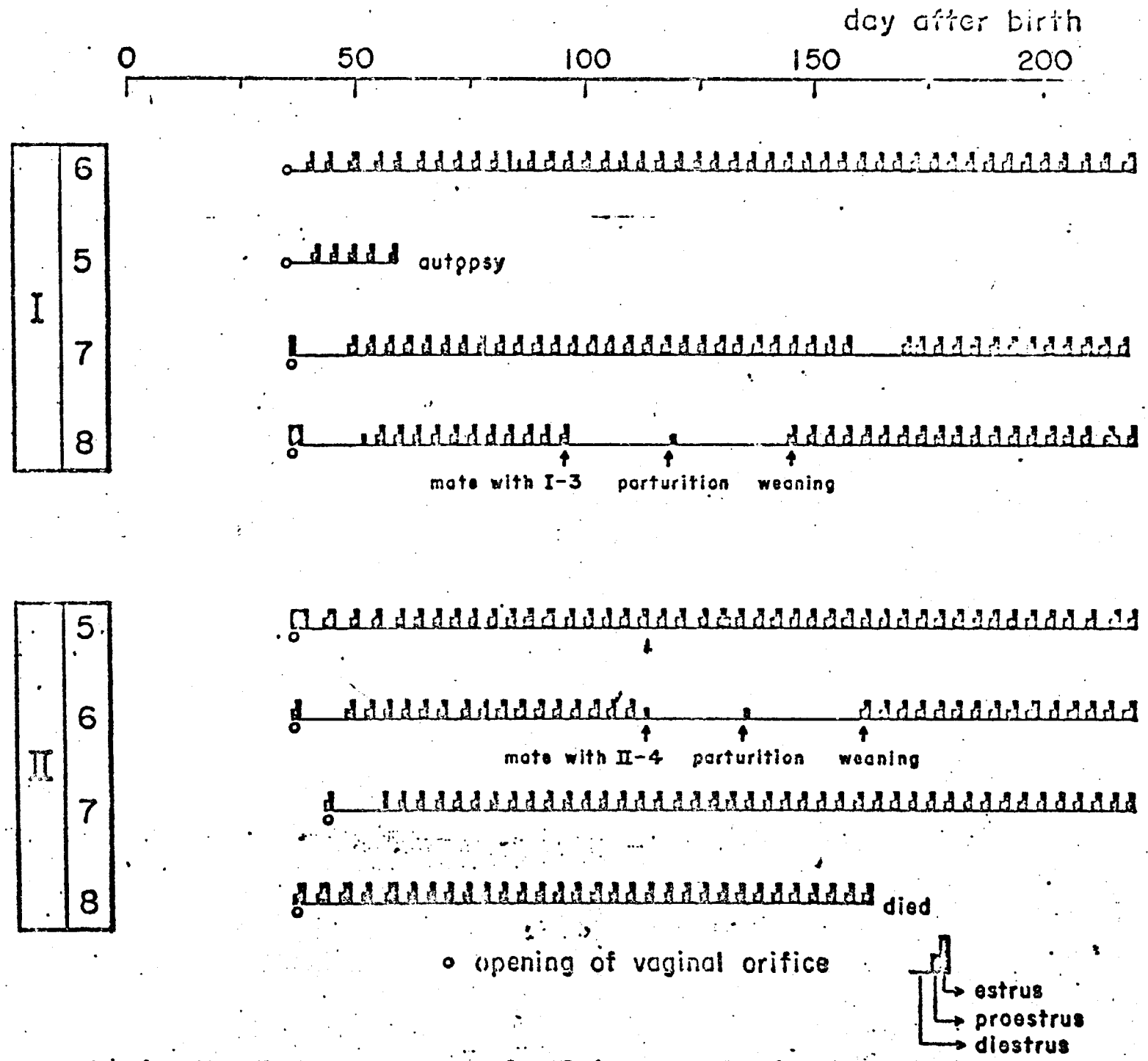


Fig 5 Reccurence of Estrous Cycle in Offspring (MSG Loaded Groups**)

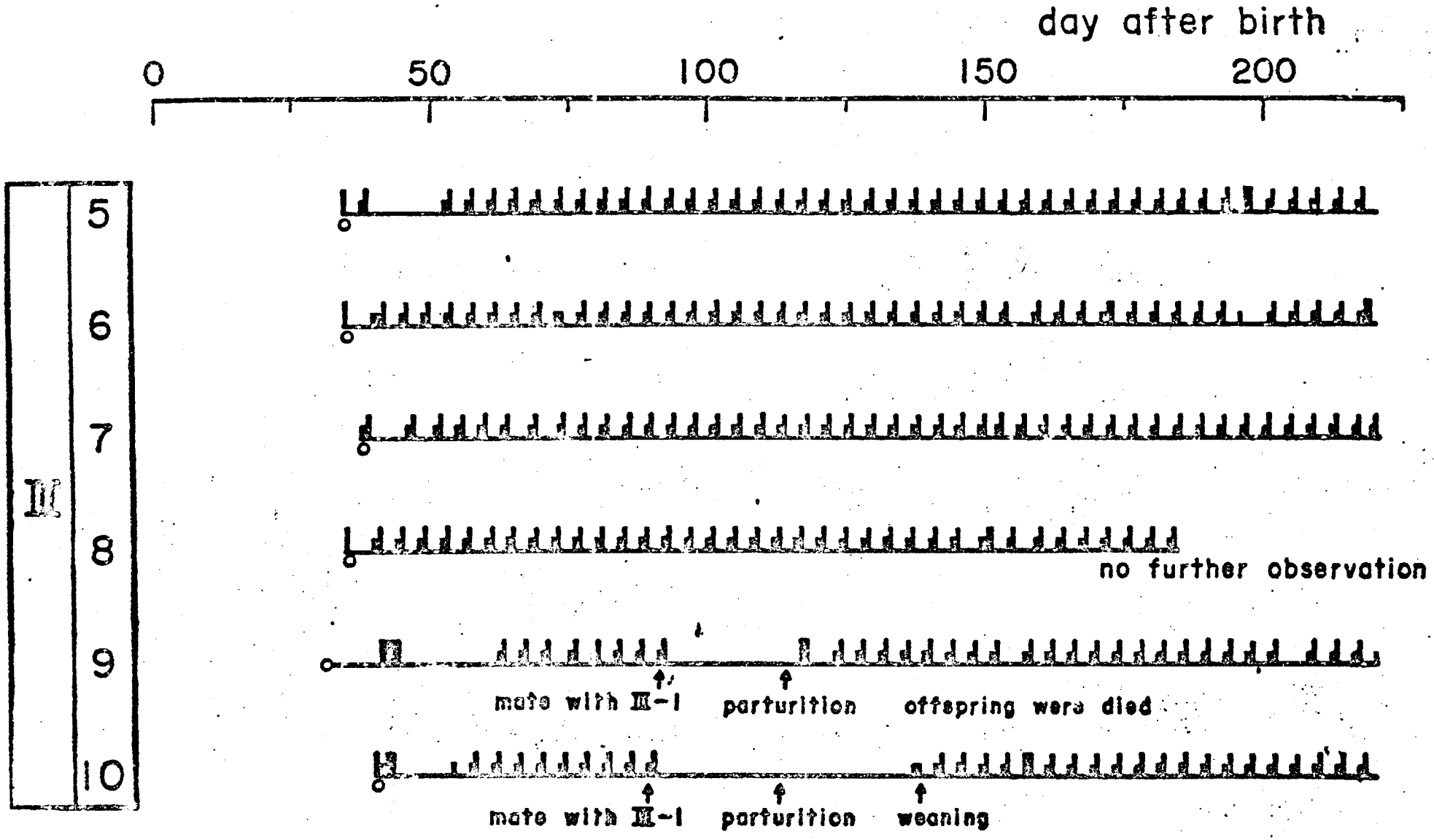


Fig 6 Reccurence of Estrous Cycle in Offspring (MSG Loaded Group**)

** See Table I

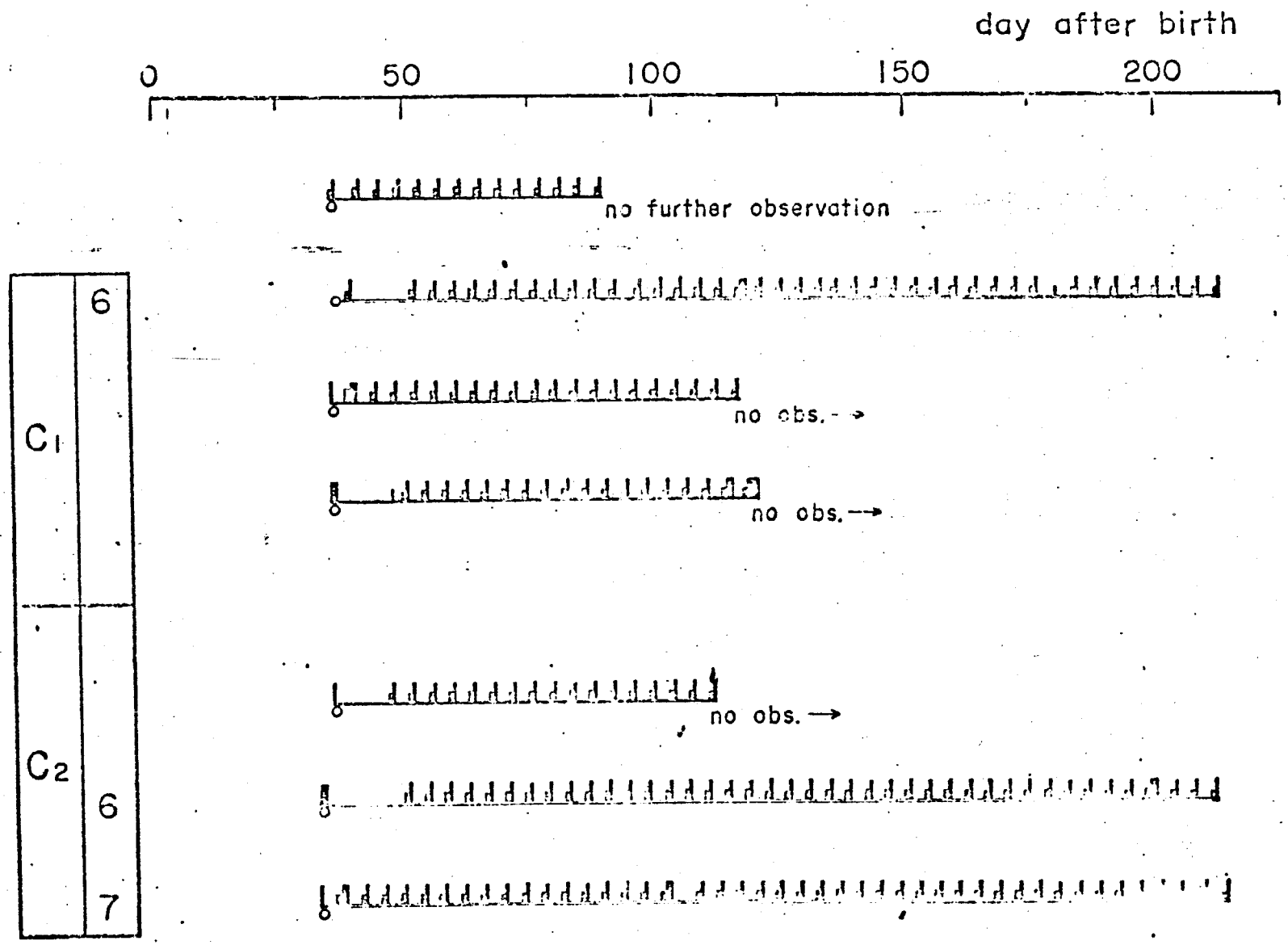


Fig 7 Reccurence of Estrous Cycle in Offspring (Intact control Group**)

** See Table I

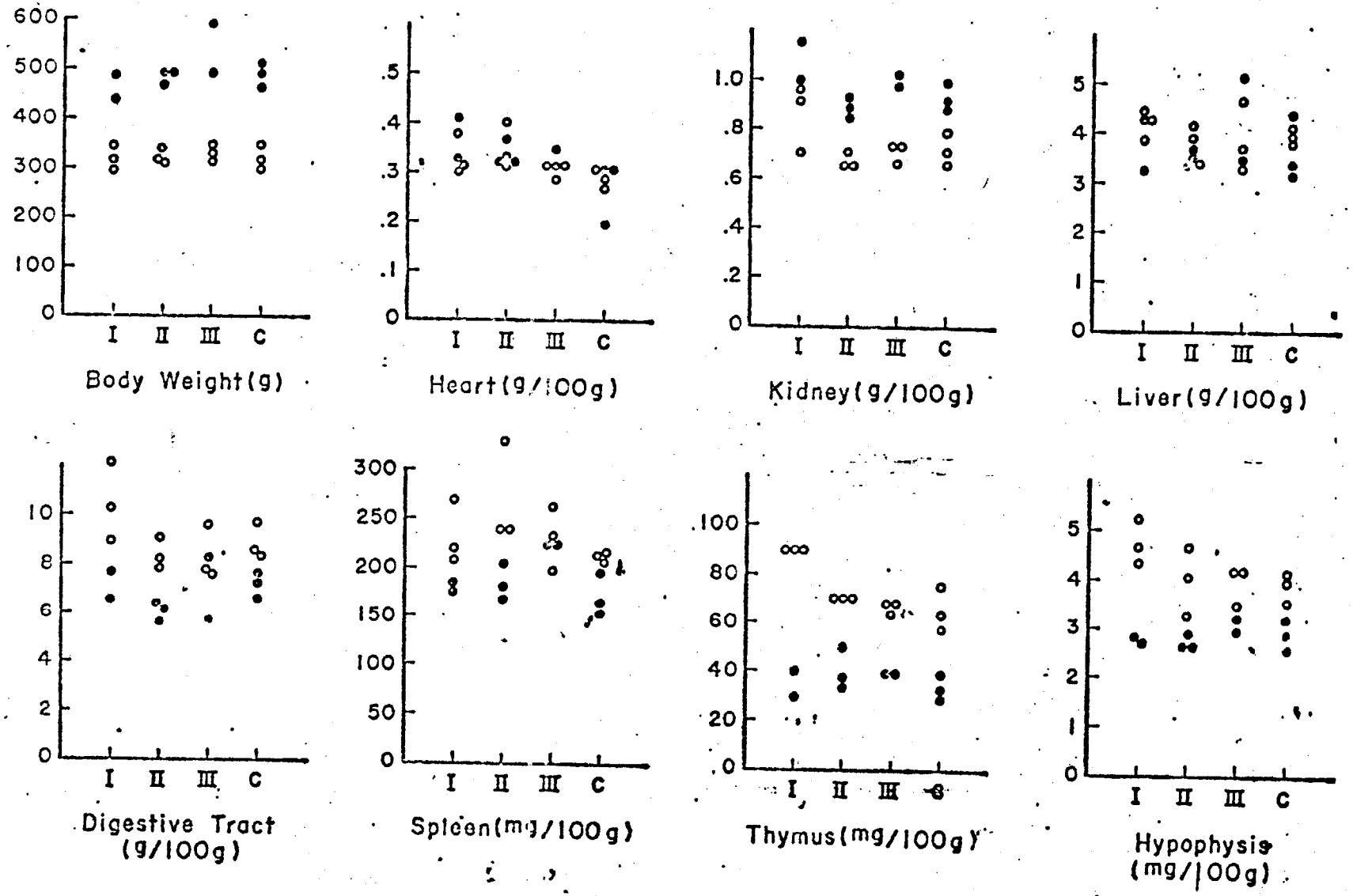


Fig 8-a Body Weight and Some Organ's Weight (Per 100g Body Wt.) at autopsy (220 day of age in Groups I~III, and 214 day of age in Group C)

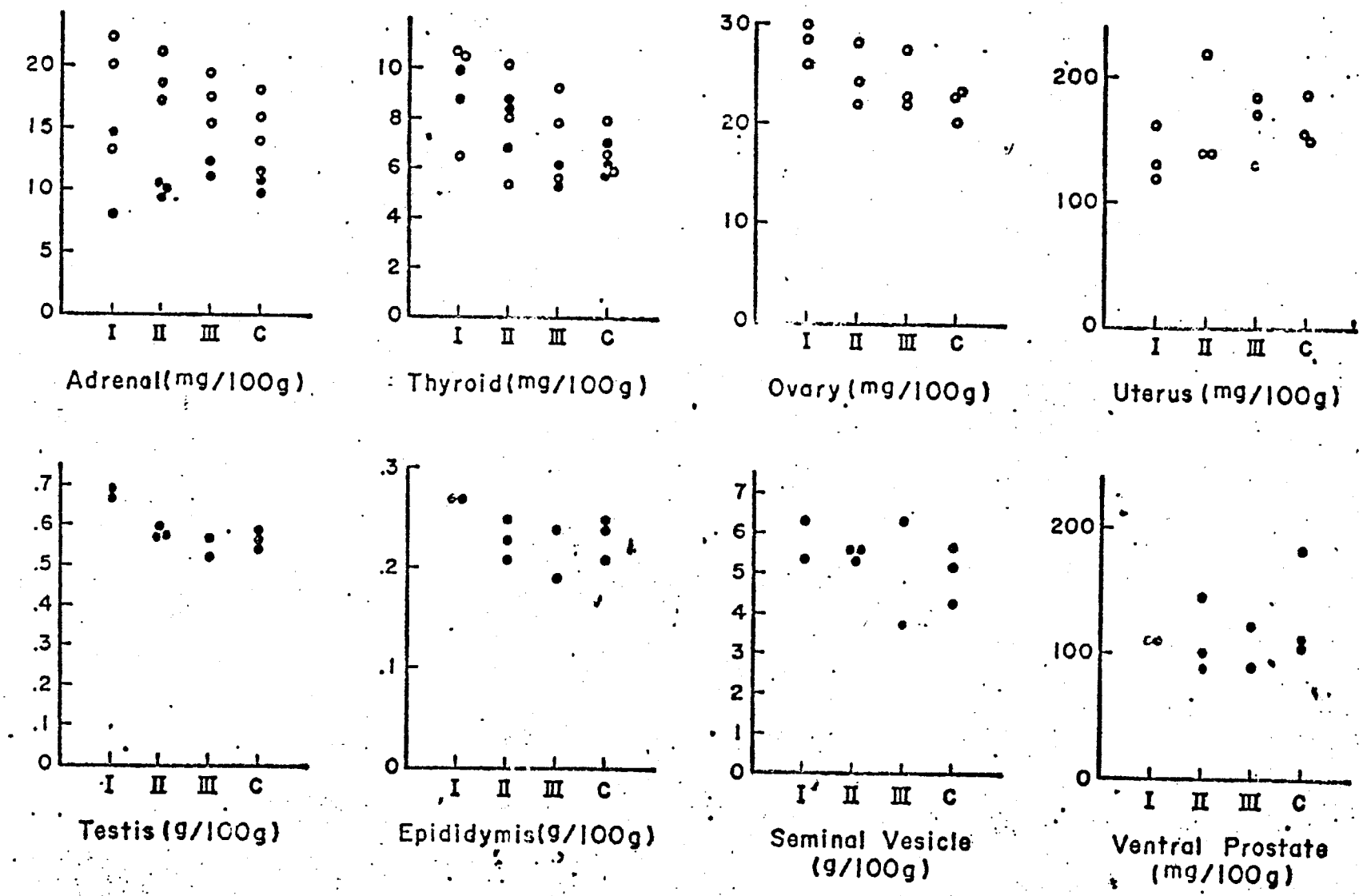


Fig 8-b Body Weight and Some Organ's Weight (Per 100g Body Wt.) at autopsy (220 day of age in Groups I~III, and 21^ day of age in Group C)

Appendix to "The Effect of Perorally Administered Monosodium Glutamate on the Reproductive Function and Somatic Growth in Rats"

— Histopathological Examinations —

Tsutomu Shimizu

Kageaki Aibara

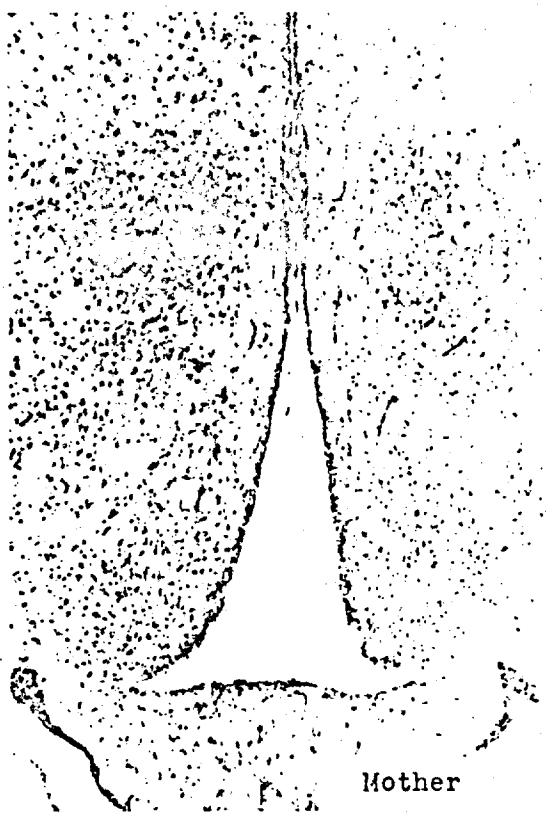
(National Institute of Health, Japan)

Appendix to "The Effect of Perorally Administered Monosodium Glutamate on the Reproductive Function and Somatic Growth in Rats"

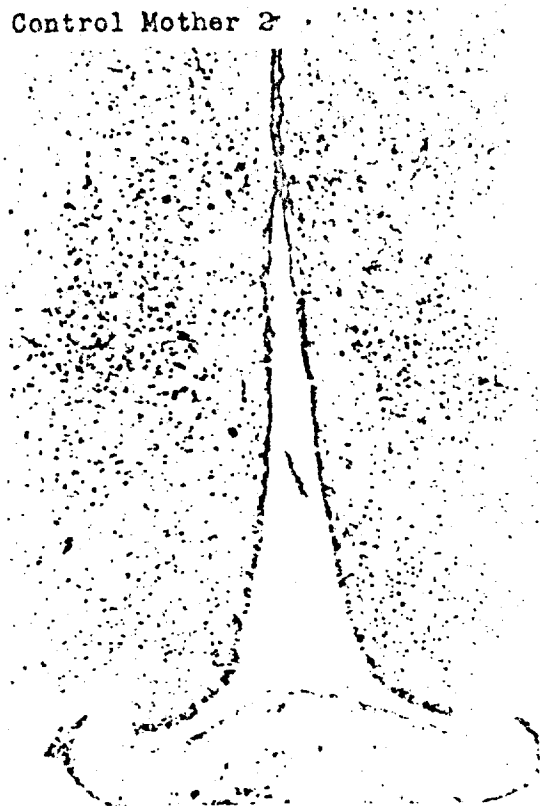
— Histopathological Examinations —

The animals explaned in page 3 of the main volume, were killed with ether, and brain, hypophysis and eye were removed, fixed with 10% neutral buffered formalin and embeded in paraffin. Sections of 8μ in thickness were prepared and then stained with Hematoxylin-Eosin and Luxol fast blue-cresyl echt violet for histopathological examination. Arcuate nuclei and median eminence in hypothalamus and retina, where olney observed acute neuronal necrosis due to the subcutaneous injection of MSG, were observed microscopically, but no finding suggesting the difference between the control and MSG-loaded groups was observed.

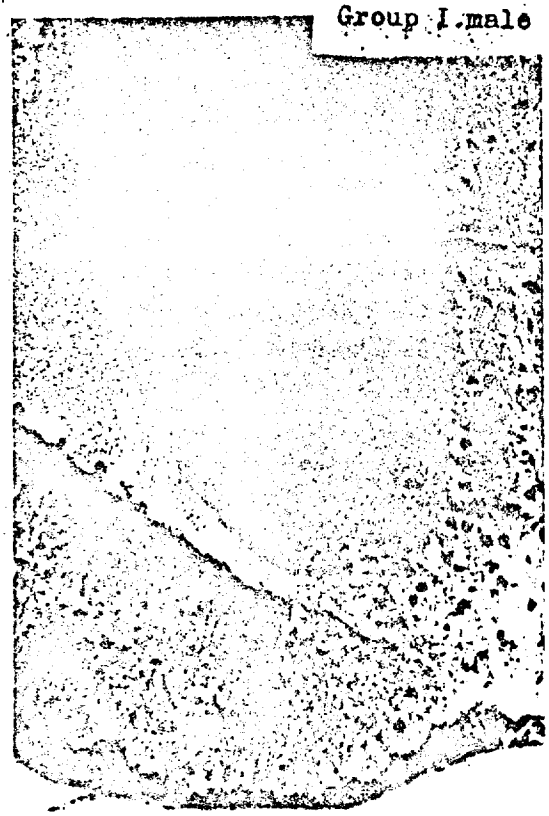
(Ref. Photographs of hypothalamic area).



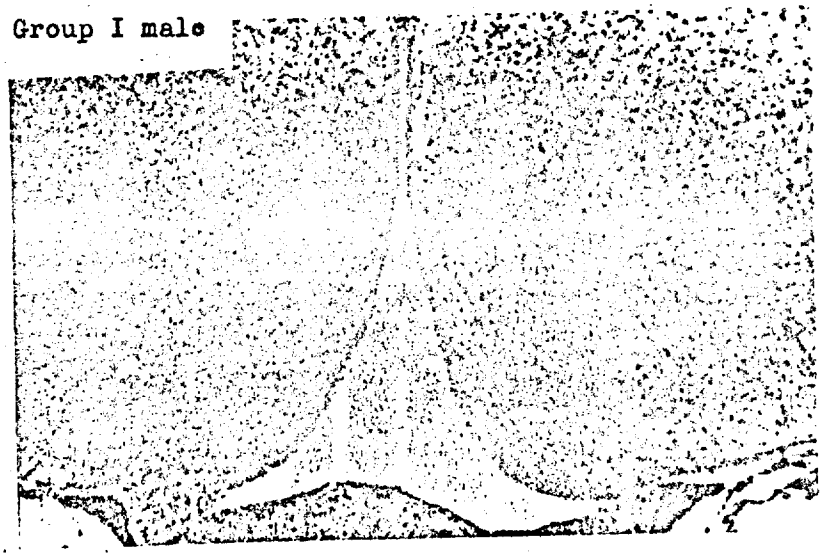
Mother



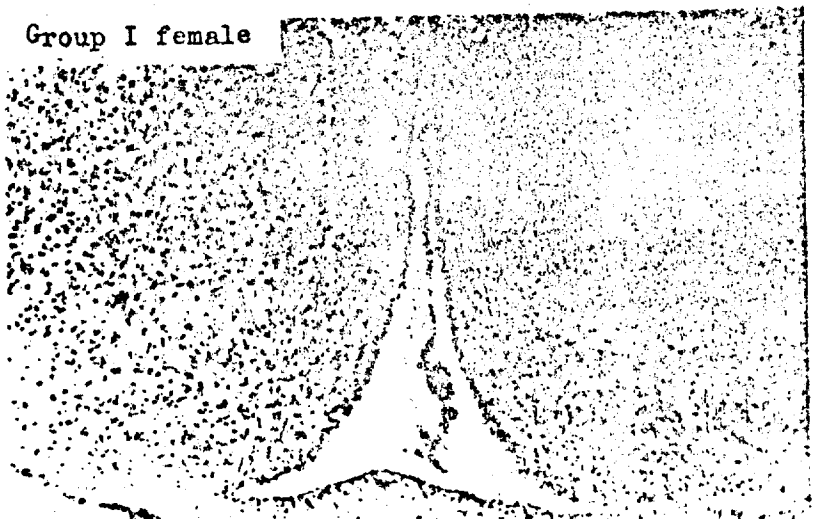
Control Mother ♂



Group I male

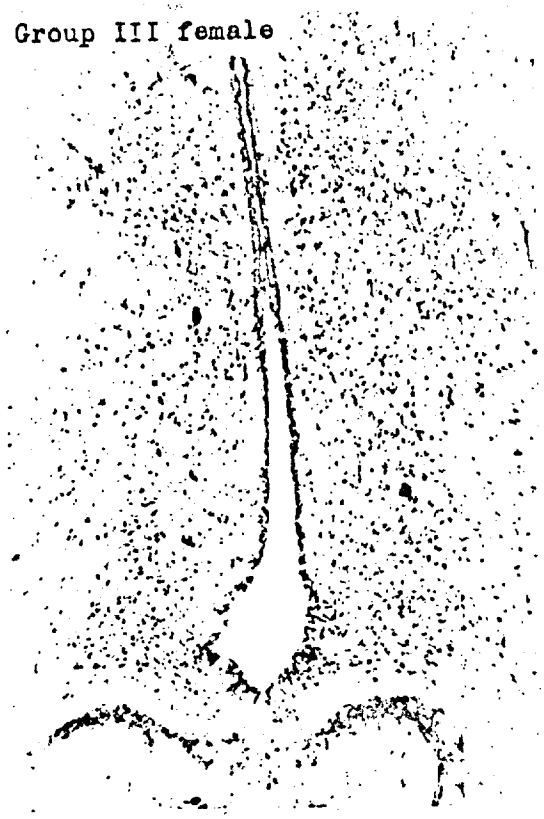


Group I male

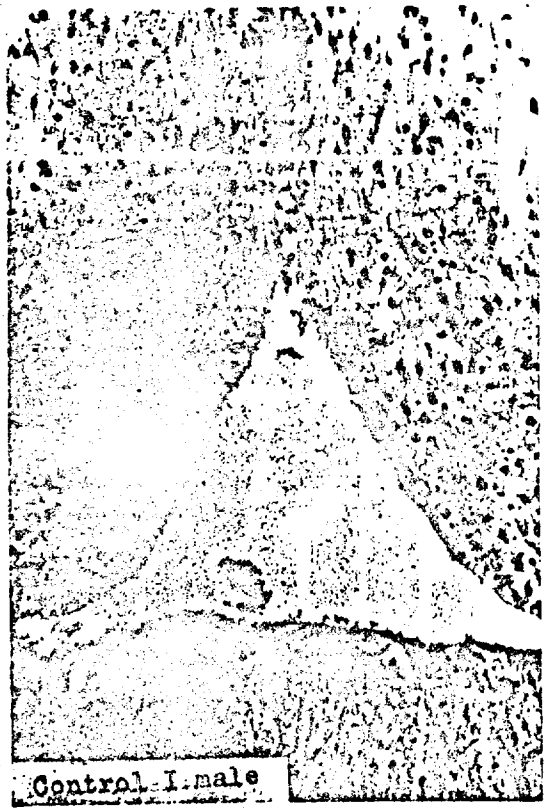


Group I female

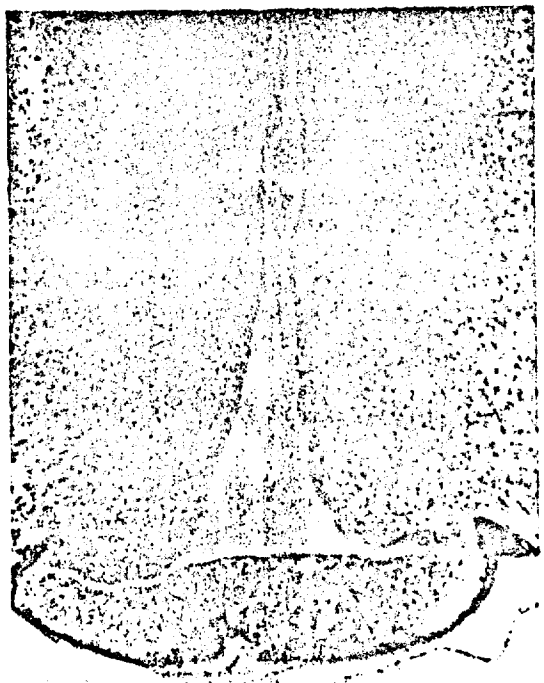
Group III female



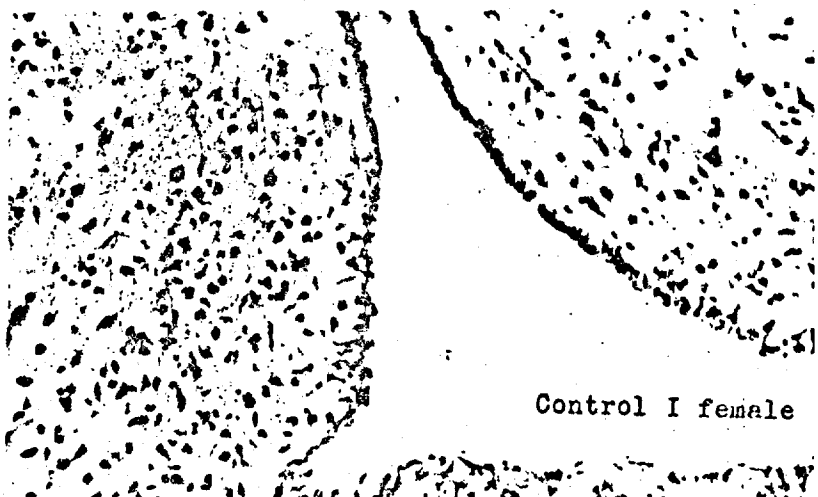
Group III female



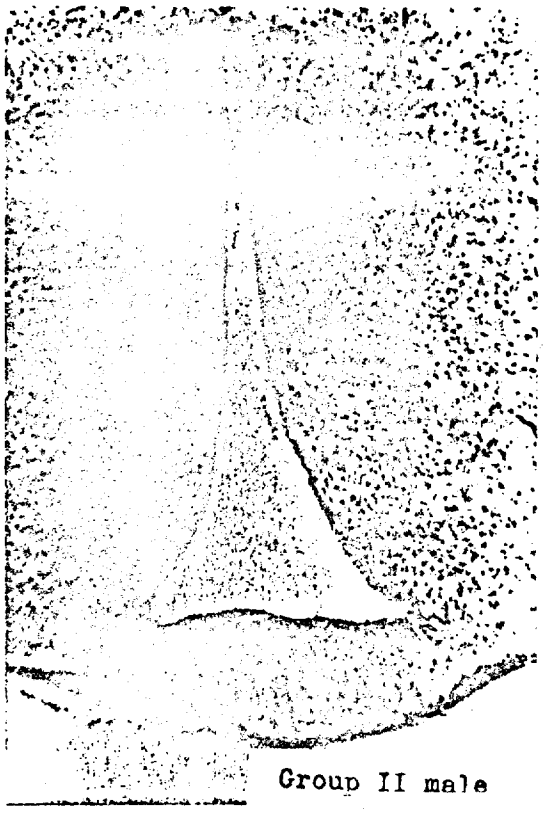
Control I male



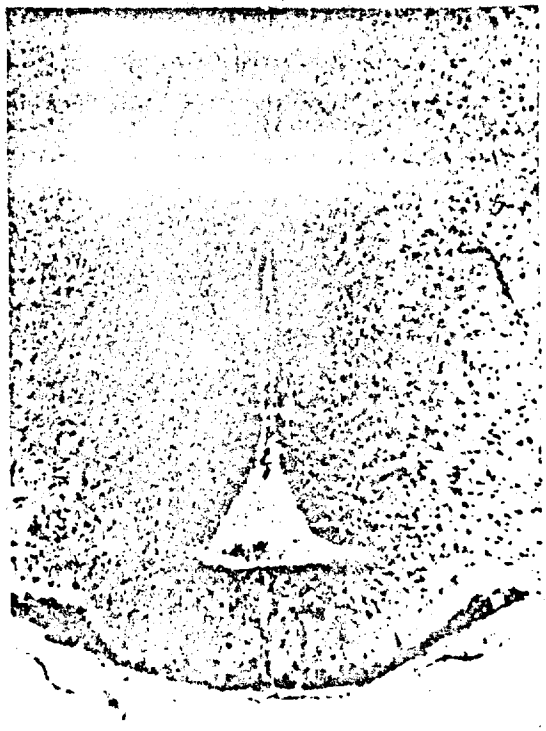
Control I male



Control I female

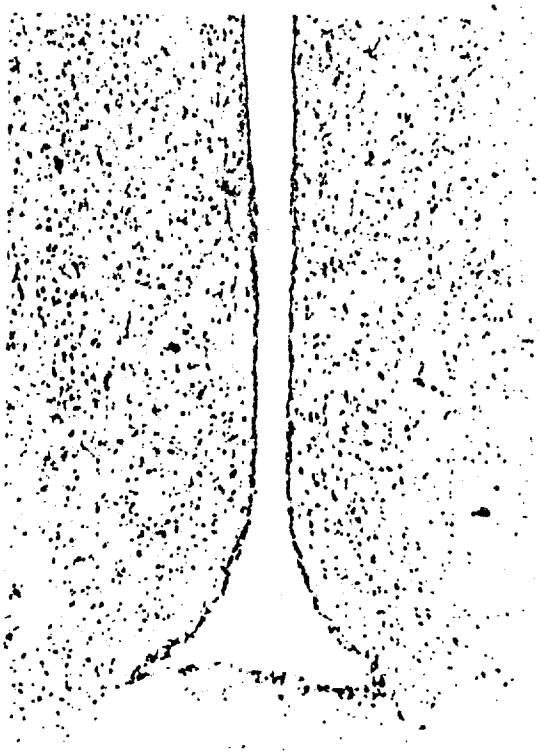


Group II male



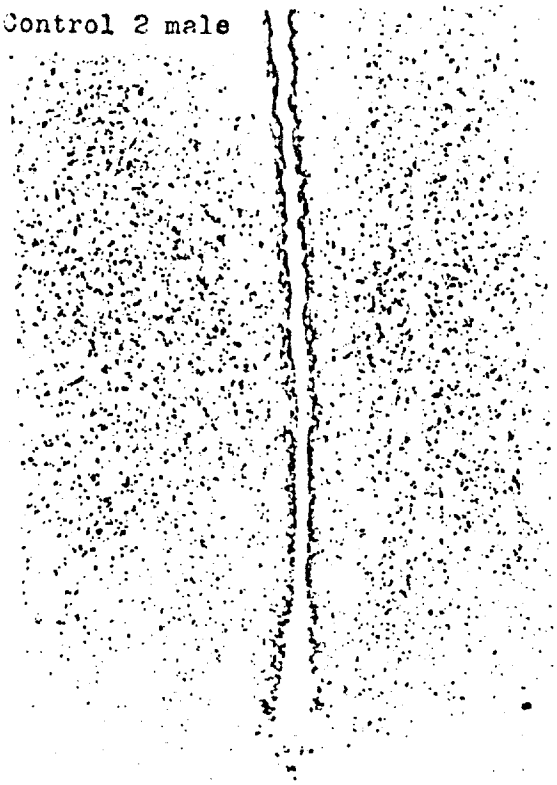
Group II female

Group III male

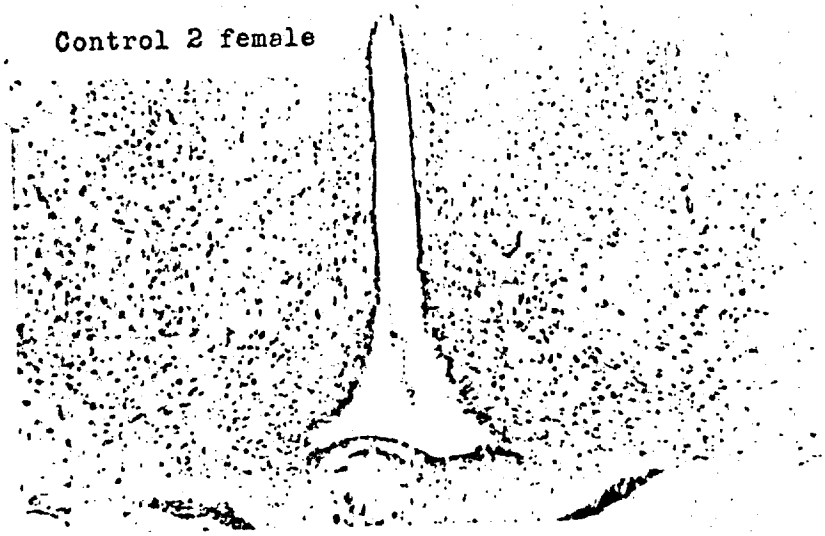


Group III male

Control 2 male



Control 2 female



Journal of Neurochemistry, 1970. Vol. 17, pp. 1591 to 1599. Pergamon Press. Printed in Northern Ireland

PIG BRAIN GLUTAMINASE: PURIFICATION AND IDENTIFICATION OF DIFFERENT ENZYME FORMS

G. SVENNEBY
Department of Neurochemistry, The University Psychiatric Clinic, Vinderen, Oslo 3, Norway

(*Received* 15 *April* 1970. *Accepted* 18 *May* 1970)

Abstract—Pig brain glutaminase (EC 3.5.1.2 L-glutamine amidohydrolase) has been purified about 5000-fold from acetone powder. Glutaminase exists in different molecular forms, dependent on the ionic composition of the buffer. The three main forms are similar to those of kidney glutaminase and therefore called the tris-HCl enzyme, the phosphate enzyme, and the phosphate-borate enzyme. The sedimentation coefficients, as estimated by sucrose gradient technique, are 7·3, 8·7, and 53, respectively. Glutaminase has a pH optimum of about 9, but the pH curves of the tris-HCl enzyme and the phosphate-borate enzyme have different shapes. The apparent pK_1 of the tris-HCl enzyme-substrate complex is similar to pK_2 of inorganic phosphate, the apparent pK_2 of both the tris-HCl and the phosphate-borate enzyme complexes is similar to pK_2 of glutamine. By use of the electron microscope we were able to see the phosphate-borate enzyme.

GLUTAMINASE (EC 3.5.1.2 L-glutamine amidohydrolase) was designated the brain type of glutaminase by KREBS (1935) to distinguish this enzyme from the liver type, which is identical with a glutamine-α-ketoacid transaminase (GREENSTEIN and CARTER, 1947). Glutaminase from pig kidney has been extensively purified (KVAMME, TVEIT and SVENNEBY, 1970). It exhibits allosteric properties (SVENNEBY, TVEIT and KVAMME, 1970) and has been shown to be a polymeric protein. Three main forms, called the tris-HCl enzyme, the phosphate enzyme, and the phosphate-borate enzyme, have different sedimentation coefficients and molecular weights, as also has been shown for glutaminase from ox brain (KVAMME, SVENNEBY and TVEIT, 1966).

Because of the central roles of glutamine, glutamate and ammonia in brain, it is of interest to compare the properties of brain glutaminase with other known glutaminases. In this paper, we describe a procedure of purification of glutaminase from pig brain, and three main forms of the enzyme. A preliminary report has been published earlier (SVENNEBY, 1969).

MATERIALS AND METHODS

Materials. L-glutamine, L-glutamate dehydrogenase (EC 1.4.1.2) (type II), alcohol dehydrogenase (EC 1.1.1.2) (crystalline, saltfree, dry preparation from yeast), and catalase (EC 1.11.1.6) (twice crystallized from Jack beans) were products of the Sigma Chemical Co. Ferritin (twice crystallized from horse spleen) was obtained from Nutritional Biochemicals. The other reagents were of highest purity, analytical grade.

In order to minimize the content of ammonia and glutamate in the glutamine solutions, either freshly prepared L-glutamine solutions or freshly prepared L-glutamine solutions stored at −90°C in small vials, which were only thawed once, were used. The spontaneous hydrolytic cleavage of glutamine was almost negligible during the assay. If necessary, a correction was made for this amount of glutamate or ammonia. Tris solutions were always neutralized with HCl to the desired pH.

Methods. Glutaminase was measured (1) by spectrophotometric assay and (2) by chromatographic assay.

(1) Spectrophotometric assay: The rate of ammonia formation at 23°C was determined spectrophotometrically (KVAMME *et al.*, 1970), and the assay system depends on the glutamate dehydrogenase reaction as an auxiliary system. The rate of NADH oxidation was followed at 340 nm and 23°C. The reaction mixture (1 ml) contained 8 mM-L-glutamine; 20 mM-α-ketoglutarate; 0·1 mM-NADH; 40 mM-Na-phosphate; 0·2 mM-EDTA; 1–2 I.U. L-glutamate dehydrogenase; and glutaminase.

The pH of the reagents except the L-glutamate dehydrogenase and NADH was adjusted to 8·0. Despite the fact that glutaminase has a pH optimum of about 9, pH 8 was used in the spectrophotometric assay because glutamate dehydrogenase has a pH optimum of about 7·6. In order to remove free ammonia, which contaminates the glutamine and glutamate dehydrogenase preparations, the reaction mixture was preincubated for 5 min at 23°C before addition of glutaminase.

(2) Chromatographic assay: This method is modified from the one described earlier (KVAMME *et al.*, 1970). The enzyme was incubated for 2 min at 23°C in a final volume of 1 ml containing 40 mM-sodium phosphate, 8 mM-L-glutamine, and 0·2 mM-EDTA at pH 9·0. The enzymic reaction was usually stopped by the addition of 3 ml of cold ethanol. When the pH was varied, the reaction was stopped by adding perchloric acid (PCA), and the PCA extracts were neutralized with KOH to a pH of about 6·5. Controls without enzyme were treated in the same manner in order to estimate non-enzymically hydrolysed glutamine. Corrections (usually negligible) were made on the basis of these data.

A portion of the ethanol or PCA extract was applied to paper (Whatman 3 MM), and chromatographed for about 20 to 36 h in butanol-acetic acid-water (100:22:50, by vol.). After dipping the dry chromatograms in 0·25% ninhydrin in acetone, containing 5 ml pyridine and 5 ml acetic acid per 100 ml acetone, the amino acids were developed by heating the papers in an oven for 30 min at 60°C. Equal squares of the glutamic acid spots were cut, the ninhydrin colour was extracted by 5 ml 50% ethanol, and 25 μl 5% $Cu(NO_3)_2$ was added. The colour was read at 510 nm in a Beckman B spectrophotometer against a paper blank.

Beer's law was followed in the range of 2 to 30 mM-glutamate in the cuvette. Although the colour for unknown reasons varied somewhat from one run to another, glutamate could be quantitatively determined when unknown and standard solutions of glutamate were chromatographed at the same time. The rate of hydrolysis of glutamine by glutaminase was usually linear with time under the conditions used.

The spectrophotometric method was used during the purification procedure and in fractions from sucrose gradients, whereas the chromatographic method was used in the other experiments.

Sedimentation coefficient and molecular weight. Sedimentation coefficient and molecular weight were determined by the method of MARTIN and AMES (1961) using sucrose gradient centrifugation. Linear sucrose gradients of 5 to 20% containing 0·1 M-tris or 0·1 M-phosphate buffer, and 5 to 40% gradients containing 0·1 M-phosphate + 30 mM-borate were used. All buffers were adjusted to pH 8·0 and contained 3 mM-EDTA. Glutaminase was applied in the phosphate-borate form. The centrifugation was performed for 2 to 14 h at 130,000 *g* (Spinco ultracentrifuge, model L, rotor SW 39). Fractions of 0·1 ml were collected and analysed for glutaminase activity. Details of the procedure were described earlier (SVENNEBY *et al.*, 1970). The following markers were used: yeast alcohol dehydrogenase $s_{20,w}$ = 7·4, molecular wt. 150,000, catalase $s_{20,w}$ = 11·3, molecular wt. 250,000, and ferritin $s_{20,w}$ = 65 (SIEGEL and MONTY, 1965).

Alcohol dehydrogenase and catalase were assayed according to the methods of RACKER (1957) and CHANCE (1957), respectively. Ferritin was determined by means of its specific absorption at 410 nm. Protein determination was determined using Lowry's method in crude preparations (LOWRY, ROSEBROUGH, FARR and RANDALL, 1951), and by spectrophotometric determination (LAYNE, 1957) in purified preparations.

For electron microscopic studies, the enzyme solution was touched with carbon film coated copper grids (200 mesh) and excess material removed by blotting with filter paper. The grids were then placed for a couple of seconds in a 1% solution of phosphotungstic acid in water, pH 8·0. Excess staining solution was removed with filter paper. The grids were examined in a Siemens Elmiskop 1A at an instrumental magnification of 40,000 times.

Purification of glutaminase. The method developed for the purification of pig brain glutaminase is a modification of the method described earlier for pig kidney glutaminase (KVAMME *et al.*, 1970) (see Table 1).

Pig brains obtained from the slaughterhouse were taken out immediately after death and cooled on ice. After having removed membranes and cerebellum, the brains were stored at −20°C for 2 weeks. The frozen brains (200 g wet wt.) were cut into small pieces with a knife and homogenized in 5 vol. cold acetone (−40°C) with a Sorwall Ominimixer. The homogenate was filtered using a Büchner funnel, and the filter cake was rehomogenized in the same volume of acetone (−40°C) as above, and refiltered. After being dried for some minutes in vacuum, the powder was allowed to dry completely at room temperature. The yield was about 40 g acetone powder from 200 g (wet wt.) of brain tissue. The acetone powder was stored at −20°C.

The acetone powder was homogenized in about 20 volumes of cold 10 mM-sodium borate buffer, pH 8·0 (borate buffer), and centrifuged for 2 h in a Sorwall centrifuge RC2-B rotor GSA at 16,000 *g*. The supernatant was carefully removed, as the precipitate was somewhat fluffy.

Sodium sulphate (2 M) was added dropwise to the supernatant (S1) to a final concentration of 1 M,

and centrifuged for 20 min at 16,000 *g*. The precipitate (B2) was homogenized in borate buffer, using a Potter-Elvehjelm homogenizer, and stored at −20°C overnight or longer. This preparation (B2) was refractionated after centrifugation. Sodium sulphate was added to the supernatant (S3) to a final concentration of 0·6 M. Following centrifugation, more sodium sulphate was added to the supernatant (S4) to a final concentration of 0·8 M. This sodium sulphate precipitate (B5) was homogenized in borate buffer, and the specific activity (μmol NH_3 formed/min/mg protein at pH 8·0 and 23°C) of this preparation was usually about 1·6 to 4·0, with a protein concentration of 12–15 mg/ml. In cases where this desired purification was not obtained, the procedure from B2 to B5 was repeated once, the last precipitate being called B7. The B5 or B7 preparations may be stored for at least one year at −20°C.

The sodium sulphate precipitated glutaminase (B5 and/or B7) was diluted with 0·05 M tris buffer, pH 8·0, to a protein concentration of about 10 mg per ml and dialysed against 150 volumes of the same tris buffer for about 20 h. The non-diffusable residue was centrifuged for 6 h in a Spinco Beckman ultracentrifuge (model L, rotor 30) at 65,000 *g*. To the supernatant (S8) was added a solution containing 1 M-potassium phosphate and 0·3 M-sodium borate at pH 8·0 to a final concentration of 50 mM-phosphate and 15 mM-borate. The pH was then adjusted to 7·6 with a few drops of another solution containing 1 M-potassium-dihydrogen-phosphate and 0·3 M-boric acid. The suspension was allowed to stand at 0°C for about 14 h and centrifuged for 6 h. The precipitate (B9) was resuspended in a buffer containing 0·1 M-sodium phosphate and 30 mM-sodium borate at pH 8·0 and homogenized with a Branson sonifier. This preparation may be stored at −90°C for one year without loss of activity.

The phosphate-borate precipitated fraction (B9) could be more highly purified by repeating the procedure of tris dialysis followed by phosphate-borate precipitation. The protein concentration was then adjusted to about 6 mg/ml, and B9 dialysed against 0·05 M-tris, pH 8·0 (100 volumes), for 14 h. The dialysis residue was centrifuged for 6 h at 65,000 *g*. The upper layer of the supernatant (S10) (about two thirds of the volume) gave a preparation (B11) with higher specific activity than the bottom layer when reprecipitated by adding the solution containing 1 M-potassium phosphate buffer and 0·3 M-sodium borate buffer pH 8·0 to a final concentration of 50 mM-phosphate and 15 mM-borate. All buffers contained 3 mM-EDTA. The purification procedure was done in the cold, except the sodium sulphate fractionation steps.

Preparation of different enzyme forms. Tris–HCl enzyme was prepared by dialysis overnight of a phosphate–borate precipitated preparation of glutaminase against 0·01 M-tris buffer containing 3 mM-EDTA, pH 8·0. The supernatant obtained after centrifugation for 1 to 6 h (depending on the volume) at 65,000 *g* in a Spinco Beckman ultracentrifuge (model L, rotor 30) contained the tris–HCl enzyme.

The phosphate-borate precipitated fraction prepared as described in the purification procedure above was used as phosphate-borate enzyme.

RESULTS

Purification. Using the purification method described (Table 1) we obtained a specific activity between 1·5 and 4·0 by sodium sulphate precipitation of glutaminase. Without the acetone powder step, the preparation obtained by sodium sulphate precipitation of an extract of homogenate in phosphate buffer did not reach a specific activity higher than 0·7.

When the specific activity of the purest preparation of brain glutaminase (B11, Table 1) was compared with that of brain homogenate in phosphate buffer, a purification of about 5000-fold was obtained. Treatment with detergents such as Triton X-100 and deoxycholate was of no use in the purification procedure. Applying acrylamide gel electrophoresis on the purest preparation following tris dialysis, several bands were observed after protein staining. Acrylamide inhibits the enzyme completely; and the different bands were not analysed for glutaminase activity for this reason.

Sucrose gradient centrifugation. The sedimentation coefficient of glutaminase is dependent on the buffer used in the sucrose gradients. Tris and phosphate buffer gave apparent molecular weights of 137,000 and 178,000 and sedimentation coefficients of 7·3 and 8·7, respectively (Figs. 1 and 2), whereas addition of borate to the phosphate buffer in the gradient increased the sedimentation coefficient to 53 (phosphate–borate enzyme) (Fig. 3 and Table 2). In the experiments reported here, we used

TABLE 1.—PURIFICATION OF GLUTAMINASE FROM PIG BRAIN

Fraction	Volume (ml)	Protein (mg)	Activity*	Specific activity†
Brain homogenate in phosphate buffer				0·02
Acetone powder in borate buffer	15,000	1,470,000	8217	0·005
Na_2SO_4 0–1·0 M (B2)	2280	21,500	9167	0·43
Na_2SO_4 0·6–0·8 M (B5)	225	2830	5083	1·80
1st tris–HCl (S8)	237	879	657	0·75
1st phosphate–borate (B9)	6	53·2	1367	25·7
2nd tris–HCl (S10)	12·5	22·7	299	13·2
2nd phosphate–borate (B11)	1·0	4·2	443	105

Starting material 3750 g brain (wet wt.). Glutaminase was assayed by the spectrophotometric method.

* Activity: μmol NH_3 formed/min.

† Specific activity: activity/mg protein.

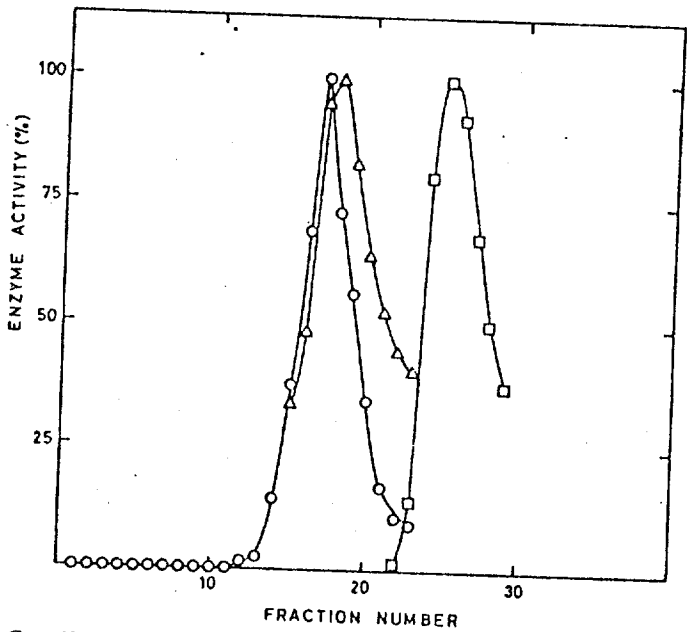


FIG. 1.—Centrifugation of pig brain glutaminase in a 5 to 20% sucrose gradient containing 0·1 M-tris and 3 mM-EDTA, pH 8·0. To this 2 mg glutaminase, 0·5 mg alcohol dehydrogenase, and 1 mg catalase were applied. The gradient was centrifuged for 14 h at 39,000 rev./min in a Spinco Beckman ultracentrifuge model L, rotor SW 39. Details of the procedure and enzyme assay are described under Methods. Enzyme activities are expressed as per cent of those at maximum.

O, glutaminase (spectrophotometric assay); Δ, alcohol dehydrogenase; □, catalase.

phosphate–borate precipitated enzyme. When the gradients contained tris or phosphate buffer, the enzyme was converted during the run from phosphate–borate enzyme (Fig. 3) to tris–HCl enzyme (Fig. 1) or phosphate enzyme (Fig. 2), respectively. The pH of the gradients was always 8·0.

The estimated values for sedimentation coefficients and apparent molecular weights for both the tris–HCl enzyme and the phosphate–borate enzyme are similar to those obtained for glutaminase from pig kidney (KVAMME *et al.*, 1970) and ox

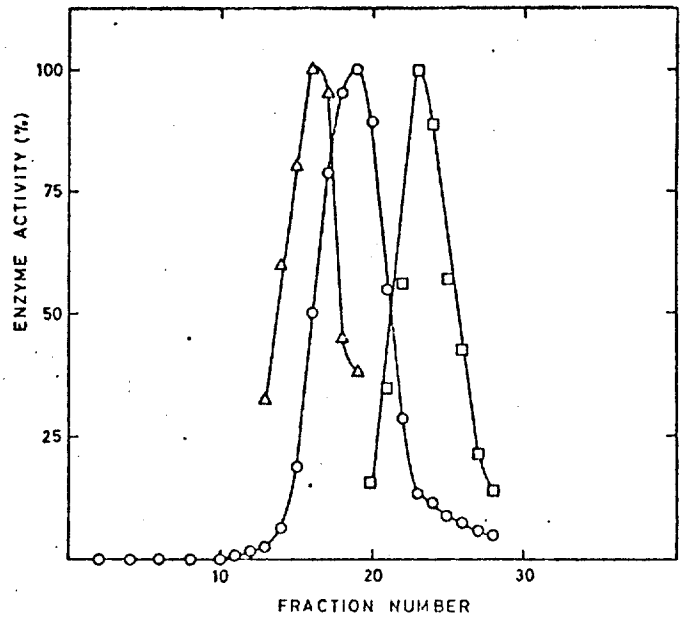


FIG. 2.—Centrifugation of pig brain glutaminase in a 5 to 20% sucrose gradient containing 0·1 M-sodium phosphate and 3 mM-EDTA, pH 8·0. Other conditions are as described in legend to Fig. 1.
O, glutaminase; △, alcohol dehydrogenase; □, catalase.

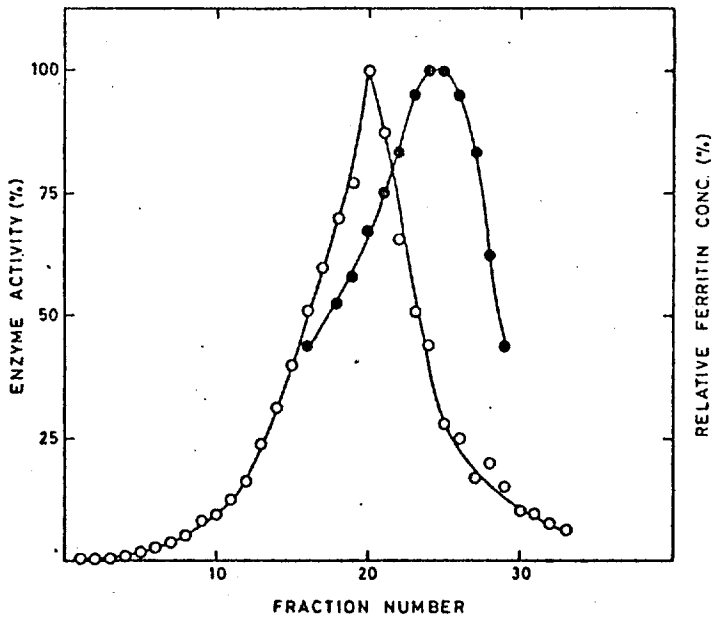


FIG. 3.—Centrifugation of pig brain glutaminase in a 5 to 40% sucrose gradient containing 0·1 M-sodium phosphate, 30 mM-sodium borate, and 3 mM-EDTA, pH 8·0. The gradient was centrifuged for 2 h, the other conditions are as described in legend to Fig. 1 with the exception that ferritin was used as marker.
O, glutaminase; ●, ferritin.

TABLE 2.—SEDIMENTATION COEFFICIENTS AND MOLECULAR WEIGHTS OF THREE DIFFERENT FORMS OF GLUTAMINASE

Buffer	$s_{20,w}$	Apparent molecular weight
Tris	7·3	137,000
Phosphate	8·7	178,000
Phosphate-borate	53	~2,000,000

The method of MARTIN and AMES (1961) was used and the centrifugations were performed as described in Figs. 1–3.

brain (KVAMME *et al.*, 1966). However, the sedimentation coefficient and apparent molecular weight for the phosphate enzyme are somewhat lower compared with preparations from pig kidney and ox brain. Thus, sedimentation coefficients and apparent molecular weights for these tissues were 10·8 and 248,000; and 11·3 and 320,000, respectively, when estimated by the method of MARTIN and AMES (1961).

pH *optimum*. The pH optimum for both the tris–HCl enzyme and the phosphate–borate enzyme was determined by the chromatographic assay. We were unable to find buffers which did not affect the rate of the reaction, and therefore we used phosphate buffer throughout, in spite of the fact that phosphate does not have buffering capacity in the whole pH range used. The final pH was checked in duplicate experiments to assure that hydrolysis of glutamine during the incubation did not change the pH. Special care was taken to ensure that the initial rate of enzyme activity was linear with time, as was the case for 2 min in the pH range from 6·5 to at least 10.

The tris–HCl enzyme has a symmetrical pH curve with optimum of about pH 8·8, whereas the phosphate–borate enzyme has an asymmetrical broader curve with the same optimum (Fig. 4). The data did not permit estimation of V_{max} in the pH range investigated. However, the shape of these curves was independent of the concentration of both glutamine (6·7 to 50 mM) and phosphate (100 to 300 mM). The apparent pK values for the enzyme–substrate complexes were estimated from a plot of log per cent activity against pH (Fig. 4B) (DIXON and WEBB, 1958), using the values in Fig. 4A. The apparent pK_1 and pK_2 for the tris–HCl enzyme complex were 7·5 and 9·3, respectively, and for the phosphate–borate enzyme complex, the apparent pK_2 was 9·5. In the latter case, only pK_2 could be estimated, because the slope of log activity against pH was 0·4 in the acidic pH range studied.

Electron microscopy. Electron microscopy of the phosphate–borate enzyme showed a predominance of helix-like structures (Fig. 5). These helices are identical with those observed in a phosphate–borate preparation of pig kidney glutaminase (KVAMME, SVENNEBY and TVEIT, 1968). In addition to helices, small structures were seen which also appeared to be similar to those of pig kidney glutaminase in the tris–HCl form. This may indicate that either we have not obtained a complete conversion of the tris–HCl enzyme to the phosphate–borate enzyme, or that there may be impurities. However, no helices were observed in the tris–HCl enzyme preparation before phosphate–borate buffer was added. In this preparation, the resolution of the instrument did not permit analysis of the exact structure.

DISCUSSION

In contrast to purification of kidney glutaminase (KVAMME *et al.*, 1970), where no impurities could be detected in the final preparation, purification of the brain enzyme

was not equally successful. Thus, the specific activity of the kidney enzyme is about three times higher than that of the brain enzyme. This discrepancy may depend on differences in the non-glutaminase protein of brain and kidney. Moreover, we obtained a preparation from brain having higher specific activity when the acetone powder step was introduced, whereas this step was of no use in the purification of kidney glutaminase.

The great change in molecular weight of glutaminase with the ionic environment is likely to be due to aggregation and disaggregation of subunits. However, we have been unable to obtain information about protein structures with lower molecular weight than the tris-HCl enzyme, because no catalytically active subunits were obtained by treating the tris-HCl enzyme with urea, guanidine-HCl or dodecyl sulphate.

The methods used for estimation of sedimentation coefficient and apparent molecular weight are inaccurate, but the values are of the same order of magnitude for corresponding forms of glutaminase in brain and kidney, except for the phosphate enzyme. In this case, values obtained for pig kidney (KVAMME *et al.*, 1970) are almost similar to those for ox brain (KVAMME *et al.*, 1966) and significantly higher than those for pig brain. It is of interest that the sedimentation behaviour in tris buffer of the

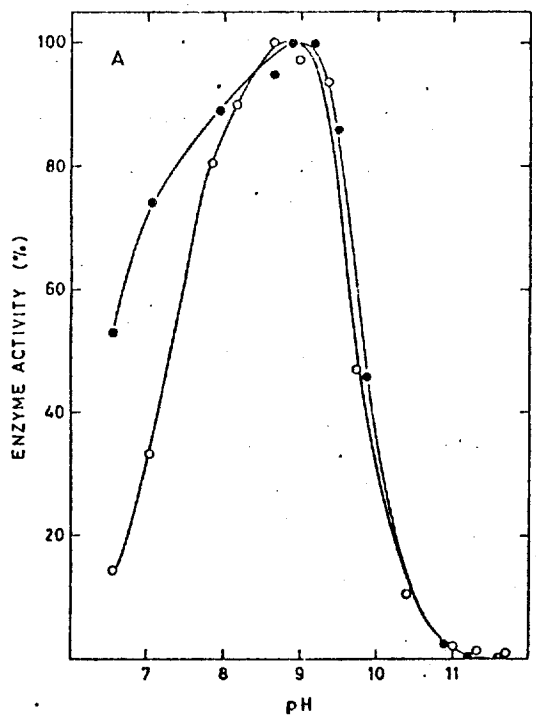


FIG. 4A.—Effect of pH on the activity of the tris-HCl enzyme O—O and the phosphate-borate enzyme ●—●. Chromatographic assay of glutaminase was performed as described in the text. The incubation mixture contained 100 mM-sodium phosphate, 0·17 mM-EDTA, and 50 mM-glutamine. Following adjustment of pH to the desired volume with NaOH, about 0·1 mg enzyme protein was added to 1 ml incubation mixture. The reaction was stopped after 2 min by adding 100 μl 40% PCA. Enzyme activities are expressed as per cent of those at the optimum pH.

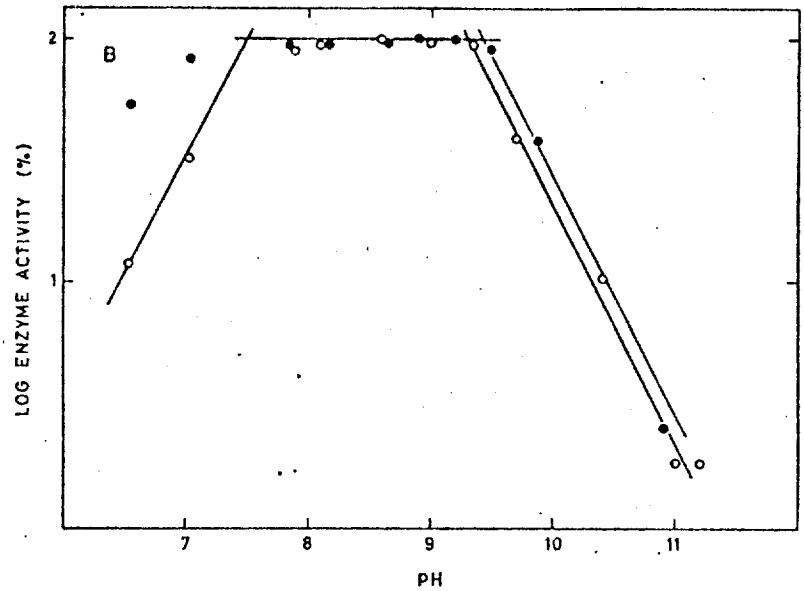


FIG. 4B.—Effect of pH on log enzyme activity. The data were obtained from Fig. 4A.

phosphate-independent glutaminase of KATSUNUMA, TEMMA and KATUNUMA (1968) corresponds to that of the tris–HCl enzyme. Moreover, the molecular weight of glutaminases from various species are different. Thus, the molecular weights of glutaminase from *Pseudomonas* (RAMADAN, EL ASMAR and GREENBERG, 1964) and from *E. coli* (HARTMAN, 1968) are only 25,000 and 110,000, respectively.

Two apparent pK values were found for the tris–HCl enzyme–substrate complex. It cannot be decided whether the apparent pK values of the enzyme–substrate complexes reveal ionisation of groups in the protein moiety or ionisation of the bound substrate. However, comparing the acidic side of the activity curve of the tris–HCl enzyme with a plot of the relative concentration of HPO_4^{2-} against pH, one finds a striking similarity, and pK_2 of phosphate is similar to pK_1 of this enzyme–substrate complex. Furthermore, pK_2 of the tris–HCl enzyme–substrate complex is similar to pK_2 of glutamine. It may therefore be assumed that the pK_1 and pK_2 values of the enzyme–substrate complex reflect the ionisation of groups on bound phosphate and glutamine, respectively.

In contrast to the tris–HCl enzyme, the phosphate–borate enzyme complex showed only one pK value in the pH range studied, which was of the same order of magnitude as pK_2 for glutamine, and no pK value similar to that of phosphate was observed. The loss of an acidic ionisation group on the enzyme–substrate complex by conversion of the tris–HCl enzyme to the phosphate-borate enzyme may be explained on the basis of aggregation of subunits.

Glutaminase from *E. coli* and *Pseudomonas* have pH optima on the acidic side (pH 3 to 5·8 and 6·6), whereas the pH optimum for the phosphate-dependent enzyme of KATSUNUMA *et al.* (1968) is in the range of 7·2 to 7·6, and the pH optimum for glutaminase from both pig brain and kidney is about 9. The difference in pH optima between glutaminases from various species may be due to several possible factors.

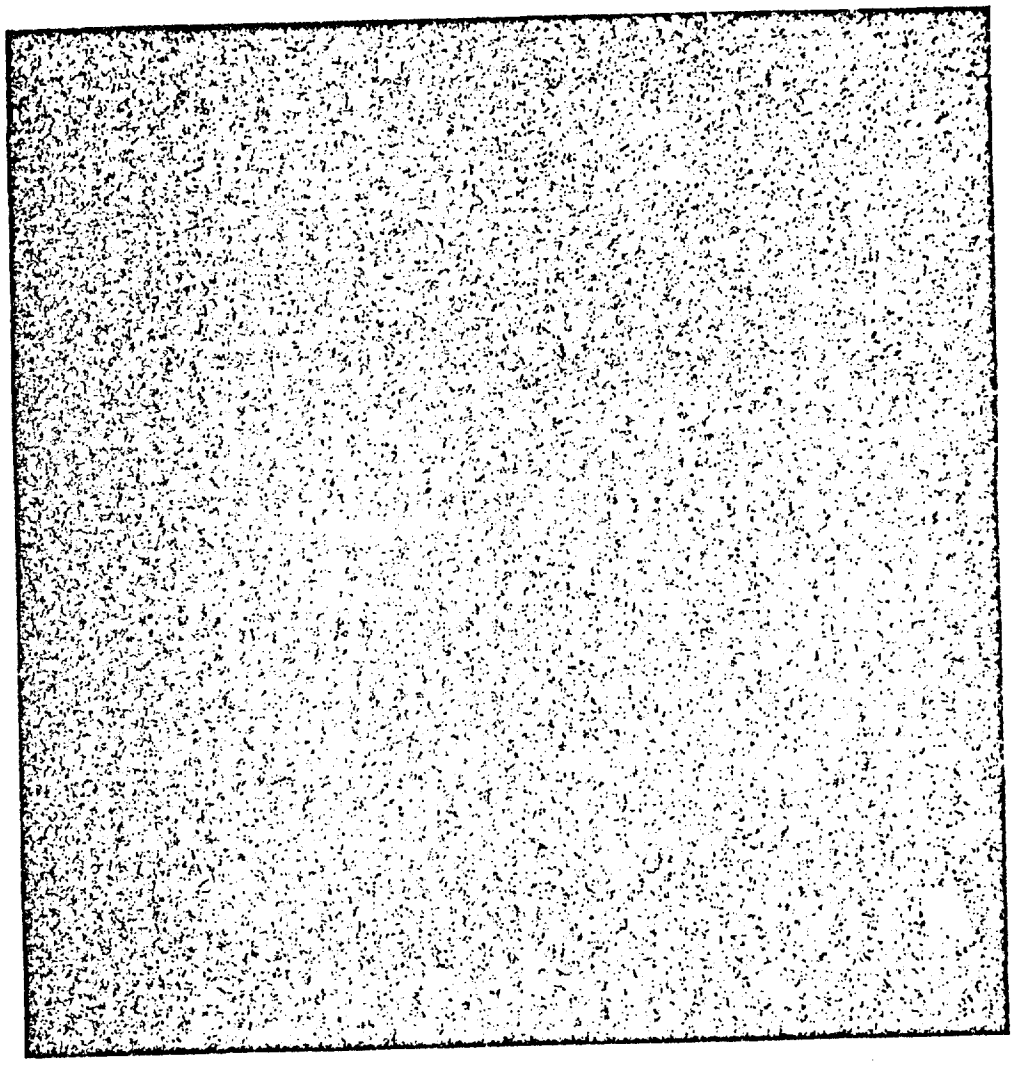


FIG. 5.—Staining with phosphowolframate, pH 8·0, of a tris–HCl enzyme (specific activity about 80, protein concentration 1·5 mg/ml) following incubation at 25°C for 30 min in a buffer containing 0·1 M-sodium phosphate + 30 mM-sodium borate, pH 8·0. The primary magnification was 40,000 times, and the total magnification was 200,000 times.

The environmental buffer system may change the pK of ionising groups in or near the active site as well as affect the subunit interaction. To the authors knowledge, nothing is known about subunit structure in glutaminases other than those of kidney and brain.

Acknowledgements—This research was supported by a grant from the Norwegian Research Council for Sciences and the Humanities. The author is indebted to dr. med. B. REINO OLSEN, Anatomical Institute, University of Oslo for preparing the electron microscopic picture, and also to dr. med. E. KVAMME for his advice throughout this work.

REFERENCES

CHANCE B. (1957) *Methods in Enzymology* **1**, 764.
DIXON M. and WEBB E. C. (1958) In *Enzymes* (Edited by DIXON M. and WEBB E. C.) p. 120, Longmans, Green and Co., London.
GREENSTEIN J. P. and CARTER C. E. (1947) *J. nat. Cancer Inst.* **7**, 57.
HARTMAN S. C. (1968) *J. biol. Chem.* **243**, 853.
KATSUNUMA T., TEMMA M. and KATUNUMA N. (1968) *Biochem. biophys. Res. Commun.* **32**, 433.
KREBS H. A. (1935) *Biochem. J.* **29**, 1951.
KVAMME E., SVENNEBY G. and TVEIT B. (1966) In *Molecular Basis of Some Aspects of Mental Activity* (Edited by WALAAS O.) p. 211, Acad. Press, London, New York.
KVAMME E., SVENNEBY G. and TVEIT B. (1968) In *Regulation of Enzyme Activity and Allosteric, Interactions* (Edited by KVAMME E. and PIHL A.) FEBS Symposium, p. 89, Universitetsforlaget Oslo.
KVAMME E., TVEIT B. and SVENNEBY G. (1970) *J. biol. Chem.* **245**, 1871.
LAYNE E. (1957) *Methods in Enzymology* **3**, 447.
LOWRY O. H., ROSEBROUGH N. J., FARR A. L. and RANDALL R. J. (1951) *J. biol. Chem.* **193**, 265.
MARTIN R. G. and AMES B. N. (1961) *J. biol. Chem.* **236**, 1372.
RACKER E. (1957) *Methods in Enzymology* **1**, 500.
RAMADAN M. E. A., EL ASMAR F. and GREENBERG D. M. (1964) *Biochim. biophys. Acta* **108**, 143.
SIEGEL L. M. and MONTY K. J. (1965) *Biochem. biophys. Res. Commun.* **19**, 494.
SVENNEBY G. (1969) *Abstract 2nd Int. Meeting Int. Soc. Neurochem.*, Milano, p. 386.
SVENNEBY G., TVEIT B. and KVAMME E. (1970) *J. biol. Chem.* **245**, 1878.

Acidity. Transfer 50 ml. of the sample into a 300-ml. Erlenmeyer flask containing 1 ml. of bromocresol purple T.S. and 50 ml. of methanol that has been previously titrated with 0.1 *N* alcoholic potassium hydroxide to the first appearance of a bluish purple color, and titrate the sample with the alkali to the same color. Not more than 4.2 ml. is required.

Monosodium L-Glutamate, page 544

Change the SPECIFICATION (*Limits of Impurities*) for *Loss on drying* to read:

Loss on drying. Not more than 0.5 percent.

Change the paragraph entitled *Loss on drying*, page 545, to read:

Loss on drying, page 931. Dry at 100° for 5 hours.

Niacin, page 549

Change *Identification test C*, page 550, to read:

C. The infrared absorption spectrum of a mineral oil dispersion of the sample, previously dried at 105° for 1 hour, exhibits maxima only at the same wavelengths as that of a similar preparation of N.F. Niacin Reference Standard.

Papain, page 574

This monograph is subsumed by the new monograph for *Enzyme Preparations*, page 18 of THIS SUPPLEMENT.

Paraffin, Synthetic

Insert the following new monograph to precede the monograph entitled *Parsley Seed Oil*, page 576:

Evaluating the Safety of Food Chemicals

FOOD PROTECTION COMMITTEE
FOOD AND NUTRITION BOARD
DIVISION OF BIOLOGY AND AGRICULTURE
NATIONAL RESEARCH COUNCIL

NATIONAL ACADEMY OF SCIENCES
WASHINGTON, D.C. 1970

ISBN 0-309-01859-5

Available from

Printing and Publishing Office
National Academy of Sciences
2101 Constitution Avenue, N.W.
Washington, D.C. 20418

Library of Congress Catalog Card Number 70-608751

Printed in the Un. States of America

Preface

The Food Protection Committee of the Food and Nutrition Board has, during the past 20 years, periodically reviewed general principles and procedures for evaluating the safety of food chemicals. During that period proposals for improving the assurance of safety have been made by many individuals and organizations. This report is an evaluation by the Subcommittee on Toxicology of the more recently proposed or adopted procedures, against a background of experience with what have become conventional procedures.

FOOD PROTECTION COMMITTEE

William J. Darby, *Chairman*
David B. Hand, *Vice Chairman*
John C. Ayres
Julius M. Coon
Kenneth P. DuBois
Lloyd J. Filer, Jr.
Leon Golberg
Wayland J. Hayes, Jr.
Lloyd W. Hazleton
Emil M. Mrak
Bernard L. Oser
Virgil B. Robinson
John H. Rust
R. Blackwell Smith, Jr.
Frank M. Strong

SUBCOMMITTEE ON TOXICOLOGY

Julius M. Coon, *Chairman*
Frank R. Blood
Kenneth P. DuBois
David W. Fassett
Lloyd W. Hazleton
Arnold J. Lehman
Bernard L. Oser
R. Blackwell Smith, Jr.
J. H. Wills
John A. Zapp, Jr.

Contents

INTRODUCTION 1

Definition of Terms 1
Bases for Evaluation of Safety 2
Current Practice in and Scope of Toxicologic Evaluation 7

EVALUATION OF CONVENTIONAL METHODS 10

SOME RECENT APPROACHES TO SAFETY EVALUATION 14

Studies on the Metabolic Fate of Chemicals *in Vivo* 14
Biochemical and *in Vitro* Studies 21
Design of Studies to Develop Tests Applicable to Man and Properly Chosen Animals Species 23
Physiologic Studies 25

EVALUATING CARCINOGENIC POTENTIAL 35

General Considerations 35

INTERPRETATION AND PREDICTION OF SAFETY 40

Factors Affecting Prediction 40
Prediction of Safety for Man from Observations on Animals 41
Selection of Species 42
Prediction of Safety for Man from Observations on Man 43
In Vitro Models 46
Evaluation of Carcinogenic Hazards of Chemicals for Man 47

APPENDIX: Guidelines for Estimating Toxicologically Insignificant Levels of Chemicals in Food 49

Introduction

The Food Protection Committee, The Joint FAO/WHO Expert Committee on Food Additives, and the U.S. Food and Drug Administration (FDA) have published complementary statements of the principles underlying evaluation of the safety of food chemicals and have set forth proposed procedures for accomplishing the evaluation.[1-10] Recently the FDA has been actively developing protocols as guides for the evaluation of safety of food, drug, and cosmetic chemicals. It is not the purpose of this report to present another set of principles and procedures, but rather to review the purposes and value of what have become conventional procedures and to evaluate in light of that review the contribution or potential contribution that more recently adopted and proposed procedures might make to ensuring the safety of the food supply.

DEFINITION OF TERMS

Natural components of foods are obviously chemical substances. However, the term *food chemicals,** as here used, relates to substances

*The term *food chemical* is used rather than *food additive* because the latter term has specific legal meanings in the United States. Food additive, according to the Food, Drug, and Cosmetic Act, may not include pesticide residues, substances "generally recognized as safe," and color additives.

added to food either directly and intentionally for a functional purpose, or indirectly during some phase of production, processing, storage, or packaging without intending that it remain in, or serve a purpose in, the final product. It does not include either the basic foodstuff itself or chance contaminants. A food chemical may be of natural or synthetic origin; it may be nutritive or nonnutritive and physiologically active or inert. The procedures for evaluating the safety of a chemical for use in food are independent of the reasons for its presence in food.

Toxicity is the capacity of the substance to produce injury. The term includes capacity to induce teratogenic, mutagenic, and carcinogenic effects.

Safety is the practical certainty that injury will not result from the substance when used in the quantity and in the manner proposed for its use.

Hazard is the probability that injury will result from the use of a substance in a proposed quantity and manner.

The evaulation of safety or hazard must take into account the conditions of use of the substance.

BASES FOR EVALUATION OF SAFETY

Safety of a food chemical should be appraised relative to the extent of its proposed use in foods, to the amount that would be consumed under all likely dietary patterns of the populations concerned, to the nature of the biologic response it may induce, and to the minimal intake that might provoke response. No use of a chemical in food should be permitted if there is reason to anticipate that the intake of the chemical from the permitted use could be so high as to produce adverse effects.

Risk–Benefit Relation The decision to use a chemical in such a way that it may remain in the food as consumed should be based on the assurance that the use will be safe (i.e., that there is virtual certainty that no injury will result), and that, directly or indirectly, the use will in some way benefit consumers. Factors to be considered include the following:

The estimated hazard to the consumer
Consumer needs and wishes
The requirements of food supply and public health

The needs of the food producer and processor
The economy, and
The availability of methods and mechanisms for regulatory control

No single formula can relate all these factors to one another under all conditions, but they should be taken into account whenever a decision is being made concerning whether to permit or deny use of a chemical. The risk–benefit relation is affected by the circumstances that pertain to a given situation. For example, if the supplies of a major food staple in a food-deficient area are threatened by pests, use of a chemical to control the pests might well be justified, even though levels of residues greater than those usually permitted remain in the food.

Chemical and Physical Properties It should be possible to characterize the substance in question in chemical and physical terms. Constancy of composition, stability, and freedom from harmful amounts of impurities should be assured. The *Food Chemicals Codex*[11] is designed to provide national standards of identity, purity, and composition for food chemicals, as has been done for drugs in the United States and elsewhere. Standards for food-grade chemicals are described in the *Codex* by chemical and physical specifications that can be attained through good manufacturing practice but that, at the same time, provide reasonable standards of identity and assurance of safety. Obviously, such a compendium is never complete, nor are its listed specifications necessarily permanent; it must be continuously reviewed and revised. Patterns of use of foods and new toxicologic knowledge may be expected to determine the chemicals included as well as their specifications.

The persistence of the chemical in the foods in which it is to be used, its reactivity with the components of these foods, and the identity of the substances to which it may be converted in foods should be known.

Methods for estimation of chemicals in foods must be sufficiently accurate and sensitive to permit estimation of quantities hazardous to health. When conversion products are likely to be hazardous, appropriately accurate and sensitive assay methods for their detection should be available. When, however, the proposed use of a chemical cannot reasonably be expected to result in hazardous amounts being present in food, it is not necessary that analytical methods practicable for routine application to foods in commerce be developed.

Biological Aspects Results of critically designed animal studies of the physiologic, pharmacologic, and biochemical behavior of a proposed food chemical provide the crucial information in the evaluation of safety at a specified level of intake by man. Because of species variation, however, judgment must be exercised in basing a safe level of use by man on data derived from studies with various species of animals. No method for establishing the safety of a food chemical with absolute certainty under all conditions of use is at hand, and none is in sight. Experience has shown, however, that properly conducted and interpreted animal experiments can provide that degree of practical assurance of safety reasonably expected in the evaluation of chemicals for use in human food. In rare instances, adverse responses in animals, discovered well after the material in question had come into regular use, have led to discontinuance of that use, even though no hazard to man had been demonstrated. Nitrogen trichloride, certain synthetic colors, and a few naturally occurring flavoring components, e.g., safrole and coumarin, are examples.

The appropriate study of responses in human beings is a valuable phase of the safety evaluation of chemicals used, proposed for use, or present in food. Occupationally or accidentally exposed subjects provide some data; another possibility is the use of volunteers in controlled studies. The latter should be carried out only after careful investigation of biochemical and toxicologic properties in animals has clearly indicated that the risk to which the subjects may be exposed will be trivial in relation to the benefit of the knowledge to be expected. Data from human studies are especially appropriate in assessing the safety of a material of exceptionally widespread potential use.

Once a food chemical is approved for use, there should be continual observation of the exposed population for deleterious effects that might emerge under prolonged and varying conditions of use, and safety of the use should be reappraised whenever warranted by experience in use or advances in knowledge.

It has been customary to permit use of a new chemical on the basis of long-term feeding studies in at least two species of animals. Chronic toxicologic studies in animals are designed to find a dosage level that will produce a deleterious effect in the animal as a whole or in one or more of its organs. It is not always possible, however, to find a deleterious effect unequivocally related to toxic properties of the substance being studied. In addition to a toxic level, a "no-adverse-effect" level

is sought. This level, adjusted by incorporating an adequate margin of safety, provides an estimated safe level for use in the human diet.

Patterns of Consumption Certain substances that have been in use for many years, either as food chemicals or as natural components of foods, are assessed as being "generally recognized as safe" (GRAS) by experts qualified by scientific training and experience to make that evaluation. Such assessments, like those for any food chemical, should take into account specified or reasonably inferrable patterns of food consumption, for which it may be desirable to consider such regional or national surveys of food consumption patterns as those reported by the U.S. Department of Agriculture.[12] A bulletin on "High Consumption of Foods," prepared by the Agricultural Research Service of the U.S. Department of Agriculture,[13] has stated that "because of increasing need for estimating chemical residues and food additives in foods, there has been growing interest and information on quantities of foods used by *high* consumers, as well as by *average* consumers." High levels of food consumption in this instance are based on data for the ninth decile of the population, i.e., the group representing the highest 10 percent of consumers as distinguished from the remaining 90 percent. These estimated levels are 1.5 to 3.5 times the mean consumption for each broad category of foods.

Adverse reactions that may result from the gross misuse of an otherwise beneficial substance in a food should not militate against its proper use.

Regulatory Aspects The regulatory control of chemicals in foods is normally based in part on the principle that there is a "safe" level of intake for any chemical. In some instances, the high toxicity of a given substance or threat of serious hazard (e.g., carcinogenesis) has led to the setting of "zero" tolerances. The concept "zero" is dependent upon developments in analytical methodology, and a zero tolerance may have little significance from the viewpoint of safety evaluation. In other cases, small quantities of chemicals are known to be present in foods but are treated administratively as "negligible residues," i.e., insignificant or inconsequential insofar as public health is concerned.

It has been proposed that a "zone of toxicologic insignificance" be recognized for administrative purposes. When the maximum level of a chemical likely to be present in food is either calculated on the basis of its proposed technologic use or is analytically determined to be an

amount sufficiently below the safe level, the possibility that injury will result approaches zero. Such a level, which can be established only by qualified experts, is within the zone of toxicologic insignificance, and therefore does not require regulatory action (see Appendix, Guidelines for Estimating Toxicologically Insignificant Levels of Chemicals in Food).

Food additives, as legally defined in the United States, embrace the entire range of substances, the intended use of which may result in their becoming components of or otherwise affecting the characteristics of any food. It is immaterial whether the effect is intentional or unintentional, direct or indirect. Taking into account the broad scope of this definition, Congress provided for certain exceptions, the principal one being to exclude those substances that scientifically qualified experts generally recognize as safe under the conditions of intended use (GRAS) on the basis of either scientific procedures or experience based on common use in food. The effect of this exemption is to use scientific judgment informally in deciding whether it is necessary to require submission of a formal petition, along with supporting evidence of safety, for a regulation to permit and limit the use of a food chemical.

In addition to such common food ingredients as salt, pepper, sugar, vinegar, and baking powder, hundreds of substances have been regarded as GRAS when used in accordance with "good manufacturing practice." The latter term implies that the substances in question are of suitable food grade and that they are used and remain in food in amounts no higher than necessary to accomplish their intended effects. Judgment that a substance is GRAS also takes into consideration the history of the occurrence or use of the substance in food, experience through its use in medicine or in industry, and biochemical, metabolic, and toxicologic evidence relating to the substance itself or to chemically or pharmacologically comparable substances.

Of paramount importance in any evaluations of safety are the conditions of intended use. In addition to the level or concentration of the food chemical, these conditions include the specific food or foods to which it will be added, the amounts and frequency of consumption, and the age, sex, and physiological state of the consuming population.

When all available information is taken into consideration, a GRAS appraisal can provide assurance of safety sufficient to obviate the need for subjecting every food chemical, irrespective of its origin, to the extensive animal testing required to support a food additive petition. Of course, as new knowledge develops relevant to safety evaluation, as patterns of food consumption change, or as technological uses of a substance in food production and processing change, its status as GRAS should be subject to review.

CURRENT PRACTICE IN AND SCOPE OF TOXICOLOGIC EVALUATION

Current practice in the toxicologic evaluation of food chemicals has evolved from experience with laboratory animals, particularly from knowledge of their nutritional requirements and biological reactions. During the early part of this century studies of the physiology, anatomy, and behavior of the albino rat led to its being selected as the species of choice for nutritional investigations. Research resulting in the discovery of vitamins, which proceeded intensively during the second, third, and fourth decades, led to the development of diets that made it possible to conduct lifetime feeding studies in the rat and, subsequently, in other species.

Toxicologic procedures have evolved further in both design and scope. It is now generally considered desirable to employ at least one nonrodent mammalian species in addition to the rat or mouse. Studies may well continue over the major part of the lifetime of the rodent and for at least one year (generally two years) in tests with the dog or monkey. The common practice is to administer at least three dosage levels of the substance under test to each species in the expectation of finding both a no-adverse-effect level and a dose that induces some abnormal response. The number of animals routinely assigned to each experimental group of rats has increased from 5 or 6 in the early days to the present practice of using 25 of each sex, with a view toward increasing the statistical validity of the findings, especially at threshold dosages. The number of available biochemical indices of effect has increased from a few simple blood and urine determinations to a score or more that include levels of metabolites, electrolytes, and enzymes in body fluids and tissues. In many instances metabolic and functional tests are conducted. Effects on all aspects of reproductive physiology, with emphasis on the possibility of teratogenesis and on lactation, are investigated intensively. Lifetime studies are conducted to detect carcinogenic potential.

Histopathologic observations are now made on biopsy material as well as on material obtained postmortem and on many more tissues and organs than were examined in the early years. Electron microscopy, in addition to light microscopy, is being employed to detect

ultrastructural changes. Studies of the absorption, storage, excretion, and biotransformation of chemicals in the animal body are often an essential feature of toxicological assessments, and behavioral studies may be of use in such evaluations. In the future it will doubtless become customary to employ an even larger number of increasingly exacting tests for adverse reactions in animal species as a prerequisite for safety evaluation, at least of food chemicals that are proposed for extensive use.

REFERENCES

1. Food Protection Committee, Food and Nutrition Board. 1954. Principles and procedures for estimating the safety of intentional chemical additives in foods. NAS–NRC Publication. National Academy of Sciences, Washington, D.C.
2. Food Protection Committee, Food and Nutrition Board. 1959. Principles and procedures for evaluating the safety of food additives. NAS–NRC Publ. 750. National Academy of Sciences, Washington, D.C.
3. Food Protection Committee, Food and Nutrition Board. 1959. Problems in evaluating the carcinogenic hazard from use of food additives. NAS–NRC Publ. 749. National Academy of Sciences, Washington, D.C.
4. Food Protection Committee, Food and Nutrition Board. 1965. Some considerations in the use of human subjects in safety evaluation of pesticides and food chemicals. NAS–NRC Publ. 1270. National Academy of Sciences, Washington, D.C.
5. Food Protection Committee, Food and Nutrition Board. 1967. Use of human subjects in safety evaluation of food chemicals: Proceedings of a conference. NAS–NRC Publ. 1491. National Academy of Sciences, Washington, D.C.
6. Joint FAO/WHO Expert Committee on Food Additives. 1957. General principles governing the use of food additives: First report. FAO Nutrition Meetings Report Series No. 15. WHO technical report.
7. Joint FAO/WHO Expert Committee on Food Additives. 1958. Procedures for the testing of intentional food additives to establish their safety for use: Second report. FAO Nutrition Meetings Report Series No. 17. WHO technical report.
8. WHO Scientific Group. 1967. Procedures for investigating intentional and unintentional food additives. WHO Technical Report Series No. 348. WHO.
9. Joint FAO/WHO Expert Committee on Food Additives. 1961. Evaluation of the carcinogenic hazards of food additives: Fifth report. FAO Nutrition Meetings Report Series No. 29. WHO technical report.
10. Division of Pharmacology, Food and Drug Administration. 1959. Appraisal of the safety of chemicals in foods, drugs and cosmetics. The Association of Food and Drug Officials in the U.S., Baltimore.
11. Committee on Specifications, Food Chemicals Codex, Food Protection Committee. 1966. Food chemicals codex. I. NAS–NRC Publ. 1406. National Academy of Sciences, Washington, D.C.

12. U.S. Department of Agriculture, Agricultural Research Service. 1968. Food consumption of households in the United States, spring 1965. U.S. Government Printing Office.
13. U.S. Department of Agriculture, Agricultural Research Service, Household Economics Research Division. Nov. 17, 1960. High consumption of foods (mimeo).

Evaluation of Conventional Methods

In 1959, the Food Protection Committee described the procedures then deemed most applicable to the evaluation of the safety of food chemicals.[1] After considering anticipated amounts and patterns of consumption of food chemicals, as well as their physical and chemical properties, the toxicologic aspects of the evaluation were described in terms of studies in rats and dogs of acute oral toxicity, subacute oral toxicity, and chronic (2-year) oral toxicity. The principal basis for the evaluation of safety became the determination of what was then termed the "no-effect" level in the 2-year study with rats and dogs. From this no-effect level, incorporating a substantial safety factor, an estimate could be made of a safe level of intake for man. In general, a no-effect level was regarded as one that produced no disturbance in growth, no clinical illness, no change in the mortality rate or pattern, no adverse biochemical or physiological effect, and no evidence, by gross and microscopic examination, of damage to body tissues or organs. Finally, this level of intake had nc observed adverse effects on the reproduction of the rat.

On *a priori* grounds there is a calculated risk in basing a judgment with respect to the human species on the responses of any two non-human species. Nevertheless, the principles and procedures described in 1959 appear to have served well. Authenticated cases of human injury resulting from the presence of chemicals in foods as a consequence of approved usage in production, processing, packaging, or storage are extremely rare, indicating that the older methods have been effective; they should not be casually abandoned in favor of approaches that may be attractive only because they are new and different.

The classic 2-year chronic toxicity study took into consideration three things: that variation is to be expected among individuals within a single species; that variation is to be expected among species; and that a lifetime, or even several generations, of the test species might be required to demonstrate certain adverse effects.

Intraspecies variation was taken into account by insisting that enough animals be employed to validate the statistical significance of the results—in practice a minimum of 25 male and 25 female rats in each experimental group. Interspecies variation was taken into account by using the dog as comparison with the rat. The time required for the development of subtle effects and for the appearance of such slowly developing lesions as tumors was taken into account by extending the study over the approximate life span of the rat. Reproductive and teratogenic effects were sought through rat reproduction studies.

Tests of this kind are expensive and time-consuming, and they yield little information on just why or how the observed effects are produced. Methods developed since 1959 offer to supply some of these answers, but the crucial question is whether the newer methodology provides greater assurance of safety with smaller expenditure of time, scientific resources, and money, or whether they merely supplement the traditional methodology.

Since 1959 methods have been devised that will provide much more specific answers than those obtainable from chronic toxicity regardless of how carefully the latter are designed and conducted. The use of larger numbers of experimental animals and of more species will increase the precision of the observations that can be made but cannot supply information on mechanism of action. Among the principal areas in which the older methods fail to provide adequate answers are the following:

- In the absence of specific collateral studies, chronic studies do not provide knowledge of either the metabolic pathways followed by a compound or the physiological response induced by metabolic overloading. Adverse effects from relatively high dosage levels (i.e., meta-

bolic overloading) may be accorded (and in practice have been so accorded) undue importance in evaluating safety whereas, in fact, normal metabolism could safely cope with exposures at realistic use levels.

- The correlation between carcinogenic activity of chemicals in rodents and known chemical carcinogenesis in man is not sufficiently consistent to permit prediction of human hazard by direct translation from the effects in animals. Nonrodent species such as dogs, cats, and primates have life spans that make total lifetime exposure impractical, and there is therefore little, if any, information on carcinogenesis in these species to correlate with effects in man.
- In the complex environment of modern life, human beings are exposed to a multiplicity of chemicals. Although few instances of specific health hazard from the interaction of chemicals in the environment have shown up, there is always this possibility. Results from conventional methods rarely, if ever, provide insight into these possibilities.
- In general, conventional methods of estimating acute, subacute, and chronic toxicity do not bear on potential effects on reproductive function or on embryonic development. Modifications have been adopted to include these aspects, but further research is needed.

The above critique of conventional methods does not impugn their value, as established by experience. It does suggest that evaluation of the safety of food chemicals should be dynamic. As new methods are demonstrated to be more reliable than earlier ones, they should be adopted. Unless they meet this criterion, however, reliance must be placed on the conventional methods as refined by improved statistical control, by using animals of uniformly high quality, and by incorporation of advanced techniques.

It is essential that whatever tests are used they be performed on a sufficiently large number of animals of each species to ensure the desired level of statistical validity. At the present time no studies of short duration can be relied upon to predict the occurrence or nonoccurrence of such long-term effects as the induction of tumors. In spite of obvious disadvantages in terms of time, effort, and money, procedures for evaluating the safety of food chemicals recommended in 1959 cannot be considered obsolete.

It would be desirable indeed to have short, simple, and safe methods for evaluating the safety of food chemicals directly in the human species, but they are not now in sight. Studies on human subjects can be done under rigidly controlled conditions with reasonable safety, and results from such studies may well contribute to the overall evaluation

of safety. It is obvious, however, that in any use of human subjects the benefit in terms of knowledge to be gained should far outweigh the anticipated risk to the subjects, a matter that has recently been discussed in some detail.[2]

REFERENCES

1. Food Protection Committee, Food and Nutrition Board. 1959. Principles and procedures for evaluating the safety of food additives. NAS–NRC Publ. 750. National Academy of Sciences, Washington, D.C.
2. Food Protection Committee, Food and Nutrition Board. 1967. Use of human subjects in safety evaluation of food chemicals: Proceedings of a conference. NAS–NRC Publ. 1491. National Academy of Sciences, Washington, D.C.

Some Recent Approaches to Safety Evaluation

STUDIES ON THE METABOLIC FATE OF CHEMICALS *IN VIVO*

The effects of chemicals in various species can be considered from the point of view of the action of the chemical on the body or, conversely, of the action of the body on the chemical. The first is the principal objective of the search for the mechanism of action of a substance; the second is concerned mainly with the overall metabolism of the substance. For the purposes of this discussion, "metabolism" comprises everything that happens to the chemical during its stay in the body, including absorption from the intestinal tract, binding to proteins, transport through cell membranes, storage in various tissues, conversion to new substances (which may be either less toxic or more toxic), and excretion of the substance or its metabolites.

Metabolic studies, in general, long antedate their introduction into experimental toxicology. As early as 1830, Liebig proved that the substance present in the urine of horses that he called hippuric acid resulted from the combination of benzoic acid with a then unknown organic compound; shortly thereafter Keller showed that hippuric acid was produced in man from ingested benzoic acid.[1] By the beginning of the present century, the basic patterns of metabolic changes for many classes of organic compounds were relatively well understood, and a major topic during the early development of biochemistry was the metabolic fate of food substances.

One of the early applications of metabolic studies to food chemicals was that contained in the report of the Board of Scientific Advisors to the Secretary of Agriculture in 1911 on the safety of saccharin in man[2]—a detailed account of the nature and rate of excretion in urine. The development of sulfa drugs and of many other new pharmacologically active substances resulted, in the 1930's, in greatly increased use of metabolic studies in pharmacologic research. It was not until relatively recently, however, that this important approach received special attention in the study of food chemicals.[3]

The early lack of emphasis on metabolic studies is perhaps understandable in that their cost is high and the duration lengthy. Newer techniques, particularly the use of isotopes, various types of chromatography, and some of the newer developments in spectrometry, make it feasible to include metabolic studies in the ordinary course of development of toxicologic information about food chemicals. Along with the great increase in knowledge of chemical metabolism during recent years has come a realization that, when studied by modern techniques, the metabolic process is frequently more complex than had been previously thought and that it is not always possible to interpret the resulting data in terms of the safety of the compound.

Barnes and Denz,[4] in a critical review of the then current experimental methods for predicting cumulative effects of substances in man, said in effect that the toxicologist should determine not only whether a substance is toxic but, if so, why. Conversely, if a material is believed to be safe, evidence should be marshaled to support that view (e.g., evidence of metabolism and excretion). They advocated relatively short-term, high-dose studies to elicit any apparent toxic effects to be followed by intensive biochemical study with attention being given to the metabolism of the substance and to confirmation by studies on man whenever feasible. Only after this information is available, according to the authors, can other types of animal studies be properly designed. The report of the Food Protection Committee on the use of human subjects in safety evaluation[3] emphasizes much the same viewpoint and points out that one of the greatest needs at present is for better knowledge of the comparative metabolism of substances in various animal species and man.

Metabolic studies do not give direct evidence of the presence or absence of a toxic effect. It is instructive, however, to compare the toxicity and metabolism of foods, including their naturally occuring toxic

constituents, to those of chemicals that may be used in the production of foods. Evidence that the living organism uses similar metabolic pathways for any given chemical regardless of whether it is encountered in natural food or as a purely synthetic substance is overwhelming.

The ability of man to ingest with apparent impunity the nearly infinite variety of chemical substances found in his normal food is closely related to his ability to use them directly or to convert them to substances that are relatively easily excreted or stored without injury. It is apparent that man's adaptation to chemicals is related not only to his ability to handle them through the normal metabolic pathways associated with food components or endogenously produced substances, but also to the fact that substances never before encountered can be metabolized because of increased microsomal or other enzyme activity that may be induced following a short period of contact.[5] Although it is recognized that *qualitative* information about absorption, transport, storage, biotransformation, and excretion is important, we emphasize the fact that *rates* at which such processes occur often determine the differences among species and individuals in their responses to toxic materials. Experience with pharmaceutical agents provides many examples in which the rates of these processes determine the toxicity, side reactions, or effectiveness under given circumstances.[5] There is no reason to suppose that differences will not occur among animals used in toxicologic studies of chemicals, and they must be recognized in considering the safety of these chemicals for use in foods.

The following are areas in which information may be useful in assessing the safety of food chemicals:

Conversion to normal metabolic products. There are many instances in which lack of toxicity can be explained by the metabolic conversion of the substance to one or more products, the further metabolism of which is already known. One example is propylene glycol, a portion of which is converted to glycogen in the liver, whereas the remainder is excreted in the urine as an ester. The safety of many simple fatty acid esters is associated with their ready hydrolysis to the component alcohols and acids, which then enter the normal metabolic pathways. Many flavoring agents are esters of this category, a fact generally considered a strong indication of their safety.

Excretion, unchanged or in less toxic form. Rapid excretion cannot stand alone as a criterion of lack of toxicity, but there are so many examples from feeding studies of the close correlation between lack

of toxicity and rapid and complete excretion that the significance is obvious. A high margin of safety frequently appears to be related to rapid excretion of a material in the urine either unchanged or as some metabolite less toxic than the original substance. The conversion of benzoic acid to hippuric acid, which is then excreted in the urine, is an example. A number of phenolic substances are acetylated or conjugated and rapidly excreted in the urine. Nitrates appear to be extremely rapidly absorbed and excreted, as such, in the urine. Nitrate may be reduced to nitrite by the flora of the upper gut. This is usually of no consequence in the adult but, because of the greater susceptibility of infant hemoglobin, may be important in causing methemoglobinemia in infants. A number of dyes and larger molecules may be excreted via the biliary system rather than through the urine. Some solvents, for example, ethyl chloride and methylene chloride, are very rapidly and completely excreted in the expired air, apparently with no biochemical change. By contrast, substances such as benzene are converted to many diverse metabolites that are disposed of in a variety of ways. There is increasing evidence that the toxicity of carbon tetrachloride may be due to its conversion in the body to a free radical or other reactive metabolite.[6]

Formation of more toxic substances. Although most biotransformations of foreign organic chemicals result in less toxic compounds, the opposite occasionally occurs. The classical example is the conversion of fluoroacetate to fluorocitrate, which effectively interrupts the Krebs cycle and results in extraordinary toxicity. Other examples are the conversion of esters of thionophosphates to the corresponding phosphates, which are the actively toxic substances, and the conversion of nitrates to nitrites.

Metabolism and carcinogenesis. Recent reviews[7, 8] of carcinogenesis have emphasized that the action of most well-known carcinogens can be theoretically associated with the formation of "proximate" carcinogenic metabolites. Although information of this kind cannot yet be used directly in predicting carcinogenicity, research in this area should lead to improved predictability.

Evidence of nonabsorption from the intestinal tract. There are a number of substances, practically devoid of toxicity when taken orally, that are known to be not absorbed from the intestinal tract. Cellulose and a variety of artificially produced cellulose derivatives such as methylcellulose,[9] sodium carboxymethylcellulose,[10] and sodium cellulose sulfate,[11] are examples. The cellulose derivatives can be fed at very high levels in the diet without toxic effects other than those

related to reduction of nutrient intake or the induction of loose or bulky stools. Lack of absorption undoubtedly accounts for the nontoxicity of many insoluble substances (e.g., polyolefins, petroleum waxes, a wide variety of nonmetabolizable and nonabsorbable polymers, and barium sulfate and cadmium sulfide,[12] as well as of certain soluble salts (e.g., magnesium sulfate). Poor absorption is not always associated with lack of toxicity, however, because in some cases even a small absorption percentage of a chemical may be sufficient to produce toxic effects.

Storage in tissues. Evidence on the storage of a substance in the body may point to deposition in a particular organ, as, for example, the localization of mercury in the kidney or of lead in bones. The fact that storage occurs does not necessarily constitute a hazard; for example, the storage of DDT in fat has not apparently been associated with toxic reactions in human subjects who daily ingest small quantities of the chemical. Where storage in tissues has been thoroughly studied, as in the case of DDT or lead, there appears to be an equilibrium among uptake, storage, and excretion such that no obvious toxic effects occur at moderate levels of intake. Nevertheless, as a general rule, evidence that a material is tenaciously held in one or another organ of the body is cause for some concern and indicates the need for additional detailed investigation.

The transport of substances across body membranes. Sufficient information has now accumulated about the absorption of materials from the intestinal tract and about penetration through various cell membranes to allow useful theoretical analysis of the properties that may be expected to facilitate it. Reviews of this subject by Schanker[13] and Brodie[14] make it clear that lipid solubility is often an important factor in penetration of body membranes. If the material is ionizable, the portion un-ionized at the pH existing at the cell membrane surface penetrates most readily. Some small water-soluble molecules and naturally occurring metabolites can pass by diffusion through pores in cell membranes or by active transport. Protein binding is important in determining the rate and extent of renal excretion. These physicochemical factors, together with metabolic processes, may be critical in predicting what will happen with various chemical compounds.

The extensive background of work on the placental transfer of drugs may be helpful in considering possible effects of food chemicals. Villee[15] has pointed out that many lipid-soluble materials and a great variety of natural compounds readily cross the placenta. The rates of transfer of different lipid-soluble materials, however, vary considerably.

Knowledge of the ability of a chemical to cross the placenta and of its rate of transfer is obviously of help in evaluating possible effects on the fetus.

Problems of antagonism and synergism. Knowledge of the details of the metabolic alterations of substances may help explain some of the known cases of synergism and antagonism between chemical substances. An example is the ability of ethanol to lessen methanol toxicity. The preferential oxidation of ethanol inhibits oxidation of methanol in the liver and thus prevents the methanol from being converted to such more toxic substances as formic acid and permits it to be excreted unchanged. In the synergism between the two organophosphate insecticides, EPN and malathion, EPN inhibits the enzyme that destroys malathion, thus limiting the detoxification of the latter.

Simple additive effects are probably most likely when the substances have similar modes of action or when they have similar chemical structures and metabolic pathways. A metabolic antagonism may occur between a foreign substance and a substrate or co-enzyme of similar structure. In the classic case of Prontosil, the conversion to *p*-aminobenzene sulfonamide results in a competitive inhibition of the enzymes utilizing *p*-aminobenzoic acid, a growth factor for many microorganisms. Similarly, there are many examples in the drug literature of compounds that bear a sufficiently close resemblance to some naturally occurring hormone or chemical transmitter to be bound at an active site and that are thus able to block the action of the natural substance. Strong binding to an active site may result in antagonistic effects even when chemical structures are dissimilar.

In addition to the value of information on metabolism in predicting specific types of toxicity, there are other general uses. One of the principal uses for detailed information of this kind is related to epidemiologic studies, as in the exposure of persons to DDT. Samples of fat obtained at autopsy or from surgical procedures have given valuable clues as to the probable intakes of DDT by individuals and of the nature of the equilibrium reactions that are so important in evaluating the safety of this substance (see Reference 3).

Knowledge of metabolism increases the value of information that can be developed through occupational, medical, and industrial hygiene programs in industries manufacturing or using food chemicals. Intake by workers exposed under various conditions can best be estimated if the metabolism of the substance is known and if methods can be found for measuring the substance or its metabolites in blood, urine, or ex-

pired air. The foregoing applies also to accidental ingestion of substances. Knowledge of metabolism of the ingested substance would allow extraction of the maximum possible information from such incidents.

Pharmacogenetics is receiving emphasis in drug studies[16, 17, 18] and may be expected to yield insights into comparative toxicology. In the case of isoniazid, for example, the main pathway for detoxication is acetylation. Studies of blood levels of populations indicate that the distribution of values is bimodal. Although strictly genetic factors are not necessarily involved, they are suspected of being important. There have been similar findings in the case of the apnea induced by succinyl chlorine. The distribution of this condition apparently results from the fact that some members of the population hydrolyze the drug at a much slower rate than do others. Genotypes that result in deficiencies in glucose-6-phosphate dehydrogenase may be responsible for some drug-induced hemolytic anemias. Most of the examples developed thus far seem to involve a difference in rates rather than in types of metabolism, but this subject will be of increasing interest in the future.

The answers to the question of *why* substances are toxic will be approached increasingly by a combination of *in vitro* and *in vivo* methods of studying the interactions of chemicals with cells, tissues, or organs. However, evidence that a substance may be safe is not derived merely from a demonstration that at use levels it does not exert any of a variety of toxic effects seen at much higher levels. Confirmation of safety should be more concerned with elucidating the means by which the body is able to handle substances at characteristically low levels of intake over long periods without undue disturbance of, or stress on, body functions.

For ease of analysis, or sheer convenience, most metabolic studies have been carried out at levels of exposure far in excess of levels at which food chemicals are used. Such high levels often involve metabolic pathways that are distinct from those involved at low levels of exposure, or they result in the occurrence of the unmetabolized substance in body fluids or tissues because the capacity to detoxify or eliminate it has been exceeded. It can reasonably be expected that the population distribution of the metabolic processes, rates, and values associated with overloading will be entirely different from that associated with the more normal metabolic pathways at lower levels of intake.

The relevance of the presence or absence of toxic effects as seen in feeding studies to the prediction of safety at use levels can be determined with greatest certainty if the nature of metabolism is known at

both the toxic level and the use level. A toxic effect seen only at a level of exposure at which aberrant metabolism is present is not as helpful in estimating a safe use level as would be one appearing at levels at which normal metabolic patterns prevail. Accordingly, the toxicologist should give prime attention to the nature of the metabolic processes at levels of exposure near those to be anticipated in use and should attempt to evaluate those factors that bear on the ability of populations to tolerate substances at these levels. It is in this respect that study of the qualitative and quantitative aspects of metabolism will be most useful in the future.

BIOCHEMICAL AND *IN VITRO* STUDIES

Significant advances have been made in recent years in knowledge of the intracellular location of metabolic pathways in mammalian cells. The nature, properties, and intracellular location of enzyme systems that catalyze the biotransformation of substances foreign to the body have received a great deal of study. Evaluation of the safety of chemical agents can become increasingly more precise by the application of these advances in knowledge to the study of the biological effects and fates of food chemicals. The scope and nature of studies along these lines will necessarily differ from one chemical agent to another, a fact that makes it impossible to designate in advance a series of measurements to be done on a given compound. Direction for the type of investigations on the mechanistic aspects of a substance may come from the observations made during toxicity tests and studies on the pharmacological actions in the intact animal. An important objective of the application of recent advances in knowledge and methodology is to increase understanding of the mechanism of the toxic action, the role of biotransformation in determining the duration of action and persistence of the chemical agent, and the possible influence of the substance being examined on the actions or fates of other chemicals given simultaneously or at a later time.

A considerable amount of exploratory work, including the development of suitable methodology, may be necessary before an *in vitro* technique can be applied to the evaluation of the toxicity of food chemicals. It is thus apparent that the initial study of a chemical should be done in a species such as the rat, whose metabolic processes have been much studied. Treatment with relatively high single doses or repeated high doses usually yields animals well suited to examination for defects in cellular metabolism. Correlations of such defects with

morphological alterations observed by conventional histologic techniques, histochemical methods, and electron microscopy can then be attempted.

Electron microscopy is particularly important in that it can demonstrate changes that are not otherwise visible. The great magnification and resolution of the electron microscope allow the observation not only of structures that are just distinguishable with the light microscope, such as mitochondria, but also of those that are completely unseen with it, such as the cristae of various intracellular organelles and the ribosomes attached to the endoplasmic reticulum. Specific examples of contributions of electron microscopy to understanding of physiologic effects are to be found in References 19–23.

When significant biochemical or morphologic changes in experimental animals are coincident with high doses of a chemical, attempts should be made to ascertain whether the same effects occur at doses approximating levels that man would be exposed to under proposed conditions of use. Consideration should be given also to the possibility of devising direct or indirect methods for determining whether a particular change observed in experimental animals occurs in man. However, the practical limitations of any such study in man must be recognized in the final assessment of safety.

Results of some of the newer *in vitro* approaches to studies of the mode of action of chemical agents cannot be interpreted clearly in terms of similar changes in the intact animal. For example, the use of tissue and cell cultures is frequently suggested as an approach to measurement of cell injury. However, the pronounced influence that one organ system—such as the liver, the kidney, or an endocrine gland—or the intact animal *in toto*, may exert on the duration, intensity, and type of action of a chemical agent *in vivo* makes it necessary to consider findings in isolated cell systems as no more than suggestive of experiments that might profitably be conducted.

Research on the mechanistic aspects of the toxicology of food chemicals should be clearly differentiated from the application of a series of routine biochemical tests to the urine and blood of animals and man. The latter procedures are primarily diagnostic aids in revealing injury to certain organ systems, but they may also point toward mechanistic studies in a particular organ. Negative findings in these routine tests indicate only that the chemical under investigation does not affect the particular processes that were examined. Intensive research on the mode of toxic action of chemical agents on a molecular, *in vitro* basis may not elucidate the exact mode of action prior to

introduction of the agent into actual use. However, such studies could in many instances provide a better understanding of the compatibility of the agent with biological systems than can be obtained from conventional methods.

In safety evaluation, consideration should be given the hepatic microsomal enzyme systems that catalyze metabolism of many relatively nonpolar chemicals. The role of these enzymes in metabolizing a food chemical can be studied, as can the ability of the chemical to induce synthesis of microsomal enzymes. Both of these types of investigations should first be conducted in experimental animals. *In vitro* studies can then be done by adding the food chemical to a microsomal enzyme system and identifying metabolites by chemical or physical methods. In this connection, a certain amount of predictability concerning the types of changes that will occur is made possible by knowledge that has been developed during the past few years. *In vitro* studies will indicate the types of metabolites that might be expected to appear in the blood, bile, and urine. The alternative, detecting and identifying metabolites in urine and blood without prior *in vitro* studies, may provide the most practical procedure for measuring the rate and extent of metabolism of a food chemical in man.

The ability of a food chemical to induce synthesis of microsomal enzymes can be determined by multiple dosing of animals for several days followed by assays for microsomal enzyme activity in the liver. The effect can then be studied in other species, including man, by determining, both before and after administration of the chemical for several days, the blood levels and rate of excretion of the same or another chemical agent known to be metabolized by these enzymes. The no-effect level for enzyme induction can be measured by performance of enzyme assays on the livers of animals fed various levels of the food chemical. This type of information will aid in ensuring that the permitted levels are incapable of altering the toxicity or duration of action of chemicals to which animals and man may be exposed.

DESIGN OF STUDIES TO DEVELOP TESTS APPLICABLE TO MAN AND PROPERLY CHOSEN ANIMAL SPECIES

During the past decade, there have been several developments bearing upon safety evaluation. One of these is the use of the electron microscope to provide detailed information about changes in structure of various organelles within cells as a result of administering chemicals to an experimental animal or human subject. In some cases, it has been

possible to show in experimental animals that a single, large oral dose of a chemical produces within one hour a recognizable change in the morphology of some type of parenchymal cells. This kind of evidence leads rapidly to the target organs or tissues affected by the chemical under study and allows the investigator so to plan his subsequent investigation as to furnish detailed knowledge of the actions of the chemical on the target sites.

The initial change is not necessarily one that results in damage to the organ or tissue affected; whether it results in a deleterious structural change will await subsequent study. If the initial change is purely functional, it may be reflected in the increased production of the drug-metabolizing enzymes within the endoplasmic reticulum of the cell or the microsomes of homogenates. Mitochondrial enzymes also may be altered.

In developing tests with animal species designed to yield results applicable to man, it is important to find a species that metabolizes and excretes a given chemical as nearly as possible in the same manner as does man. In many cases, however, an ideal experimental animal cannot readily be identified, and studies must be made in less desirable species. Of the many species of laboratory animals now available, the most useful are probably the mouse, rat, hamster, guinea pig, rabbit, dog, pig, and monkey. No precise rule for choosing animal species for study can be stated.

To select an animal species suitable for detailed study, a number of test species may be given repeated sublethal doses of the test chemical during a period of several days, their urine and feces being analyzed for metabolites. It may then be feasible to employ a few conservative tests in man, using single oral doses, to allow comparative examination of urine and feces. If the metabolic pattern in man is similar to that of one of the species tested, then that species may well be chosen for intensive studies of its reactions, both immediate and delayed, to repeated administration of the test compound, as evidenced by biochemical or ultrastructural changes. Prediction that the toxic effects observed in the test species might occur in man may be made on the basis of blood or tissue concentrations after exposure of man and the test species to various doses of the chemical, as suggested by Brodie.[24]

After completion of the toxicity studies, usually within 3 to 6 months after the animal species is selected for intensive study, feeding tests in man may be undertaken. In these tests, the chemical should be used as in its projected role in the diet. The subjects should be given thorough physical examinations. Base-line values should be established in appropriate tissue specimens and body fluids for those factors that underwent significant alterations in the test animals. The trials in man should last for at least 3 to 6 weeks, and can be expected to establish whether the previous choice of an animal species as being reasonably similar to man was correct and to indicate the likelihood of significant effects in man from the new chemical if used in food.

PHYSIOLOGIC STUDIES

Reproduction Conventional studies on fertility and reproduction are carried out in rodents. However, since the female monkey or chimpanzee, like the human, has a menstrual rather than an estrus cycle, they may be of particular value.

It is important to study the effects of proposed food chemicals on the maturation of both ovum and sperm. Effects on reproductive efficiency can be exercised also through changes, for example, in libido, receptiveness, vaginal or prostatic pH, and implantation. These factors can be studied experimentally in laboratory animals, and some of them can be studied in man during the trials discussed earlier.

Females becoming pregnant during the study should be examined near or at term to assess implantation, resorption, and other aspects of embryonic development. Females not exposed to the chemical and made pregnant at known times should be dosed during various crucial stages of pregnancy, e.g., for 1-month periods, starting 2, 6, 10, 14, or 18 weeks after fertilization, in the monkey, or for 1-week periods, starting 1, 8, 16, or 25 days after copulation, in the rabbit.

Placental Transfer If pregnancy proceeds normally despite administration of the test chemical, appropriate studies to define the placental transmission of the material to the fetus may be undertaken. Such studies might involve nothing more than estimation of the rate of accumulation of the chemical or its metabolites in fetuses, or they might involve more sophisticated studies of simultaneous levels of the chemical and its metabolites in maternal and fetal bloods or of the rate of appearance of radioactively labeled chemical in fetal blood following its injection into the maternal circulation.

Lactation Studies of the effects of the chemical on the amount and quality of milk produced may be carried out. Transmission of the chemical through milk to the newborn animal is an important factor for study.

The possibility of increased susceptibility of the lactating animal to a chemical should be considered. These toxicity determinations should be made in previously unexposed females at intervals during lactation. When the chemical is administered as a proportion of a diet fed *ad lib.*, the response to increased intake because of increased food consumption should not be confused with enhanced toxicity.

Teratogenesis Teratogenic chemicals act on the developing embryo in such a way as to produce congenital malformations that are ordinarily not heritable. Though only a few substances have been demonstrated to be teratogenic for man, a large number have been shown under experimental conditions to produce fetal abnormalities in laboratory animals.[25, 26] Variations in experimentally induced teratogenic response in rats, rabbits, and mice, and the lack of correlation with human experiences raise serious questions concerning species and strain specificity. Recent knowledge regarding the mechanisms of congenital malformations was presented in 1968 at the Second International Workshop in Teratology.[27]

Assuming that an ingested chemical has teratogenic potential and gains access to the embryo, the main factors determining whether teratogenesis will, in fact, occur are (a) dosage, (b) the species of animal, and (c) the stage of development of the embryo at the time of contact with the chemical.

Experience in experimental teratology has shown that a substance that is teratogenic in one species may not be so in another and that not all strains of a given species respond alike, nor do all individuals of the same litter. Hence the presence or absence of a teratogenic effect in one or more animal species exposed to a chemical is of limited value in predicting whether the chemical will be teratogenic for man. As in all toxicologic work, however, the consistent finding of such an effect in several species strengthens the suspicion (but cannot prove) that man, too, would be susceptible. Conversely, consistent absence of the effect in laboratory animals gives some assurance, but does not prove, that man would not be affected.

The sensitivity of the embryo to teratogenic change is restricted to the period of cellular differentiation and organogenesis. Different tissues or embryonic sites are susceptible to changes at different stages of development of the embryo, and the times of the peak sensitivity vary within the narrow limits of only a few days during gestation. When organogenesis is completed, true teratogenesis can no longer occur, though the fetus is still subject to toxic effects that may influence its later growth or survival.

Most experimental teratology has employed the mouse, the rat, and the rabbit. Since the chemical under investigation must be given to the pregnant female before and during the period when fetal cell differentiation and organogenesis are in progress, the time of conception must be precisely known. Interpretation of the results should be conditioned by knowledge of the time course of absorption, distribution, metabolism, and excretion of the chemical. The relation between the concentrations of the chemical in the maternal blood and in the embryo are critical in evaluating its teratogenicity.

The chick embryo is also used for teratology studies. In this case, the embryo is exposed to controlled concentrations of the chemical by injecting the chemical into the incubating egg. Although this test may reveal a teratogenic potential of a food chemical, the results obtained may have but limited relevance to the evaluation of teratogenic hazard for man, not only because of species variation but also because of the likely differences in the concentration of the acutely administered and chronically ingested chemical at the critical stage and sites of embryogenesis.

The initial observations of possible teratogenic effect are generally made during appropriate multigeneration reproduction studies in mammals. These studies should be designed with adequate numbers of females so that some can be delivered by cesarean section to permit observation of implantations and resorptions and examination of intact fetuses. If any teratogenic effect or other aberration is indicated, specifically designed tests in which the timing of conception and dosage are controlled should be carried out. The most critical test involves the administration of the chemical to the pregnant animal at the period of maximum sensitivity during embryogenesis. The rate of *in vivo* biotransformation of many chemicals may either increase or decrease during the course of repeated intake. For this reason, the teratogenic potential of a food chemical administered continuously during a reproduction study may differ from that in a teratology test in which the chemical is given only at the estimated time of maximum embryonic sensitivity.

Little is yet known about toxicity to the developing embryo, the transport of chemicals to it, the biochemical mechanisms controlling embryogenesis, and the factors that influence the sensitivity of the processes of cellular differentiation to disruption by foreign chemicals.

Considerable progress in this area is necessary before the teratogenic hazard of food chemicals for man can be reliably evaluated.

Mutagenesis Mutagenesis differs from the other categories of embryotoxicity in that the defect, if not lethal, has the potential for reappearing in generations succeeding that in which it is first seen. Chemicals may alter not only fertilization and intrauterine development of fertilized ova, but also the hereditary material itself (mutagenesis). Although they may be manifested in similar ways, genetic aberrations are to be distinguished from other types of impairment of reproductive function, including blastotoxicity, resorption, abortion, teratogenesis, and impaired regulatory or functional mechanisms (e.g., abnormal enzyme or protein constitution, hormone imbalance, and carcinogenesis).

Although radiation has been the most commonly recognized mutagen, a number of chemicals have been found to be mutagenic *in vitro* to cultured cells or *in vivo* to experimental species. No instance of chemical mutagenesis in man seems to be definitely known. Nevertheless, the similarity of the hereditary materials in different animal species and the ability of nonlethal mutations to affect generations following the one in which the change is first observed raise the question of whether mutations may not be induced in man by chemicals added to food.

Tests for mutagenic action in mammals are in an early state of development. An *in vitro* procedure that can be used as an initial screening test consists of exposing human leukocytes in a culture fluid to graded concentrations of the chemical. After a period of incubation, mitosis is stopped at metaphase by the addition of colchicine. The cells are then stained and examined for altered chromosomes. Whether to accept such changes as evidence of mutation is subject to sharp differences of opinion.

The intact animal possesses mechanisms that afford protection against toxic substances, for example, limited absorbability or detoxication within the gastrointestinal tract or after absorption. Because cells *in vitro* lack these protective mechanisms, the substances found to induce chromosomal change by an *in vitro* test should be examined for possible mutagenic effects in the intact animal. The host-mediated assay, which has attracted considerable attention, is an attempt to bridge this gap. A microbiological indicator (*Neurospora candida* spores or bacterial cells) is administered intraperitoneally to a host mammal while the host is exposed by a different route to the chemical under consideration. The microorganism is withdrawn after a suitable period and compared with *in vitro* grown cultures for evidence of induction of mutations.

If reproduction studies, carried out through three or more offspring generations, are negative, they tend to rule out mutagenic as well as other embryotoxic effects. If the indexes of reproductive performance are abnormal in the multigeneration reproduction test, definitive tests should be carried out to identify the cause.

With respect to mutagenesis, a widely used first test is the dominant lethal test in which treated and untreated males are mated with untreated females. The pregnant females are then sacrificed and the uteri examined for the number of implantation sites, dead fetuses, and living fetuses. Present evidence indicates that, at least in mice, rats, and guinea pigs, there is no correlation between the number of corpora lutea in the ovaries and the number of implantation sites.

A variation of the dominant lethal test that has gained considerable support involves treating maturing male mice (8 to 9 weeks of age) with the chemical under test and mating them with normal females during four successive weeks of spermatogenesis. Ten days after mating, the females are autopsied and the number of viable fetuses counted.[28,29] The evaluation of potential mutagenic effects during spermatogenesis is believed to have special value for detecting antifertility effects that are not associated with apparent structural damage to testicular cells.[29]

A more critical application of the multigeneration test involves feeding the initial F_0 generation throughout its life cycle and observing it for a dominant effect, and following the second litter of each generation to at least F_6. If a recessive effect appears, progeny are backcrossed with parent generations to confirm it.[30]

It is obvious that improved methods are needed for assessing mutagenesis, which can produce such conditions as alcaptonuria, phenylketonuria, galactosemia, sickle cell anemia, mongolism, and dizygotic twinning in man. As a corollary, research should be performed not only on the possible mutagenic effects of chemicals to be added to foods but also on ways whereby their mutagenic activities in man can be predicted accurately.

Environmental Stresses That various environmental stresses may have additive effects on the toxicities of chemical agents has been recognized for many years, but it has not been practical to study the possible relationships extensively. However, since most chemical agents are studied in several laboratories that may differ from each other in the method

of housing, in the strains of animals used, and even in the composition of the diets used, similarity of results obtained in different laboratories indicates that at least subtle to moderate differences in the environment do not affect the toxicity of a chemical agent. Special consideration of stress conditions is indicated, however, when it is known that the food chemical would be used under conditions unusual with respect to diet, environment, or disease.

Age Differences A comparison of the susceptibility of animals of different ages should be a regular part of toxicity evaluation. Particular attention should be given to measuring differences between infants and adults, because incomplete development of enzymes in the very young of some species is known to affect their responses to chemical agents. The time relationships have been less clearly established for other species, including man, but age differences in susceptibility have been observed in enough different species to indicate the advisability of carefully considering this factor.

Psychopharmacology Many chemicals affect the central nervous system. The effects range from local (interference with the perception of some particular sensory input) to general intoxication (hallucination, aimless and perseverative activity, coma, etc.).

Routine psychopharmacologic testing of food chemicals is not suggested, but this is an area of active research, and where such study is indicated new food chemicals may be examined not only for direct psychotropic action but also for ability to enhance the effects of other compounds.[31]

Methods of studying psychotropic actions in experimental animals are extremely varied. They range from the use of chronically implanted electrodes to record from, or to stimulate, particular loci within the central nervous system, to sophisticated tests of ability to perform alternatively different sequences of responses (see References 32–44).

A thorough study of the behavioral effects of a chemical requires the use of more than one animal species in a statistically adequate design. Because genetic and cultural backgrounds of animal populations are important determinants of responses to chemicals, the rat has distinct advantages over most other species of laboratory animals for psychopharmacologic research. Other species, however, have been used.[45, 46]

Whatever species of laboratory animal is used, the research should be planned to relate change in behavior quantitatively to the independent

variable (dose of chemical or time between administration of the chemical and observation). To do this, the independent variable must be presented in at least two (preferably three) values and the behavioral (dependent) effects must be measured quantitatively. The psychopharmacologist must keep in mind also that behavior can be altered by nonspecific, peripheral actions.

When human studies of psychopharmacologic effects have been undertaken, the following tests, among others, have been used:

Visual acuity
Estimation of elapsed time
Dynamometer
Romberg's sign
Simple reaction time
Manual dexterity
Sequential addition or subtraction
Repetition of a series of numbers

Other tests, such as canceling certain numbers within arrays of random numbers, circling a specified type of word on pages of print, or drawing lines to connect serially numbered points, may replace the last two items in the battery of tests or may be added to it.

Both animal and human tests must be adjusted to the properties of the particular compound under study. Certain tests may have to be altered or replaced by others after preliminary studies have been made. It is obvious that the methods employed in psychopharmacological study must be as varied as the behavioral responses to administered chemicals.

REFERENCES

1. Williams, Richard Tecwyn. 1959. Detoxication mechanisms. 2nd ed. Wiley, New York.
2. U.S. Department of Agriculture. Report No. 94. November 15, 1912.
3. Food Protection Committee, Food and Nutrition Board. 1965. Some considerations in the use of human subjects in safety evaluation of pesticides and food chemicals. NAS–NRC Publ. 1270. National Academy of Sciences, Washington, D.C.
4. Barnes, J. M., and F. A. Denz. 1954. Experimental methods used in determining chronic toxicity. Pharmacol. Rev. 6:191.
5. National Academy of Sciences–National Research Council. 1969. Application of metabolic data to the evaluation of drugs. Report of Committee on Problems of Drug Safety, Drug Research Board. Clin. Pharm. Ther. 10:607.

6. Fassett, D. W. 1963. Toxicology of organic compounds: A review of current problems. Ann. Rev. Pharmacol. 3:267.
7. Miller, Elizabeth C., and James A. Miller. 1966. Mechanisms of chemical carcinogenesis. Pharmacol. Rev. 19:805.
8. Weisburger, John N., and Elizabeth K. Weisburger. 1966. Chemicals as causes of cancer. Chem. Eng. News 44:124.
9. Knight, H. F., Jr., H. C. Hodge, E. P. Samsel, R. E. DeLap, and D. D. McCollister. 1952. Studies on single oral doses of a high gel point methylcellulose. J. Amer. Pharm. Ass. Sci. Ed. 41:427.
10. Frawley, J. P., A. K. Wielie, and E. D. Klug. 1964. Studies on the gastrointestinal absorption of purified sodium carboxymethylcellulose. Fed. Cosmet. Toxicol. 2:539.
11. Morrow, Paul E., H. C. Hodge, W. F. Neurman, E. A. Maynard, H. J. Blanchet, Jr., D. W. Fassett, R. E. Birk, and S. Manrodt. 1952. The gastrointestinal non-absorption of sodium cellulose sulfate labeled with S^{35}. J. Pharmacol. Exp. Ther. 105:273.
12. Gabby, J. L. 1950. Toxicity of cadmium sulfide and cadmium sulfoselenide pigments. Arch. Ind. Hyg. Occup. Med. 1:677.
13. Schanker, Lewis S. 1962. Passage of drugs across body membranes. Pharmacol. Rev. 14:501.
14. Brodie, Bernard B. 1964. Physico-chemical factors in drug absorption, p. 16. *In* T. B. Binns, ed., Absorption and distribution of drugs. Williams and Wilkins Co., Baltimore.
15. Villee, Claude A. 1965. Placental transfer of drugs. Ann. N.Y. Acad. Sci. 123:237 Art. 1.
16. Kalow, W. 1965. Dose–response relationships and genetic variation. Ann. N.Y. Acad. Sci. 123:212 Art. 1.
17. LaDu, Bert N. 1965. Pharmacogenetics. Toxicol. Appl. Pharmacol. Suppl. II, 7:27.
18. Meier, Hans. 1963. Experimental pharmacogenetics. Academic Press, New York.
19. Bruni, C. 1960. Hyaline degeneration of rat liver cells studied with the electron microscope. Lab. Invest. 9:209.
20. Herman, M. M., and K. G. Bensch. 1967. Light and electron microscopic studies of acute and chronic thallium intoxication in rats. Toxicol. Appl. Pharmacol. 10:199.
21. Papadimitriou, J. M., R. B. Shilkin, J. M. Archer, and M. N.-I. Walters. 1967. Inflammation induced by dimethylsulfoxide (DMSO). II. Ultrastructural investigation of the inflammatory phase. Exp. Mol. Path. 6:347.
22. Benitz, K. F., G. K. S. Roberts, and A. Yusa. 1967. Morphologic effects of minocycline in laboratory animals. Toxicol. Appl. Pharmacol. 11:150.
23. Imai, H., and F. Coulston. 1968. Ultrastructural studies of absorption of methoxychlor in the jejunal mucosa of the rat. Exp. Mol. Path. 8:135.
24. Brodie, Bernard B. 1964. Of mice, microsomes, and man. Pharmacology 6:12.

25. Goldstein, A., L. Aronow, and S. Kalman. 1969. Chemical teratogens, Chap. 12. *In* Principles of drug action. Hoeber Medical Division, Harper and Row, New York.
26. Karnofsky, D. A. 1965. Drugs as teratogens in animals and man. Ann. Rev. Pharmacol. 5:447.
27. Nishimura, H., J. R. Miller, and M. Yasuda. 1969. Methods for teratological studies in experimental animals and man. Medical Examination Publishing Co., Inc., Flushing, New York.
28. Hershberger, L. G., D. M. Hansen, and L. M. Hansen. 1969. Effects of antifertility agents on male mice as determined by a serial mating method. Proc. Soc. Exp. Biol. Med. 131:667.
29. Jackson, H. 1966. Antifertility compounds in the male and female. Thomas, Springfield, Illinois.
30. Biology of the laboratory mouse. 1968. McGraw Hill Book Co., New York.
31. Carlton, P. L. 1961. Potentiation of the behavioral effects of amphetamine by imipramine. Psychopharmacologia 2:364.
32. Miller, N. E., and H. Barry. 1960. Motivational effects of drugs: methods which illustrate some general problems in psychopharmacology. Psychopharmacologia 1:169.
33. Anderson, E. E. 1938. The interrelationship of drives in the male albino rat: II. Intercorrelations between 47 measures of drives and of learning. Comp. Psychol. Monogr. 14, No. 6.
34. Dennis, W. 1939. Spontaneous alternation in rats as an indicator of the persistence of stimulus effects. J. Comp. Psychol. 28:305.
35. Brown, J. S. 1942. The generalization of approach responses as a function of stimulus intensity and strength of motivation. J. Comp. Psychol. 33:209.
36. Bugelski R., and N. E. Miller. 1938. A spatial gradient in the strength of avoidance responses. J. Exp. Psychol. 23:494.
37. Dews, P. B. 1956. Modification by drugs of performance on simple schedules of positive reinforcement. Ann. N.Y. Acad. Sci. 65:268.
38. Sidman, M. 1956. Drug-behavior interaction. Ann. N.Y. Acad. Sci. 65:282.
39. Weissman, A. 1959. Differential drug effects upon a three-ply multiple schedule of reinforcement. J. Exp. Anal. Behav. 2:271.
40. Sidman, M. 1956. Time discrimination and behavioral interaction in a free operant situation. J. Comp. Physiol. Psychol. 49:469.
41. Heistad, G. T. 1958. Effects of chlorpromazine and electroconvulsive shock on a conditioned emotional response. J. Comp. Physiol. Psychol. 51:209.
42. Kreezer, G. L. 1942. Technics for the investigation of psychological phenomena in the rat. *In* J. Q. Griffith and E. J. Farris, eds., The rate in laboratory investigation. Lippincott, Philadelphia.
43. Nissen, H. W., and T. L. McCulloch. 1937. Equated and non-equated stimulus situations in discrimination learning by chimpanzees. III. Prepotency of response to oddity through training. J. Comp. Psychol. 23:377.

44. Hunt, H. F. 1960. Some applications of conditioned fear. *In* L. Uhr and J. G. Miller, eds., Drugs and behavior. Wiley, New York.
45. Kelleher, R. T., C. A. Gill, W. C. Riddle, and L. Cook. 1963. On the use of the squirrel monkey in behavioral and pharmacological experiments. J. Exp. Anal. Behav. 6:249.
46. Smith, R. P., A. I. Wagman, W. Wagman, C. C. Pfeiffer, and A. J. Riopelle. 1957. Effects of some tranquilizing and depressant drugs on conditioned avoidance behavior in monkeys. J. Pharmacol. Exp. Ther. 119:317.

Evaluating Carcinogenic Potential

In many, if not most cases, the carcinogenic potential of a chemical is assessed using the same animals employed for assessing other toxic hazards. Carcinogenicity deserves special treatment, however, because of the frequently long induction periods required before tumors can be detected and, in the United States at least, because of the special emphasis given to it by the Food, Drug, and Cosmetic Law.

The Food Protection Committee[1] and the FAO/WHO Joint Expert Committee on Food Additives[2] reviewed the matter of evaluation of carcinogenic potential of food chemicals in 1959 and 1961, respectively. The following presents contemporary views on this matter.

GENERAL CONSIDERATIONS

Routes of Application Although chemical carcinogens can be administered in a variety of ways, the three routes that have been most used are subcutaneous, oral, and topical. Parenteral routes, such as intraperitoneal and intravenous injection, and the direct injection of compounds into, or their implantation within, specific tissues have also been employed. Insofar as possible, tests to assess the carcinogenic hazard of a chemical for man should utilize conditions similar to those that might obtain during human exposure. Compounds that might

enter via the alimentary tract should be tested by oral administration, and those that would come into contact with the skin or lungs should be tested by topical application or inhalation. Except in special situations, the latter two routes are of little relevance to human exposure to food chemicals.

Carcinogens that are administered in the ultimate carcinogenic form, or that can be metabolized to such a form at the injection site, are usually administered by single or repeated subcutaneous injection. However, the results of subcutaneous injection in the commonly used rodents must be treated with caution. In many cases production of sarcomata in these tests seems to be related to physiochemical properties of the substance injected and to special conditions of the experiment. For example, "smooth surface carcinogenesis" occurs in rats and some other species in connective tissue cells near intact implants of thin pieces of sufficient area of almost every substance tried (cellophane, nylon, Teflon, ivory, silver, platinum, etc.), and the weak carcinogenic activity of repeated injections of cholesterol in the rat has been ascribed to the surfaces of the cholesterol crystals so deposited. In general, the production of sarcomata at injection site appears not to be related to the chemical composition of the implants and is considered not to be evidence of carcinogenicity by the oral route.[3]

The oral route is the most likely mode of entry into man of food chemicals (including pesticide and herbicide residues), many drugs, and water pollutants. The carcinogenicity of aromatic amines has been most readily demonstrated by chronic oral administration. The total amounts of compounds administered often have had to be large, probably because of limited conversion to the ultimate carcinogenic metabolite and limited delivery to the critical targets.

Dose Response In general, the incidence of tumors increases and the latent period decreases as the dose of a carcinogenic chemical is increased. Even when the carcinogenic potential of a chemical at a given dose level has been demonstrated, with smaller dose levels the latent period may be too long to permit gross tumors to develop within the life span of the animal, and a "no carcinogenic level" may thus be shown for that species. Conversely, high doses may kill the animal before tumors become apparent.

Combined Effects of Two or More Carcinogens Doses of the same or of different carcinogens administered either simultaneously or sequentially may produce additive, synergistic, or inhibitory effects. Inhibitory effects can result from altered metabolic patterns that reduce the effective levels of one or both carcinogens (inhibition of carcinogenesis by amines through administration of polycyclic hydrocarbons is an example). Competition for reactive sites may be important in other situations. The best documented synergistic responses are found in mouse skin tumorigenesis. Thus, initiating agents (e.g., urethan) and promoting agents (e.g., croton oil) may induce very few tumors when applied alone, but application of an initiator and then a promoter results in high tumor yields.

Rapid Tests for Carcinogenic Activity At present only the production of tumors in the intact animal is recognized as a valid test of carcinogenic activity. Since these tests require many months or years, more rapid assays to predict the possible carcinogenic hazard of chemicals have been sought. To date no test with good predictive value for chemicals of different types has been developed, although in some cases correlations have been achieved within restricted classes of compounds. Chemical transformation of cells cultured *in vitro* has been studied in this context, since at least some clones from such transformed cells grow as tumors *in vivo*. Assays for destruction of sebaceous glands of mouse skin and various mutagenicity and toxicity tests in plants, in *Drosophila*, and in microorganisms have also been proposed as short-term tests for carcinogenicity.

Two major questions are pertinent to all these tests: Is the same reactive form the key intermediate in the assay under consideration and in the carcinogenic action of the chemical? and Are the capabilities of the test cells and the various tissues of man to make the reactive metabolite similar? These questions are difficult enough to answer with known chemical carcinogens, but impossible with compounds whose carcinogenic potential is unknown.

Test Procedures in the Detection of Carcinogenic Activity of Food Chemicals In view of differences in susceptibility, it is generally recommended that at least two species be used for tests on each chemical. Furthermore, special conditions of immaturity, pregnancy, sex, other drugs, etc. that might be involved in human exposures should be simulated in at least some of the tests. The number of people with tumors of any type at any one time in the United States approximates 3 per 1,000 population. Most tumors occur in older individuals who have been at risk for extended periods. Experimental groups of animals large enough to detect tumor incidences of this order of magnitude

are clearly not feasible. Hence, lifetime experimental dosage levels far exceeding the probable human exposure levels are employed to compensate partially for this logistical problem. In general, it is recommended that the doses administered be 10 to 1,000 times those that could be achieved in human exposure, if this is pharmacologically and practically possible. However, it must be recognized that compounds administered in massive doses may be metabolized by pathways different from those that characterize low levels of intake.

In view of the importance often attached to low incidence of tumors in the treated animals, it is essential that the number of animals assigned to each group be as large as practicable and that most of the nontumor-bearing animals survive for most of their expected life span. Several dosage levels of each test compound should be used so that information on the relationship between tumor incidence and dose is available, especially when spontaneous tumors occur in the animal strain employed.

It is critical that all animals be autopsied at death or upon the termination of the experiment and that they be subjected to careful gross and microscopic examination. Of equal importance is the maintenance at the same time of large groups of control animals that receive treatment identical to that of the experimental groups except for administration of the compound under test; this includes feeding the same diet and applying the vehicle used for administering the test compound. The control animals must be autopsied comparably to those of the experimental group.

Oral Administration Compounds can be administered by incorporation into the diet, dosage being controlled by weighing food intake, or by gastric intubation, which permits more accurate dosage and is preferable for labile compounds. Stable water-soluble compounds can also be administered in the drinking water. Diets containing test compounds should be made up frequently and stored so as to avoid rancidity or destruction of the test compound. When compounds are administered in the drinking water, the water should be changed daily. In both cases records of consumption should be kept so that total intakes can be calculated.

The highest dosage employed should be one that will produce a minimal to moderate amount of short-term toxicity but that will permit most of the animals to survive for at least 1 year. At least one or two lower doses that will not cause significant reduction in the life span should also be administered. Unless the survival of the animals is threatened, administration of the compounds should continue for most of the life span.

The rat, mouse, and hamster have been widely used in studies of the carcinogenicity of orally administered compounds; because of this and their relatively short life spans, they are probably the animals of choice.

Pathological Examinations Regardless of the species, compound, or route of administration, pathological examination of all animals is of paramount importance. For this purpose it is essential that moribund animals or those with large tumors be sacrificied and that dead animals be removed for necropsy as soon as possible. All animals should receive complete necropsies that include careful gross examination of the skin and its underlying structures; the mammary tissue; the abdominal, thoracic, pelvic, and oral glands; and endocrine and nervous systems. All tumors or possible tumors should be fixed and examined by a competent pathologist familiar with the species under study. Control animals must receive comparable examinations to ascertain the significance of the tumors observed in the experimental groups.

REFERENCES

1. Food Protection Committee, Food and Nutrition Board. 1959. Problems in the evaluation of carcinogenic hazard from use of food additives. NAS–NRC Publ. 749, National Academy of Sciences, Washington, D.C.
2. Joint FAO/WHO Expert Committee on Food Additives. 1961. Evaluation of the carcinogenic hazards of food additives. FAO Nutrition Meeting Report Series No. 29. WHO Technical Report Series No. 220.
3. Joint FAO/WHO Expert Committee on Food Additives. 1969. Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents and certain other substances. FAO Nutrition Meeting Report Series No. 46A. WHO Technical Report Series, in press.

Interpretation and Prediction of Safety

FACTORS AFFECTING PREDICTION

The principles and procedures for safety evaluations recommended in 1959 have, insofar as we know, served well in protecting the public from health hazards that might otherwise have resulted from use of chemicals in food production, processing, storage, and packaging. It has been widely felt, however, that these conventional procedures are overly cumbersome, time-consuming, wasteful of manpower and resources, and expensive, and that they do not provide the basic information needed to advance the efficiency and dependability of safety evaluation.

Although toxicologic knowledge and understanding increased greatly in the 10 years since 1959, the requirements for reaching a judgment of safety became, at the same time, more rigorous, with the result that no savings in time, manpower, and money were realized. Although there has been a trend toward streamlining of the conventional acute, subacute, and chronic toxicity tests, there has appeared no reliable alternative to the test for carcinogenic potential, which is the chief time-consumer. In addition, reproduction studies are now almost routinely required, and it is increasingly urged that observations on teratogenicity and mutagenicity be made. One can anticipate that clinical tests will be expanded and that histological examination will

become more extensive and detailed than is now generally the case.

The increased complexity of the methodology of safety evaluations has come about because of our continuing ignorance of the basic mechanisms of the actions of chemicals in the body, and is enhanced by our ignorance of the significance of the results of many of the new tests for the body economy. However, recently developed knowledge of the basic biochemical actions of chemicals and new insights into these actions promise an improvement in both the economy and reliability of safety evaluation. As more knowledge is gained about the comparative metabolism of chemicals in various species and about the basic biochemical, structural, and functional changes induced by chemicals, there should be a concomitant increase in the usefulness of data derived from study of one species for guiding judgments of safety in another.

PREDICTION OF SAFETY FOR MAN FROM OBSERVATIONS ON ANIMALS

Physiological and biochemical similarities among mammals permit one to measure many indexes in laboratory species that can then be predictively transposed to man. The opportunity to exaggerate both the dosage and duration of administration or exposure in animal studies has special value as contrasted to human tests and makes such studies mandatory whether or not tests or observations in man are undertaken. Despite the interspecies similarities, however, there are many metabolic and organic differences that cast a shadow of uncertainty on species-to-species extrapolations. Although the uncertainty inherent in such extrapolation tends to diminish as the number of animal species showing identical responses increases, it never vanishes completely. In the last analysis, experience and scientific judgment must be relied upon in applying experimental data to conditions of use of food chemicals.

Present knowledge does not ensure absolute protection against injury from food chemicals—or indeed from any environmental hazard—for all individuals in the population. The population includes hypersensitive or allergic individuals, persons with bizarre food habits, and persons in different age groups and physiologic and disease states. Most safety evaluation studies are conducted in healthy animals receiving normal diets, and the results are probably most applicable to prediction of events in healthy human beings on good diets. There is much need for research to extend the validity of conclusions to the more extreme or abnormal human situations.

The determination of a dosage level, ideally the maximum dosage

level, that is without discernible adverse effect in animal species is of decisive importance in safety evaluations since it is this dose (expressed in relation to body weight or surface) that is translated, after applying a suitable "safety factor," into a tolerable (or maximum acceptable) intake for man. The no-adverse-effect dose can be estimated to a degree sufficient to provide virtual certainty of safety but can never be absolute. The reasons for the residual uncertainty are numerous and include the following:

- The size of the test groups is small relative to the population to which the data are to be applied.
- A certain degree of risk (probability factor) is inherent in any findings depending on a finite number of animals.
- No-adverse-effect levels vary with the species, strain, age, sex, physiologic state, etc., of the test animals, hence the failure to observe an adverse effect under one set of defined experimental conditions does not preclude the possibility of an effect under other conditions.
- No matter how many types of response may be observed in toxicologic studies, it is always conceivable that one or more tests not employed in a particular study, or not even devised, might reveal an effect not previously observed.
- Although no adverse effect means no toxic effect, it is difficult sometimes to decide whether an aberration, i.e., a difference from a "normal" or control response, is indeed an adverse effect. For example, an elevated level of a normal constituent in blood or tissue, an increase in organ weight, or a so-called nonspecific histomorphologic alteration, unaccompanied by dysfunction, may or may not be indicative of disease or injury.

SELECTION OF SPECIES

After acute and short-term toxicity measurements in several animal species and preliminary studies of the fate of a food chemical in these species and in man have been completed, species for the special and long-term evaluation procedures might be selected, based on three considerations: the most sensitive species, the most convenient species, and the species with a metabolic pattern most similar to that of man.

Sensitivity Use of results from tests in the most sensitive species for predicting how man will be affected has little scientific merit unless it is already known that man resembles that species in his reaction to the chemical. In setting tolerances and in regulatory control, however, it seems sensible to use the no-effect level in the most sensitive of the species appropriately tested, even though the practice is conservative, involving uncertainties in extrapolating information to man and the assumption that man may be equally as sensitive as, or more sensitive than, the experimental animal.

Convenience Selection of species on the basis of convenience, i.e., because of availability, familiarity, ease of handling, length of life span, size, cost, etc., has been widely resorted to. In the first place, it is highly practical and from such a selection one can choose the most sensitive species for the long-term tests and for the computation of tolerance. Furthermore, after studies of the fate of the chemical have been accomplished in the various species chosen, the one most closely simulating man in this regard can then be subjected to long-term and other special studies.

Similarity in Metabolism Choice of an animal species with a metabolic pattern similar to that of man has substantial scientific merit for reasons noted above, but it is likely to present difficulties.[1] It presupposes that sufficient animal studies have already been done to justify investigating the fate of the chemical in man. Hopefully, one of the species selected for the first stages of the evaluation will prove to simulate man in this regard. If none does, then further exploration would have to be done. If a suitable species is thus identified for use in special and long-term studies, before such studies are undertaken acute and short-term toxicity tests would have to be done. Should the species thus identified turn out to be a relatively infrequently used animal or a large animal, such problems as procurement and housing will in many cases outweigh the scientific advantages. Enthusiasm for the concept of pairing an animal species with man on the basis of metabolic similarities must be tempered by the realization that both may exhibit marked variability in the metabolism of chemicals, depending on age, sex, genetic factors, and physiologic state.

PREDICTION OF SAFETY FOR MAN FROM OBSERVATIONS ON MAN

The ideal species in which to study safety of food chemicals for man is man himself. The nature, usefulness, and limitations of using man in such studies have been succinctly discussed.[2,3]

The ultimate assessment of the safety of a food chemical derives from years of widespread consumption by man under given conditions of use. Even here, however, the absence of known adverse effects does not, by itself, constitute adequate assurance of safety. The possibility always exists of adverse effects that, because of their subtle or slowly developing nature, are not recognized as being caused by the chemical in question.

Observations on occupationally exposed workers and on accidentally overexposed individuals can provide reasonable support for a judgment of safety, especially if the exposures are excessive and still cause no detectable adverse effects. Under these circumstances, however, the conditions do not simulate the proposed use of the food chemical in respect either to the route of intake or the schedule and duration of exposure.

Carefully controlled epidemiological studies in which the observed subjects consume the food chemical under conditions of its proposed use are notably difficult to carry out. They are subject to the pitfalls inherent in the complex interplay of multiple factors—intake, age, sex, race, dietary habits, and variable physiological, social, and environmental circumstances. To conduct a meaningful survey requires large numbers of subjects and of physiological measurements. If the distribution of products containing a new food chemical could be initially restricted to a limited geographic area, epidemiologic studies could be done much more easily than is true under the more customary practice of nearly universal distribution. Practically, however, restricted distribution would, in most instances, not be long enough to provide reliable information on the possible development of infrequently occurring or slowly developing ill effects.

Controlled experimental studies in man, though desirable, have limited predictive value for a number of reasons:

- The feasible duration of the experiment is but a relatively short segment of man's total life span. Thus, observations relating to carcinogenesis are impossible in the light of present knowledge and methodology.
- The number of subjects is usually not adequate to reveal serious reactions that occur but rarely.
- In extended studies, the low levels of a food chemical administered, as dictated by ethical considerations, do not provide data from which a range of safety can be computed. No degree of risk can be measured. Neither the toxicity nor the degree of safety of food chemicals

can readily be measured quantitatively in man in a controlled experiment that meets accepted ethical standards.
- To control accurately the intake of the chemical, it is frequently given by capsule or in some other manner not precisely that proposed for use in foods.
- Lapses in the cooperation of the subject weaken the dependability of the data.
- Tests are usually carried out on a specific category of subjects, such as healthy adult males, and do not make allowances for the variable susceptibilities that are related to sex, pregnancy, age, race, and environmental factors.
- The variety of experimental tests that can legitimately be used is limited in comparison to those that can be employed in experimental animals.
- Because the subjects are informed of the experiment and of any potential risk, psychological reactions may distort the results, unless the experiment is carefully designed.

It is clear that appropriate and well-executed animal studies are more likely to provide a substantial background of biological data from which to judge safety than are studies in man alone.

At the same time, recent developments in the study of the fate of chemicals in man promise to provide data than can be of prime predictive value. A comparison of the patterns of disposition of a food chemical in man and in several animal species might well bring to light an ideal experimental animal in which to carry out the necessary extensive toxicologic studies from which data may be obtained that can be extrapolated back to man with a comparatively high degree of reliability.

Man himself may exhibit individual quantitative or qualitative variations in disposing of a chemical, variations resulting from differences in age, sex, physiologic state, inheritance, or environment. In general, however, if an animal with a relatively short life span and a pattern of metabolism and excretion similar to that of man can be identified, the results of a well designed life-span toxicity test in that species can be accorded greater value for the prediction of safety in man than can the results of a test of simular duration and best possible design in man.

In spite of the limited predictive value of studies in man, the maximum level of assurance of safety would be achieved by the combination of the following: (1) extensive studies of toxicology, mechanism of action, and metabolic fate in appropriately selected animal species;

(2) suitably controlled testing procedures in man; (3) epidemiological studies; and (4) several years of monitored exposure of man. But even the conscientious exploitation of this program of study will not completely dispel the uncertainty about harmful effects that are difficult to attribute to specific causes, because they develop slowly, are of a subtle nature, or occur infrequently.

IN VITRO MODELS

Chemicals have been applied *in vitro* directly to living cells, to tissues or organs, or to various simple organisms, in seeking to elucidate their toxicity and mechanisms of action. Chicken eggs and embryos have been used in studies of teratology and toxicology, animal and human leukocytes; *Drosophila* in testing for mutagenicity; tissue slices and homogenates for enzyme studies; and cell and tissue cultures for study of growth and structural changes. These techniques have frequently been of value in certain assay procedures and in the study of the basic mechanisms of interaction between chemicals and cells or cell constituents. Results serve to guide the investigators' observations of the whole animal, particularly when the *in vitro* system is prepared from the same species of animal as that used for *in vivo* study. In evaluating the safety of a new food chemical, data obtained from an *in vitro* system cannot be directly extrapolated to the *in vivo* situation in either qualitative or quantitative terms with any degree of certainty. Hence, the results of *in vitro* toxicity tests cannot be said to have direct predictive value, because of the following factors:

- The effects of the gastrointestinal environment upon the chemical are absent.
- Factors that would limit absorption of the chemical from the intestine in the intact animal are missing.
- The *in vitro* system may not metabolize (activate or inactivate) the chemical.
- Excretory mechanisms are lacking.
- Responses to stress and homeostatic and adaptive mechanisms are missing.
- Distribution and transport factors are missing, and the *in vitro* model may not simulate the critical target organ.
- Duration of exposure to the chemical in the *in vitro* system cannot accurately simulate chronic exposure of the whole animal.

Notwithstanding these limitations, the potential of *in vitro* investigations as a guide to more definitive tests justifies continued search for ways in which they can be of value.

EVALUATION OF CARCINOGENIC HAZARDS OF CHEMICALS FOR MAN

On both ethical and practical grounds, the possible carcinogenic effects of chemicals to which man might be exposed must be determined in experimental animals, generally in short-lived species. The extrapolation of the results of these determinations to man rests on two basic findings:

1. Despite a great variety of studies, no significant aspect of the natural occurrence, induction, and properties of cancer has been shown to differ fundamentally between man and experimental animals.
2. Considerable assurance of the general applicability of the tests can be derived from the fact that the chemicals generally known to be carcinogenic in man also induce neoplasia in experimental animals.

Many clear differences in the activities of chemical carcinogens in various species exist, however, and the unpredictable nature of species differences is the principal limitation to the extrapolation of results from studies with experimental animals to man.

In order to minimize the species (i.e., metabolic) differences between man and experimental animals in their responses to a chemical compound, tests for carcinogenicity must be conducted with dosages at the highest practical levels—i.e., at the highest level that does not overload the normal metabolic pathway or greatly decrease life span—and in more than one test species. As knowledge of the mechanisms of chemical carcinogenesis increases, information derived from metabolic experiments with tissue preparations from human beings and experimental animals may make it possible to select the best species for the lifetime test of a given chemical. At present, however, the uncertainties make it necessary to evaluate both positive and negative results in these tests with considerable caution.

Evidence that a substance is carcinogenic in one or more experimental species raises the suspicion that it would be carcinogenic in man. Although there are probably noncarcinogenic dosage levels for an experimental species in which a given compound is carcinogenic, there is

as yet no generally accepted way of quantitatively extrapolating dose-response data in predicting a noncarcinogenic level for man. This fact is the principal basis for urging that such compounds be permitted for use in foods only if an explicit judgment has been made that demonstrable benefit greatly exceeds the risk.

There is unavoidable uncertainty about the safety of a substance not found to be carcinogenic in experimental species, the degree of which must be judged for each compound on the basis of the stringency of the tests used and the expected exposure of man to the compound in question. This uncertainty must be balanced against the benefits to be gained from the proposed uses of the substance.

Unless there is prior knowledge of the carcinogenic potential of a substance that is proposed for addition to the environment, particularly if large numbers of human beings of all ages might be exposed to it for appreciable portions of their life spans, tests adequate to demonstrate safety in this regard are necessary. Safety of each substance must be evaluated on results of such tests and a knowledge of the projected uses.

REFERENCES

1. Beyer, K. H., Jr. 1967. Limitations of animal data for predicting safety for man, pp. 43–49. *In* Proceedings of a conference on use of human subjects in safety evaluation of food chemicals. Food Protection Committee, Food and Nutrition Board. NAS–NRC Publ. 1491. National Academy of Sciences–National Research Council, Washington, D.C.
2. Food Protection Committee, Food and Nutrition Board. 1965. Some considerations in the use of human subjects in safety evaluation of pesticides and food chemicals. NAS–NRC Publ. 1270. National Academy of Sciences, Washington, D.C.
3. Frazer, A. C. 1967. Limitations in the value of studies of human subjects, pp. 63–69. *In* Proceedings of a conference on use of human subjects in safety evaluation of food chemicals. Food Protection Committee, Food and Nutrition Board. NAS–NRC Publ. 1491. National Academy of Sciences, Washington, D.C.

Appendix: Guidelines for Estimating Toxicologically Insignificant Levels of Chemicals in Food*

In 1958, the Food Protection Committee issued a statement[1] that pointed out that for every chemical there is some finite level, sometimes called the "safe level,"[2] at or below which it can be present in food without prejudicing safety. The safe level of a substance is usually established through extensive toxicological study and expert evaluation. It is generally set by applying some factor, called the "safety factor," to the highest dietary intake that is found not to injure experimental animals exposed under close observation for extended periods. Thus, the safe level is frequently expressed as 1/100 of the experimentally determined "no-adverse-effect level." This ratio, 1:100, provides a conservative estimate of the safety factor needed to afford adequate protection, even to persons whose dietary patterns or individual susceptibilities are unusual. This procedure has been so widely adopted by national and international bodies concerned with food safety that the term "safe level" as applied to chemicals in food is generally understood in the sense set forth here.

*Printed separately in 1969 as an unnumbered publication of the National Academy of Sciences–National Research Council.

[1] Insignificant levels of chemical additives in foods. 1958. Food Drug Cosmetic Law J. 13:477–479.

[2] The "safe level" in the sense here discussed refers to the maximum acceptable daily intake in the total diet. It is not to be equated with "tolerances," which are legally established limits to the concentration of chemicals permitted in agricultural products or foods compatible with good agricultural or manufacturing practice. Tolerances are established with due consideration of safe levels.

Uses of a chemical that in total contribute a level to the diet close to the "safe evel" should be regulated to prevent risk to the consumer from avoidable or ad-entitious excesses. The 1958 statement referred to above recognized that when ny particular use results in a dietary level well below the safe level, the possibility f hazard from use or misuse becomes so remote that regulatory activity to pro-ect the public from the chemical in question is superfluous. Such low levels are ufficiently presumptive of safety that they may reasonably be termed "toxicologi-ally insignificant." No attempt was made in 1958 to define "toxicologically in-ignificant" in quantitative terms. In this statement we now propose guidelines for uantitatively defining levels of chemicals in food that can be administratively onsidered toxicologically insignificant.

It is axiomatic that no substance should be allowed to enter food unless there convincing evidence that the amount used will be safe beyond reasonable doubt. his principle is embodied in food regulations (21 CFR 121.1 i) that define safety s the reasonable certainty that no harm will result from the intended use of the ood additive.

In the period since the Committee's 1958 statement, a considerable body of ew or additional toxicological information has accumulated about many chemi-als added to foods, the metabolic processes by which the body deals with them, nd their acceptable levels of intake. For administrative and regulatory purposes, e safe level has generally been derived in the manner outlined above as a con-ervative fraction of the "no-adverse-effect level" found in animal testing. However, a level in the feeding test that permits survival of at least some of the animals for e duration of the test is found to produce cancer, it may not, according to the od, Drug, and Cosmetic Law, be used in any concentration in or on food. An bservation of any such serious condition makes it prudent to consider the nature f the dose–response relationship and the physiologic, metabolic, or pathologic rocesses involved to ensure against the possibility that the same effect might ccur in man.

The vast majority of the chemical entities that we consume are present naturally nd unavoidably in foods. Only a small proportion of these substances have so far een identified, and an even smaller proportion have been investigated by conven-onal toxicological procedures. The levels at which most of them are usually con-med are assumed to be toxicologically insignificant because (1) they have been onsumed by man for generations without apparent harm; (2) they are present in ods at extremely low concentrations; and (3) insofar as is known, they are not lated chemically to substances of known high toxicity. Thus, based on these cri-ria, there is a body of empirical knowledge deemed sufficient on which to base a dgment of the safety of virtually all these substances at the levels found in food. e latter two criteria are equally valid when applied to evaluation of safety for se of synthetic substances.

THE NEED FOR PRIORITIES

Because of the thousands of natural and synthetic substances present in food at low and intermediate levels, it becomes necessary to establish a reasonable system of priorities for the further study of those substances not yet fully evaluated. To study every chemical to the same extent as those that must be used at close to their safe levels would represent an unjustifiable expenditure of effort not contributing significantly to protection of public health. It is neither practicable nor necessary to undertake experimental toxicological studies of every chemical to which man is exposed; to do so would be to assign equal importance to problems of unequal risk. This would deny the value of experience in assessing probable risk. All environmental exposures must be subjected to scientific evaluation, but not all exposures require experimental toxicological study.

To provide optimum assurance of public safety within the limitations of capabilities available, toxicological facilities for evaluating safety must be concentrated on environmental situations in which there is a reasonable expectation that exposure to chemicals may cause real hazards. The definition of a "toxicologically insignificant" level simply as one well below an established safe level is too indefinite and too limiting to be of practical value, since it requires in every case the establishment of a safe level by experiment. To insist that nothing can be assumed to be safe without direct experimental toxicological evidence implies that safety must be proved experimentally before the proof of safety can be considered unnecessary. This denies the value of establishing criteria for insignificance. Thus, there is urgent need to arrive at more specific guidelines for estimating dietary levels that can be considered toxicologically insignificant.

This report endorses and extends the application of the term "negligible" as employed by a previous committee of the National Academy of Sciences–National Research Council,[3] which recommended that uses contributing only a small fraction of the safe dietary level, as previously defined, be ignored administratively. But it is concerned in addition with dietary levels of chemicals for which safe levels have not been determined in the laboratory.

THE APPLICATION OF EXPERIENCE

Our search for increasingly toxic pesticides or antipersonnel agents, for drugs active at low dosages, and for naturally occurring toxins has had the expected result of revealing substances that demonstrate toxicity at lower levels than any substances previously known. Thus, if distinction among probable hazards is ignored, the

[3] Report of the Pesticide Residues Committee, June 1965. A Report of the National Academy of Sciences–National Research Council to the Food and Drug Administration and the U.S. Department of Agriculture.

concept of an insignificant level would drift downward to essentially zero. For the practical reasons outlined above, however, we cannot afford to ignore the distinctions, outlined in the following, that increasing experience now permits us to make.

Tabulation and examination of compounds in commercial use that may have deleterious effects at low levels reveal that they fall into four general categories:

1. Certain impurities of contaminants of natural origin;
2. Certain essential nutrients and hormones;
3. Certain heavy metals and their compounds; and
4. Certain organic compounds employed for their biological activity.

In the first category are such substances as aflatoxin, botulinus toxin, and tetrodotoxin, which are toxic at concentrations as low as 0.001 ppm. In categories 2, 3, and 4 are a large number of pesticides, pharmaceuticals, and antipersonnel agents that may have biological activity at levels as low as 0.1 ppm. Aside from these classes of compounds, no commercial compound has been demonstrated to produce toxic reactions below a dietary concentration of 40 ppm.[4]

Obviously, the level that can be considered toxicologically insignificant for one category may not, for that reason, be so considered for another category. The criteria for insignificance will vary for different classes of compounds and may change with further research on compounds within classes. Chemicals that exert significant biological effects and that are useful or unavoidable in food or any other part of the environment will continue to be subjected to laboratory investigations to establish safe levels, and the experience gained thereby will constantly provide the basis for confirming or modifying the earlier conclusions.

Synthetic organic chemicals that are not manufactured specifically for their biological activity must be sharply distinguished from naturally occurring toxins or trace contaminants. During development and production of a new commercial chemical, some form of toxicological testing is a commonplace precaution. Additionally, a degree of biological knowledge is gained through human exposures in development and production of a new chemical that is more extensive than the knowledge we have of most trace contaminants. It is virtually certain that unpredicted effects of extremely toxic compounds would be revealed through exposure in the work environment, and such compounds would then be placed in category 4 of the above tabulation.*

The toxicology of substances intentionally used for their biological activity is always investigated experimentally to establish limits of their safe usage.

ESTIMATION OF TOXICOLOGICALLY INSIGNIFICANT LEVELS

It is generally true that exposure by nonoral routes may not be a reliable basis for predicting a maximum safe dietary level. Nevertheless, unavoidable industrial experience provides useful guidance that can be applied to evaluating safety in the diet, including judgment as to toxicological insignificance for various classes of substances, based on available information concerning their safety or that of related compounds.

Except for the categories of compounds cited under 2, 3, and 4, no single organic chemical that has advanced from the laboratory, through development, into general commercial use has been demonstrated to be toxic to experimental animals at a dietary level of 40 ppm or less. Compounds that possess greater toxicity have either been developed specifically for use as economic poisons (as defined by the law), as drugs, or as chemical warfare agents, etc., or were found to possess such biological activity during development and were diverted to these uses.

A. Chemicals in Commercial Production

If a chemical has been in commercial production for a substantial period, e.g., 5 years or more, without evidence of toxicological hazard incident to its production or use, if it is not a heavy metal or a compound of a heavy metal, and if it is not intended for use because of its biological activity, it is consistent with sound toxicological judgment to conclude that a level of 0.1 ppm of the chemical in the diet of man is toxicologically insignificant.

B. Pesticide Degradation Products

Most degradation products of pesticides are less toxic than the parent material. A few exceptions have been experienced, but even in these cases the toxicity of the degradation products is only slightly greater than that of the parent compound. If the safe level of the pesticide is 1 ppm or above, it appears to be a safe working guideline that dietary levels of degradation products below 0.1 ppm are insignificant and undeserving of laboratory investigation.

C. Organic Chemicals Lacking Toxicological Data, but Meeting Special Structural Restrictions

For many substances that are functionally effective in food at dietary concentrations above 0.1 ppm, but still much below any reasonable judgment as to their maximum safe level, as previously defined, there is need to arrive at estimates of toxicologically insignificant levels. For these substances, it is justifiable to employ accumulated scientific experience and to recognize their structural analogy to other chemicals whose metabolism or toxicity is known. Reasoning by analogy may be used to arrive at conclusions of toxicological insignificance. If a substance meets *all* the following criteria, it may be presumed to be toxicologically insignificant at a level of 1.0 ppm or less in the human diet:

1. The substance in question is of known structure and purity;

[4] J. P. Frawley. 1967. Scientific evidence and common sense as a basis for food-packaging regulations. Food Cosmet. Toxicol. 5:293–308.

*That is, if they are commercially developed.

2. It is structurally simple[5];

3. The structure suggests that the substance will be readily handled through known metabolic pathways; and

4. It is a member of a closely related group of substances that, without known exception, are, or can be presumed to be, low in toxicity.[6]

D. Organic Chemicals with Minimal Toxicological Data and Less Closely Related Structures

In cases nearly, but not precisely, meeting the above criteria, a level of insignificance may be established if

1. There are available adequate scientific studies that establish safe levels of similar magnitude for at least two analogous substances.

2. The acute or subacute toxicity of the new substance and the analogous substances is of the same nature and degree.

A sound estimate of the safe level of the new substances that meets the two foregoing conditions is the lowest safe level of all the analogous substances that have been studied. If the safe levels for all the structurally analogous substances are essentially identical, 1/10 of the estimated safe level may be taken as a toxicologically insignificant level. In the event of appreciable differences among the safe levels of the analogous substances, the insigificant level should be taken as 1/20 of the estimated safe level.

In conclusion, it is important to protection of public health that responsible and reasonable priorities be established to guide research efforts in toxicology. The principle of toxicological insignificance is a valid concept for separating potential health hazards from predictably safe applications. Guidelines on toxicological insignificance are needed to eliminate wasteful diversion of scientific resources in university, industry, and government laboratories. The levels judged to be insignificant by this Committee are conservatively derived from accumulated toxicological experience. They are intended to guide and stimulate—not replace—informed professional and administrative judgment. Like all other judgments of toxicologic hazards, they should not be considered infallible against future evidence; and, as with any experimentally derived toxicologic information applied to evaluation of safety for man, continuous awareness of possible unexpected effects must be maintained. However, in light of today's knowledge, application of these guidelines will protect public health and simultaneously allow greater attention to be devoted to projects of greater potential significance to health.

[5] Examples that are intended to be illustrative, not limiting, are

(a) Straight-chain or simply branched aliphatic alcohols, acids, and esters.

(b) Linear polymers of ethylene or ethylene oxide.

(c) Cellulose ethers.

(d) Mononuclear aromatic compounds containing only carbon, hydrogen, and oxygen, and equipped with one or more functional groups that include hydroxyl, aldehyde, and keto.

[6] "Closely related" shall be understood to mean

(a) Near members of a homologous series.

(b) Geometric or positional isomers that would not be expected to present serious differences in chemical reactivity or steric effects.

(c) Substances of identical basic structure or differing only by (a) or (b) above, and possessing additional functional groups readily accommodated by known metabolic mechanisms.

(d) Compounds readily metabolized into substances meeting the other criteria here listed.

SAFETY AND SUITABILITY OF MONOSODIUM GLUTAMATE FOR USE IN BABY FOODS

Subcommittee on Safety and Suitability of
MSG and Other Substances in Baby Foods

Food Protection Committee

Food and Nutrition Board

National Academy of Sciences - National Research Council

July 1970

PREFACE

The National Academy of Sciences was requested by the U.S. Food and Drug Administration to advise it on the safety and suitability of monosodium glutamate (MSG), salt, and modified starches for use in baby foods. A special advisory committee, under the general direction of the Food Protection Committee, was appointed by NAS for this purpose. Reported here is a review and evaluation of information concerning MSG prepared by that committee.

This work was performed under terms of contract CPF 70-10.

Subcommittee on Safety and Suitability of
MSG and Other Substances in Baby Foods

Lloyd J. Filer, Jr., Chairman

Pierre M. Dreyfus

Kenneth P. DuBois

Lloyd W. Hazleton

George M. Owen

Virgil B. Robinson

John A. Zapp, Jr.

CONTENTS

Summary and Evaluation 1

Introduction 3

Human Experience 4

As a Seasoning or Flavor-Enhancer 4

As a Drug 5

Metabolism of MSG 9

Experimental Toxicology 17

Neuropathologic Effects on Neonatal Mammals 23

Possible Effects of MSG on the Retina 30

Pediatric Aspects 34

SUMMARY AND EVALUATION

Animal studies designed to evaluate the safety of MSG and extensive observations of human subjects receiving MSG as a therapeutic agent or ingesting it as a flavor-enhancer confirm the high degree of safety of this food chemical as ordinarily used.

Of specific concern to the present review is the safety of MSG for use in foods for babies, a concern prompted by published reports that high doses of MSG administered orally or parenterally to neonatal laboratory animals produced specific lesions in the arcuate nucleus and in the retina. These reports can probably be considered as confirmed insofar as mice and rats are concerned whenever the level of administration is somewhat greater than 1 g/kg, and the animals are no more than ten days of age. The effect has not been confirmed for dogs or monkeys.

The greater resistance of neonatal monkeys, and perhaps of neonatal dogs, and the resistance of rodents past ten days of age is thought to be associated with the development of effective barriers to entrance of glutamate into the brain and retina. At birth the development of the nervous system of the human infant is much farther advanced than is that of the rodent, and this is probably also true of the blood-brain and blood-retina barriers. This

view appears to be supported by observations on human neonates fed intravenously over prolonged intervals with protein hydrolysates furnishing as much as 0.3 g glutamate/kg/day.

In any event, in considering the safety of added MSG in baby foods, one must remember that the levels added are small--not higher than 0.6 percent, that these foods are not likely to be consumed before the child is two months of age--certainly not by the neonate, and that they supply only a minor part of the total dietary intake of children of this age.

Thus, the Committee concludes that the risk associated with using MSG in foods for infants is extremely small. The Committee cannot find, however, that the usage confers <u>any</u> benefit to the child and therefore recommends that MSG not be added to foods specifically designated for infants.

The Committee found no evidence of hazard from the reasonable use of MSG in foods for older children and adults, except for those who are individually sensitive to the substance. The flavor-enhancing property of MSG is considered to be beneficial to the general consumer in these age groups. The Committee therefore recommends that use of MSG be permitted in processed foods for these groups and that such foods be clearly labeled to indicate the presence of added MSG for the information of those who wish to avoid it. Sale of MSG in packages for institutional and home consumer use need not be curtailed.

SAFETY AND SUITABILITY OF MONOSODIUM GLUTAMATE FOR USE IN BABY FOODS

INTRODUCTION

Glutamic acid is a naturally occurring amino acid and a constituent of all common food proteins. It is estimated that the average human adult ingests about 15 grams, and the three-week old infant about 1.5 grams, of glutamic acid daily from food sources as the free acid and in combination as a natural constituent of foods. It is understandable that a substance that has always been a part of the normal diet of man, that is a normal constituent of the protein building blocks of human tissue, and that produces usable energy, would be generally recognized as safe.

A constituent of a food when used separately as a component of the diet or as a drug increases the total amount of that component ingested above the amount otherwise available. So long as the increase is moderate, little, if any, adverse effect is to be expected; if the increase is large, an adverse effect might result. Indeed, for almost any substance there is probably a level at which some adverse effects will occur.

Glutamic acid, isolated from protein or synthesized, has been used, usually in the form of MSG, as a seasoning or flavor-enhancer for foods and as a drug. The question here is whether such usage, particularly in foods for infants, is safe.

HUMAN EXPERIENCE

As a Seasoning or Flavor-Enhancer

Soy sauce, prepared from hydrolyzed soybean protein, has long been used in the Orient as a seasoning or flavor-enhancer. In 1908, it was recognized in Japan that MSG had unique properties of its own, and that MSG was probably the primary "active ingredient" of soy sauce. Monosodium glutamate was sold in Japan under the name "Aji No Moto," and relatively small amounts were exported to the United States. American production began about 1940. In 1967, the U.S. production was 45 million pounds, about 2/3 of which was used for commercial food processing and in institutions, and about 12 percent in the home. The rest was exported or remained in inventory. The amount of MSG added to commercially processed foods in which it is used is at a level to provide about 0.1 to 0.6 percent by weight as consumed.

There are no reported instances of lasting or significant adverse effects attributable to MSG in persons eating commercially processed foods containing this compound. In 1968, however, Kwok[1] described a syndrome that he experienced when he ate "Chinese" food. The syndrome occurred 15 to 20 minutes after eating the first dish and lasted about two hours "without any hangover effects." Kwok named the syndrome "Chinese Restaurant Syndrome" and speculated that it might be caused by cooking wine, MSG, or the high sodium content of Chinese foods.

Additional letters-to-the-editor confirmed Kwok's observations[2]. Two further letters-to-the-editor[3] in 1968 identified MSG as the cause of the Chinese restaurant syndrome. Schaumburg et al.[4] expanded these observations, concluding that "the oral dose-effect curves of MSG indicate that the Chinese restaurant syndrome is a normal response to this agent. All subjects tested could be made to experience the sensory phenomena if they ingested enough MSG or if they had an intravenous injection of as little as 125 mg." The syndrome occurred only if the MSG was taken on an empty stomach by a susceptible individual. The "susceptible individuals" in this study had oral thresholds of 3 grams or less. "Because prior ingestion of food, which delays absorption of the agent, will protect the most susceptible individual from this phenomenon, it is not surprising that soups, which are usually eaten on an empty stomach and contain large amounts of MSG, provoke the symptoms."

It appears likely that the Chinese restaurant syndrome occurs when the concentration of MSG in the blood exceeds a threshold level--differing among individuals--either from rapid absorption of a large oral dose or from parenteral injection. No lasting adverse effects have been reported in those experiencing the syndrome.

As a Drug

Monosodium glutamate has been clinically investigated as a drug for the treatment of ammonia accumulation in hepatic failure, and the

treatment of behavioral disorders, epilepsy, and mental retardation, and has been used as an ingredient of salt substitutes and as a nutritional supplement. The significance of these uses for the present discussion lies only in the doses used and the effects attributed to the MSG itself.

Himwich et al.[5] investigated the effect on the plasma levels of glutamic acid of feeding MSG to elderly individuals. After blood was drawn in the basal state the MSG was administered in 125 ml of tomato juice. Blood samples were collected at 30, 60, 120, and 180 minutes. Although the response to a single dose varied with different individuals, plasma levels of glutamic acid generally reached a maximum within 60 minutes after administration of the MSG and had returned to basal levels within 180 minutes.

In a chronic study MSG was administered three times daily in a daily dose of 15 grams (glutamic acid equivalent) the first week, 30 grams the second week, and 45 grams for the next ten weeks. Continued ingestion of MSG for twelve weeks had no effect on the basal plasma level of glutamic acid or glutamine. No mention was made of MSG effects on the patient in this study.

In a subsequent study[6], in which MSG was fed to a group of patients hospitalized for a long time with varying psychiatric disorders, it was noted: "However, the monosodium salt in 15 gram doses also evoked side effects in four patients whose dose had to be reduced from 15 grams T.I.D. to 10 grams T.I.D."

An earlier paper by Loeb and Tuddenham[7] described feeding MSG to patients 6 to 29 years of age as part of a study of the effect of glutamic acid on mental function. The MSG was given by mouth three times daily, after meals. The total dose was 12 grams per day the first week, 18 grams per day the following week, and 24 grams per day for the next 16 weeks. The authors state: "The MSG solution was mildly unpleasant in the concentrations used, but no serious untoward reactions were observed."

It might be added that no long-term adverse effects were observed in the many early studies that involved the feeding of l-glutamic acid or its hydrochloride for various therapeutic purposes. Since chronic toxic effects, if any, would likely be attributable to the glutamate ion, these studies reinforce the impression from studies involving MSG that long-term toxic effects in humans have not been observed.

A recent study of human reactions to MSG has been reported by Rosenblum et al.[8] According to their abstract, 98 male volunteers of varying ages and backgrounds were fed a single 5 gram dose of MSG dissolved in either tap water or diluted chicken soup stock. Each volunteer filled out a questionnaire pertaining to 14 symptoms. Approximately one half of the subjects affirmed one or more symptoms, but only one of these appeared to have "reacted" to MSG.

References

1. Kwok, R.H. 1968. Chinese restaurant syndrome. N. Engl. J. Med. 278:796.

2. Correspondence. 1968. N. Engl. J. Med. 278:1122-1124.

3. Correspondence. 1968. Ibid. 279:105

4. Schaumburg, H.H., R. Byck, R. Gerstl, and J.H. Mashman. 1969. Monosodium l-glutamate: its pharmacology and role in the Chinese restaurant syndrome. Sci. 163:826.

5. Himwich, W.A., and I.M. Peterson. 1954. Ingested sodium glutamate and plasma levels of glutamic acid. J. Appl. Physiol. 7:196.

6. Himwich, H.E., K. Wolff, A.L. Hunsicker, and W.A. Himwich. 1955. Some behavioral effects associated with feeding sodium glutamate to patients with psychiatric disorders. J. Nerv. Ment. Disord. 121:40.

7. Loeb, H.G., and R.D. Tuddenham. 1950. Does glutamic acid administration influence mental function? Pediat. 6:72.

8. Rosenblum, I., D.M. Serrone, J.C. Killeen, Jr., J. Bradley, J.H. Wills, and F. Coulston. An Evaluation of the Effects of Monosodium Glutamate in Man and Animals. Abstracts of Papers, 9th Ann. Meet., Soc. Toxicol., March 15-19, 1970, p. 85.

METABOLISM OF MONOSODIUM GLUTAMATE

Monosodium glutamate is a sodium salt of glutamic acid. Glutamic acid is not an essential amino acid, but it functions as a necessary amino donor for the synthesis of other amino acids. The normal metabolic pathways involving glutamic acid have been studied extensively, and it is well established that the metabolism of glutamate involves oxidative deamination, transamination, decarboxylation, and amidation[1].

Oxidative deamination of glutamic acid is catalyzed by glutamate dehydrogenase and results in the formation of alpha-ketoglutarate. Glutamate is also converted into alpha-ketoglutarate by transaminase, which catalyzes transfer of amino groups of glutamic acid to alpha-keto acids such as oxalacetic acid and pyruvic acid[2]. The reaction is reversible, proceeds with great rapidity, and occurs in almost every tissue. However, the different tissues vary in the details of the transamination process. In muscle tissue, three different enzymes function for transamination of glutamic acid, aspartic acid, and alanine. By combination of these three systems, glutamic acid can be formed from alpha-ketoglutarate plus alanine or aspartic acid, and aspartic acid can be formed from oxalacetic acid plus alanine[2].

The main degradative metabolism of glutamate in mammals involves deamination or transamination followed by oxidation of the resulting alpha-ketoglutarate in the tricarboxylic acid cycle[3].

Decarboxylation of glutamate to γ-aminobutyric acid (GABA) is a significant pathway of glutamate metabolism in mammalian brain. Thus Roberts and associates[4,5] reported relatively large amounts of free GABA in brain and demonstrated[5] the existence of an enzyme that can form this compound from glutamic acid. Roberts and Frankel[6] studied the properties of glutamic decarboxylase in mouse brain and found that it is a typical decarboxylase that utilizes pyridoxal phosphate as a coenzyme.

Formation of glutamine from glutamate and ammonia was demonstrated by Krebs[7] in slices of the kidney, brain, and retina of various species. Synthesis of this amide was generally dependent on the respiration of the tissue and the respiration seemed to supply the necessary energy. However, in retina, which possesses a very active glycolytic system, glutamine was produced anaerobically, provided glucose was present. In further studies on the mechanism of glutamine synthesis, Speck[8,9] concluded that the reaction requires glutamic acid, ammonia, phosphate, and magnesium ions and that the energy is supplied by respiratory processes. Adenosine triphosphate was considered to be the source of energy because glycolysis can support anaerobic glutamine synthesis by retina. Proof of the role of adenosine triphosphate as a source of energy for this reaction was obtained by Speck[9] using fortified extracts of liver, brain, and kidney.

Numerous studies on the metabolism of glutamate have been conducted. A variety of doses, different routes of administration, and species

have been used, and the objectives of the experiments have differed widely, reflecting the individual interests of investigators in the metabolic fate, tissue distribution, or rate of absorption of glutamate. The initial stimulus for the unusual amount of attention given to the metabolism of glutamic acid was the report by Albert et al. in 1951[10] that large doses of glutamate had a beneficial effect in mental retardation.

Studies with glutamate 2-^{14}C have shown[11] that conversion to succinate via alpha-ketoglutarate is the primary route of catabolism when glutamate is injected intraperitoneally into rats. When glutamic acid is administered orally it is partly converted to succinate and partly metabolized in such a manner that the 2-carbon becomes the methyl carbon atom of acetate. Instillation of glutamic acid into the cecum resulted in conversion of the 2-carbon to a methyl carbon of acetate. The action of intestinal bacteria was considered responsible for this conversion.

Lajtha et al.[12] conducted detailed studies on the metabolism of ^{14}C labeled glutamic acid in rats and mice. The entry of the ^{14}C into various organs was very rapid and within minutes the glutamic acid, glutamine, and glutathione fraction of the brain, liver, and kidney as well as the GABA fraction of brain were significantly labeled. Within two minutes after the administration of labeled glutamic acid, the specific activity of glutamine in the organs equaled that of labeled glutamic acid, indicating rapid intraconversion of the glutamic acid-glutamine system. In experiments of longer duration

the specific activity of glutamic acid, glutathione, and GABA decreased, as would be expected from their rapid metabolism. Balazs[13] found that transamination is the main pathway for glutamate metabolism by brain and by liver mitochondria. The oxidation of glutamate by pathways other than transamination was much lower in brain that in liver mitochondrial preparations.

Further interest in the metabolism of glutamic acid was stimulated in 1957 by the finding of Lucas and Newhouse[14] that subcutaneous injection of 4 to 8 g/kg of sodium l-glutamate into neonatal mice produced changes in the retina. They found that large doses of the immediate metabolites of glutamate were ineffective in producing the retinal changes, except in the case of aspartate, which is readily converted to glutamate by transamination. These investigators found that anaerobic glycolysis in the excised retina of mice killed one hour after glutamate injection was lower than normal by about one third. Respiration of retinal tissue with pyruvate as the substrate was unaffected.

Potts _et al._[15] confirmed the retinal lesion produced by monosodium glutamate, and Freedman and Potts[16] found that the lesion in rats was associated with a reduction in the level of retinal glutaminase I, an enzyme that forms glutamate from glutamine by deamination. The possibility was considered[17] that this reduction in enzyme activity was due to a feedback inhibition by glutamate of the activity of the enzyme that forms glutamate from

glutamine (glutaminase I), but experimentation did not support this idea. A 40 percent increase in glutamic oxalacetic transaminase in the injured retina was also observed by these investigators. It was suggested[16] that product (glutamate) repression of enzyme formation was the mechanism responsible for decreased levels of retinal glutaminase I and that glutamate increases glutamic oxalacetic transaminase by substrate induction of enzyme synthesis. Although these enzyme changes occur in the retina following administration of large doses of glutamate, there is no evidence that they are causally related to the injury. No biochemical information on enzyme changes associated with hypothalamic lesions in neonatal animals has been reported.

Numerous studies have been conducted on the rate of absorption, tissue distribution and excretion of glutamic acid and its monosodium salt. The most significant studies in relation to retinal and hypothalamic lesions are those on animals a few days old. In one such investigation[17] a single dose of 2-^{14}C-d, l-monosodium glutamate plus unlabeled glutamate (3.2 g/kg) was given intraperitoneally to five-day-old rats. The highest plasma level of glutamate occurred at 1.5 hours after injection and the retina and brain levels were maximal at 3 hours. At all times the concentration in retina exceeded that in brain. After the peak concentration occurred, the level of glutamate in retina exceeded the amount in plasma. Repetition of this experiment with ten-day old rats demonstrated that the peak retinal concentration of

to glutamate develops appears to be of considerable significance in determining whether hypothalamic or retinal lesions will occur. Although the exact mechanism responsible for these lesions has not been elucidated, the observations that one or both lesions occur in rats and mice, if high doses of glutamate are given during the first few days after birth, strongly suggest that information is needed on the susceptibility of various other species to retinal and hypothalamic injury by monosodium glutamate in relation to time of maturation of the blood-brain and blood-retinal barriers.

References

1. Von Euler, H., E. Adler, G. Gunther, and N.B. Das. 1938. Ueber den enzymatischen Abbau und Abbau der Glutaminsaeure. II. In tierischen Geweben. Z. Physiol. Chem. 254:61.

2. Cohen, P.P. 1949. Transamination in pigeon breast muscle. Biochem. J. 33:1478.

3. Meister A. Biochemistry of the Amino Acids. Vol. I and II, 2nd ed. Academic Press, N.Y. 1965.

4. Roberts E., S. Frankel, and P.J. Harman. 1950. Amino acids of nervous tissue. Proc. Soc. Exper. Biol. and Med. 74:383-387.

5. Roberts E., and S. J. Frankel. 1950. Aminobutyric acid in brain, its formation from glutamic acid. J. Biol. Chem. 187:55-63.

6. Roberts, E., and S. J. Frankel. 1951. Glutamic acid decarboxylase in brain. J. Biol. Chem. 188:789-796.

7. Krebs, H.A. 1935. The metabolism of glutamic acid. Biochem. J. 29: 1951.

8. Speck, J.F. 1949. The synthesis of glutamine in pigeon liver dispersions. J. Biol. Chem. 179:1387-1403.

9. Speck, J.F. 1949. The enzymatic synthesis of glutamine, a reaction utilizing adenosine triphosphate. J. Biol. Chem. 179: 1405-1426.

10. Albert K., P. Hock, and H. Waelsch. 1951. Glutamic acid and mental deficiency. J. Nerv. Ment. Disord. 114:471-491.

11. Wilson, W. E., and R. E. Koeppe. 1959. Variation of glutamate metabolism with the site of injection. J. Biol. Cham. 234:1186-1187.

12. Lajtha, A., A. Berl, and H. Waelsch. 1959. Amino acid and protein metabolism of the brain. IV. The metabolism of glutamic acid. J. Neurochem. 3:332.

13. Balazs, R. 1965. Control of glutamate oxidation in brain and liver mitochondria systems. Biochem. J. 95:497.

14. Lucas, R. R., and J.P. Newhouse. 1957. The toxic effects of sodium glutamate on the inner layers of the retina. A.M.A. Arch. Ophthal. 58: 193-201.

15. Potts, A.M., R.W. Modrell, and C. Kingsbury. 1960. Permanent fractionation of the electroretinogram by sodium glutamate. Am. J. Ophthal. 50:900-907.

16. Freedman, J.K., and A.M. Potts. 1962. Repression of glutaminase I in the rat retina by administration of sodium-l-glutamate. Invest. Ophthal. 1:118.

17. Freedman, J.K., and A.M. Potts. 1963. Repression of glutaminase I in the rat retina by administration of sodium-l-glutamate. II. Invest. Ophthal. 2:252-258.

EXPERIMENTAL TOXICOLOGY

Safety evaluation studies directed at the use of MSG as a food additive were conducted in the early 1950's[1,2]. Reproduction and teratology studies designed to supplement data from these acute and chronic studies were subsequently done[3]. These chronic and teratology studies were recently reviewed by Ebert[4]. (See Table 1.)

These studies confirmed that l-monosodium glutamate, dl-monosodium glutamate, and l-glutamic acid possess a very low order of acute and chronic toxicity and no teratological properties. The mouse studies were designed specifically to evaluate carcinogenic potential. Under the conditions of study the results were negative.

In the teratology studies, pregnant rabbits were fed diets high in MSG. Fetuses were taken by Caesarean section at gestation day 29-30 for fixation. There was no postnatal exposure to MSG. Histological and special teratological examinations showed no deviation from normal. No evidence of brain damage was seen in hemotoxylin and eosin stained sections of paraffin-embedded material.

Unpublished reports from investigators at the U.S. Food and Drug Administration[5] indicate that no obvious toxicity or teratogenicity was observed in chick embryo studies. Tissue culture studies in the rat kangaroo cell line indicate no toxic effects on the mitotic index nor the incidence of chromosomal breaks.

Table 1

OUTLINE OF SOME CHRONIC TOXICITY AND TERATOLOGY STUDIES WITH MSG*

COMPOUND	ANIMALS	LEVELS IN DIET (%)	TEST	REFERENCE
1-monosodium glutamate·H_2O	Rats, Sprague-Dawley n = 75/level ♂ & ♀ + 50 controls begin 3 months	0.1 (50 mg/kg/Day)[1] 0.4 (200 mg/kg/Day)	2 yr feeding	1
dl-monosodium glutamate·H_2O 1-glutamic acid	Mice, C-57 Black (Jackson) n = 100/level ♂ + 100 controls begin 6 weeks	1 (1.65 g/kg/Day)[2] 4 (6.60 g/kg/Day)	2 yr feeding	2
1-monosodium glutamate	Rabbits, New Zealand White n = 40 + 40 controls ♂ + ♀	0.1 (30 mg/kg/Day)[3] 0.825 (250 mg/kg/Day) 8.25 (2.5 g/kg/Day)	Teratology (6-7 wk feeding)	3

* Ref. 4

[1] Assuming 200 g rat eating 10 g food/day.

[2] Assuming 30 g mouse eating 5 g/day.

[3] Assuming 2 kg rabbit eating 60 g/day.

In 1965, Tugrul[6] conducted studies in rabbits to determine whether glutamic acid had teratological properties. Numerous adverse effects on both dam and offspring were recorded, but lack of detail makes evaluation of this report difficult. In 1967, Yonetani[7] attempted to reproduce the Tugrul results using glutamic acid hydrochloride and MSG. The results of these studies directly contradicted the Tugrul results, showing no difference between controls and animals fed glutamic acid-HCl or MSG.

Khera et al.[8] report that oral administration of MSG in doses of 0.07, 0.7, or 7.0 g/kg/day to pregnant rats produced no adverse effects, including no evidence of neuropathologic damage, on the dam or offspring when the doses were given from day 6-15 of pregnancy. Under the same conditions, when the MSG was given on days 15-17, there was no observed effect on the dam or the fetus, or on the subsequent growth of the young. The progeny, however, had impaired ability to reproduce. At a dosage of 0.7 g/kg/day during days 15-17 of pregnancy, there was an apparent slight impairment; at 7 g/kg/day, there was total impairment. It has not been determined whether the impaired fertility was due to an effect in the male or the female embryo. Although the groups were small and the reproductive efficiency of the controls averaged only 53 percent, these results suggest that the 15- to 17-day-old rat embryo may be affected when large doses of MSG are administered orally to the mother.

Recent studies at the Food and Drug Administration[5] indicate that:

- There is growth suppression when MSG constitutes 20 percent of the diet of rats.
- The addition of 10 percent MSG or glutamic acid to the diet has no effect on the growth of rats.
- Litter size and birth weights are unaffected when 10 percent MSG or glutamic acid is added to the diet before mating and throughout gestation and lactation.
- Brains from newborn rats from MSG-fed mothers show no evidence of a reduction in DNA content.
- There is increased vascularization of the iris and supression of electroretinogram β-wave in newborn rats given 5 g/kg MSG orally on days 5 - 10.
- GABA content is reduced in brains of rats fed diets containing 1, 5, 10, or 20 percent MSG for 15 weeks. (No other biochemical changes were observed in this study.)

References

1. Report on an investigation of the toxicity of l-monosodium glutamate, dl-monosodium glutamate and l-glutamic acid. July 30, 1952. Unpublished report submitted by Arthur D. Little, Inc., to International Minerals and Chemicals Corporation. Copy on file in the Food Protection Committee office.

2. Report on a study of l-monosodium glutamate, dl-monosodium glutamate and l-glutamic acid with respect to potential carcinogenicity. July 21, 1952. Unpublished report submitted by Arthur D. Little, Inc., to International Minerals and Chemicals Corporation. Copy on file in the Food Protection Committee office.

3. Reproduction study - rabbits, monosodium glutamate, final report, November 3, 1966. Unpublished report submitted by Hazleton Laboratories, Inc., to International Minerals and Chemicals Corporation. Copy on file in the Food Protection Committee office.

4. Ebert, A.G. 1970. Chronic toxicity and teratology studies of l-monosodium glutamate and related compounds. Presented at 9th Ann. Meet. Soc. Toxicol., Atlanta, Georgia. Abstract published in Toxicol. Appl. Pharmacol. 16:3.

5. Various unpublished reports submitted by the Food and Drug Administration to the Food Protection Committee advisory committee on safety and suitability of MSG, salt, and modified starches for use in food for infants, and on file in the Food Protection Committee office.

6. Tugrul, S. 1965. The monster-producing action of glutamic acid. Arch. Int. Pharmacodya. 153:2, 323-333.

7. Yonetani S. Effects of some salts of glutamic acid on pregnant rabbits and their fetuses. June 7, 1967. Unpublished report from Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan. Copy on file in the Food Protection Committee office.

8. Khera, K.S., L.L. Whitta, and E.A. Nera. 1970. Development in progeny of monosodium glutamate treated pregnant rats. Paper prepared for publication and privately communicated to the Committee from Research Laboratories, Food and Drug Directorate, Canada.

NEUROPATHOLOGIC EFFECTS OF MSG ON NEONATAL MAMMALS

Neuropathologic, growth, and endocrine changes have been described in neonates of certain animal species receiving large parenteral or oral doses of MSG[1-6]. Olney[1] injected single subcutaneous doses of from 0.5 to 4 g/kg of MSG into Swiss mice. Mice 1 to 9 days of age were so treated and killed 3 to 48 hours after treatment. Tissues were fixed by whole-body perfusion with glutaraldehyde through the ascending aorta. Stained coronal brain sections were prepared that included the arcuate nucleus and median eminence of the hypothalamus. Preparations were studied by light and electron microscopy.

A symmetrical lesion was described on each side of the third ventricle in the arcuate nucleus and sometimes at the base of the ventricle in the median eminence. Neurons were described as swollen and necrotic, and nuclei were pyknotic three hours after treatment. Forty-eight hours after treatment the majority of neurons in the arcuate nucleus had disappeared, yet no glial response or phagocytosis could be observed. Lesions were reported in mice at all dose levels studied. Maps of the lesion were prepared by means of composite electron photomicrographs. Similar lesions were reported in adult mice receiving 5 to 7 g/kg but not at lower doses. Similar lesions were also described in neonatal C 57BL/6 mice and albino rats treated with MSG.

Extreme obesity and underdeveloped skeletons in both sexes, and sterility in females, developed in Swiss mice receiving a series of daily

injections of MSG during the first ten days of life according to the schedule used by Cohen[7].

Olney and Sharpe[2] gave 2.7 g/kg of MSG to a premature rhesus monkey three days after birth, killed the animal three hours after treatment, and fixed the whole body by means of glutaraldehyde perfusion. Small lesions similar to those seen in mice were reported in the arcuate nucleus and median eminence of the hypothalamus. Olney[3] has also observed lesions in two one-week-old rhesus monkeys receiving 1 and 2 g/kg, respectively, of MSG orally in skim milk. Lesions were described as less severe than those observed in young mice receiving comparable doses.

Olney[4] has reported lesions in mice treated orally with single doses of MSG similar to those seen in animals receiving subcutaneous injections. Hypothalamic lesions were reported in 54 percent of animals receiving 0.5 g/kg of MSG, in 82 percent of those receiving 0.75 g/kg, and in all the animals receiving 1 and 2 g/kg of body weight. Olney states that similar lesions also occurred in young chicks and rabbits treated with MSG.

Coulston[8] and co-workers have studied the effects of a single dose of 4 g/kg of MSG orally or subcutaneously administered to three-day-old C 57 strain mice. The mice were killed 3 or 24 hours after treatment and the brains fixed in cold Zenker's formalin solution. Coronal brain sections that included the arcuate nucleus and median eminence were studied by both the light and the electron microscope.

These workers reported no significant differences between the appearance of brains from the mice killed at three hours after oral or parenteral treatment and those of controls. Lesions, the description of which indicates that they differ from those described by Olney, were reported in the arcuate nucleus of 12/20 mice killed 24 hours after parenteral treatment and in 5/38 mice killed 24 hours after oral treatment. Deterioration and probably destruction of neurons were described. Small dense chromatin-like structures, which were interpreted as glial cell nuclei when observed by either light or electron microscopy, were present in the lesion. Lesions observed after oral treatment appeared less severe than those seen following parenteral injection.

Four three-day old rhesus monkeys were studied following a single dose of 4 g/kg of MSG. Two animals were treated orally, two subcutaneously, and an additional control monkey received physiological saline subcutaneously. Stained sections from the brain, including the arcuate nucleus and median eminence of the hypothalamus, were studied with the light and electron microscopes. Electron photomicrographic maps were prepared of the area from the third ventricle through the arcuate nucleus. No pathologic changes were observed.

Oser and associates have studied the effects of MSG on neonatal Swiss Webster mice, Wistar rats, beagle puppies[9], and rhesus monkeys[10]. Groups of mice and rats were given single doses of 1 g/kg of MSG orally or

subcutaneously at three days of age and killed 24 hours after treatment. Other mice were given 1 g/kg MSG subcutaneously at 12 days of age and killed 24 hours later. Beagle puppies and rhesus monkeys, three days old, were given single doses of 1 g/kg MSG either orally or subcutaneously, and killed 6 or 24 hours later. Other puppies of 35 days of age have been similarly treated and examined. Fixation was accomplished by perfusing the upper body with glutaraldehyde through the ascending aorta or, for some of the rodents, rapid removal of the brain and immersion in formalin. Stained coronal sections of the brain that included the arcuate nucleus and the median eminence of the hypothalamus were prepared.

The microscopic appearance of tissue sections from the treated animals in each species did not vary from the controls. No lesions attributable to MSG were reported in any of the animals studied.

Studies are continuing with variations in age at treatment and interval between treatment and the time the animal is killed.

Discussion

Data available to the committee on the effects of MSG in mice indicate that hypothalamic lesions may result in some neonates treated with 4 g/kg of body weight when administered orally or parenterally. Lesions resulting from parenteral administration seem most severe. The various investigators do not agree that the lesions occur invariably in mice and rats with doses of 1 g/kg of body weight. The lesions have not been seen in dogs given 1 g/kg MSG. The preponderance of data available from studies of young

rhesus monkeys indicates that lesions are not produced with doses as high as 4 g/kg body weight. The committee has no explanation for the apparent differences in results from different laboratories.

The hematoencephalic exchange[11] of sodium glutamate is thought to be a function of age in the rat and other animals. Glutamic acid was found to enter the brain of rats readily during the first 24 hours of life while it does not penetrate readily at other age levels. Three-day-old infant rhesus monkeys appear resistant to high doses (up to 4 g/kg body weight) of MSG given subcutaneously and orally. Whether this is due to advanced myelination is not known.

It is probable that maturity of the neonatal human brain equals or exceeds that of the neonatal rhesus. In any event, the human infant does not normally get food containing added MSG until at least four weeks of age.

References

1. Olney, J.W. 1969. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Sci. 164:719.

2. Olney, J.W., and L.G. Sharpe. 1969. Brain lesions in an infant rhesus monkey treated with monosodium glutamate. Sci. 166:386.

3. Olney, J.W. Private communication.

4. Olney, J.W. Brain damage in infant mice following oral intake of monosodium glutamate or aspartate. (Manuscript seen by the Committee before publication.)

5. Redding, T.W., and A.V. Schally. 1970. Effect of monosodium glutamate on the endocrine axis in rats. Fed. Proc. 29:2, Abstr. 755.

6. Knittle, J.L., and F. Ginsberg-Fellner. 1970. Cellular and metabolic alterations in obese rats treated with monosodium glutamate during the neonatal period. Abstract in program of the Am. Pediat. Soc.

7. Cohen, A.I. 1967. An electron microscopic study of the modification by monosodium glutamate of the retinas of normal and "rodless" mice. Am. J. Anat. 120:319.

8. Coulston, Frederick. Private communication.

9. Oser, B.L., Steven Carson, Eugene E. Vogin, and George E. Cox. 1970. Evidence for the non-toxicity of monosodium glutamate in infant rats, mice and dogs. Manuscript prepared and privately communicated to the Committee.

10. Oser, B.L. Private communication.

11. Himwich, W.A., JoAnn C. Petersen, and Mary L. Allen. 1957. Hematoencephalic exchange as a function of age. Neurol. 7:705.

POSSIBLE EFFECTS OF MONOSODIUM GLUTAMATE ON THE RETINA

Histopathologic changes in the retina of immature mice and rats following large doses of MSG have been described. Lucas and Newhouse[1] reported retinal changes as seen by light microscopy in two- to four-day-old mice after a series of five injections of MSG. The inner layer of neuronal and the adjacent ganglion cells were affected. Pyknotic nuclei were observed in both cell types a few hours after injection, and the number of cells remaining intact were noticeably reduced by the eighth day. The outer layers of cells were essentially unaffected. The retinal effect decreased as the age of the animal at the time of injection increased. No such lesions were seen in mice born of females injected parenterally with 40 mg MSG daily throughout gestation and lactation.

Potts et al.[2] confirmed the observations of Lucas and Newhouse and reported permanent alterations in the β-wave of the electroretinogram (ERG). In this study, two-day-old mice received daily intraperitoneal injections beginning with 2.2 g/kg body weight with gradually increasing doses to 5.8 g/kg body weight on the 18th day of age. Lesions and ERG changes were most consistent in those animals treated for more than seven days. The decreased number of inner layer cells of the retina was interpreted by these workers as an inhibition of cellular development by the large doses of MSG.

Freedman and Potts[3] reported a similar decrease in number of cells of the inner retinal layers and alterations of ERG in rats treated with MSG.

Daily increasing doses were given intraperitoneally beginning with 2.2 g/kg body weight on day 2 and reaching 4.6 g/kg on day 12. Cohen[4,5] studied the retina of mice with the light and the electron microscopes at intervals of two months and one year after giving daily subcutaneous doses of MSG from day 1 through day 10 and day 1 through day 18. He followed the same dosage schedule used by Potts and co-workers[2]. Two months after treatment he reported that the neuronal cells of the inner layer had been reduced from five to seven nuclei deep to about one to two layers, but residual synaptic relationships appeared normal.

Olney[6] reported vacuolization and distension by edema of ganglion cells and neurons of the inner layers of the retinas of small animals three hours after treatment at days 1 to 3 or days 9 to 10 with a single intraperitoneal dose of 4 g MSG/kg body weight. Similar results were observed in mice given daily doses (4g/kg) from day 1 to day 10. The intracellular edema progressed to necrosis, and cell debris was almost completely removed from the area in 48 hours. By this time the inner nuclear layer of cells seemed to be reduced from the normal of about six to seven layers to two or three layers, and the ganglion cells also appeared reduced in number. Intracellular degenerative changes were demonstrated in the neuronal cells of the inner retinal layer and ganglion cells by electron microscopic studies of acutely evolving lesions. After the 10th day of life, mice became resistant to induction of this lesion.

Oser et al.[7] found no lesions referable to treatment in the eyes of mice, rats, and dogs administered 1 g MSG/kg orally or subcutaneously at

three days of age and examined between 3 and 24 hours after treatment, or in eyes of mice and rats similarly dosed at 12 days of age or dogs dosed at 35 days of age and examined between 3 and 24 hours after treatment. These results are not necessarily in conflict with the others reviewed since dosage sizes and numbers differed.

In Olney and Sharpe's study of a neonatal rhesus monkey following subcutaneous administration of MSG, retinal lesions were not observed[8,9].

Discussion

The mechanics of the development of retinal lesions in immature rats and mice may be related to the maturation of the blood-retinal barrier which appears to be intact by the age of 10 days in these animals[5]. The human retina, even in the premature infant, is considered to be much further differentiated than that of the newborn mouse or rat[10].

References

1. Lucas, D.R., and J.P. Newhouse. 1957. The toxic effect of sodium l-glutamate on the inner layers of the retina. AMA Arch. Ophth. 58:193.

2. Potts, A.M., K.W. Modrell, and C. Kingsbury. 1960. Permanent fractionation of the ERG by sodium glutamate. Am. J. Ophth. 50:900.

3. Freedman, J. K., and A.M. Potts. 1962. Repression of glutaminase I in rat retina by administration of sodium l-glutamate. Invest. Ophthal. 1:118.

4. Cohen, A.I. 1967. An electron microscopic study of the modification by monosodium glutamate of the retinas of normal and "rodless" mice. Am. J. Anat. 120:319.

5. Cohen, A.I. Personal communication.

6. Olney, J.W. 1969. Brain lesions in an infant rhesus monkey treated with monosodium glutamate. Sci. 166:386.

7. Oser, B.L., Steven Carson, Eugene E. Vogin, and George E. Cox. 1970. Evidence for the non-toxicity of monosodium glutamate in infant rats, mice and dogs. Manuscript prepared and privately communicated to the Committee.

8. Olney, J.W., and L.G. Sharpe. 1969. Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. J. Neuropath. & Exper. Neurol. 28:455.

9. Olney, J.W. Personal communication.

10. Patz, Arnall. Personal communication.

PEDIATRIC ASPECTS

In considering the safety of MSG for use in infant foods, it seems advisable to focus upon pregnancy, lactation, and the early months of life as initial periods for review. It has been demonstrated in both man[1] and the rhesus monkey[2] that the placenta functions to maintain by an active transport mechanism a higher blood level of amino acids in the fetus than in the mother. Kerr and Waisman[2] compared the serum amino acid concentrations of non-pregnant rhesus monkeys to those of pregnant monkeys at term and showed that pregnancy results in a lowered serum concentration of most amino acids, including glutamic and asparatic acids, and also of glutamine. Thus the ratio of cord to maternal serum levels of amino acids is greater than one because of the lowered maternal serum concentrations associated with pregnancy. According to Kerr and Waisman the hemochorial placenta does not function to protect the fetus in utero from excessive levels of amino acids. If a pregnant primate has an elevated serum level of free amino acids, the fetal blood concentrations are also increased with the cord to maternal ratio remaining greater than one.

The placenta of the mouse and rat differs from the placenta of man and monkey, and thus the relationship of maternal to fetal blood levels of amino acids may also differ. It is, however, perhaps significant that the retinal and arcuate nucleus lesions seen in the neonatal mouse or rat following

subcutaneous or oral administration of MSG have not been produced in young of these species born to dams fed large amounts (up to 7 g/kg) of MSG[3,4].

Rats, rabbits, and mice differ from man as to when myelination takes place. The myelination process in man starts in utero and reaches maximum just before birth[5,6]. In the rat, myelination starts approximately 10 to 15 days after birth, with a rapid phase from 10 to 21 days. Biochemical changes including penetration of substrate into brain tissue must, a priori, differ between man and the mouse, rat, or rabbit. In 1950, Schwerin, Bessman, and Waelsch[7] demonstrated that the intravenous administration of glutamic acid at a level of 1.2 to 1.3 g/kg body weight to young adult rats or mice produced no significant change in brain concentration of glutamic acid or glutamine. These investigators concluded that the high concentration of glutamic acid in brain represented its synthesis within the brain or its formation from glutamine. McLaughlan et al.[8] have also shown that glutamic acid and glutamine concentrations in the brains of young rats are not elevated, even when blood concentrations of glutamic acid are 25 times greater than normal. These results have been interpreted as indicating a very effective blood-brain barrier or very rapid metabolism of glutamate by brain tissue.

A limited number of samples of human milk, cow's milk, and infant formula products have been analyzed for free amino acid concentrations by Ghadimi and Pecora[9]. Colostrum contains three to four times more free

amino acids than mature milk and human milk contains four to seven times more free amino acids than cow's milk (Table 2). The three-week-old breast-fed infant will receive about 50 mg of free glutamic acid per day, and the infant fed a formula prepared from cow's milk about 4 mg. Studies of the mammary transfer of MSG by lactating women given a single 6 gram oral dose of MSG now in progress indicate only a slight rise in aspartic acid and no rise in glutamic acid, alanine or glutamine[10]. The concentrations in breast milk of all free amino acids vary considerably. Changes in concentration of aspartic and glutamine acids, glutamate, and alanine occurring after MSG loading do not exceed normal variation of these compounds.

Until December 1969, monosodium glutamate (MSG) was added to various strained foods prepared especially for infants. Manufacturers of infant foods had used this material for some 20 years[11], primarily as a flavor-enhancing agent in soups, vegetables, meats and combinations of meats and vegetables. The levels of MSG added ranged from 0.1 percent or less to 0.6 percent. Analysis of representative infant foods for total (not only that added) free MSG gave the following results[11]:

Food	Free MSG %	Size Container g	MSG per Container mg
Strained chicken noodle dinner	0.11	127.6	140
Strained creamed spinach	0.12	127.6	153
Strained dinner vegetables, egg noodles, and chicken	0.20	127.6	255

A food containing 0.6 percent MSG would provide 765 mg per 127.6 g container as added MSG. Thus the breast-fed human infant might ingest

about 350 mg/kg of added MSG per day. It is highly unlikely, however, that in any one day the infant would get this much of the one or two foods that contain this upper level of added MSG. The more likely food pattern would supply the bulk of nutrient intake from milk and the rest from a great variety of canned foods containing 0 to 0.4 percent of added MSG. Only occasionally would the foods containing the upper level of added MSG appear in the diet. Apparently no studies have been made to determine whether MSG-treated foods are more acceptable to infants than similar foods with no added MSG. It is probably fair to conclude that MSG was added to infant foods to influence their acceptance by the mother rather than by the child.

Normal values for plasma amino acids in the human infant have been established in a number of studies. The neonate, like adult man, rapidly develops a circadian periodicity in blood amino acids (12). Ghadimi and Pecora have reported that doubling the concentration of dietary protein fed premature infants was without affect on plasma concentrations of glutamic or aspartic acids, glutamine, alanine, or GABA (13).

Within recent years a large number of infants, including gestationally young infants, have been maintained in positive nitrogen balance by the administration of high caloric solutions administered by central venous catheter (14,15,16). Such therapy involves the use of protein hydrolysates that provide between 80 and 300 mg of glutamate and 70 to 105 mg of asparate per kilogram body weight per day. Stegink and Baker (17) have

determined plasma amino acid patterns in five such infants (a total of seven studies) and found plasma concentrations of glutamic and asparatic acids within normal limits(Table 3). One infant was known to have moderate to severe liver damage (giant cell hepatitis). Yet plasma levels of glutamate and aspartate were within normal limits. Patients followed for as long as 18 months post-infusion have not demonstrated detectable evidence of CNS damage or changes in growth patterns.

Table 2

Selected Free Amino Acid Concentrations

in Human Milk and Cow's Milk*

mg/100 ml

Amino Acid	Human Milk			Cow's Milk
	2nd day	3 week	2 mo	2 mo
Glutamic Acid	12.88	7.66	4.20	0.64
Glutamine	9.48	1.79	1.75	0.41
Aspartic Acid	2.92	1.40	0.53	0.08
Alanine	2.65	0.88	0.80	0.18
GABA	0.22	1.19	0.84	0.65

* From Ref. 9.

Table 3

Plasma Glutamic Acid, Aspartic Acid, and Glutamine Levels

in Infants Treated with Protein Hydrolysates

(μ moles/100 ml plasma)*

								Mean values for normal subject	
Subject	1	2	3	4	5	5	5	1 to 2 yr olds overnight fast N=15	1 month-old inf 2 hrs post-pran N=15
Age (days)	50	13	68	92	6	10	12		
Hydrolysate			Casein(1)			Fibrin(2)			
Plasma Level									
Glutamine	47.7	25.5	41.5	36.9	47.8	45.3	57.3	54.9±3.7	70.3±4.6
Glutamate	9.4	7.8	8.0	1.3	5.0	5.8	5.0	2.7±1.5	7.7±2.5
Aspartate	1.7	1.7	0.4	1.4	0.5	0.2	0.6	0.5±0.4	2.1±2.3

* From Ref. 17

(1) Glutamic acid concentration in Casein hydrolysate = 1960 μ moles/100 ml (Amigen)

(2) Glutamic acid concentration in Fibrin hydrolysate = 358 μ moles/100 ml (Aminosol)

References

1. Ghadimi, H., and P. Pecora. 1964. Free amino acids of cord plasma as compared with maternal plasma during pregnancy. Pediat. 33:500.

2. Kerr, G.R., and H.A. Waisman. Transplacental ratios of serum free amino acids during pregnancy in the rhesus monkey, p. 429. In W.L. Nyhan, ed., Amino Acid Metabolism and Genetic Variation. McGraw-Hill Book Co., New York, 1967.

3. Olney, J. W. Personal Communication.

4. Khera, K.S., L.L. Whitta, and E.A. Nera. 1970. Development in progeny of monosodium glutamate treated pregnant rats. Paper prepared for publication and privately communicated to the Committee from Research Laboratories, Food and Drug Directorate, Canada.

5. Davison, A.N., and J. Dobbing. 1966. Myelination as a vulnerable period in brain development. Brit. Med. Bull. 22:40.

6. Dobbing, J. 1965-66. The effect of undernutrition on myelination in the central nervous system. Biol. Neonat. 9:132.

7. Schwerin P., S.P. Bessman, and H. Waelsch. 1950. The uptake of glutamic acid and glutamine by brain and other issues of the rat and the mouse. J. Biol. Chem. 184:37.

8. McLaughlan, J.M., F.J. Noel, H.G. Botting, and J.E. Knipfel. 1970. Blood and brain levels of glutamic acid in young rats given monosodium glutamate. Nutr. Rep. Int. 1:2, p. 131-138.

9. Ghadimi, H., and P. Pecora. 1963. Free amino acids in different kinds of milk. J. Clin. Nutr. 13:75

10. Stegink, L.D. Private communication.

11. From information supplied by FDA to the Committee for review.

12. Feigin, R.D., and M.W. Haymond. 1970. Circadian periodicity of blood amino acids in the neonate. Pediat. 45:782.

13. Ghadimi, H., and P. Pecora. 1964. Plasma amino acids after birth. Pediat. 45:782.

14. Dudrick, S.J., D. W. Wilmore, H.M. Vars, and J.E. Rhoads. 1968. Long-term total parenteral nutrition with growth, development, and positive nitrogen balance. Surg. 64:134.

15. Wilmore, D.W., and S.J. Dudrick. 1968. Growth and development of an infant receiving all nutrients exclusively by vein. J.A.M.A. 203:860.

16. Filler, R.M., A.J. Eraklis, V.G. Rubin, and J.B. Das. 1969. Long-term total parenteral nutrition in infants. N. Engl. J. Med. 281:589.

17. Stegink, L.D., and G.L. Baker. 1970. Infusion of protein hydrolysates into the newborn: Plasma amino acid patterns. Paper prepared for publication and privately communicated to the Committee for review.

Biochem. J. (1969) 113, 281
Printed in Great Britain

Compartmentation of Glutamate Metabolism in Brain

EVIDENCE FOR THE EXISTENCE OF TWO DIFFERENT TRICARBOXYLIC ACID CYCLES IN BRAIN

By C. J. VAN DEN BERG,* LJ. KRŽALIĆ, P. MELA AND H. WAELSCH†
New York State Psychiatric Institute and Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032, U.S.A.

(*Received* 17 *December* 1968)

1. ^{14}C from [1-^{14}C]glucose injected intraperitoneally into mice is incorporated into glutamate, aspartate and glutamine in the brain to a much greater extent than ^{14}C from [2-^{14}C]glucose. This difference for [1-^{14}C]glucose and [2-^{14}C]glucose increases with time. The amount of ^{14}C in C-1 of glutamate increases steadily with time with both precursors. It is suggested that a large part of the glutamate and aspartate pools in brain are in close contact with intermediates of a fast-turning tricarboxylic acid cycle. 2. ^{14}C from [1-^{14}C]acetate and [2-^{14}C]acetate is incorporated to a much larger extent into glutamine than into glutamate. An examination of the time-course of ^{14}C incorporated into glutamine and glutamate reveals that glutamine is not formed from the glutamate pool, labelled extensively by glucose, but from a small glutamate pool. This small glutamate pool is not derived from an intermediate of a fast-turning tricarboxylic acid cycle. 3. It is proposed that two different tricarboxylic acid cycles exist in brain.

After intracerebral injection of labelled glutamate the specific radioactivity of glutamine in the brain is found to be higher than that of glutamate for at least 30min. (Berl, Lajtha & Waelsch, 1961). Intravenous injection of radioactive bicarbonate (Waelsch, Berl, Rossi, Clarke & Purpura, 1964) and leucine (Roberts & Morelos, 1965; Berl & Frigyesi, 1968; Patel & Balázs, 1969) leads to similar results. When [U-^{14}C]glucose is used as a precursor, however, the specific radioactivity of glutamine is never observed to be higher than that of glutamate (Cremer, 1964; Gaitonde, Dahl & Elliot, 1965; C. J. Van den Berg & P. Mela, unpublished work). The pathway leading from leucine to glutamate–glutamine probably involves acetyl-CoA (see Meister, 1965), whereas glucose carbon atoms must pass through acetyl-CoA before entering the tricarboxylic acid cycle. Taken together, all the above results suggest that at least two metabolically different acetyl-CoA pools and two segregated, but presumably identical, pathways from acetyl-CoA and oxaloacetate to glutamate–glutamine occur in brain.

Since multiple turns of the tricarboxylic acid cycle lead to the preferential elimination of C-1 of acetyl-CoA as carbon dioxide, in comparison with C-2 (Weinman, Strisower & Chaikoff, 1957), it is apparent that metabolites derived directly from tricarboxylic acid-cycle intermediates should be more highly labelled by [2-^{14}C]acetyl-CoA than by [1-^{14}C]acetyl-CoA. The ratio of ^{14}C incorporation from [2-^{14}C]acetyl-CoA to ^{14}C incorporation from [1-^{14}C]acetyl-CoA in a metabolite of acetyl-CoA is therefore dependent on the contribution of cycling in the tricarboxylic acid cycle to the labelling of this metabolite.

^{14}C from [1-^{14}C]acetate is incorporated to a larger extent into glutamine than into glutamate (O'Neal & Koeppe, 1966). A comparison of the time-course of incorporation of ^{14}C from specifically labelled glucose with that from acetate should therefore give some information of the relation between the two glutamate pools and the tricarboxylic acid cycle. In this paper we report data on the incorporation of ^{14}C from [2-^{14}C]glucose, [1-^{14}C]glucose, [1-^{14}C]acetate and [2-^{14}C]acetate into glutamate, aspartate and glutamine in the mouse brain *in vivo*. The results obtained strongly support the hypothesis that two different tricarboxylic acid cycles exist in brain. This hypothesis has been suggested by Garfinkel (1966) on the basis of a computer analysis of literature data and by O'Neal & Koeppe (1966), who reported that acetate and some related compounds were preferentially incorporated into glutamine in brain. A preliminary note has been published (Van den Berg, Mela & Waelsch, 1966).

* Present address: Netherlands Central Institute for Brain Research, IJdijk 28, Amsterdam, The Netherlands.
† Deceased, 22 March, 1966.

MATERIALS AND METHODS

[1-^{14}C]Glucose and [2-^{14}C]glucose (specific radioactivity of each compound 3mc/m-mole) and sodium [1-^{14}C]acetate and sodium [2-^{14}C]acetate (specific radioactivity of each compound 2mc/m-mole) were obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.). Some experiments were done with [1-^{14}C]glucose and [2-^{14}C]glucose obtained from Nuclear-Chicago Corp. (Des Plaines, Ill., U.S.A.) and gave equivalent results.

Mice (Swiss Webster Albino, males, 24–26g. body wt.) were injected intraperitoneally with the labelled compounds. Fed animals were given 10μc of glucose or 15μc of acetate, and a group of starved animals (18hr. without food, resulting in a weight decrease from 25 to 21g.) received 5μc of glucose. The labelled substances were all dissolved in 0·2ml. of 0·9% NaCl. No carrier was added.

The animals were killed by immersion in liquid N_2. The brains were then removed, pulverized in solid CO_2 and extracted with 0·25M-trichloroacetic acid (1·5ml./300mg. of brain). Care was taken to keep the brain samples at solid-CO_2 temperature until the trichloroacetic acid was added. The samples were kept overnight in a refrigerator; 8ml. of water was added to each and the tubes were centrifuged. The precipitate was then washed twice with 8ml. of water. The combined extracts were neutralized with NaOH and fractionated on Dowex 1 (X4; acetate form; 200–400 mesh) (AG1-X4; Bio-Rad Laboratories, Richmond, Calif., U.S.A.) as described by Cheng & Waelsch (1963) and Cheng & Mela (1966). The neutral fraction was hydrolysed with 2M-HCl (1hr. at 100°) and the glutamate formed from glutamine was isolated on Dowex 1. The neutral fraction obtained in this step was passed through a column of Dowex 50 (X4; 200–400 mesh) (AG50W-X4; Bio-Rad Laboratories) to isolate glucose. The Dowex 50 was converted into the pyridinium form by washing first with 2M-HCl, then with 2M-pyridine and finally with 0·2M-pyridine–formate buffer, pH 2·90. The neutral fraction from the Dowex 1 procedure was adjusted to pH 2·90 with HCl and then applied to a column of Dowex 50. The first fraction eluted with the pyridine–formate buffer, pH 2·90, contains glucose. The radiochemical purity of the fractions from several representative experiments was confirmed by one-dimensional paper chromatography in three different solvent systems. The radiochemical purity of the isolated amino acids was greater than 95%, that of the glucose greater than 90%. The recovery of standard radioactive substances carried through the whole procedure was greater than 95%. Samples were counted at infinite thinness in a Nuclear-Chicago end-window counter, with an efficiency of about 30%. Glutamate and aspartate were determined by the method of Cheng & Mela (1966) and glucose by the method of Lowry, Passonneau, Hasselberger & Schulz (1964).

The results obtained were multiplied by appropriate factors, so that they correspond to a uniform injection of 10μc of glucose and 15μc of acetate in an animal of 25g. body wt. All values are expressed as c.p.m./μmole.

The amount of ^{14}C in the C-1 of glutamate (and the glutamate obtained from glutamine) was determined with glutamate decarboxylase (from *Clostridium welchii*, Worthington Biochemical Corp., Freehold, N.J., U.S.A.). The enzyme was dissolved in a 0·2M-sodium acetate buffer, pH 5. Enough enzyme was added to decarboxylate 49–50% of synthetic DL-[1-^{14}C]glutamate (obtained from New England Nuclear Corp.). After an incubation of 1hr. at 37° the reaction was stopped with trichloroacetic acid and the amount of ^{14}C remaining in the solution measured in a Packard liquid-scintillation counter, together with the appropriate controls.

In a number of experiments we measured the concentration of lactate in the brain samples by the method of Lowry *et al.* (1964). The lactate concentration was found to be 1·50μmoles/g. wet wt. (S.D. 0·28; *n* 8) and the glucose concentration 2·01 μmoles/g. wet wt. (S.D. 0·42; *n* 11). These results indicate that post-mortem artifacts were minimal (Lowry *et al.* 1964).

RESULTS

Experiments with [1-^{14}C]- *and* [2-^{14}C]-*glucose*

Specific radioactivities of glucose, glutamate and aspartate. The rate of uptake of radioactive glucose in the brain is fast (Table 1). The specific radio-

Table 1. *Specific radioactivity of glucose and the specific radioactivity of glucose relative to the specific radioactivity of glutamate in the brain at various time-intervals after the injection of* [1-^{14}C]*glucose and* [2-^{14}C]*glucose*

Fed animals were injected with 10μc and starved animals with 5μc of the labelled compound. The specific radioactivity is expressed as c.p.m./μmole for an injected dose of 10μc of the labelled compound in an animal of 25g. body wt. Results for the fed animals are the means ± S.D. of four experiments.

	10^{-3} × Specific radioactivity of glucose (c.p.m./μmole)		Specific radioactivity of glucose/specific radioactivity of glutamate	
Time (min.)	[1-^{14}C]Glucose	[2-^{14}C]Glucose	[1-^{14}C]Glucose	[2-^{14}C]Glucose
Fed animals				
5	19·1 ± 3·3	17·5 ± 0·8	9·5	6·9
15	15·9 ± 5·0	29·0 ± 5·8	1·3	2·4
30	10·1 ± 3·1	23·3 ± 13·9	0·6	1·4
Starved animals				
5	—	42·4		9·6
15	49·7, 31·9	47·8, 48·9, 61·5	1·8	3·6
30	35·9, 27·2	37·8, 18·0, 17·3	1·0	1·5
45	19·3	14·7, 16·2	0·6	1·3

Table 2. *Specific radioactivity of glutamate and specific radioactivity of aspartate and glutamine relative to the specific radioactivity of glutamate in the brain after the injection of* [1-^{14}C]*glucose and* [2-^{14}C]*glucose*

Values for glutamate and aspartate in parentheses were obtained after the subtraction of the amount of ^{14}C in C-1 of glutamate, as given in Table 5. The animals were starved overnight. Results are expressed as means ± S.D. of the numbers of experiments indicated, for an injected dose of 10 μc of the labelled compound.

Time (min.)	Specific radioactivity of glutamate (c.p.m./μmole)	Relative specific radioactivity of aspartate	Relative specific radioactivity of glutamine	No. of expts.
Precursor: [1-^{14}C]glucose				
5	2500; 9300	0·52, 0·59	0·37, 0·26	
15	20400 ± 4600	0·88 ± 0·04 (0·92)	0·50 ± 0·08	4
30	28700 ± 4200	0·92 ± 0·05 (1·01)	0·62 ± 0·04	4
45	25900	0·94	0·82	
Precursor: [2-^{14}C]glucose				
5	4400	0·57	0·37	
15	14800 ± 3800 (12600)	0·85 ± 0·04 (1·01)	0·63 ± 0·16	3
30	15300 ± 4700 (11100)	0·80 ± 0·03 (1·09)	0·88 ± 0·19	3
45	12000, 12300	0·63, 0·66	0·83, 0·70	

activity of glucose in the brain of fed animals at 15 and 30 min. after the injection of [2-^{14}C]glucose is higher than that in the brain of animals injected with [1-^{14}C]glucose (Table 1). This difference between [2-^{14}C]- and [1-^{14}C]-glucose in the degree of labelling of glucose in the brain decreased when starved animals were used (Table 1).

The incorporation of radioactivity into glutamate from both precursors is rapid and extensive (Table 2). The ratio of the specific radioactivity of glucose to the specific radioactivity of glutamate decreases with the length of the experimental period with both precursors in the fed and starved animals (Table 1). This ratio decreases faster when [1-^{14}C]glucose was the precursor than when [2-^{14}C]glucose was the precursor. This faster decrease indicates that more radioactivity from [1-^{14}C]glucose than from [2-^{14}C]-glucose accumulates in glutamate.

The results of the experiments with both precursors can be compared directly by calculating the ratio of the specific radioactivity of the amino acid after [1-^{14}C]glucose to the specific radioactivity of the same amino acid after [2-^{14}C]glucose ([1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio). This ratio increases continuously with time for glutamate and aspartate in the starved animals (Table 3). The [1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio for glutamate and aspartate increases only slightly in the fed animals. The much larger specific radioactivity of the glucose-precursor pool in the brain of animals injected with [2-^{14}C]glucose as compared with the animals injected with [1-^{14}C]glucose might explain the observation that the [1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio for glutamate and aspartate does not increase very much with time in the fed animals. As the specific radioactivity of glucose in the brain of animals injected with [2-^{14}C]-glucose is approximately twice that in animals injected with [1-^{14}C]glucose, the [1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio for glutamate in the fed animals should be about 0·5 at 15 and 30 min., when equal amounts of [1-^{14}C]glucose and [2-^{14}C]-glucose are incorporated into glutamate. The observed values of the [1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio are 0·91 and 1·03 (Table 3).

Despite the considerable variation of the results and the differences in the extent of labelling of the glucose precursor pool in brain we may conclude that more radioactivity from [1-^{14}C]glucose than from [2-^{14}C]glucose accumulates in glutamate and aspartate in the brain. The increase in the [1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio for glutamate and aspartate with time is therefore very likely a result of turning of the tricarboxylic acid cycle within the brain and not of processes occurring outside the brain.

Comparison of the specific-radioactivity curves for glucose and glutamate. The maximal specific radioactivity of glucose in the [2-^{14}C]glucose experiments occurs at about 15 min. (Table 1). The carboxyl group of acetyl-CoA is the precursor of C-5 of α-oxoglutarate (and glutamate). The oxaloacetate formed from α-oxoglutarate is labelled in both carboxyl groups. After condensation of this oxaloacetate with acetyl-CoA, one carboxyl group is lost in the conversion of isocitrate into α-oxoglutarate; the other carboxyl group becomes C-1 of α-oxoglutarate (or glutamate). Thus: glucose C-2 → acetyl-CoA C-1 → α-oxoglutarate C-5 → oxaloacetate C-1 and C-4 → carbon dioxide and α-oxoglutarate C-1 → carbon dioxide.

The sequence of reactions for acetyl-CoA C-2 is very complicated and it is impossible to analyse these results properly in terms of precursor-

Table 3. [1-^{14}C]*Glucose*/[2-^{14}C]*glucose labelling ratio and* [2-^{14}C]*acetate*/[1-^{14}C]*acetate labelling ratio of glutamate, aspartate and glutamine*

Labelling ratios were calculated from the average specific radioactivities of the amino acids with each of the precursors. Values for glutamate in parentheses were obtained after subtraction of the amount of ^{14}C in C-1 of glutamate, as given in Table 5. Fed and starved animals were used for the glucose experiments and fed animals only for the acetate experiments.

	No. of expts.		[1-^{14}C]Glucose/[2-^{14}C]glucose labelling ratio		
Time (min.)	[1-^{14}C]Glucose	[2-^{14}C]Glucose	Glutamate	Aspartate	Glutamine
Fed animals					
5	4	4	0·79	0·90	0·97
15	4	4	0·91	1·00	0·91
30	4	4	1·03	1·37	1·13
Starved animals					
5	2	1	1·34	1·27	1·25
15	4	3	1·38 (1·54)	1·41	1·15
30	4	3	1·87 (2·32)	2·16	1·35
45	1	2	2·14	3·12	2·29
	[2-^{14}C]Acetate	[1-^{14}C]Acetate	[2-^{14}C]Acetate/[1-^{14}C]acetate labelling ratio		
5	3	3	1·53 (1·70)	1·46	1·11 (1·19)
10	1	1	1·71	1·94	1·29
15	3	3	1·95 (2·69)	2·27	0·90 (1·04)
20	1	1	2·56	4·37	1·23
25	1	1	3·15	5·41	1·36
30	3	3	3·47 (4·85)	4·30	1·66 (1·97)

product relations. A valid comparison of glucose with glutamate can thus only be made between the specific radioactivity of glucose and the specific radioactivity of C-5 of glutamate in the [2-^{14}C]-glucose experiments. Subtraction of the amount of ^{14}C in C-1 of glutamate gives a reasonable estimate of the specific radioactivity of C-5 of glutamate. The amount of ^{14}C in C-2, C-3 and C-4 of glutamate in the brains of rats injected with [2-^{14}C]glucose is 8–15% (O'Neal & Koeppe, 1966). This amount is not large enough to affect appreciably the analysis of the results. The maximum specific radioactivity of C-5 of glutamate occurs at about 15 min. (Table 2). The glucose and glutamate maxima are thus very close. The maximal specific radioactivity of C-5 of glutamate is much lower than the maximal specific radioactivity of glucose. It should be realised that the specific radioactivity of acetyl-CoA will be half the specific radioactivity of glucose from which this acetyl-CoA is formed, as two molecules of acetyl-CoA are formed from one molecule of glucose. In the starved animals the maximal specific radioactivity of acetyl-CoA entering the tricarboxylic acid cycle is therefore about 26000 c.p.m./μmole (Table 1), which is about twice the maximal specific radioactivity of C-5 of glutamate (Table 2). It follows directly from these results that more than half of the glutamate pool is labelled by glucose. This is a minimal estimate, as glucose is not the immediate precursor of glutamate. The maximal specific radioactivity of glutamate in the [1-^{14}C]-glucose experiments is even higher than the calculated maximal specific radioactivity of the acetyl-CoA, showing in a dramatic way that ^{14}C accumulates in glutamate as a consequence of the turning of the tricarboxylic acid cycle and that a proper analysis of these data is very complicated.

Percentage of ^{14}C *in* C-1 *of glutamate and glutamine.* There is always a larger amount of ^{14}C in C-1 of glutamate isolated from the brain of animals injected with [2-^{14}C]glucose than in that from the brain of animals injected with [1-^{14}C]glucose (Table 5). The rate of glucose utilization in the brain of adult mice is 0·76 μmole/g. wet wt./min. (Lowry *et al.* 1964). The amount of acetyl-CoA entering the tricarboxylic acid cycle is twice this amount or 1·52 μmoles/g. wet wt./min. The combined concentration of tricarboxylic acid-cycle intermediates in mouse brain is about 1·7 μmoles/g. wet wt. (Goldberg, Passonneau & Lowry, 1966). The amount of tricarboxylic acid-cycle intermediates relative to the rate of acetyl-CoA entering the cycle is therefore not very large. One would therefore expect almost equal fractions of an intermediate in the first, second, third and following turns of the cycle after the entrance of acetyl-CoA.

In the first turn of the cycle after the entrance of [1-^{14}C]acetyl-CoA all the radioactivity present in α-oxoglutarate (glutamate) is located in C-5 of α-oxoglutarate. In the second turn 50% of the ^{14}C is lost and 50% is present in C-1 of α-oxoglutarate (glutamate). Of the radioactivity present in

α-oxoglutarate 33·3% should therefore be in C-1 of α-oxoglutarate when [2-^{14}C]glucose is used as a precursor. When intermediates of the tricarboxylic acid cycle are in rapid equilibrium with compounds related to these intermediates, the pool of intermediates present (pool of carbon compounds through which acetyl-CoA carbon atoms have to move before conversion into carbon dioxide) is much larger. This slows down the rate of movement of carbon atoms, entering as acetyl-CoA, from the first to the second cycle, resulting in a time-dependent increase in the amount of ^{14}C in C-1 of α-oxoglutarate. Dilution of intermediates of the cycle by compounds related to these intermediates will therefore result in a time-dependent increase of the amount of radioactivity in C-1 of α-oxoglutarate. The time-course of the increase in amount of radioactivity is, of course, dependent on the time-course of the specific radioactivity of the precursor entering the cycle and the rate of equilibrium between cycle intermediates and the compounds related to them. If equilibrium is fast, then it is clear that the value of 33·3% of the radioactivity in C-1 of α-oxoglutarate will be reached when the specific radioactivity of the precursor entering the cycle (acetyl-CoA) starts to decrease. The value of 33·3% will be lower when there is carbon dioxide fixation at the pyruvate level in the brain. Carbon dioxide fixation has been shown to occur in the brain by Berl, Takagaki, Clarke & Waelsch (1962*a*,*b*). O'Neal & Koeppe (1966) found indeed 8–15% ^{14}C in C-2, C-3 and C-4 of glutamate isolated from the brains of rats injected with [2-^{14}C]glucose. The theoretical value of 33·3% of ^{14}C in C-1 of α-oxoglutarate or glutamate will therefore be actually a little lower, say about 30%.

We have no information on the time-course of the specific radioactivity of acetyl-CoA, but the time-course of the specific radioactivity of glucose is probably almost the same. In a number of experiments (results not shown) we found that the specific radioactivity of lactate in the brain was always about 50% of the specific radioactivity of glucose, indicating that the assumption with regard to acetyl-CoA is valid. The specific radioactivity of glucose in the [2-^{14}C]glucose experiments starts to decrease between 15 and 30min. (Table 1). The amount of radioactivity in C-1 of glutamate is about 30% at 30min. after the injection of [2-^{14}C]glucose (Table 5). The results reported as analysed in this section strongly suggest therefore that glutamate and other compounds related to tricarboxylic acid-cycle intermediates are in a very rapid equilibrium with intermediates of the cycle. A rapid equilibrium of glutamate and α-oxoglutarate *in vitro* has been found by Balázs & Haslam (1965).

Comparison of results for glutamate and aspartate. The rate of incorporation of ^{14}C into aspartate is somewhat lower than the corresponding rate for glutamate (Fig. 1 and Table 2). The rapid equilibrium of labelled α-oxoglutarate and glutamate mentioned before offers itself as a reasonable explanation. Dilution by other compounds related to tricarboxylic acid-cycle intermediates might conceivably occur as well.

The relative specific radioactivity of aspartate is about 0·9 for both precursors at 15 and 30min. It is better to compare the specific radioactivity of aspartate with the specific radioactivity of C-2, C-3, C-4 and C-5 of glutamate. The amount of ^{14}C in C-2, C-3, C-4 and C-5 of glutamate is a reasonable estimate of the amount of ^{14}C in C-5, as it has been shown that the amount of ^{14}C in C-2, C-3 and C-4 is 8–15% (O'Neal & Koeppe, 1966). The specific radioactivity of aspartate, relative to the specific radioactivity of C-2, C-3, C-4 and C-5 of glutamate is about 1·0 (Table 2). In the experiments with [2-^{14}C]glucose it is also possible to compare the specific radioactivity of aspartate with the specific radioactivity of the C-1, C-2 and C-3 of glutamate,

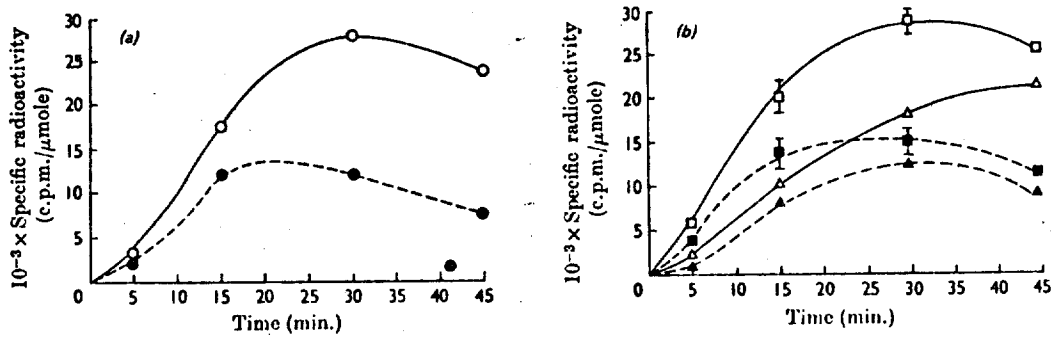


Fig. 1. Time-course of the specific radioactivities of (*a*) aspartate (○,●), (*b*) glutamate (□, ■) and glutamine (△, ▲), in the brain of starved mice, injected intraperitoneally with [2-^{14}C]glucose (■, ●, ▲) or [1-^{14}C]glucose (□, ○, △). Vertical bars indicate the standard deviations.

multiplied by 2, because one carboxyl group of oxaloacetate (or aspartate) is lost in the conversion of isocitrate into α-oxoglutarate (or glutamate). The specific radioactivity of aspartate is about three times the calculated specific radioactivity of the C-1, C-2 and C-3 of glutamate at 15min. This value is, however, decreased to 1·4 at 30min. Complete isotopic equilibrium between glutamate and aspartate and between aspartate and glutamate would, if the complete glutamate and aspartate pools are involved, lead to values of about 1.

The [1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio for aspartate increases faster with time than the same ratio for glutamate (Table 3). But again we should compare C-2, C-3, C-4 and C-5 of glutamate with aspartate. The ratio for C-2, C-3, C-4 and C-5 of glutamate increases now a little faster than the ratio for aspartate (Table 3).

Glutamine. The specific radioactivity of glutamine increases with time, but more slowly than the specific radioactivity of glutamate (Fig. 1 and Table 2). The [1-^{14}C]glucose/[2-^{14}C]glucose labelling ratio does not increase very much between 5 and 30min. (Table 3). This latter finding suggests that isotopic equilibrium between glutamate and glutamine is less rapidly established than between glutamate and α-oxoglutarate.

Experiments with [1-^{14}C]*acetate and* [2-^{14}C]*acetate*

Rate of labelling of glutamate, aspartate and glutamine. [1-^{14}C]Acetate corresponds to [2-^{14}C]-glucose and [2-^{14}C]acetate corresponds to [1-^{14}C]-glucose with respect to the labelling of the carboxyl group and methyl group respectively of acetyl-CoA. The specific radioactivity of glutamate is highest at between 5 and 15min. after the introduction of [1-^{14}C]acetate into the animal (Fig. 2 and Table 4). The maximum is reached later in the [2-^{14}C]acetate experiments. Aspartate follows glutamate closely. Glutamine is exceptional, a maximum being reached at about 5min. with both precursors. The specific-radioactivity curves of glutamine for both precursors are almost identical. This is not so with glutamate and aspartate. A striking difference between the glucose and the acetate experiments is that the specific radioactivity of glutamine is much higher than the specific radioactivity of either glutamate or aspartate, with acetate as a precursor, whereas the specific radioactivity of glutamine is always lower than the specific radioactivity of glutamate and aspartate with glucose as a precursor. Acetate is therefore a specific precursor of glutamine and of the glutamate pool labelled by bicarbonate, leucine and other compounds.

The rapid incorporation of ^{14}C from [^{14}C]acetate is not surprising in view of the fact that the acetate pool in brain is probably very small. Busch & Baltrush (1954) found, a few minutes after the injection of labelled acetate, almost no radioactivity in the volatile acid fraction from the brain; most of the radioactivity was already in non-volatile acid compounds, mostly glutamate and aspartate. We also found ^{14}C in glucose in the brain, but the specific radioactivity of glucose in the first 15min. was much lower than the specific radioactivity of the amino acids (results not shown). At 30min.,

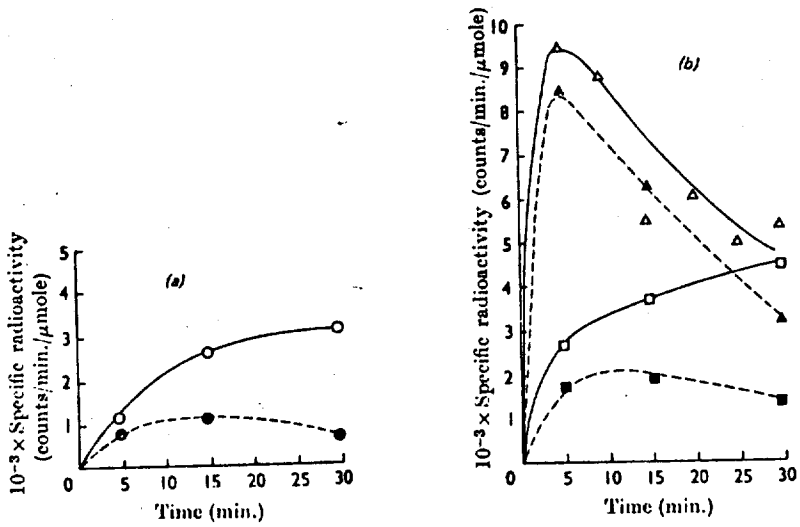


Fig. 2. Time-course of the specific radioactivities of (*a*) aspartate (○, ●), (*b*) glutamate (□, ■) and glutamine (△, ▲), in the brain of mice, injected intraperitoneally with [1-^{14}C]acetate (▲, ●, ■) or [2-^{14}C]acetate (○, △, □).

Table 4. *Specific radioactivity of glutamate and the relative specific radioactivity of aspartate and glutamine in the brain of mice at various time-intervals after the intraperitoneal injection of 15μc of [1-^{14}C]acetate and [2-^{14}C]acetate*

Values in parentheses for the relative specific radioactivity of aspartate were obtained after the subtraction of the amount of ^{14}C in C-1 of glutamate, as given in Table 5. Results are expressed as means of the numbers of experiments indicated.

Time (min.)	Specific radioactivity of glutamate (c.p.m./μmole)	Relative specific radioactivity of aspartate	Relative specific radioactivity of glutamine	No. of expts.
Precursor: [1-^{14}C]acetate				
5	1779	0·46 (0·61)	4·81	3
10	2310	0·57	3·00	1
15	1946	0·59 (0·89)	3·27	3
20	1721	0·45	2·85	1
25	1283	0·40	2·92	1
30	1339	0·47 (0·89)	2·45	3
Precursor: [2-^{14}C]acetate				
5	2726	0·44 (0·46)	3·55	3
10	3960	0·64	2·25	1
15	3800	0·70 (0·75)	1·46	3
20	4398	0·78	1·37	1
25	4039	0·68	1·26	1
30	4647	0·70 (0·79)	1·17	3

however, the specific radioactivity of glucose, especially in the experiments with [2-^{14}C]acetate, is of the same order of magnitude as the specific radioactivity of the amino acids. Although we have not proved that acetate enters the brain as such, we may safely assume that most of the ^{14}C enters the brain in the first 15min., in the form of acetate, and that acetate is converted into acetyl-CoA in the brain. Acetyl-CoA synthetase is present in the brain (Schuberth, 1965).

Comparison of results for [1-^{14}C]acetate and [2-^{14}C]acetate. The ratio of the specific radioactivity of each of the amino acids after [2-^{14}C]acetate to the specific activity after [1-^{14}C]acetate is given in Table 3. This [2-^{14}C]acetate/[1-^{14}C]acetate labelling ratio increases rapidly for glutamate and aspartate, whereas it stays constant for almost 20min. for glutamine. Radioactivity from [1-^{14}C]acetate and [2-^{14}C]acetate is thus incorporated into glutamine in about the same ratio, or the glutamate precursor pool of glutamine is not formed from an intermediate of a rapidly turning tricarboxylic acid cycle. The glutamate labelled by acetate is certainly derived from an intermediate of a rapidly turning cycle. This follows directly from the increase of the [2-^{14}C]acetate/[1-^{14}C]acetate labelling ratio for glutamate. The immediate precursor of glutamine is supposed to be glutamate, but the glutamate that is labelled by acetate does not seem to be the precursor of glutamine. It is thus probable that the glutamate precursor pool of glutamine is very small.

Percentage of ^{14}C in C-1 of glutamate and glutamine. If it is true that glutamine is derived from an intermediate of a slowly turning cycle, then we should expect a small amount of ^{14}C in C-1 of glutamine. As is shown in Table 5, the percentage of ^{14}C in the C-1 of glutamine is indeed much lower than the corresponding value for glutamate. However, it is clear that there is an appreciable incorporation of ^{14}C into C-1 of glutamine. Some glutamine is probably derived from the glutamate labelled after acetate. This explains partly the incorporation of ^{14}C into C-1 of glutamine.

Comparison of results for glutamate and aspartate. The rates of labelling of glutamate and aspartate are almost the same (Fig. 2 and Table 4). The [2-^{14}C]acetate/[1-^{14}C]acetate labelling ratio for aspartate increases with time somewhat faster than the ratio for glutamate (Table 3). As with glucose this situation is reversed when the [2-^{14}C]acetate/[1-^{14}C]acetate labelling ratio for aspartate is compared with this ratio for C-2, C-3, C-4 and C-5 of glutamate (Table 3). The relative specific radioactivity of aspartate remains always lower than 1·0 (Table 4), even when the specific radioactivity of aspartate is compared with the specific radioactivity of C-2, C-3, C-4 and C-5 of glutamate (Table 4). Aspartate or oxaloacetate derives carbon atoms from tricarboxylic acid-cycle intermediates, but also directly from pyruvate. In unpublished experiments we found an appreciable incorporation of ^{14}C from [3,4-^{14}C$_2$]glucose into aspartate (approximately 10% of the incorporation of ^{14}C from [2-^{14}C]glucose into aspartate). There is, of course, no direct pathway from acetate to aspartate. This then might explain the difference in the relative

Table 5. *Percentage of the amount of ^{14}C present in C-1 of glutamate and glutamine isolated from the brain of animals injected with [1-^{14}C]- and [2-^{14}C]-glucose or [2-^{14}C]- and [1-^{14}C]-acetate*

Results are expressed as means of the numbers of experiments indicated in parentheses.

Time (min.)	[1-^{14}C]Glucose				[2-^{14}C]Glucose			
	^{14}C in C-1 of glutamate (%)		^{14}C in C-1 of glutamine (%)		^{14}C in C-1 of glutamate (%)		^{14}C in C-1 of glutamine (%)	
	Fed	Starved	Fed	Starved	Fed	Starved	Fed	Starved
5	—	—	—	—	2·0 (1)	9·4 (1)	7·2 (1)	2·6 (1)
15	3·7 (1)	5·0 (2)	6·1 (1)	5·7 (2)	18·2 (1)	14·9 (3)	18·6 (2)	15·0 (3)
30	10·3 (2)	10·0 (2)	8·3 (2)	10·3 (2)	24·6 (2)	27·3 (3)	20·6 (2)	19·5 (3)
45	—	—	—	—	—	33·3 (1)	—	25·5 (1)

	[2-^{14}C]Acetate		[1-^{14}C]Acetate	
	^{14}C in C-1 of glutamate (%)	^{14}C in C-1 of glutamine (%)	^{14}C in C-1 of glutamate (%)	^{14}C in C-1 of glutamine (%)
5	4·6 (1)	1·9 (1)	14·1 (1)	7·6 (2)
15	7·9 (1)	4·1 (1)	33·1 (2)	18·7 (2)
30	13·7 (1)	14·6 (1)	38·3 (3)	27·9 (2)

specific radioactivity for aspartate in the glucose and the acetate experiments.

Conversion of glutamine into glutamate. In the [1-^{14}C]acetate experiments the specific radioactivity of glutamate is always lower than the specific radioactivity of glutamine, even in the decreasing part of the curve. When one compares the total amount of ^{14}C present in both amino acids, a different picture emerges: the total amount of ^{14}C in glutamate is higher than the total amount of ^{14}C in glutamine between 15 and 30 min. after the injection of labelled acetate. It is thus possible that glutamine is a precursor of glutamate. The total contribution of glutamine to glutamate is not very large; this follows from the low specific radioactivity of glutamate. The larger amount of ^{14}C in C-1 of glutamate than in C-1 of glutamine is easily explained by the effect on the large glutamate pool of turning of the tricarboxylic acid cycle. The relatively high labelling of aspartate is consistent with this explanation. [^{14}C]Glutamine that is injected directly into the brain is in fact converted into [^{14}C]glutamate at an appreciable rate (Berl *et al.* 1961).

DISCUSSION

Two glutamate pools: two-cycle model. Two glutamate pools (a large and a small one) exist in brain. The results obtained with [1-^{14}C]glucose and [2-^{14}C]glucose further suggest that the large glutamate pool is in close contact with α-oxoglutarate from a rapidly turning tricarboxylic acid cycle. The acetate results on the other hand suggest that the small pool has a different relation to the tricarboxylic acid cycle.

We may conclude that glucose and acetate are not the precursors of the same homogeneous acetyl-CoA pool in brain. The assumption is made, of course, that all the glucose and acetate carbon atoms pass through acetyl-CoA before being incorporated into glutamate, aspartate and glutamine. Compartmentation of acetyl-CoA with respect to the tricarboxylic acid cycle and the synthesis of acetylcholine has been postulated by Cheng, Nakamura & Waelsch (1967).

Leucine (Roberts & Morelos, 1965; Berl & Frigyesi, 1968), butyrate (O'Neal & Koeppe, 1966) and acetate (O'Neal & Koeppe, 1966) are effective precursors of the small glutamate pool and therefore of glutamine. These compounds are precursors of acetyl-CoA. Bicarbonate (Waelsch *et al.* 1964), aspartate (Berl *et al.* 1961) and propionate (O'Neal, Koeppe & Williams, 1966; C. J. Van den Berg & P. Mela, unpublished work) are also effective precursors of the small glutamate pool. These substances are all precursors of C_4 compounds and thus of oxaloacetate. The relative specific radioactivity of glutamine is about 2–4 with all these precursors, suggesting that they are precursors of the same small glutamate pool.

These results suggest that there are in brain two segregated, but presumably identical, pathways from acetyl-CoA and oxaloacetate to α-oxoglutarate and glutamate. We might therefore conclude that two different tricarboxylic acid cycles occur in the brain.

Berl *et al.* (1962*a*) have shown that the α-amino

group of glutamine incorporated more ^{15}N from [^{15}N]ammonium acetate than does the α-amino group of glutamate. This implicates a role for glutamate dehydrogenase in the formation of the small glutamate pool. The intracellular localization of glutamate dehydrogenase, as studied with sucrose density gradients of mitochondrial preparations from brain, is not identical with the localization of succinate dehydrogenase (Salganicoff & De Robertis, 1965; Neidle, Van den Berg & Grynbaum, 1969). Acetyl-CoA synthetase has almost the same intracellular localization as glutamate dehydrogenase (Neidle *et al.* 1969). It seems thus that the two tricarboxylic acid cycles are localized in physically distinct particles. Baxter (1968) found that γ-aminobutyrate is also a specific precursor of the small glutamate pool. γ-Aminobutyrate transaminase is not localized in the particles where most of the cytochrome oxidase is present (Van Kempen, Van den Berg, Van der Helm & Veldstra, 1965) but has almost the same intracellular localization as glutamate dehydrogenase (Salganicoff & De Robertis, 1965). This argues again for the hypothesis that two different tricarboxylic acid cycles are present in the brain.

Are both glutamate pools present in the same cell? Different types of cells, neurones and glial cells, are present in the brain. The two tricarboxylic acid cycles might well occur in different cells. Glutamate dehydrogenase (Lowry, Roberts & Chang, 1956) and γ-aminobutyrate transaminase–succinate semialdehyde dehydrogenase (Van Gelder, 1965*a*,*b*) are present both in neurones and glial cells. If we take these enzymes as criteria for the presence of the small pool, then we can conclude that the small pool is not present in one cell type only. This follows also from the finding that both populations of mitochondria are present in cerebral cortex and in corpus callosum of the rat (Neidle *et al.* 1969). The argument is weak, however, since the presence of an enzyme at a certain site does not prove that it is operating, nor is there any proof that all the glutamate dehydrogenase has a function in the formation of the small glutamate pool.

Large glutamate and aspartate pools. A large fraction of the glutamate present in the brain is labelled by glucose, and most of the carbon atoms from glucose pass through the glutamate pool before being converted into carbon dioxide. The same is true for aspartate. The rapid equilibrium between glutamate, aspartate and tricarboxylic acid-cycle intermediates, and the time-course of labelling of the amino acids, all suggest that a large fraction of both amino acids are almost intermediates of the tricarboxylic acid cycle. The concentration of glutamate, aspartate, α-oxoglutarate and oxaloacetate are approximately those demanded for the equilibrium of glutamate–aspartate transaminase (Goldberg *et al.* 1966). The α-amino group of glutamate is rapidly incorporated into aspartate, although complete equilibrium was not attained (Berl *et al.* 1962*a*). Rapid isotopic equilibrium between glutamate and α-oxoglutarate *in vitro* was found by Balázs & Hasham (1965). Lindsay & Bachelard (1966) found *in vivo* a higher specific radioactivity of α-oxoglutarate than glutamate in the first 5–10min. after the injection of [U-^{14}C]-glucose into rats. Gaitonde (1965), however, found a lower specific radioactivity of α-oxoglutarate than of glutamate. The specific radioactivity of α-oxoglutarate is always lower than the specific radioactivity of glutamine in experiments with labelled bicarbonate (Waelsch *et al.* 1964). The reported data for α-oxoglutarate are thus contradictory. In some experiments we isolated succinate; its specific radioactivity, from the succinate concentration as determined by Goldberg *et al.* (1966), was always of the same order of magnitude as the specific radioactivity of glutamate and aspartate.

The incorporation of ^{14}C from [U-^{14}C]glucose into glutamate and aspartate is decreased to almost the same extent by pentobarbitone sodium (Bachelard & Lindsay, 1966), Pentothal (C. J. Van den Berg, Lj. Kržalić, P. Mela & H. Waelsch, unpublished work) and γ-hydroxybutyrate (Godin, Mark & Mandel, 1968). The slowing down of the flow of carbon atoms through the tricarboxylic acid cycle thus affects the labelling of glutamate and aspartate in about the same way. This is what one would expect if a large fraction of glutamate and aspartate pools are in rapid equilibrium with tricarboxylic acid-cycle intermediates.

General remarks. The brain uses mainly glucose as its source of energy, most of which is used for the maintenance of its functional activity (McIlwain, 1966). The metabolic pathways leading to the production of energy are probably under direct control of this 'functional activity'. It would therefore not be surprising if the metabolism of compounds not directly involved in energy production were separated from the reaction pathways involved in energy production. Metabolic pathways used for biosynthetic reactions or for the removal of waste material would then be separated from those pathways that should be critically and highly controlled by the need of energy for the functional activity of the brain.

Compartments certainly exist in other organs as well. Liver mitochondria are probably heterogeneous (Swick, Stange, Nance & Thomson, 1967). Incorporation of radioactive bicarbonate into amino acids in liver suggests compartmentation of amino acid metabolism (Berl *et al.* 1962*a*,*b*). Fritz (1967) proposed a compartmentation of acetyl-CoA metabolism in liver; he assumes that these compartments are located in different spaces within

the mitochondrion. In brain it seems that metabolically different acetyl-CoA pools are located in different particles. Fritz (1967) postulated these compartments to account for peculiarities observed with the metabolism of fatty acids. Butyrate and acetate are lower fatty acids, and are also precursors of the small glutamate pool in brain.

We thank Dr H. A. Naruse and Dr Neidle, without whom this work could not have started or finished. C.J.V.d.B. and Lj.K. received a Fulbright Travel Grant. This research was supported by U.S. Public Health Service Grant NB 00557 from the National Institutes of Health.

REFERENCES

Bachelard, H. S. & Lindsay, J. R. (1966). *Biochem. Pharmacol.* **15**, 1053.
Balázs, R. & Haslam, R. J. (1965). *Biochem. J.* **94**, 131.
Baxter, C. F. (1968). In *Progress in Brain Research*, vol. 29, p. 429. Ed. by Lajtha, A. & Ford, D. H. Amsterdam: Elsevier Publishing Company.
Berl, S. & Frigyesi, T. L. (1968). *J. Neurochem.* **15**, 965.
Berl, S., Lajtha, A. & Waelsch, H. (1961). *J. Neurochem.* **7**, 186.
Berl, S., Takagaki, G., Clarke, D. D. & Waelsch, H. (1962*a*). *J. biol. Chem.* **237**, 2562.
Berl., S., Takagaki, G., Clarke, D. D. & Waelsch, H. (1962*b*). *J. biol. Chem.* **237**, 2570.
Busch, H, & Baltrush, H. A. (1954). *Cancer Res.* **14**, 448.
Cheng, S.-C. & Mela, P. (1966). *J. Neurochem.* **13**, 281.
Cheng, S.-C., Nakamura, R. & Waelsch, H. (1967). *Biochem. J.* **104**, 52P.
Cheng, S.-C. & Waelsch, H. (1963). *Biochem. Z.* **338**, 643.
Cremer, J. E. (1964). *J. Neurochem.* **11**, 165.
Fritz, I. B. (1967). *Perspectives Biol. Med.* **10**, 643.
Gaitonde, M. K. (1965). *Biochem. J.* **95**, 803.
Gaitonde, M. K., Dahl, D. R. & Elliott, K. A. C. (1965). *Biochem. J.* **94**, 345.
Garfinkel, D. (1966). *J. biol. Chem.* **241**, 3918.
Godin, Y., Mark, J. & Mandel, P. (1968). *J. Neurochem.* **15**, 1085.
Goldberg, N. D., Passonneau, J. V. & Lowry, O. H. (1966). *J. biol. Chem.* **241**, 3997.
Lindsay, J. R. & Bachelard, H. S. (1966). *Biochem. Pharmacol.* **15**, 1045.
Lowry, O. H., Passonneau, J. V., Hasselberger, F. X. & Schulz, D. W. (1964). *J. biol. Chem.* **239**, 18.
Lowry, O. H., Roberts, N. R. & Chang, M. L. W. (1956). *J. biol. Chem.* **222**, 97.
Mellwain, H. (1966). *Biochemistry and the Central Nervous System*, 3rd ed., pp. 6–9. London: J. and A. Churchill Ltd.
Meister, A. (1965). *Biochemistry of the Amino Acids*, 2nd ed. vol. 2, p. 753. New York: Academic Press Inc.
Neidle, A., Van den Berg, C. J. & Grynbaum, A. (1969). *J. Neurochem.* **16**, 225.
O'Neal, R. M. & Koeppe, R. E. (1966). *J. Neurochem.* **13**, 835.
O'Neal, R. M., Koeppe, R. E. & Williams, E. I. (1966). *Biochem. J.* **101**, 591.
Patel, A. J. & Balázs, R. (1969). *Biochem. J.* **111**, 17P.
Roberts, S. & Morelos, B. S. (1965). *J. Neurochem.* **12**, 373.
Salganicoff, L. & De Robertis, E. (1965). *J. Neurochem.* **12**, 287.
Schuberth, J. (1965). *Biochem. biophys. Acta*, **98**, 1.
Swick, R. W., Stange, J. L., Nance, S. L. & Thomson, J. F. (1967). *Biochemistry*, **6**, 737.
Van den Berg, C. J., Mela, P. & Waelsch, H. (1966). *Biochem. biophys. Res. Commun.* **23**, 479.
Van Gelder, N. M. (1965*a*). *J. Neurochem.* **12**, 231.
Van Gelder, N. M. (1965*b*). *J. Neurochem.* **12**, 239.
Van Kempen, G. M. J., Van den Berg, C. J., Van der Helm, H. J. & Veldstra, H. (1965). *J. Neurochem.* **12**, 581.
Waelsch, H., Berl, S., Rossi, C. A., Clarke, D. D. & Purpura, D. P. (1964). *J. Neurochem.* **11**, 717.
Weinman, E. G., Strisower, E. H. & Chaikoff, I. L. (1957). *Physiol. Rev.* **37**, 252.

Zeit. physiolo. Chemie. 254 (1938)

Über den enzymatischen Abbau und Aufbau der Glutaminsäure*).

II. In tierischen Geweben**).

Von

Hans v. Euler, Erich Adler, Gunnar Günther und Nalin Bandhu Das.

Mit 15 Figuren im Text.

(Aus dem Biochemischen Institut der Universität Stockholm.)

(Der Schriftleitung zugegangen am 16. Mai 1938.)

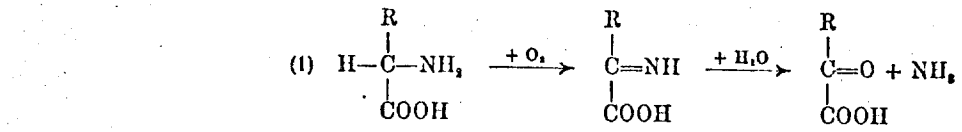
A. Einleitung (S. 62). Geschichtliches. Fragestellung. Kurzer Überblick über die Ergebnisse der vorliegenden Arbeit. — B. Präparate und Methoden (S. 67). — C. Ergebnisse. I. Die „dehydrierende Desaminierung" der l(+)-Glutaminsäure (S. 68). 1. Nachweis durch den Thunberg-Versuch. 2. Sauerstoff als Endacceptor. a) Die Komponenten des aeroben Systems; b) Der Wasserstofftransport bei der aeroben Dehydrierung; c) Die summarische Reaktionsgleichung der aeroben Dehydrierung. 3. Spektrophotometrischer Nachweis der primären Dehydrierungsreaktion und deren Mechanismus. — II. Die Reversibilität der „dehydrierenden Desaminierung" der Glutaminsäure (S. 76). 1. Hemmung der Glutaminsäuredehydrierung durch die Reaktionsprodukte. 2. Hemmung der Cozymase-Hydrierung durch die Reaktionsprodukte. 3. Das Gleichgewicht der primären Dehydrierungsreaktion. — III. Die „hydrierende Aminierung" der α-Ketoglutarsäure (Synthese der Glutaminsäure) (S. 81). 1. Hydrierung von Iminoglutarsäure durch Dihydro-cozymase. a) Spektrophotometrische Versuche. — p_H-Abhängigkeit der „hydrierenden Aminierung"; b) Nachweis der gebildeten Glutaminsäure. 2. Hydrierung von Iminoglutarsäure durch Wasserstoffliefernde Dehydrasesysteme. — IV. Die Spezifität der Glutaminsäure-apodehydrase (S. 88). 1. Codehydrase-Spezifität. 2. Substrat-Spezifität. — V. Die Verteilung der Glutaminsäure-apodehydrase im Tierkörper (S. 93). — D. Über die physiologische Rolle des Systems der Glutaminsäuredehydrase (S. 95). Spezifische Fixierung von NH_3 an Ketoglutarsäure (Glutaminsäuresynthese) als Aufgabe der Dehydrase. Hintereinanderschaltung von Glutaminsäuresynthese und Umaminierung als Weg der Synthese von Aminosäuren. Schema des Aminosäureumsatzes im Tierkörper. Andere Wege der Aminosäuresynthese. — E. Zusammenfassung (S. 102).

*) Zugleich XXI. Mitteilung über die Komponenten der Dehydrasesysteme.

**) Als I. Mitteilung dieser Reihe soll die Arbeit von v. Euler, Adler, u. Steenhoff Eriksen [Diese Z. 248, 227 (1937)] über die Glutaminsäuredehydrase aus Hefe bezeichnet werden.

A. Einleitung.

Durch die Untersuchungen von Neubauer[1]) und Knoop[1a]) sowie von Krebs[2]) ist der chemische Mechanismus der Desaminierung von Aminosäuren im Tierkörper klargelegt worden. Die Desaminierung geschieht oxydativ, wobei Iminosäure als Intermediärprodukt anzunehmen ist und Ketosäure und Ammoniak als Endprodukte auftreten:



Was die Synthese der Aminosäuren betrifft, so konnte Knoop[3]) durch Fütterungsversuche zeigen, daß aus Ketosäuren Aminosäuren gebildet werden, ein Befund, der von Embden[4]) sowie von Dakin[5]) im Durchströmungsversuch an überlebender Leber bestätigt wurde. Neuerdings wurde von Neber[6]) eine Bildung von Aminosäure aus Brenztraubensäure und Ammoniak in Gegenwart von Leberschnitten nachgewiesen.

Für den Mechanismus der Synthese stellte Knoop vor allem zwei Hypothesen zur Diskussion. Die eine wird ausgedrückt durch die einfache Umkehrung des Schemas (1), wonach die Synthese durch die enzymatische Reduktion der (nichtenzymatisch gebildeten) Iminosäure erfolgt. Diese Hypothese wurde von Knoop und Oesterlin[7]) durch rein chemische Modellversuche gestützt, bei denen die Bildung von Aminosäuren durch Reduktion von Iminosäuren (d. h. eines Gemisches von Ketosäuren und NH_3) mittels katalytisch erregten Wasserstoffs oder mit Hilfe von Reduktionsmitteln wie Cystein oder Ferrosulfat[8]) gezeigt wurde.

Eine andere Hypothese Knoops stützte sich auf die interessante Beobachtung, daß nach Verfütterung von γ-Phenyl-α-ketobuttersäure im Harn neben anderen Umwandlungsprodukten optisch

---

[1]) Neubauer u. Gross, Diese Z. 67, 219 (1910); Neubauer u. Fromherz, Diese Z. 70, 326 (1911).

[1a]) Diese Z. 67, 489 (1910).

[2]) Diese Z. 217, 191 (1933); 218, 157 (1933); Biochemic. J. 29, 1620 (1935).

[3]) Knoop u. Kertess, Diese Z. 71, 252 (1911); Knoop u. Blanco, Diese Z. 146, 267 (1925).

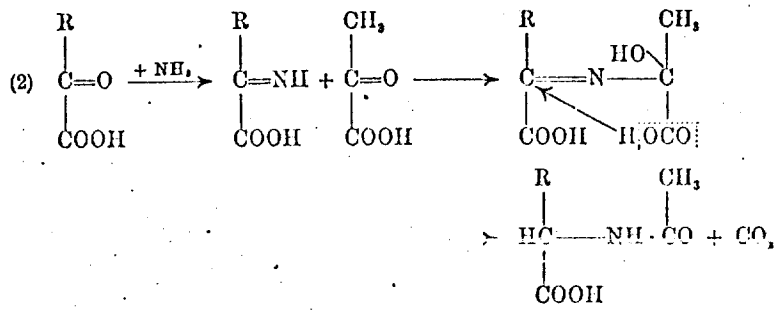
[4]) Embden u. Schmitz, Biochem. Z. 29, 423 (1910); 38, 393 (1912).

[5]) Dakin u. Dudley, J. of Biol. Chem. 18, 29 (1914).

[6]) Diese Z. 234, 83 (1935). [7]) Diese Z. 148, 294 (1925).

[8]) Knoop u. Oesterlin, Diese Z. 170, 186 (1927).

aktive N-Acetyl-γ-phenyl-α-amino-buttersäure auftrat, und zwar in gesteigerter Ausbeute bei gleichzeitiger Verfütterung von Brenztraubensäure. Knoop nahm an, daß die Acetylgruppe entstanden sei durch Dehydrierung eines Brenztraubensäuremoleküls, und daß der dabei verfügbar werdende Wasserstoff zur reduktiven Fixierung des Ammoniaks an die Phenyl-keto-buttersäure diente; dieser Vorgang wäre analog der aus rein chemischen Versuchen[9]) bekannten Bildung von Alanin aus Brenztraubensäure und NH_3 und kann nach Knoop[7]) schematisch folgendermaßen formuliert werden:



Verläuft dieser Vorgang an natürlichen Ketosäuren, so ist eine rasche Spaltung der Acetylaminosäure zur Aminosäure zu erwarten, so daß es nicht — wie beim Phenylderivat — zu einer Ausscheidung der Acetylverbindung im Harn kommt.

Diese Hypothese der „acetylierenden Aminierung" ist von Knoop zuerst aufgestellt und jetzt von Du Vigneaud und Irish[10]) durch neue Versuche gestützt worden [vgl. dazu Knoop, Diese Z. 253, 281 (1938)]. Von Krebs[11]) wird auch die von Neber[6]) mit Leberschnitten nachgewiesene Aminosäurebildung aus Brenztraubensäure als „acetylierende Aminierung" gedeutet. Die Brenztraubensäure spielt hierbei nach Krebs eine spezifische Rolle als Wasserstoffdonator für die Iminosäure, denn sie konnte durch andere Ketosäuren, wie Ketoglutarsäure, nicht ersetzt werden.

Was nun die Kenntnis der Enzymsysteme betrifft, die den Aminosäureumsatz katalysieren, so ist man zwar über die Enzyme der oxydativen Desaminierung schon recht gut unterrichtet, war

[9]) Erlenmeyer u. Kunlin, Liebigs Ann. 307, 146 (1899); 316, 145 (1901); Ber. chem. Ges. 35, 2438 (1902); De Jong, Rec. Trav. chim. Pays-Bas 19, 259 (1900).

[10]) J. of Biol. Chem. 122, 349 (1938).

[11]) Annual Rev. Biochem. (Am.) 5, 247 (1936).

es dagegen bisher noch kaum über den enzymatischen Apparat der Synthese.

Die oxydativ desaminierenden Enzyme sind in Niere, Leber und Darmschleimhaut angereichert und kommen in den übrigen Geweben höchstens in kleiner Konzentration vor. Schnitte von Geweben, die Aminosäuredehydrase enthalten, vermögen beide optischen Antipoden der Aminosäuren anzugreifen, während durch verdünnte Suspensionen oder zellfreie Extrakte eigentümlicherweise nur die nichtnatürlichen Formen desaminiert werden. Als Acceptor wird molekularer Sauerstoff verwertet — weshalb diese Enzyme der Gruppe der „Aero-Dehydrasen"[12]) zuzurechnen sind — und Wasserstoffsuperoxyd tritt als Reaktionsprodukt auf[12a]). Redoxfarbstoffe sind viel schlechtere Acceptoren als Sauerstoff.

Von N. B. Das[13]) wurde der Nachweis geführt, daß die aus Niere isolierte Dehydrase des Prolins und Alanins durch einen in tierischem Gewebe sowie in Hefe vorkommenden hitzestabilen Stoff aktiviert wird, der mit den bekannten Co-Fermenten nicht identisch war. Warburg und Christian[14]) sowie Straub[15]) konnten die Aufteilung der Alanindehydrase in Apo- und Co-Enzym kürzlich exakt beweisen, und haben in dem neuen Co-Enzym eine flavinhaltige Substanz erkannt (Flavin-Adenin-nucleotid)[14]).

Bei älteren Versuchen über die Dehydrierbarkeit von Aminosäuren war häufig die Glutaminsäure besonders hervorgetreten durch ihre gute Donatoreigenschaft[15a]). Die Vermutung, daß diese Aminosäure durch ein spezifisches Enzym dehydriert werde, wurde gestützt durch die Tatsache, daß Cozymase gerade die Dehydrierung von Glutaminsäure, aber nicht die anderer Aminosäuren beschleunigte[16,17]). (Die zur damaligen Zeit verfügbaren Cozymase-

---

[12]) Zur Nomenklatur vgl. z. B. Franke u. Lorenz, Liebigs Ann. 532, 1 (1937).

[12a]) Keilin u. Hartree, Proc. roy. Soc., Lond. Ser. B: Biol. Sci. 119, 114 (1936).

[13]) Biochemic. J. 30, 1080, 1617 (1936); Naturw. 26, 168 (1938).

[14]) Biochem. Z. 295, 261 (1938); Naturw. 26, 201 u. 235 (1938).

[15]) Nature (Brit.) 141, 603 (1938).

[15a]) Vgl. z. B. Thunberg, Skand. Arch. Physiol. 40, 1 (1920); Quastel u. Mitarb., Biochemic. J. 26, 725 (1932). — Weitere Literatur vgl. z. B. W. Franke in H. v. Euler, Chemie der Enzyme, München, Bergmann 1934, S. 592.

[16]) B. Andersson, Diese Z. 217, 191 (1933).

[17]) C. G. Holmberg, Akad. Avhandl., Lund (1933).

präparate waren allerdings unreine Gemische aus den beiden Codehydrasen I und II.)

Vor kurzem haben v. Euler, Adler und Steenhoff Eriksen[18]) in Hefe eine auf (die natürliche) l(+)-Glutaminsäure spezifisch eingestellte Apodehydrase aufgefunden, die sich mit Codehydrase II (Warburgschem Co-Ferment, Triphospho-pyridinnucleotid) zur Holodehydrase vereinigt. Die dehydrierende Funktion des Enzyms wurde durch Acceptorversuche (Methylenblau-Reduktion und Sauerstoffzehrung) und weiterhin durch direkte optische Messung der in der Primärreaktion zwischen Glutaminsäure und Codehydrase II in Gegenwart der Apodehydrase gebildeten Dihydrocodehydrase II nachgewiesen.

In der eben genannten Arbeit[18]) wurde die Absicht ausgesprochen, zu untersuchen, ob die Hefedehydrase „in der Zelle auch an dem umgekehrten Vorgang, der Bildung von Glutaminsäure aus ihrem Dehydrierungsprodukt teilnimmt". Es war ja zu erwarten, daß die Primärreaktion zwischen Glutaminsäure und der Holodehydrase wie die vieler anderer analogen Systeme reversibel sei. Diese Erwartung hat sich in neueren Versuchen, über die demnächst berichtet werden soll, bestätigt.

In der Zwischenzeit haben wir das Studium der Glutaminsäuredehydrase in tierischen Geweben aufgenommen und konnten den Mechanismus der durch dieses Enzym katalysierten reversiblen Reaktion aufklären. Über diese Ergebnisse soll in der vorliegenden Arbeit berichtet werden.

Aus tierischen Geweben können Apodehydrasepräparate hergestellt werden, die nach Ergänzung durch Codehydrase I (Cozymase) spezifisch l(+)-Glutaminsäure dehydrieren. Apodehydrasepräparate aus Leber werden außer durch Codehydrase I auch durch Codehydrase II wirksam ergänzt; ob diese Unspezifität bezüglich des Co-Enzyms für alle Gewebe gilt, soll noch untersucht werden.

Es ist bemerkenswert, daß Glutaminsäure-apodehydrase in verschiedenen Organismen verschiedene Codehydrasespezifität aufweist. Im Gegensatz zur tierischen Dehydrase ist die Hefedehydrase, wie oben erwähnt, Codehydrase II-spezifisch, und das gleiche zeigte sich in Versuchen von V. Hellström[18a]) für die Dehydrase von Bacterium coli. Dagegen ist die Glutaminsäuredehydrase höherer Pflanzen Codehydrase I-spezifisch[19]). Die

---

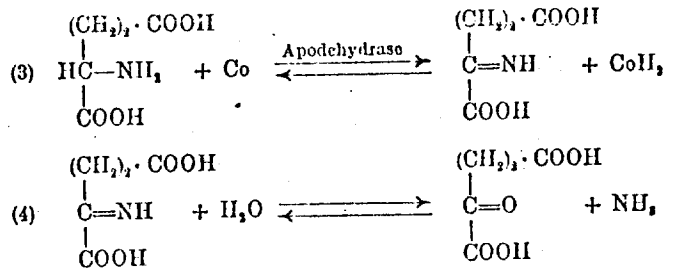
[18]) Diese Z. 248, 227 (1937).

[18a]) Unveröffentlichte Versuche.

[19]) Adler, Das, v. Euler u. Heyman, C. r. Trav. Labor. Carlsberg (Dän.), Sér. chim. 22, 15 (1938) (Sörensen-Festband).

eigentümliche Tatsache, daß Apodehydrasen, welche die gleiche Reaktion katalysieren, sich mit verschiedenen Codehydrasen spezifisch verbinden, zeigt nicht nur, daß verschiedene Proteine vorliegen, sondern auch, daß Substratspezifität und Codehydrasespezifität an zwei verschiedenen Stellen des Apodehydrasemoleküls lokalisiert sein müssen. Die Frage, wodurch die Spezifität der Apodehydrasen zur einen oder anderen Codehydrase überhaupt bedingt ist, kann noch nicht exakt beantwortet werden. Auf die weitere Frage, in welcher Weise die Auswahl der Codehydrase von Bedeutung für die Einordnung des Glutaminsäureumsatzes in den Gesamtstoffwechsel des betreffenden Organismus sein kann, werden wir in einem späteren Abschnitt (S. 96) noch zurückkommen.

Die Primärreaktion der Glutaminsäuredehydrierung in tierischen Geweben besteht nach den Ergebnissen der vorliegenden Arbeit in einer an der spezifischen Apodehydrase verlaufenden Dehydrierung der Glutaminsäure durch Codehydrase unter Bildung von Iminoglutarsäure und Dihydro-codehydrase, worauf die Iminosäure spontan in Ketoglutarsäure und Ammoniak zerfällt [Gleichungen (3) und (4), Co-Codehydrase I oder II]:



Von besonderem Interesse für die biologische Funktion der Glutaminsäuredehydrase ist jedoch der Befund, daß Reaktion (3) reversibel ist.

Aus diesem Befund, zusammen mit der weiteren Tatsache, daß auch Reaktion (4) reversibel ist, ergibt sich folgendes: geht man aus von Ketoglutarsäure, Ammoniak und Dihydro-cozymase, so muß — in Gegenwart der Glutaminsäure-apodehydrase — eine Reduktion der mit Ketoglutarsäure und NH_3 im Gleichgewicht stehenden Imino-glutarsäure zu Glutaminsäure stattfinden. Diese enzymatische Synthese der Glutaminsäure, die „hydrierende Aminierung" der Ketoglutarsäure, ließ sich experimentell leicht verwirklichen [20]). Sie erhält eine besondere biologische

[20]) Vorläufige Mitt. über diese Ergebnisse vgl. [19]) sowie G. Günther, Sv. Vet. Akad. Arkiv f. Kemi 12 A, Nr. 23 (1937); Adler, Das u. v. Euler, Ebenda 12 B, Nr. 40 (1937); E. Adler, Ebenda 12 B, Nr. 42 (1937).

Bedeutung dadurch, daß nach Braunstein und Kritzmann[21]) in den Zellen die Aminogruppe der Glutaminsäure auf die verschiedensten Ketosäuren übertragen wird („Umaminierung").

Von Weil-Malherbe[21a]) wurde vor kurzem bereits beschrieben, daß im Gehirn Glutaminsäure als einzige Aminosäure oxydativ desaminiert wird, wobei Ketoglutarsäure entsteht; der Autor fand ferner, daß Ketoglutarsäure in Gegenwart von Gehirnschnitten und Glucose oder Brenztraubensäure NH_3 bindet, woraus er auf die Reversibilität der Glutaminsäuredesaminierung schloß.

B. Präparate und Methoden.

Glutaminsäure-apodehydrase. Zu den meisten Versuchen wurden Apodehydrasepräparate aus Rinder- oder Schweineleber angewandt. Die Leber wurde in der Fleischhackmaschine zerkleinert, 2 mal mit der doppelten Gewichtsmenge eiskaltem Aceton verrührt und abgepreßt, schließlich mit Äther gewaschen und im Vakuum über Paraffin und Schwefelsäure oder auch in dünner Schicht auf Filtrierpapier an der Luft getrocknet. Das Acetonpräparat wurde im Mörser zerkleinert und von gröberen Bestandteilen durch Sieben befreit. Es ist monatelang haltbar.

Zur Herstellung der Apodehydraselösung wurden 10 g Acetonleber 2 mal mit 40 ccm Wasser und Sand verrieben, und der durch Zentrifugieren erhaltene Extrakt 8—12 Stunden aus Cellophan gegen fließendes Wasser dialysiert. Das durch Zentrifugieren von ausgeflocktem Eiweiß befreite, als „Rohlösung" bezeichnete Präparat war für Routineversuche meist völlig ausreichend. Extraktion des Acetonpulvers mit m/10-Na_2HPO_4 und nachfolgende Dialyse liefert noch aktivere Lösungen.

Reinigung: Glutaminsäure-apodehydrase war von T. H. Quibell[22]) in Lösungen von Glucose-dehydrase aus Leber nachgewiesen worden, die im wesentlichen nach Harrison[23]) gereinigt waren. Man kann also durch wiederholte Ammoniumsulfatfällung eine Reinigung der Glutaminsäure-apodehydrase erzielen. In derart gereinigten Lösungen muß jedoch immer mit der Anwesenheit von Resten von Ammonsalz gerechnet werden, die unerwünscht sind, wenn der Einfluß des NH_3 gesondert untersucht werden soll. Im Gegensatz zu Glucose-dehydrase wird die Glutaminsäure-dehydrase durch Fällung mit Aceton oder Alkohol nicht wesentlich geschädigt. Es wurden so haltbare Enzym-Trockenpräparate gewonnen. Aus den wäßrigen Lösungen solcher Präparate haben wir die Apodehydrase durch Adsorption an Aluminiumhydroxyd C_y

[21]) Nature 140, 503 (1937); Enzymologia (Nd.) 2, 129 (1937).
[21a]) Biochemic. J. 30, 665 (1936). [22]) Diese Z. 251, 102 (1938).
[23]) Biochemic. J. 27, 382 (1933).

(nach Willstätter und Kraut) und Elution mit m/10-Na_2HPO_4 weiter gereinigt.

Eine systematische Reinigung der Apodehydrase wurde noch nicht unternommen.

Dihydro-cozymase. Aus Cozymase entweder durch enzymatische Hydrierung[24]) oder durch Hydrierung mit Hydrosulfit dargestellt und als Bariumsalz mit Aceton ausgefällt, im letzteren Fall nach vorangehender Abscheidung von Bariumsulfit und -sulfat in der Hitze[25]). Zur Darstellung der Versuchslösungen wurde das Bariumsalz mit Na_2SO_4 umgesetzt.

Substrate. l(+)-Glutaminsäure von Hofmann-La Roche sowie von Fränkel und Landau. Eine Probe von d(—)-Glutaminsäure verdanken wir Herrn Dr. V. Bruckner (Szeged). [Vgl. Diese Z. 247, 281 (1937).] Für die Überlassung von α-Ketoglutarsäure sind wir Herrn Prof. P. Karrer zu besonderem Dank verpflichtet.

Die Messung der Dehydrierung geschah a) durch den Thunberg-Versuch in der üblichen Weise, b) durch die Messung der Sauerstoffzehrung im Warburg-Apparat.

Die reversible Primärreaktion wurde durch spektrophotometrische Verfolgung der Dihydro-cozymase-Absorption bei $\lambda = 334$ mμ studiert.

Ammoniak wurde nach Embden, Carstensen und Schumacher[26]), Aminostickstoff nach van Slyke bestimmt.

C. Ergebnisse.

I. Die „dehydrierende Desaminierung" der l(+)-Glutaminsäure.

1. Nachweis durch den Thunberg-Versuch.

Tab. 1 zeigt, daß l(+)-Glutaminsäure in Gegenwart von Dehydraselösung aus Leber und von Cozymase Methylenblau reduziert.

Tabelle 1.

Glutaminsäuredehydrierung. Substrat: 0,5 m l(+)-Na-Glutaminat. — Enzym: Dehydraselösung aus Leber („Roblösung"). — Cozymase: 1 mg/ccm. — m/2-Phosphatpuffer $p_H = 7,6$. — Methylenblau 1 : 5000. — Jeder Ansatz enthält 0,25 ccm Puffer, 0,5 ccm Mb (im Einsatzröhrchen) und ist mit Wasser auf 2,0 ccm ergänzt; übrige Komponenten vgl. Tabelle.

---

[24]) Adler u. v. Euler, Sv. Vet. Akad. Arkiv f. Kemi 12 B, Nr. 36 (1937).
[25]) v. Euler u. Hellström, Diese Z. 252, 33 (1938).
[26]) Diese Z. 179, 191 (1928).

Versuch Nr.	Glutaminsäure	Enzym	Cozymase	Entfärbungszeit Minuten
1	0,25	0,25	0,25	6
2	—	0,25	0,25	170
3	0,25	0,25	—	> 180
4	0,25	0,25 *)	0,25	> 180

*) hitzeinaktiviert.

Cozymase ist also eine notwendige Komponente des Systems. Dagegen ist ein Zusatz von Flavinenzym nicht erforderlich. Die Wasserstoffübertragung von der Dihydro-cozymase auf das Methylenblau muß also durch eine bereits in der Enzymlösung enthaltene Komponente bewirkt werden. In Versuchen mit „Rohlösungen", wie in Tab. 1, bewirkt Flavinenzymzusatz im allgemeinen eine gewisse Verkürzung der Spontanentfärbung, beeinflußt aber die Entfärbungszeit im Ansatz mit Glutaminsäure nur ganz unbedeutend. Bei Versuchen mit gereinigten Enzympräparaten dagegen verkürzt Flavinenzym die Entfärbungszeit, woraus hervorgeht, daß solche Präparate in bezug auf die Wasserstoff-übertragende Komponente nicht mehr voll aktiviert sind. Die Natur des in den Enzympräparaten enthaltenen Wasserstoffüberträgers wird weiter unten diskutiert. Tab. 2 zeigt die Flavinenzymwirkung im System mit gereinigter Apodehydrase.

Tabelle 2.

Flavinenzym-Effekt. Apodehydrase: Nach Quibells[22]) Angaben für die Reinigung von Glucose-dehydrase dargestellt, durch Acetonfällung sowie Adsorption und Elution weiter gereinigt. Flavinenzym aus Hefe. Im übrigen wie Versuche zu Tab. 1.

Versuch Nr.	Glutaminsäure	Apodehydrase	Cozymase	Flavinenzym	Entfärbungszeit Minuten
1	—	0,25	0,25	0,25	> 180
2	0,25	0,25	0,25	—	25
3	0,25	0,25	0,25	0,25	15
4	0,25	—	0,25	0,25	> 180

2. Sauerstoff als Endacceptor.

a) Die Komponenten des aeroben Systems. Das System Glutaminsäure + Leberenzym + Cozymase + Mb nimmt, wie schon auf Grund der Thunberg-Versuche zu erwarten war, Sauerstoff auf. Fig. 1 zeigt die Unerläßlichkeit der Cozymase auch für diesen Vorgang.

Aus Fig. 1 ergibt sich außerdem, daß auch der Zusatz von Methylenblau notwendig ist, um eine rasche Sauerstoffaufnahme zu erzielen. Flavinenzym (allerdings in viel kleinerer Konzentration als das Mb angewandt) aktiviert die Sauerstoffaufnahme nur sehr wenig. Diese beiden Befunde stehen zwar nicht in unmittelbarer Beziehung zum Mechanismus der Dehydrierung und der Synthese von Glutaminsäure; da sie jedoch von allgemeinerem

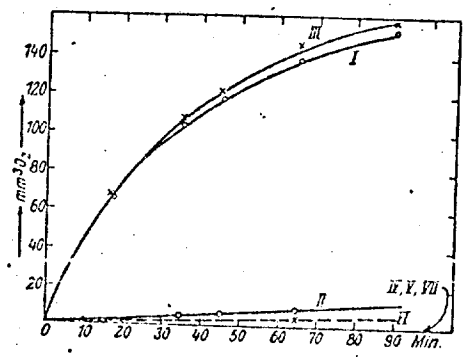


Fig. 1.
Aerobe Dehydrierung von Glutaminsäure.
Kurve I: 0,25 ccm 0,5 m l(+)-Na-Glutaminat; 1,0 ccm Enzym (Rohlösung); 0,25 ccm Methylenblau 1 : 500; 0,2 ccm m/2-Phosphatpuffer p_H = 7,6; 0,2 ccm Cozymase entsprechend 1 mg aus Anhang eingekippt. — Kurve II: Wie I, + 0,5 ccm Flavinenzym aus Hefe, entsprechend etwa 6 γ gebundenem Lactoflavin, jedoch kein Methylenblau. — Kurve III: Wie II, jedoch mit Methylenblau. — Kurve IV: Wie I ohne Methylenblau. — Kurve V: Wie I ohne Cozymase. — Kurve VI: Wie I ohne Glutaminsäure. — Kurve VII: Wie I mit erhitztem Enzym. — Alle Ansätze sind mit Wasser auf 2,4 ccm ergänzt. Warburg-Apparat, 30°.

Interesse für die Frage des Wasserstofftransportes bei aeroben Dehydrierungen sind, so sollen sie im folgenden Abschnitt etwas näher beleuchtet werden.

b) Der Wasserstofftransport bei der aeroben Dehydrierung. Aus den Untersuchungen über isolierte Hefedehydrasen, z. B. die Alkoholdehydrase, ist bekannt, daß Flavinenzym die Übertragung des Wasserstoffs von Dihydro-cozymase auf Sauerstoff vermitteln kann. In Analogie dazu hatten wir auch gefunden, daß die aerobe Dehydrierung von Glucose durch das nach Harrison gereinigte Leberenzym durch Flavinenzym stark aktiviert wird[27]). Mit der in den Versuchen zu Fig. 1 verwendeten rohen Glutaminsäuredehydrase aus Leber finden wir jedoch

a) daß zur Erzielung der raschen Sauerstoffaufnahme Mb nötig ist,
b) daß Flavinenzym das Mb nicht ersetzen kann.

[27]) Adler u. v. Euler, Diese Z. 232, 6 (1935).

Zur weiteren Klärung der Wasserstoffübertragung durch das Leberenzym haben wir einige Versuche angestellt, in denen das System insofern vereinfacht war, als wir direkt von Dihydrocozymase ausgingen und deren Oxydation durch den Luftsauerstoff spektrophotometrisch verfolgten (Fig. 2).

In Übereinstimmung mit den Ergebnissen, die bei der aeroben Glutaminsäuredehydrierung erhalten wurden, finden wir, daß das Leberenzym die Übertragung des Sauerstoffs auf Dihydro-cozymase nur mit sehr geringer Geschwindigkeit katalysiert (Kurve Ia), daß

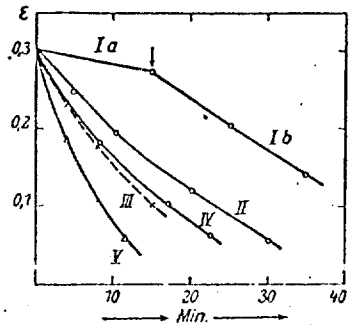


Fig. 2.
Der Wasserstofftransport bei der aeroben Dehydrierung.
Dihydro-cozymase, 0,1 mg per Ansatz. — Enzyme: Leberenzym („Rohlösung"), enthält 14 γ gebundenes Lactoflavin per ccm. Flavinenzym aus Hefe, enth. 16 γ gebund. Lactoflavin per ccm. — Die Bestandteile der Ansätze wurden in der Reihenfolge gemischt, wie im folgenden angegeben. — Kurve I: 3,2 ccm Wasser; 0,5 ccm m/2-Phosphatpuffer $p_H = 7,4$; 0,1 ccm m/10-KCN; 0,1 ccm Dihydro-cozymase; 0,05 ccm Leberenzym entsprech. 0,7 γ Flavin. (Kurventeil Ia). — Beim ↓ Zusatz von 0,1 ccm Flavinenzym, entsprech. 0,4 γ Flavin (Kurventeil Ib). — Kurve II: Wie Kurve I, mit 0,1 ccm Flavinenzym, jedoch ohne Leberenzym. — Kurve III: Wie Kurve Ia, + 0,05 ccm Methylenblau 1 : 1000. — Kurve IV: Wie Kurve II, jedoch mit 0,05 ccm Mb. — Kurve V: Wie Kurve Ib, jedoch mit 0,05 ccm Mb.

jedoch die Reaktion durch das System Leberenzym + Methylenblau (Kurve III) zustande kommt*). Diese Tatsachen weisen darauf hin, daß das Leberenzym praktisch kein Flavinenzym enthält, sondern, daß die von der Gegenwart des Methylenblaus abhängige Oxydation durch Diaphorase[28]) hervorgerufen wird, die ja be-

*) Die im Versuch zu Fig. 2 angewandte Enzymlösung war aus einem Acetonpräparat von Schweineleber dargestellt; mit anderen Präparaten (aus Rinderleber) fanden wir überhaupt keine aerobe CoH_2-Dehydrierung ohne Zusatz von Mb.

[28]) Adler, v. Euler u. Hellström, Sv. Vet. Arkiv f. Kemi 12 B, Nr. 38 (1937); Dewan u. Green, Nature 140, 1097 (1937); Biochemic. J. 32, 626 (1938); Adler, v. Euler u. Hughes, Diese Z. 252, 1 (1938); v. Euler u. Hellström, Diese Z. 252, 31 (1938); v. Euler u. Hasse, Naturw. 26, 187 (1938).

kanntlich nicht direkt mit Sauerstoff reagiert. Die Bezeichnung „Diaphorase" (von διαφορεῖν, übertragen) wurde vorgeschlagen [Adler, Euler u. Günther, Sv. Vet. Akad. Ark. f. Kemi 12 B, Nr. 54 (1938)] für das neue Enzym, welches den labilen Wasserstoff der Dihydro-cozymase auf Acceptoren wie Cytochrom oder Methylenblau überträgt.

Überraschenderweise zeigte sich jedoch, daß die zu den Oxydationsversuchen verwendete Enzymlösung aus Leber große Mengen schwer dialysierbares, d. h. hochmolekular gebundenes Flavin enthält. Nach Spaltung mit heißem 50%igem Aceton und Überführung in Lumiflavin wurden 14 γ Lactoflavin pro Kubikzentimeter Enzymlösung gefunden. Die in den Versuchen zu Fig. 2 verwendete Enzymmenge enthält also 0,7 γ gebundenes Flavin. Aus Kurve II in Fig. 2 geht hervor, daß 0,4 γ Flavin in Form von Flavinenzym aus Hefe bereits ausreichen, um eine ziemlich rasche Oxydation der Dihydro-cozymase zu katalysieren. Setzt man die gleiche Menge Flavinenzym zum Ansatz mit Leberenzym hinzu (Kurve Ib), so findet ebenfalls eine Oxydation der CoH_2 statt.

Aus diesen Befunden muß gefolgert werden, daß die im Leberenzym enthaltene hochmolekulare Verbindung des Flavins nicht mit Flavinenzym identisch, d. h. nicht imstande ist, CoH_2 zu dehydrieren. In einem besonderen Versuch stellten wir fest, daß auch die Dihydro-codehydrase II durch das Leberenzym nicht reoxydiert wird. Man kann vermuten, daß das Flavin in der Leber als (hochmolekular gebundenes) Flavin-Adenin-Dinucleotid vorliegt, welches kürzlich von Warburg und Christian[29]) sowie von Straub[30]) aus tierischen Geweben isoliert worden ist. Die obigen Befunde würden dann bedeuten, daß dieses „Flavin-Adenin-Enzym" nicht die Funktion hat, die Weiterleitung des Wasserstoffs der Dihydro-codehydrasen I und II zu vollziehen. In bezug auf Dihydro-codehydrase I wird diese Aufgabe in den tierischen Geweben offenbar durch die Diaphorase erfüllt. In welchem Ausmaße das hochmolekular gebundene Flavin in den tierischen Geweben überhaupt als eigentliches Flavinenzym vorliegt, kann gegenwärtig noch nicht beurteilt werden.

Von Dewan und Green[28]) wird in der kürzlich erschienenen Arbeit über das Wasserstoff-übertragende Enzym der tierischen Gewebe unsere früher wiederholt hervorgehobene Auffassung von der Funktion des Flavinenzyms als spezifischem Dihydro-coenzym-dehydrierendem Faktor in pole-

[29]) Biochem. Z. 295, 261 (1938); Naturw. 26, 201 u. 235 (1938).
[30]) Nature 141, 603 (1938).

mischer Form kritisiert. Die Bezeichnung „Flavinenzymsysteme" hatten wir früher für isolierte Dehydrasesysteme angewandt, zu denen Codehydrase I oder II notwendig waren und in welchen infolgedessen Flavinenzym als Wasserstoffüberträger zwischen der hydrierten Codehydrase und dem Endacceptor wirksam war. Wir fanden eine Flavinenzymwirkung auch in Dehydrasesystemen aus tierischen Geweben — besonders ausgeprägt bei der Glucosedehydrase aus Leber[27]) —, so daß auch hier die Gruppenbezeichnung „Flavinenzymsysteme" formell nicht unberechtigt war. Daß sie im Fall der tierischen Gewebe den biologischen Verhältnissen nicht gerecht wird, stellte sich erst mit der Entdeckung der „Diaphorase" heraus, die unabhängig im hiesigen Institut und durch Dewan und Green erfolgte[28]). Es ist nunmehr sehr wahrscheinlich, daß in tierischen Geweben die Wasserstoffleitung von den Dihydro-codehydrasen aus nicht durch Flavinenzym, sondern durch Diaphorase erfolgt. Gleichzeitig wird aber nun offenbar, daß Greens wiederholt vertretene Auffassung, daß die Sauerstoff-übertragende Wirkung von Methylenblau bei Dehydrasesystemen aus tierischen Geweben auf einer direkten Reaktion zwischen CoH_2 und dem Farbstoff beruhe, unrichtig ist, worauf wir schon früher[21]) hingewiesen hatten. Es besteht also unseres Erachtens von seiten Greens keine Veranlassung, gegen frühere Unvollständigkeiten bzw. Vereinfachungen der Darstellung in diesen Fragen zu polemisieren.

c) Die summarische Reaktionsgleichung der aeroben Dehydrierung.

In aeroben Versuchsansätzen wurde gefunden, daß bei der Glutaminsäuredehydrierung *Ketoglutarsäure und Ammoniak als Reaktionsprodukte* auftreten.

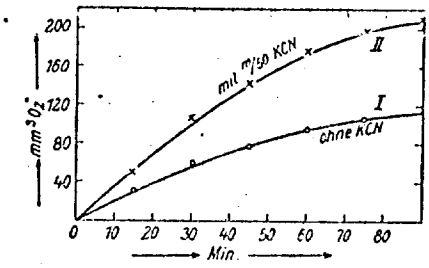
α-Ketoglutarsäure: 5 gleichartige Ansätze von derselben Zusammensetzung wie zu Kurve I in Fig. 1 angegeben, wurden nach 120 Minuten Reaktionsdauer langsam durch Kieselgur filtriert, wobei das Mb adsorbiert wird, und hierauf mittels Trichloressigsäure enteiweißt. Aus dem Filtrat fällt auf Zusatz von salzsaurem 2,4-Dinitrophenylhydrazin ein Hydrazon in gelben, seidenglänzenden Nadeln aus, die nach 12stündigem Stehen im Eisschrank abgesaugt wurden. Schmelzp. 222°; Schmelzpunkt von reinem α-Ketoglutarsäure-2,4-Dinitrophenylhydrazon 223°; Mischschmelzp. 222° (unkorr.). Es wurden 22 mg Hydrazon isoliert. Die Sauerstoffaufnahme in den 5 Ansätzen betrug insgesamt 895 cmm O_2. Daraus berechnet sich das Verhältnis $^1/_2\ O_2 : 1$ Ketoglutarsäure $= 1 : 0{,}8$.

Ammoniak: In einem aeroben Ansatz waren nach 60 Minuten 133 cmm O_2 aufgenommen und 0,18 mg NH_3 gebildet worden, d. h. $^1/_2\ O_2 : NH_3 = 1 : 0{,}9$.

Somit ergibt sich als bilanzmäßige Reaktionsgleichung

$$\text{(5)}\quad \text{Glutaminsäure} + {}^1/_2\,O_2 = \text{Ketoglutarsäure} + NH_3 + H_2O\,.$$

Diese Gleichung stellt indessen die Summe von zwei Teilreaktionen dar. Der Wasserstoff wird — wie stets bei solchen Dehydrierungen — durch die Codehydrase vom Substratmolekül paarweise abgelöst, wird von der Dihydro-codehydrase aus durch das Überträgerenzym (Diaphorase) zum Methylenblau befördert, dessen Leukoverbindung schließlich mit molekularem Sauerstoff unter Bildung von H_2O_2 reagiert. Die eigentliche Bilanzgleichung der aeroben Dehydrierung lautet also:



(6) Glutaminsäure + O_2 = Ketoglutarsäure + NH_3 + H_2O_2.

Das angewandte Enzympräparat aus Leber enthält nun, wie wir fanden, sehr viel Katalase, wodurch das nach Gleichung (6) gebildete H_2O_2 sofort zersetzt wird nach der Gleichung

(7) $H_2O_2 = H_2O + {}^1/_2 O_2$,

so daß $^1/_2$ Mol. O_2 wieder in die Atmosphäre zurückgelangt. Die Summe der Gleichungen (6) und (7) ergibt Gleichung (5).

Fig. 3.
Erhöhung der Sauerstoffaufnahme durch KCN.
Kurve I: 0,25 ccm 0,5 m-Glutaminat; 0,5 ccm Leberenzym; 0,25 ccm Mb 1 : 500; 0,2 ccm m/2-Phosphatpuffer $p_H = 7{,}6$; 0,2 ccm Cozymase (1 mg) eingeklppt. — Kurve II: Wie I, jedoch mit KCN (Endkonzentration m/50).

Die Richtigkeit dieser Ableitung wird bewiesen durch den Versuch, der in Fig. 3 dargestellt ist: Zusatz von m/50-KCN erhöht die Sauerstoffaufnahme auf beinahe das Doppelte, d. h. die Katalase wird gehemmt und an die Stelle der Reaktionsgleichung (5) tritt Gleichung (6).

3. Spektrophotometrischer Nachweis der primären Dehydrierungsreaktion.

Die spektrophotometrische Untersuchung der Hydrierung von Cozymase bzw. der Dehydrierung von Dihydro-cozymase im System der Glutaminsäure-dehydrase führte zur Aufklärung des Reaktionsmechanismus.

Fig. 4 zeigt, daß Cozymase durch l(+)-Glutaminsäure in Gegenwart des Leberenzyms (der Glutaminsäure-apodehydrase) zu Dihydro-cozymase reduziert wird.

Daß der Extinktionsanstieg tatsächlich durch die Bildung von CoH_2 verursacht ist, läßt sich leicht dadurch beweisen, daß auf Zusatz von Acetaldehyd die Extinktion sehr rasch auf den Ausgangswert fällt. Das Leberenzym enthält ja auch Alkohol-apodehydrase, an welcher sich die Dehydrierung der CoH_2 durch den Aldehyd vollzieht.

Ohne Enzym oder mit erhitztem Enzym bzw. ohne Glutaminsäure erfolgt keine Hydrierung der Cozymase.

Die Cozymasehydrierung ist, wie aus Fig. 4 weiterhin hervorgeht, abhängig vom p_H. Die Geschwindigkeit wächst mit steigendem

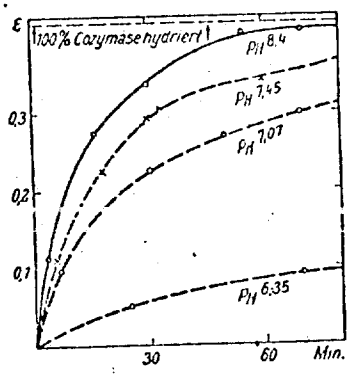


Fig. 4.
Hydrierung von Cozymase durch Glutaminsäure + Apodehydrase. — p_H-Abhängigkeit. 2,0 ccm m/2-Phosphatpuffer von verschiedenem p_H; 1,0 ccm m/2-Na-Glutaminat; 0,8 ccm Wasser; 0,05 ccm Leberenzym („Rohlösung"); 0,15 ccm Cozymase (0,85 mg/ccm).

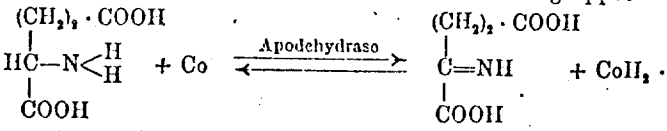
p_H-Wert. Die Hydrierung verläuft nicht zu Ende, sondern bleibt bei einem Gleichgewicht stehen, welches ebenfalls vom p_H bedingt ist. Bei $p_H = 8,4$ wird die eingesetzte Cozymase bis auf einen kleinen Rest, bei p_H 7,07 nur zu etwa 71 % hydriert. Schon dies weist darauf hin, daß, in Analogie zu anderen Dehydrierungsreaktionen, *die Reaktion zwischen Glutaminsäure und Cozymase eine Gleichgewichtsreaktion* ist.

Wenn wir sie formulieren wollen, so können wir annehmen, daß die Glutaminsäure durch die Cozymase — richtiger gesagt, durch die Holodehydrase D-Co — zur Iminosäure dehydriert wird. Letztere ist bekanntlich unstabil und zerfällt spontan durch Hydrolyse in α-Ketoglutarsäure und NH_3, d. h. wir kommen zu der Formulierung der Primärreaktion der Glutaminsäuredehydrierung, wie sie bereits in den Gleichungen (3) bis (4) dargestellt ist.

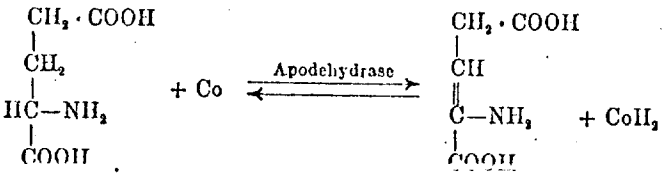
Die im folgenden zu beschreibenden Versuche stehen mit dieser Formulierung in bestem Einklang.

Was den Mechanismus des primären Angriffs des Holoenzyms auf die Glutaminsäure betrifft, so können zwei Möglichkeiten in Betracht gezogen werden:

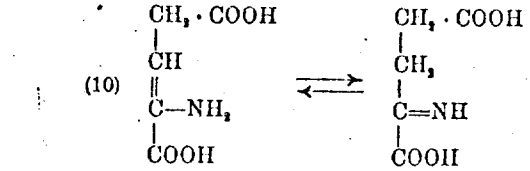
a) Die Dehydrierung erfolgt durch Herausnahme des am α-C-Atom gebundenen H-Atoms sowie des einen H-Atoms der Aminogruppe:



b) Die Dehydrierung erfolgt durch Herausspaltung je eines H-Atoms am α- und β-C-Atom:



So würde eine Enamino-säure entstehen, die sich wahrscheinlich rasch in die Iminosäure umlagern und mit ihr ins Gleichgewicht setzen würde:



Zwischen diesen beiden Möglichkeiten ist eine experimentelle Entscheidung noch nicht getroffen. Sie könnte vielleicht angegangen werden durch die Prüfung der Frage, ob die Dehydrierung auch an in β-Stellung disubstituierten, oder ob sie nur an monosubstituierten Derivaten der Glutaminsäure stattfindet. Für die Beurteilung der folgenden Versuche ist die Entscheidung dieser Frage jedoch unwesentlich und es wird der Einfachheit halber bis auf weiteres die Alternative a) angenommen.

Der folgende Abschnitt bringt den Beweis für die Reversibilität der durch die Gleichungen (3) bis (4) dargestellten Primärreaktion der Glutaminsäuredehydrierung.

II. Die Reversibilität der „dehydrierenden Desaminierung" der Glutaminsäure.

1. Hemmung der Glutaminsäuredehydrierung durch die Reaktionsprodukte.

In den Acceptorversuchen (Thunbergversuch, Atmungsversuch) deutet die Hemmung der Reaktionsgeschwindigkeit durch

die Reaktionsprodukte, NH_3 und Ketoglutarsäure, auf die Umkehrbarkeit der Dehydrierung: Tab. 3 zeigt die Hemmung der Methylenblauentfärbung, Fig. 5 die Hemmung der Sauerstoffaufnahme. Besonders im aeroben Versuch ist es deutlich, daß die Hemmung

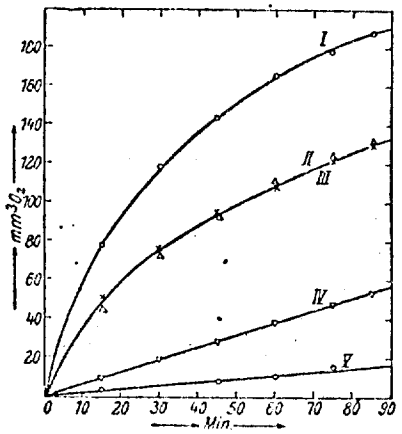


Fig. 5.

Hemmung der aeroben Glutaminsäuredehydrierung durch Ketoglutarsäure und NH_4Cl. Kurve I: Wie Kurve I zu Fig. 1. — Kurve II: Mit 0,2 ccm 0,1 m-Natrium-keto-glutarat. — Kurve III: Mit 0,2 ccm 0,1 m-NH_4Cl. — Kurve IV: Mit je 0,2 ccm 0,1 m-Ketoglutarat und NH_4Cl. — Kurve V: Wie I ohne Glutaminat.

durch die Kombination beider Reaktionsprodukte größer ist, als der Summe der Einzelwirkungen entspricht.

Tabelle 3.

Hemmung der Dehydrierung durch NH_3 und Ketoglutarsäure. „Rohlösung" als Enzym; 0,1 m NH_4Cl, 0,1 m Na-Ketoglutarat, je 0,25 ccm per Ansatz. Im übrigen wie Versuche zu Tab. 1.

Versuch Nr.	Substrat	NH_4Cl	Ketoglutarat	Entfärbungszeit Minuten
1	Glutaminsäure	–	–	7,5
2	„	+	–	12
3	„	–	+	40
4	„	+	+	150
5	Alkohol	–	–	7,5
6	„	+	–	8

Die Ursache für dieses Verhalten ergibt sich ohne weiteres aus der Betrachtung der beiden Gleichungen (3) und (4). Der Grad der Hemmung ist bedingt durch die Konzentration an Iminoglutarsäure, die ihrerseits von der Konzentration ihrer beiden

Spaltprodukte, Ketoglutarsäure und NH_3, abhängig ist. Bei Zusatz dieser beiden Komponenten wird Gleichung (4), und damit auch Gleichung (3) mehr nach links verschoben als durch Zusatz jeder Komponente für sich. Verschiebung des Gleichgewichts (3) nach links bedeutet langsamere CoH_2-Bildung und damit langsamere Mb-Entfärbung bzw. O_2-Aufnahme.

Der Vergleichsversuch mit Alkohol zeigt, daß die Ammoniakhemmung spezifisch ist für die Glutaminsäuredehydrierung.

2. Hemmung der Cozymase-Hydrierung durch die Reaktionsprodukte.

Setzt man dem aus Glutaminsäure und Leberenzym bestehenden Reaktionsgemisch vor Hinzufügung der Cozymase Ketoglutarsäure zu, so wird die Hydrierungsgeschwindigkeit der Cozymase

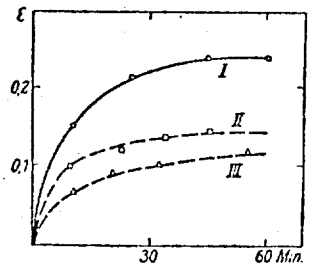


Fig. 6.

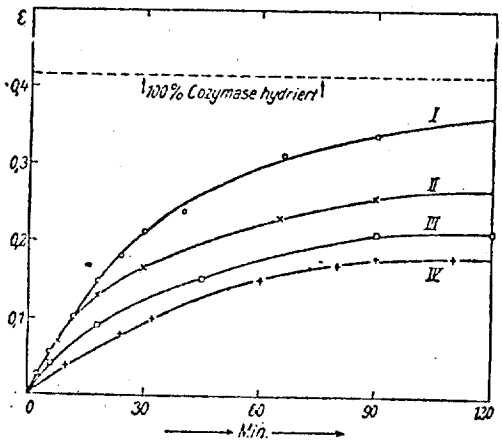


Fig. 7.

Fig. 6. Hemmung der Cozymase-Hydrierung durch Ketoglutarsäure.
Kurve I: Ansatz wie zu Fig. 4 (Puffer p_H 7,45), jedoch nur 0,1 ccm Cozymase. — Kurve II: Wie I, + 0,05 ccm m/10-Ketoglutarsäure. — Kurve III: Wie I, + 0,1 ccm m/10-Ketoglutarsäure.

Fig. 7. Hemmung der Cozymase-Hydrierung durch Ammoniumchlorid.
Kurve I: 3,5 ccm m/2-Phosphatpuffer, p_H 7,45; 0,5 ccm m/2-Na-Glutaminat; 0,2 ccm Wasser; 0,2 ccm Leberenzym; 0,15 ccm Cozymase (1 mg/ccm). — NH_4Cl-Konzentration im Ansatz zu Kurve II: 0,0022 m, Kurve III: 0,0066 m, Kurve IV: 0,011 m.

gegenüber dem Versuch ohne Ketoglutarsäure verlangsamt und die Reaktion bleibt bei einem wesentlich niedrigeren Gleichgewicht stehen (Fig. 6). In ganz analoger Weise äußert sich ein Zusatz von Ammoniumchlorid (Fig. 7).

Steigender Zusatz von Ammoniumsalz drückt den Hydrierungsgrad auf ein Niveau, welches schließlich, wie aus Fig. 7 ersichtlich

ist, und in besonderen Versuchen eingehender untersucht wurde, durch noch höhere Ammoniumsalzmengen nicht weiter erniedrigt wird. Dies bedeutet, daß dann der Überschuß an NH_3 gegenüber der entstandenen Ketoglutarsäure bereits so groß ist, daß eine weitere Erhöhung der NH_3-Konzentration die Menge der Iminoglutarsäure im Gleichgewicht (4) nicht mehr wesentlich beeinflußt. So erhält man bei der betreffenden Ammonsalzkonzentration ein Hydrierungsgleichgewicht, welches annähernd dem der eigentlichen Primärreaktion (3) entspricht, da die Störung dieses Gleichgewichts praktisch aufgehoben ist durch die Blockierung der Reaktion (4).

Wir bedienten uns dieser Tatsache, um die Abhängigkeit des Gleichgewichts (3) von der Konzentration der Reaktionsteilnehmer sowie vom p_H zu untersuchen, und um festzustellen, ob sich eine Gleichgewichtskonstante berechnen läßt, die mit der postulierten Gleichung (3) in Einklang steht.

3. Das Gleichgewicht der primären Dehydrierungsreaktion (3).

Zur Bestimmung der Gleichgewichtskonstante der Primärreaktion (3) wurden zwei verschiedene Enzympräparate verwendet. Das zu den Versuchen Nr. 1—4 der Tab. 4 angewandte Enzym war eine „Rohlösung"; bei diesen Versuchen wurde der Reaktionsmischung NH_4Cl in einer Gesamtkonzentration von 0,025 Mol/Liter zugesetzt, die ausreichend war, um nach dem oben Gesagten eine praktisch vollständige Stabilisierung des Gleichgewichtes (3) zu erzielen. In den übrigen Versuchen der Tab. 4 kam eine durch Ausfällen mit gesättigtem Ammoniumsulfat, Wiederauflösen und 12stündiges Dialysieren durch Cellophan gereinigte Apodehydrase zur Verwendung. Trotz der Dialyse enthielt diese Enzymlösung noch mehr als die zur Stabilisierung des Gleichgewichtes (3) mindest notwendige Ammonsalzmenge, so daß ein besonderer Zusatz überflüssig war.

Bei den in Tab. 4 zusammengestellten Versuchen ist die Konzentration der Reaktionsteilnehmer sowie die nach Stillstand der Hydrierung gemessene Konzentration der gebildeten Dihydrocozymase (x) angegeben. Das p_H wurde nach dem Versuch elektrometrisch bestimmt.

Wenn c die Anfangskonzentration der Cozymase und g die der Glutaminsäure war, so ist unter Zugrundelegung der Reaktionsgleichung (3) die im Gleichgewicht vorhandene Cozymasekonzentration $(c - x)$ und die Glutaminsäurekonzentration $(g - x)$, und es errechnet sich die Gleichgewichtskonstante $K = \frac{(g - x)(c - x)}{x^2}$.

Aus Tab. 4 geht hervor,

1. daß K in Versuchen mit ganz verschiedener Konzentration der Reaktionsteilnehmer in befriedigender Übereinstimmung *eine Konstante* ist, vorausgesetzt, daß das p_H in den betreffenden Versuchen gleich ist. Damit ist die Richtigkeit der Gleichung (3) bewiesen;

2. daß K mit steigendem p_H kleiner wird, d. h., daß die Glutaminsäuredehydrierung bei höheren p_H-Werten rascher verläuft als bei niedrigen und daß auf der anderen Seite die Hydrierung der Iminoglutarsäure bei niedrigen p_H-Werten begünstigt ist.

Tabelle 4.

Gleichgewichtskonstante der Primärreaktion (3).

Versuch Nr.	p_H	Molare Konzentrationen				Gleichgewichtskonstante K
		Glutaminsäure (g)	Cozymase			
			total (c)	CoH_2 (x)	Co ($c-x$)	
1	7,01	0,222	$13,33 \cdot 10^{-5}$	$3,06 \cdot 10^{-5}$	$10,27 \cdot 10^{-5}$	$2,43 \cdot 10^4$
2	7,36	0,111	$6,66 \cdot 10^{-5}$	$3,00 \cdot 10^{-5}$	$3,66 \cdot 10^{-5}$	$0,46 \cdot 10^4$
3	7,40	0,111	$8,33 \cdot 10^{-5}$	$3,48 \cdot 10^{-5}$	$4,85 \cdot 10^{-5}$	$0,44 \cdot 10^4$
4	7,57	0,017	$10,00 \cdot 10^{-5}$	$2,16 \cdot 10^{-5}$	$7,84 \cdot 10^{-5}$	$0,28 \cdot 10^4$
5	6,05	0,167	$10,00 \cdot 10^{-5}$	$0,84 \cdot 10^{-5}$	$9,16 \cdot 10^{-5}$	$21,66 \cdot 10^4$
6	6,63	0,167	$10,00 \cdot 10^{-5}$	$2,52 \cdot 10^{-5}$	$7,48 \cdot 10^{-5}$	$1,96 \cdot 10^4$
7	7,26	0,167	$10,00 \cdot 10^{-5}$	$4,20 \cdot 10^{-5}$	$5,80 \cdot 10^{-5}$	$0,55 \cdot 10^4$
8	7,39	0,167	$10,00 \cdot 10^{-5}$	$4,44 \cdot 10^{-5}$	$5,56 \cdot 10^{-5}$	$0,47 \cdot 10^4$
9	7,43	0,167	$10,00 \cdot 10^{-5}$	$5,04 \cdot 10^{-5}$	$4,96 \cdot 10^{-5}$	$0,33 \cdot 10^4$
10	7,45	0,167	$8,33 \cdot 10^{-5}$	$4,68 \cdot 10^{-5}$	$3,65 \cdot 10^{-5}$	$0,28 \cdot 10^4$
11	7,60	0,083	$13,33 \cdot 10^{-5}$	$5,16 \cdot 10^{-5}$	$8,17 \cdot 10^{-5}$	$0,26 \cdot 10^4$
12	7,66	0,011	$10,00 \cdot 10^{-5}$	$2,16 \cdot 10^{-5}$	$7,84 \cdot 10^{-5}$	$0,19 \cdot 10^4$
13	7,82	0,111	$6,67 \cdot 10^{-5}$	$4,08 \cdot 10^{-5}$	$2,59 \cdot 10^{-5}$	$0,17 \cdot 10^4$
14	8,01	0,028	$10,00 \cdot 10^{-5}$	$3,96 \cdot 10^{-5}$	$6,04 \cdot 10^{-5}$	$0,11 \cdot 10^4$
15	8,05	0,167	$10,00 \cdot 10^{-5}$	$6,96 \cdot 10^{-5}$	$3,04 \cdot 10^{-5}$	$0,10 \cdot 10^4$

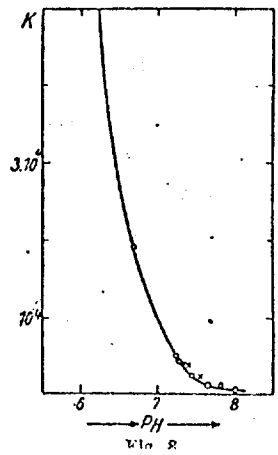
Die Abhängigkeit der Größe K vom p_H ist, unter Verwendung der in Tab. 4 wiedergegebenen Werte, in Fig. 8 aufgetragen. Die mit $\times$ bezeichneten Punkte entsprechen den Versuchen 1—4, die übrigen Punkte den folgenden Versuchen der Tab. 4.

Der numerische Wert der Gleichgewichtskonstante ist von der gleichen Größenordnung wie bei den übrigen bekannten reversiblen Dehydrasesystemen; z. B. ist bei $p_H = 7,26$

$$K = 0,55 \cdot 10^4.$$

Dies bedeutet, daß das Gleichgewicht der Reaktion (3) sehr weit nach links verschoben ist. Demnach muß das System im biologischen Milieu besonders geeignet sein zur Katalyse der in

der Richtung von rechts nach links verlaufenden Reaktion, der „hydrierenden Aminierung" der Ketoglutarsäure.



Abhängigkeit der Gleichgewichtskonstante K vom pH.

III. Die „hydrierende Aminierung" der Ketoglutarsäure.

Der folgende Abschnitt gibt Aufschluß über den Mechanismus der enzymatischen Glutaminsäuresynthese durch hydrierende Aminierung von Ketoglutarsäure, deren Möglichkeit sich schon aus den vorangehenden Abschnitten ergibt.

Die reduktive Synthese wurde vollzogen

1. mittels Dihydro-cozymase als Wasserstoffdonator, wobei das Verschwinden derselben spektrophotometrisch verfolgt und die gebildete Glutaminsäure analytisch bestimmt wurde, und

2. durch Vorlagerung eines Wasserstoff-liefernden Dehydrasesystems, aus welchem Cozymase den Wasserstoff auf das System der Iminoglutarsäure-Reduktion überträgt.

1. Hydrierung von Iminoglutarsäure durch Dihydro-cozymase.

a) Spektrophotometrische Versuche. — Die Umkehrung der gekoppelten Reaktion (3) + (4) ergibt sich aus den Versuchen, die in Fig. 9 und 10 dargestellt sind.

In Fig. 9 ist zunächst die Hydrierung von Cozymase durch das System Glutaminsäure + Apodehydrase vollzogen worden. Die Hydrierung verläuft bei dem angewandten p_H zu etwa 90%, was darauf hindeutet, daß die nach Gleichung (3) entstandene Iminosäure sehr weitgend in NH_3 und Ketoglutarsäure gespalten ist [entsprechend Gleichung (4)]. Wurde nun (bei a)

der Richtung von rechts nach links verlaufenden Reaktion, der „hydrierenden Aminierung" der Ketoglutarsäure.

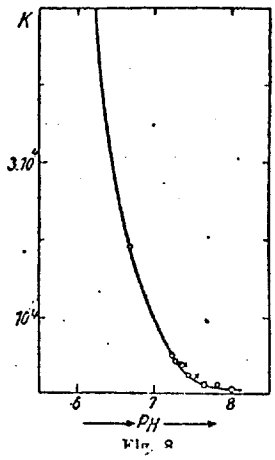


Fig. 8

Abhängigkeit der Gleichgewichtskonstante K vom p_H.

III. Die „hydrierende Aminierung" der Ketoglutarsäure.

Der folgende Abschnitt gibt Aufschluß über den Mechanismus der enzymatischen Glutaminsäuresynthese durch hydrierende Aminierung von Ketoglutarsäure, deren Möglichkeit sich schon aus den vorangehenden Abschnitten ergibt.

Die reduktive Synthese wurde vollzogen

1. mittels Dihydro-cozymase als Wasserstoffdonator, wobei das Verschwinden derselben spektrophotometrisch verfolgt und die gebildete Glutaminsäure analytisch bestimmt wurde, und

2. durch Vorlagerung eines Wasserstoff-liefernden Dehydrasesystems, aus welchem Cozymase den Wasserstoff auf das System der Iminoglutarsäure-Reduktion überträgt.

1. Hydrierung von Iminoglutarsäure durch Dihydro-cozymase.

a) Spektrophotometrische Versuche. — Die Umkehrung der gekoppelten Reaktion (3) + (4) ergibt sich aus den Versuchen, die in Fig. 9 und 10 dargestellt sind.

In Fig. 9 ist zunächst die Hydrierung von Cozymase durch das System Glutaminsäure + Apodehydrase vollzogen worden. Die Hydrierung verläuft bei dem angewandten p_H zu etwa 90 %, was darauf hindeutet, daß die nach Gleichung (3) entstandene Iminosäure sehr weitgend in NH_3 und Ketoglutarsäure gespalten ist [entsprechend Gleichung (4)]. Wurde nun (bei a)

eine gewisse Menge Ketoglutarsäure zugesetzt, so verschwand etwas CoH_2 und es stellte sich ein neues Gleichgewicht ein. Dieses ist bedingt durch Verschiebung des Gleichgewichts (4) nach links, d. h. eine Mehrbildung von Iminoglutarsäure, welche ihrerseits eine Verschiebung des Gleichgewichts (3) nach links zur Folge hat. Wurde hierauf NH_4Cl zugesetzt, so fiel die CoH_2-Extinktion rasch weiter ab. Dieser Effekt wird ebenfalls aus den Gleichungen (3) und (4) verständlich. Die Folge der vorgenommenen Zusätze von Ketoglutarsäure und NH_3 ist ja die Bildung von überschüssiger Iminoglutarsäure, welche ausreicht, um das Gleichgewicht (3) praktisch vollständig nach links zu verschieben trotz der hohen Glutaminsäurekonzentration.

Fig. 9.

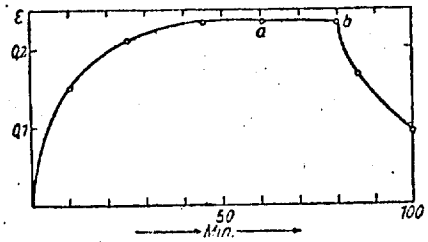


Fig. 10.

Fig. 9. Umkehrung der Glutaminsäuredehydrierung.
Versuchsansatz wie zu Fig. 6, Kurve I; nach Erreichung des Gleichgewichts (bei *a*) Zusatz von 0,05 ccm m/10-Ketoglutarat, bei *b* 0,05 ccm NH_4Cl.

Fig. 10. Umkehrung der Glutaminsäuredehydrierung.
Wie Fig. 9, jedoch bei *a* Zusatz von 0,05 ccm 0,1 m-NH_4Cl, bei *b* 0,05 ccm 0,1 m-Ketoglutarat.

Fig. 10 zeigt den analogen Versuch, in welchem jedoch gegenüber (8) die Reihenfolge des Zusatzes der beiden Iminosäurekomponenten vertauscht ist: der Zusatz von NH_4Cl allein bewirkt hier keine meßbare Änderung der Extinktion. Offenbar ist der Anteil des als NH_3, nicht als $NH_4^{\cdot}$-Ion vorliegenden Ammoniaks zu klein, um das Gleichgewicht merklich zu beeinflussen. Dies erfolgt dagegen sofort nach Zusatz der zweiten Komponente, der Ketoglutarsäure.

Die einfachste Versuchsanordnung zum Studium der Synthesereaktion besteht in der Dehydrierung von Dihydro-cozymase durch die Iminosäure in Gegenwart der Apodehydrase. Sie ist in Fig. 11 dargestellt, welche gleichzeitig die p_H-Abhängigkeit der hydrierenden Aminierung wiedergibt.

Die Versuche zu Fig. 11 sind folgendermaßen ausgeführt: Nachdem die Extinktion des Gemisches von Puffer und Apodehydrase gemessen war, wurde CoH_2 zugesetzt und deren Extinktion bestimmt. Letztere blieb dann nahezu konstant, da ein CoH_2-dehydrierendes, autoxydables Enzym im Apodehydrasepräparat nicht enthalten ist (vgl. S. 71). Hierauf wurde, z. B. im Versuch zu Kurve IV, α-Ketoglutarsäure zugesetzt, worauf innerhalb der

folgenden 10 Minuten kein Abfall der Extinktion erfolgte; wurde aber nun NH_4Cl zugefügt, so verschwand die CoH_2 rasch und vollständig. Wurde in einem analogen Versuch (z. B. Kurve II) die Reihenfolge der Zusätze vertauscht, so trat bei Zusatz von NH_4Cl keine Änderung, beim darauffolgenden Zusatz von Ketoglutarsäure der vollständige Abfall ein.

Aus diesen Befunden geht auch deutlich hervor, daß das Leberenzym eine Apodehydrase, welche zusammen mit Dihydrocozymase Ketoglutarsäure zu Oxyglutarsäure reduziert, nicht

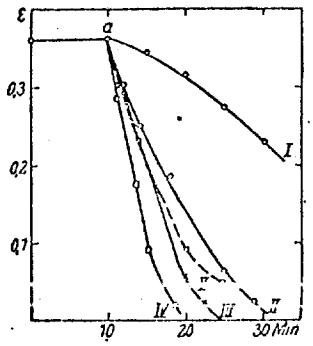


Fig. 11.

Dehydrierung von Dihydro-cozymase durch Ketoglutarsäure + NH_3 (Iminoglutarsäure). p_H-Abhängigkeit.

4,0 ccm m/2-Phosphatpuffer von verschiedenem p_H; 0,1 ccm Leberenzym; 0,15 ccm CoH_2; 0,1 ccm m/10-Ketoglutarat. Bei *a* Zusatz von 0,2 ccm m/10-NH_4Cl. Das p_H betrug im Versuch zu Kurve I: 5,85, Kurve II: 6,85, Kurve III: 7,1, Kurve IV: 7,15, Kurve V: 8,4. (Bei Kurve II ist die Reihenfolge der Zusätze von Ketoglutarat und NH_4Cl vertauscht: zuerst NH_4Cl, bei *a* Ketoglutarat.)

enthält. Da die Enzymlösung jedoch Lactico- und Malico-apodehydrase enthält, so ergibt sich gleichzeitig, daß die Wirkung dieser Apodehydrasen sich nicht auf α-Oxyglutarsäure erstreckt. Von Weil-Malherbe[31]) wurde vor kurzem eine nicht von Cozymase abhängige α-Oxyglutarsäure-Dehydrase aus Herzmuskel beschrieben, die, wie von dem Autor aus indirekten Versuchen geschlossen wird, allerdings imstande sein soll, den Wasserstoff von CoH_2 auf Ketoglutarsäure zu übertragen.

In unserem System *tritt der CoH_2-Schwund nur bei gleichzeitiger Gegenwart von Ketoglutarsäure und NH_3 ein. Die hier verlaufende Reaktion ist demnach die an der Glutaminsäure-apodehydrase katalysierte Hydrierung der α-Iminoglutarsäure durch CoH_2 zu Glutaminsäure.*

---

[31]) Biochemic. J. 31, 2080 (1937).

p_H-Abhängigkeit der hydrierenden Aminierung. Ein Vergleich der in Fig. 11 dargestellten Kurven miteinander zeigt die Abhängigkeit der Synthesegeschwindigkeit vom p_H. Man sieht, daß die Geschwindigkeit am kleinsten ist bei $p_H = 5{,}85$, am größten bei $p_H = 7{,}45$, jedoch wieder deutlich geringer bei $p_H = 8{,}30$. In Analogie zu den bisher bekannten enzymatischen Hydrierungen mit CoH_2, wie z. B. der Hydrierung von Acetaldehyd oder Brenztraubensäure durch Alkohol- bzw. Lactico-Dehydrase, sollte man auch hier eine stetige Abnahme der Hydrierungsgeschwindigkeit mit steigendem p_H erwarten. Die kompliziertere p_H-Abhängigkeit der vorliegenden Reaktion findet ihre einfache Erklärung in der Tatsache, daß hier 2 Reaktionen von gerade entgegengesetzter p_H-Abhängigkeit hintereinander geschaltet sind:

Die Bildung der Iminosäure

$$\text{(4a)}\quad \text{Ketoglutarsäure} + NH_3 \rightleftharpoons \text{Iminoglutarsäure} + H_2O$$

ist begünstigt bei höheren p_H-Werten, wo das freie NH_3 gegenüber NH_4-Ion in den Vordergrund tritt. Die Verlangsamung der Reaktion bei saurem p_H (Kurve IV → V) wird dadurch erklärt.

Auf der anderen Seite ist die eigentliche Hydrierungsreaktion

$$\text{(3a)}\quad \text{Iminoglutarsäure} + CoH_2 \xrightleftharpoons{\text{Apodehydrase}} \text{Glutaminsäure} + Co$$

bei saurer Reaktion begünstigt. Dies erklärt die Verlangsamung der Reaktion auf der alkalischen Seite (Kurve IV → V).

Die Einstellung des Gleichgewichtes (4a) erfolgt, wie ebenfalls aus den Kurven der Fig. 11 hervorgeht, sehr rasch. Die Reaktion setzt jeweils nach Zusatz der zweiten Komponente sofort mit maximaler Geschwindigkeit ein. Lediglich bei dem niedrigsten p_H-Wert macht sich eine langsamere Einstellung des Gleichgewichtes (4a) bemerkbar, indem die Reaktion langsam beginnt und mit steigender Geschwindigkeit weiterläuft.

Fig. 12 zeigt die Abhängigkeit der Reaktionsgeschwindigkeit von der Ammonsalzkonzentration bei konstanter Ketoglutarsäuremenge. In den Versuchen zu Fig. 13 ist die Ammonsalzmenge konstant gehalten und die Ketoglutarsäuremenge variiert. In allen Versuchen sind zu Beginn $13{,}5 \cdot 10^{-5}$ Millimol Dihydrocozymase vorhanden. Der Versuch zu Kurve I (Fig. 13) enthält $7 \cdot 10^{-4}$ Millimol Ketoglutarsäure und man sieht, daß diese Menge noch nicht ausreicht, um eine vollständige Dehydrierung der CoH_2 zu bewirken, sondern daß es zur Einstellung eines Gleichgewichts

kommt. Die 10fache Menge Ketoglutarsäure (Kurve II) genügt aber bereits, um die gesamte Dihydro-cozymase zu reoxydieren.

b) Nachweis der gebildeten Glutaminsäure. Der durch die vorangehenden spektrometrischen Versuche geführte Nachweis der reduktiven Aminierung von Ketoglutarsäure wurde durch die analytische Bestimmung des gebildeten Aminostickstoffs ergänzt.

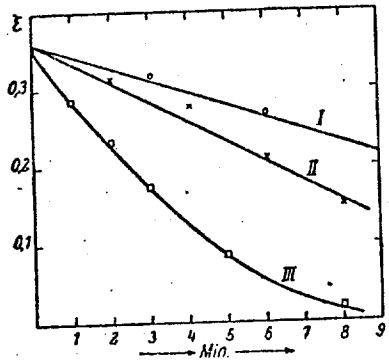


Fig. 12.

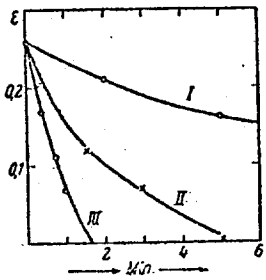


Fig. 13.

Fig. 12. Einfluß der NH_4Cl-Konzentration.

Versuchsansatz wie zu Kurve III in Fig. 11, jedoch wechselnde Konzentration. Kurve I: 0,0011 mol., Kurve II: 0,0022 mol., Kurve III: 0,0045 mol.

Fig. 13. Einfluß der Ketoglutarsäure-Konzentration.

Versuchsansatz wie zu Fig. 11 und 12, jedoch NH_4Cl zuerst zugesetzt, in allen Versuchen 0,0022 mol. (Gesamtkonz.). Bei Beginn der Kurven Zusatz der Ketoglutarsäure. Kurve I: $7 \cdot 10^{-4}$ Millimol, Kurve II: $7 \cdot 10^{-3}$ Millimol, Kurve III: $7 \cdot 10^{-2}$ Millimol.

Zu diesem Zweck mußte natürlich der Reaktionsansatz vergrößert werden.

Tab. 5 gibt die Versuchsansätze und Ergebnisse eines typischen Versuches wieder.

Tabelle 5.

Hydrierende Aminierung von Ketoglutarsäure; Dihydro-cozymase als Wasserstoffdonator.

Reaktionsmischung	Apodehydrase („Rohlösung")	CoH_2	Na-Ketoglut.	NH_4Cl	m/2-Phosph.-puffer $p_H = 7,6$	H_2O	mg Aminostickstoff		
							Gef.	Gebild. (I–II)	Ber.
I) Versuch	0,5 ccm	20 mg	20 mg	8 mg	0,5 ccm	2,5 ccm	0,85	0,23	0,42
II) Kontrolle	0,5 ccm	20 mg	—	8 mg	0,5 ccm	2,75 ccm	0,62	—	—

Zur Ausführung des Versuches zu Tab. 5 ist folgendes zu bemerken:

Die Dihydro-cozymase wurde in 4 Portionen, in Abständen von je 10 Minuten dem übrigen Reaktionsgemisch zugesetzt. Durch spektrophoto-

metrische Kontrolle abpipettierter Proben wurde festgestellt, daß im Versuch (I) die CoH_2 schon jeweils nach wenigen Minuten verschwunden war: es trat auf Zusatz von Acetaldehyd kein Extinktionsabfall ein. Im Kontrollversuch (II) ohne Ketoglutarsäure lag jedoch die Hauptmenge der eingesetzten CoH_2 noch am Ende des Versuches unverändert vor. Um für die Bestimmung des Aminostickstoffs in Versuch und Kontrolle gleiche Voraussetzungen zu schaffen, wurde nach Ablauf der Versuchszeit die Dihydrocozymase des Kontrollansatzes durch Zusatz eines Überschusses von Acetaldehyd (0,5 ccm m/2-Lösung) sowie von 0,1 ccm einer Alkohol-apodehydrase-Lösung aus Hefe zu Cozymase reoxydiert. Die gleichen Zusätze wurden beim Versuchsansatz vorgenommen. Unverbrauchtes Ammoniak und Acetaldehyd wurden dann aus Versuchs- und Kontrollansatz nach Alkalisieren mit NaOH auf $p_H = 11$ im Vakuum bei 30° quantitativ abdestilliert, worauf die van Slyke-Bestimmung vorgenommen wurde. Der in der Tabelle angegebene berechnete Wert für den Aminostickstoff bezieht sich auf eine Ausbeute von 1 Mol. Glutaminsäure pro 1 Mol. CoH_2.

In mehreren Versuchen wurden so etwa 50% derjenigen Menge an Aminostickstoff gefunden, die auf Grund der eingesetzten Dihydro-cozymase zu erwarten waren.

Daß die Ausbeute an Glutaminsäure nicht quantitativ ist, kann verschiedene Ursachen haben; z. B. ist daran zu denken, daß die gebildete Glutaminsäure weiteren enzymatischen Umwandlungen unterliegt (Peptidbildung?)

2. Hydrierung von Iminoglutarsäure durch Wasserstoff-liefernde Dehydrasesysteme.

Mit der voranstehend beschriebenen Hydrierung der Iminoglutarsäure durch Dihydro-cozymase ist unseres Erachtens der biologische Weg der Glutaminsäuresynthese experimentell realisiert. Jedoch wird in vivo die Dihydro-cozymase, welche in diesen Versuchen direkt als Wasserstoffdonator verwendet wurde, durch ein Cozymase-bedingtes Dehydrasesystem ständig nachgeliefert werden. Das heißt, die Glutaminsäuresynthese kommt dadurch zustande, daß das reversible System $Co \rightleftharpoons CoH_2$ den Wasserstoff aus einem Dehydrasesystem, dessen Substrat verschieden sein kann, auf das System der Iminoglutarsäure-Reduktase (Glutaminsäure-Dehydrase) überträgt.

Wir haben auch diese Koppelung von Wasserstoff-liefernder Dehydrierung und reduktiver Aminierung experimentell verwirklicht. Als „Donatorsystem" verwendeten wir sowohl das System Alkohol + Alkoholdehydrase wie das System Glucose + Glucosedehydrase. In beiden Fällen wurde in Gegenwart von Ketoglutarsäure und NH_3 (sowie Leberenzym und Cozymase) Aminostickstoff

gebildet. Fehlten entweder Ketoglutarsäure oder Ammoniak, so blieb die Aminostickstoffbildung aus (Tab. 6 und 7).

Tabelle 6.

Hydrierende Aminierung von Ketoglutarsäure; Alkohol als Wasserstoffdonator. Apodehydrase aus Leber und Phosphatpuffer wie zu Tab. 5. Gesamtvolumen 3,4 ccm.

Reaktionsgemisch	Äthylalkohol	Alkoholapodehydrase	Cozymase	Natrium-Ketoglutarat	NH_4Cl	mg Aminostickstoff	
						Gef.	Gebildet
I) Versuch	150 mg	12 mg	4 mg	20 mg	20 mg	1,04	(I—II): 0,59 (I—III): 0,55
II) Kontrolle	150 mg	12 mg	4 mg	—	20 mg	0,45	—
III) Kontrolle	150 mg	12 mg	4 mg	20 mg	—	0,49	—

Zur Ausführung des Versuches (Tab. 6): Um eine kräftige Wasserstofflieferung zu erzielen, wurde dem Reaktionsgemisch Alkohol-apodehydrase aus Hefe (Acetonfällung aus Lebedewsaft) zugesetzt. Die Reaktionsmischung befand sich in einem kleinen Rundkolben, der in Wasser von 30° tauchte und über eine Vorlage (mit Eintauchrohr) an die Wasserstrahlpumpe angeschlossen war. Um die Dehydrierung des Alkohols nicht durch den entstehenden Acetaldehyd zu hemmen, wurde in Abständen von 20—30 Minuten für 5 Minuten Vakuum angelegt. In der Vorlage befand sich eine salzsaure Lösung von 2,4-Dinitrophenylhydrazin. Beim Versuchsansatz machte sich das Zustandekommen der Oxydoreduktion dadurch bemerkbar, daß der gebildete und in die Vorlage überdestillierte Acetaldehyd als Hydrazon ausfiel. Im Kontrollversuch wurden nur Spuren von Acetaldehyd gebildet, da der Wasserstoffacceptor (Iminoglutarsäure) fehlte und eine aerobe Oxydation des Alkohols mangels Flavinenzym nicht in größerem Ausmaße stattfand. Einer Verminderung der Alkoholkonzentration durch die Destillationen wurde durch nachträgliche Zusätze von Alkohol vorgebeugt. Nach 90 Minuten Reaktionsdauer wurde mit NaOH alkalisiert, das überschüssige Ammoniak im Vakuum bei 30° vollständig abdestilliert (Schwefelsäure und Indicator in der Vorlage!) und hierauf die van Slyke-Bestimmung vorgenommen.

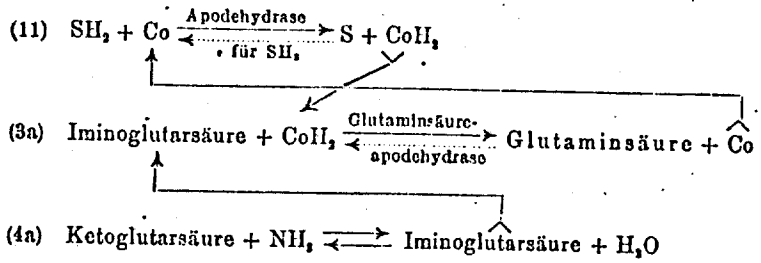
Der gebildete Aminostickstoff entspricht etwa 5 mg Glutaminsäure.

Tabelle 7.

Hydrierende Aminierung von Ketoglutarsäure; Glucose als Wasserstoffdonator. Enzympräparat: 8 g zerkleinerte Rattenleber mit 4 ccm Wasser im Mörser verrieben und zentrifugiert; die Flüssigkeit wurde als Enzymlösung verwendet. Reaktionsdauer 3 Stunden bei 30°. Die Versuchsmischung enthält 0,5 ccm Phosphatpuffer $p_H = 7,6$, Gesamtvolumen 2,5 ccm.

Reaktionsmischung	Enzym	Glucose	Cozymase	Ketoglutarsäure	NH_4Cl	mg Aminostickstoff	
						Gef.	Gebildet (I—II)
I) Versuch	0,5 ccm	50 mg	2 mg	5 mg	10 mg	0,85	0,24
II) Kontrolle	0,5 ccm	50 mg	2 mg	—	10 mg	0,61	—

Die durch die voranstehenden Versuche verwirklichte Synthese der Glutaminsäure kann schematisch folgendermaßen formuliert werden (SH_2 = Wasserstoffdonator, S = Dehydrierungsprodukt):



Sowohl Alkohol- wie Glucose-apodehydrase sind in Leber vorhanden, ja im tierischen Organismus sogar für Leber charakteristisch, und können deshalb — hauptsächlich Glucosedehydrase — gerade in diesem Organ als Wasserstoff-liefernde Systeme für die Glutaminsäuresynthese tatsächlich in Betracht kommen.

IV. Spezifität der Glutaminsäure-apodehydrase.

1. Codehydrase-Spezifität.

Alle bisher beschriebenen Versuche sind mit Cozymase (Codehydrase I) ausgeführt. Früher angestellte Versuche mit der Methylenblaumethodik schienen darauf hinzuweisen, daß Codehydrase II die Cozymase nicht ersetzen könne[19]). Dagegen lieferten spätere Versuche Ergebnisse, die auf eine Wirksamkeit beider Codehydrasen deuteten. Um die Frage der Codehydrase-Spezifität zu entscheiden, mußte aber vor allem die Möglichkeit ausgeschlossen werden, daß im Reaktionssystem eine Umwandlung von Codehydrase II in Codehydrase I (durch Dephosphorylierung) stattfindet. Wie aus der Beschreibung des Versuches zu Fig. 14 hervorgeht, gelang die Lösung der Frage mit Hilfe der spektrophotometrischen Methodik:

Versuch zu Kurve I: Codehydrase II (20%iges Präparat aus roten Pferdeblutzellen) wurde mittels Hexosemonophosphat und Hexosemonophosphat-apodehydrase aus Hefe zur Dihydroform reduziert; die Hydrierung wurde spektrophotometrisch verfolgt. Nach beendeter Reaktion wurde die Apodehydrase durch 2 Minuten langes Erhitzen auf 70° inaktiviert und die Lösung vom ausgefallenen Eiweiß durch Filtration befreit. Die somit erhaltene Lösung von CoH_2 II besaß eine Extinktion $\varepsilon = 0{,}3$ bei $\lambda = 334\ m\mu$. Hierauf wurden 0,05 ccm Leberenzym (verdünnte „Rohlösung") zugesetzt (Beginn der Kurve), wodurch die Extinktion infolge der Eigenfarbe der Enzymlösung um einen gewissen Betrag anstieg, für welchen Kurve I wie

alle übrigen Kurven korrigiert sind. Während der nächsten 6 Minuten trat kein meßbarer Extinktionsabfall ein, da die Enzymlösung kein Flavinenzym enthält (vgl. S. 71). Bei *a* wurden nun 0,05 ccm m/2-Acetaldehyd zugesetzt. Die Extinktion blieb auch während der folgenden 6 Minuten konstant. Dies bedeutet, daß während der nunmehr 12 Minuten dauernden Inkubation der CoH_2 II mit dem Leberenzym eine Dephosphorylierung zu CoH_2 I nicht stattgefunden hat. Es hätte nämlich sonst durch den Acetaldehyd eine Dehydrierung der CoH_2 I, d. h. ein Extinktionsabfall, eintreten müssen, da das Leberenzym auch Alkohol-apodehydrase enthält, wie durch den Kontrollversuch (Kurve III) bewiesen wird. Auch auf Zusatz von Ketoglutarsäure (bei *b*) blieb die Extinktion konstant. Sie fiel jedoch ab, als hierauf (bei *c*) NH_4Cl zugesetzt wurde. Die Größe des Abfalls entsprach ziemlich genau dem bei der Robisonester-Dehydrierung erhaltenen Anstieg, d. h. der vorhandenen Dihydro-codehydrase II.

Über das Verhältnis der Reaktionsgeschwindigkeiten mit CoH_2 I und CoH_2 II gibt Fig. 14 keinen Aufschluß, da im Vergleichsversuch mit CoH_2 I (Kurve II) ein aktiveres Leberenzym

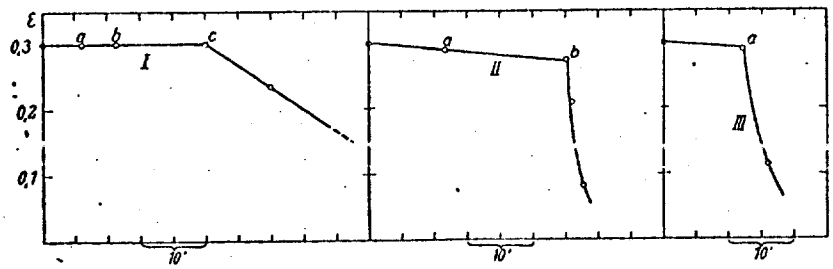


Fig. 14.

Codehydrase II als Co-Enzym der Glutaminsäuredehydrase.

Versuch zu Kurve I: Codehydrase II wird mit Robisonester und Hexosemonophosphatapodehydrase in CoH_2 II übergeführt und die Apodehydrase durch Erhitzen und Filtrieren abgetrennt. 3,0 ccm dieser CoH_2 II-Lösung werden gemischt mit 0,4 ccm m/2-Phosphatpuffer, p_H = 7,4 und 0,05 ccm Leberenzym („Rohlösung"). Der Beginn der Kurve I gibt die Extinktion der CoH_2 II in dieser Mischung an. Bei *a* Zusatz von 0,05 ccm m/2-Acetaldehyd, bei *b* 0,05 ccm m/10-Ketoglutarsäure, bei *c* 0,05 ccm m-NH_4Cl. — Kurve II: Beginn der Kurve Reaktionsmischung wie bei I, jedoch CoH_2 I statt CoH_2 II. Bei *a* Ketoglutarsäure, bei *b* NH_4Cl. — Kurve III: Beginn wie Kurve II, bei *a* Acetaldehyd.

verwendet worden ist als im Versuch mit CoH_2 II (Kurve I). Weitere Versuche deuten jedoch darauf hin, daß die Reaktion unter vergleichbaren Bedingungen mit beiden Co-Enzymen etwa gleich rasch verläuft.

Es ergibt sich also, daß beide Codehydrasen I und II als Co-Enzym der Glutaminsäure-apodehydrase wirksam sind.

Dieser Umstand kann zwei verschiedene Ursachen haben:

1. Die Glutaminsäure-apodehydrase bildet mit beiden Codehydrasen wirksame Holodehydrasen.

2. Leber enthält zwei verschiedene Glutaminsäure-apodehydrasen, von denen die eine spezifisch auf Codehydrase I, die andere spezifisch auf Codehydrase II eingestellt ist.

Welche dieser beiden Möglichkeiten zutrifft, kann noch nicht entschieden werden. Die hier vorliegenden Verhältnisse sind jedenfalls ganz analog denen bei der Glucosedehydrase der Leber (vgl. z. B.[22]). Die Alkohol-apodehydrase der Leber ist dagegen, wie Fig. 14 zeigt, streng spezifisch auf Codehydrase I eingestellt.

Die Glutaminsäure-apodehydrase der höheren Pflanzen, die ebenfalls mit Co I die wirksame Holodehydrase bildet, ist im Gegensatz zur tierischen Dehydrase mit Co II nicht wirksam. Andererseits sind die Glutaminsäure-apodehydrasen in Hefe und Bacterium coli streng spezifisch auf Co II eingestellt.

Aus diesen Tatsachen geht hervor, daß für die Fähigkeit der Codehydrasen, sich mit Apodehydrasen zum wirksamen Enzym zu verbinden, wahrscheinlich nicht allein ihre Basizität — im Verhältnis zur Basizität der Apodehydrase — ausschlaggebend sein kann, sondern daß auch andere Faktoren, wohl räumlicher Natur, eine Rolle spielen.

2. Substrat-Spezifität

Im Thunberg-Versuch wurde geprüft, ob außer l(+)-Glutaminsäure auch andere Aminosäuren als Wasserstoffdonatoren für die Leberdehydrase fungieren können, in allen Fällen jedoch mit negativem Ergebnis. Geprüft wurden: Glykokoll, d,l-Alanin, l(+)-Norvalin, l(+)Norleucin, l(—)-Leucin, d,l-Valin, l(+)-Arginin, l(—)-Asparaginsäure, d,l-Asparaginsäure, Asparagin, d(—)-Glutaminsäure, l(—)-Tyrosin, Phenylalanin, d,l-Prolin, l-Histidin. Ferner Glycyl-glycin, d,l-Alanyl-glycin, l-Leucyl-glycin, Glutathion. Höchstens Andeutungen einer Donatorwirkung zeigten Glykokoll, Alanin und Alanyl-glycin, aber es ist wahrscheinlich, daß hier nicht die Glutaminsäure-apodehydrase, sondern die Krebssche d-Aminosäure-dehydrase wirksam war.

Die Spezifität der tierischen Glutaminsäure-apodehydrase ist also in sterischer wie in konstitutioneller Hinsicht äußerst scharf ausgeprägt. Besonders auffallend ist, daß Asparaginsäure nicht angegriffen wird, die sich von der Glutaminsäure nur um eine Methylengruppe unterscheidet. Aber auch Aminosäuren mit 5 C-Atomen, wie Norvalin, Valin und Arginin, werden nicht dehydriert. Die Nichtangreifbarkeit des Norvalins, welches sich von der Glutaminsäure nur dadurch unterscheidet, daß die δ-Carboxyl-

gruppe durch Methyl ersetzt ist, zeigt, daß die Anwesenheit der beiden Carboxylgruppen in der C_5-Kette eine notwendige Voraussetzung für die Dehydrierbarkeit der Aminogruppe ist. So ist zu verstehen, daß auch Glutathion, in welchem die eine der beiden Carboxylgruppen der Glutaminsäure peptidartig gebunden ist, nicht dehydriert wird.

Die Nichtangreifbarkeit der genannten, der Glutaminsäure nahestehenden Verbindungen bedeutet jedoch offenbar nicht, daß diese Verbindungen überhaupt nicht von der Apodehydrase gebunden werden. Man findet nämlich, daß ein Zusatz von l(—)-Asparaginsäure im Thunberg-Versuch die Glutaminsäuredehydrierung hemmt, und es ist am einfachsten, diese Hemmung als eine kompetitive, d. h. als Substratverdrängung zu betrachten. Auch Dicarbonsäuren haben Affinität zur Apodehydrase, was daraus hervorgeht, daß sie die Glutaminsäuredehydrierung hemmen. Die Hemmung wird um so geringer, je kürzer die Kette ist (vgl. Tab. 8).

Tabelle 8.

Kompetitive Hemmung der Glutaminsäuredehydrierung. Thunberg-Methodik. Enzym: Verdünnte „Rohlösung". Reaktionsmischung wie zu Tab. 1. Von den Hemmungsstoffen werden je 0,25 ccm einer 0,5 mol. Lösung des Natriumsalzes angewandt. In der Tabelle sind an Stelle der Entfärbungszeiten die relativen Geschwindigkeiten angegeben, wobei die Geschwindigkeit der ungehemmten Glutaminsäuredehydrierung = 100 gesetzt ist.

Versuch Nr.	Zusatz	Relative Reaktionsgeschwindigkeit	
		mit Glutaminsäure	ohne Glutaminsäure
1, 2	—	100	13
3, 4	Glutarsäure	55	13
5, 6	Bernsteinsäure	74	14
7, 8	Malonsäure	96	10
9, 10	Asparaginsäure	45	8
11, 12	Valeriansäure	100	13

Die Spezifität der Glutaminsäure-apodehydrase bzw. des rohen Leberenzyms als solchem kommt nicht nur darin zum Ausdruck, daß keine andere Aminosäure als l(+)-Glutaminsäure dehydriert, sondern erwartungsgemäß auch darin, daß keine andere Ketosäure als α-Ketoglutarsäure hydrierend aminiert wird. Diese Form der Spezifitätsprüfung (Dehydrierung von CoH_2 durch die betreffende Ketosäure + NH_3) stößt allerdings insofern auf Schwierig-

keiten, als gewisse Ketosäuren, wie Brenztraubensäure, Oxalessigsäure, α-Ketobuttersäure*) imstande sind, mittels der im Leberenzym enthaltenen Oxysäuredehydrasen CoH_2 zu dehydrieren. In solchen Fällen wurde aber durch NH_3-Zusatz keine Beschleunigung der Reaktion beobachtet. γ-Phenyl-α-ketobuttersäure*), die sich von der Ketoglutarsäure nur dadurch unterscheidet, daß die eine COOH-Gruppe durch Phenyl ersetzt ist, vermag weder für sich allein noch in Gegenwart von NH_3 Dihydro-cozymase zu dehydrieren. Dieser Befund besitzt insofern besonderes Interesse, als er zeigt, daß die von Knoop entdeckte Bildung von N-Acetyl-γ-phenyl-α-amino-buttersäure aus der genannten Ketosäure im Tierkörper nicht an der Glutaminsäure-apodehydrase erfolgt, mit anderen Worten, daß die „acetylierende Aminierung" eines anderen enzymatischen Apparates bedarf.

Die Glutaminsäure-apodehydrase ist nicht identisch mit einer der bekannten Cozymase-bedingten Dehydrasen, was daraus hervorgeht, daß bei den verschiedenen Enzympräparaten keine Parallelität zwischen der Dehydrierungswirkung gegenüber verschiedenen Substraten besteht. Zum Beispiel enthalten gereinigte Glutaminsäuredehydrase-Präparate häufig keine Glucosedehydrase mehr, und andererseits können aus Leber nach der von Dixon und Lutwak-Mann[32]) für „Aldehydmutase" angegebenen Methode als Alkoholdehydrase wirksame Präparate[33]) hergestellt werden, die praktisch frei von Glutaminsäuredehydrase sind.

Die strenge Spezifität der Glutaminsäure-apodehydrase erstreckt sich nicht nur auf das Kohlenstoffgerüst, sondern auch auf die Amino- bzw. Iminogruppe: Verwendet man nämlich im spektrometrischen Versuch zur hydrierenden Aminierung der Ketoglutarsäure (vgl. Fig. 11) an Stelle des Ammonsalzes Monomethylamin, so tritt keine Dehydrierung der CoH_2 ein, d. h. N-Methyliminoglutarsäure wird nicht hydriert. Es ist von Interesse, daß die von Knoop durchgeführte Hydrierung der Iminosäuren mit nichtenzymatischen Reduktionsmitteln auch an dem Methyliminoderivat vollzogen werden konnte.

Auch andere Derivate bzw. Analoga der α-Iminoglutarsäure wurden an der Apodehydrase durch CoH_2 nicht hydriert, nämlich

*) Wir danken Herrn Everett für die Darstellung dieser Ketosäuren.

[32]) Biochemic. J. 31, 1347 (1937).

[33]) Adler, v. Euler u. Günther, Sv. Vet. Akad. Arkiv. f. Kemi 12 B, Nr. 54 (1938).

weder das Oxim noch das Semicarbazon noch das Hydrazon der Ketoglutarsäure.

Die Glutaminsäuredehydrase wird durch m/30-Natriumfluorid, m/100-Jodacetat und m/100-Natriumarsenit nicht gehemmt.

V. Die Verteilung der Glutaminsäure-apodehydrase im Tierkörper.

Für eine Beurteilung der biologischen Funktion des reversiblen Systems der Glutaminsäuredehydrase war eine Orientierung über den relativen Gehalt der verschiedenen Gewebe an der Apodehydrase von Interesse. Eine genaue quantitative Bestimmung des Apodehydrasegehaltes ist sehr schwer durchführbar, weil einerseits beim Arbeiten mit Gewebeschnitten Permeabilitätsverhältnisse eine unübersichtliche Rolle spielen und weil andererseits bei der Bestimmung in Extrakten das Ausmaß der Extrahierbarkeit sowie der Einfluß der Eigendonatoren in weiten Grenzen variieren können. Als Bestimmungsmethoden sind die Sauerstoffzehrungs- bzw. Mb-Reduktionsmethode nur mit großem Vorbehalt anwendbar, da die Geschwindigkeit hier nicht nur durch die Apodehydrase und das Co-Enzym, sondern auch durch den dazugehörigen Wasserstoffüberträger bestimmt ist.

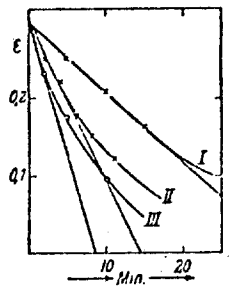


Fig. 15.

Abhängigkeit der Geschwindigkeit der CoH_2-Dehydrierung von der Enzymmenge.

2,5 ccm Wasser; 1,0 ccm m/2-Phosphatpuffer $p_H = 7,4$; 0,1 ccm Dihydro-cozymase, entsprech. etwa 0,1 mg; 0,1 ccm Enzym aus Frischleber, dargestellt wie zu Tab. 9 angegeben; 0,1 ccm m/10-α-Ketoglutarat. Während 5 Min. kein Extinktionsabfall, hierauf Zusatz von 0,2 ccm m-NH_4Cl (Anfangspunkt der Kurven). Die ursprüngliche Enzymsuspension war zu Versuch I 20 fach, zu Versuch II 10 fach, zu Versuch III 7,5 fach verdünnt, d. h. die Enzymmengen in den 3 Versuchen verhielten sich untereinander wie 1 : 2 : 2,6. Die Anfangsgeschwindigkeiten (vgl. die an den Anfangsast der Kurven gelegten Tangenten) verhalten sich wie 1 : 2 : 3,5. Bei den Vergleichsbestimmungen mit anderen Organen wurden stets solche Enzymmengen gewählt, die (wie Versuche I und II) über mehrere Minuten geradlinig verlaufende Kurven gaben, und dann stets Proportionalität zeigten.

Wir wählten als Extraktionsmethode das Zerreiben des fein zerkleinerten Gewebes mit sekundärem Phosphat und Filtrieren

der zerriebenen Zellmasse durch Verbandgaze, wodurch eine feine Suspension erhalten wird.

Zur Bestimmung der Apodehydrasekonzentration verwendeten wir die Tatsache, daß *die Geschwindigkeit, mit welcher Dihydrocozymase durch Iminoglutarsäure dehydriert wird, innerhalb gewisser Grenzen der Apodehydrasemenge proportional* ist, wie aus Fig. 15 hervorgeht.

In Tab. 9 sind die nach diesem Prinzip bestimmten relativen Konzentrationen an Glutaminsäure-apodehydrase in verschiedenen Geweben von Ratten angegeben.

Tabelle 9.

Verteilung der Glutaminsäure-apodehydrase im Rattenorganismus. Extraktion: 1 Gewichtsteil Gewebe wurde mit der Schere möglichst fein zerkleinert und mit 3 Gewichtsteilen m/10-Na_2HPO_4 und Quarzsand während 15 Minuten energisch verrieben. Nach $1^1/_2$ stündigem Stehen bei 4° wurde auf einem Büchnertrichter über doppelt gelegte Verbandgaze abgesaugt und ausgepreßt. — Das Prüfsystem enthält 0,2 ccm Dihydro-cozymase mit einer Extinktion $\varepsilon = 0{,}3$, 1,0 ccm m/2-Phosphatpuffer $p_H = 4{,}7$, 0,1 ccm Enzym (verschieden verdünnter Originalextrakt) und 2,5 ccm Wasser. Nach Messung der Extinktion werden 0,2 ccm m-NH_4Cl und 0,1 ccm 0,1 m-α-Ketoglutarsäure zugesetzt. Von jedem Gewebeextrakt wurden 2—3 verschiedene Konzentrationen geprüft und die erhaltenen Geschwindigkeiten des CoH_2-Schwundes waren — wenn die Enzymmenge nicht zu hoch gewählt war — stets proportional der Enzymmenge.

Extrakte aus manchen Geweben enthielten Wasserstoffacceptoren, welche einen Verbrauch an CoH_2 bereits ohne Zusatz von Ketoglutarsäure + NH_4Cl bewirkten. Solche Enzyme wurden durch 30—120 Minuten lange Dialyse durch Kollodium gereinigt. In der Tabelle sind die auf gleiche Mengen der verschiedenen Gewebeextrakte berechneten relativen Geschwindigkeiten des CoH_2-Schwundes wiedergegeben, wobei der mit Leberextrakt erhaltene Wert = 100 gesetzt ist.

Gewebe	Relative Apodehydrasekonzentrationen
Leber	100
Niere	41
Darmwand	7
Skelettmuskel	6
Gehirn	6
Herzmuskel	< 5
Milz	< 5
Jensen-Sarkom	6

Wenn wir die Annahme machen, daß die mit den möglichst gleichmäßig hergestellten Extrakten erhaltenen Werte ein im großen und ganzen richtiges Bild über die relativen Konzentrationen in den intakten Geweben darstellen, so ergibt sich, daß

Leber in weitem Abstand am meisten Dehydrase enthält, während Niere nur 40 % und die anderen Organe erheblich weniger Dehydrase enthalten.

Es ist interessant, die Verteilung der Glutaminsäuredehydrase mit der Verteilung der Krebsschen Aminosäuredehydrasen zu vergleichen. Die letzteren sind am stärksten in Niere und Leber, und etwas schwächer in Darmschleimhaut, während die übrigen Gewebe wahrscheinlich gar keine Dehydrase enthalten. Wenn wir, wie im folgenden Abschnitt des näheren diskutiert werden soll, die Funktion der Glutaminsäuredehydrase in der Katalyse der Glutaminsäuresynthese aus Ketoglutarsäure und Ammoniak erblicken, so ergibt sich aus den oben gefundenen Zahlen, daß diese Synthese zwar vorzugsweise in Leber und Niere, aber in geringerem Ausmaß auch in den meisten anderen Geweben stattfinden kann, während der oxydative Abbau der Aminosäuren in den meisten Geweben wohl überhaupt nicht zustande kommt, sondern in einigen wenigen lokalisiert ist.

D. Über die physiologische Rolle des Systems der Glutaminsäuredehydrase.

Wie sich aus der vorliegenden Arbeit ergibt, verfügt der Organismus in der „Glutaminsäuredehydrase" über ein Enzym, welches freies NH_3 an Ketoglutarsäure reduktiv fixiert unter Bildung von Glutaminsäure. Das bei physiologischem p_H weit nach der Seite der Glutaminsäure verschobene Gleichgewicht der reversiblen Dehydrasereaktion („Acceptorsystem"!) macht die Annahme, daß die Synthesereaktion die in der Zelle bevorzugte Richtung darstellt, sehr wahrscheinlich. Die Reaktion besteht in einer Hydrierung der α-Iminoglutarsäure durch Dihydro-codehydrase, und ihr Zustandekommen in der Zelle ist demnach nicht nur abhängig von der Anwesenheit von Ketoglutarsäure und NH_3, sondern auch von der Lieferung des Wasserstoffs in Form der Dihydro-codehydrase. Wir fanden, daß die Apodehydrase sowohl CoH_3 I wie auch CoH_2 II als Wasserstoff-beladene prosthetische Gruppe zu verwerten vermag. Somit wird der für die Reduktion der Iminoglutarsäure notwendige Wasserstoff stets in dem Maße zur Verfügung stehen, wie er durch die Dehydrierung von Nährstoffen mittels Co I- oder Co II-bedingter Dehydrasesysteme (Triosephosphat, Glucose usw.) geliefert wird. Die Tatsache, daß die hydrierten Codehydrasen mit den betreffenden Apodehydrasen nicht fest verbunden sind, sondern mit ihnen im Dissoziationsgleichgewicht stehen, hat eine große Beweglichkeit des labilen Wasserstoffs zur Folge und gewährleistet eine rasche Zuleitung desselben zum System der Glutaminsäuresynthese.

Die Glutaminsäure-apodehydrase wurde in allen bisher untersuchten Geweben (der Ratte) aufgefunden, ein Zeichen dafür, daß die Glutaminsäuresynthese eine allgemeine Zellreaktion ist. Die überragend hohe Konzentration in der Leber deutet aber darauf hin, daß dieses Organ der Hauptort der Synthese ist.

Der Mechanismus der enzymatischen Glutaminsäuresynthese, der in der vorliegenden Arbeit aufgeklärt wurde, bestätigt die Hypothese von Knoop, daß die Bildung der Aminosäuren im Tierkörper durch reduktive Aminierung der Ketosäuren, d. h. Reduktion der Iminosäuren erfolgen könne, eine Hypothese, die von Knoop und Oesterlin[7]) durch rein chemische Modellversuche gestützt wurde.

Die enzymatische Aminierung der Ketoglutarsäure an der Glutaminsäure-apodehydrase ist jedoch gegenüber der rein chemischen durch ihre Spezifität ausgezeichnet:

a) Von allen Ketosäuren wird von dieser Dehydrase nur α-Ketoglutarsäure als NH_3-Acceptor verwendet.

b) Nur NH_3, nicht monosubstituiertes NH_3 wird verwertet.

c) Als Reduktionsmittel verwendet das Enzym nur die hydrierten Codehydrasen I und II.

Apodehydrasen, die mit Hilfe von CoH_2 auch andere Ketosäuren außer Ketoglutarsäure reduktiv aminieren, haben wir nicht angetroffen.

Es wäre von Interesse zu untersuchen, was die bei verschiedenen Lebewesen verschiedene Spezifität der Glutaminsäure-apodehydrase in bezug auf die Codehydrase bedeutet. Die gleichzeitige Verwertbarkeit von Co I und Co II in der Leber verleiht jedenfalls der Iminoglutarsäure eine erhöhte Konkurrenzfähigkeit gegenüber anderen Wasserstoffacceptoren, wie z. B. Brenztraubensäure, die nur auf CoH_2 I eingestellt sind.

Die Spezifität der Hefedehydrase gegenüber CoH_2 II läßt vermuten, daß in der Hefezelle die Wasserstofflieferung gerade durch diejenigen Dehydrasesysteme erfolgt, die mit Co II fungieren. Bekanntlich sind dies die Dehydrasen, welche den dehydrierenden Abbau der Hexosemonophosphorsäure vollziehen. Für das Schicksal der dabei auftretenden CoH_2 II gibt es drei Möglichkeiten: 1. Reoxydation durch O_2 (über Flavinenzym); 2. Umwandlung der CoH_2 II in CoH_2 I und Reoxydation der letzteren; 3. Anaerobe Reoxydation der CoH_2 II durch ein Acceptorsystem. Für die Erfüllung der unter 3. genannten Möglichkeit kommt nach unserer gegenwärtigen Kenntnis nur die reduktive Aminierung der Ketoglutarsäure in Frage, da die Glutaminsäuredehydrase die einzige bisher bekannte Dehydrase ist, die Co II-spezifisch und reversibel ist.

Will man die eben skizzierte Annahme weiter verfolgen, so kommt man zu der Vorstellung, daß in der Hefezelle die Abbauwege des Kohlehydrats durch die beiden Codehydrasen in folgender Weise geleitet sein

können: Cozymase katalysiert die über Hexosediphosphat verlaufende alkoholische Gärung, Codehydrase II den über Hexosemonophosphat verlaufenden Abbau, bei dem der verfügbar werdende Wasserstoff durch oxydoreduktive Koppelung mit dem System der Glutaminsäure-dehydrase zur Synthese der Glutaminsäure, d. h. letzten Endes zur Eiweißsynthese (vgl. unten) verwertet wird.

Es erhebt sich nun die Frage, welche Bedeutung dem Umstande zugeschrieben werden kann, daß der Organismus mit einem Enzym ausgerüstet ist, welches spezifisch die Synthese einer einzigen Aminosäure, der Glutaminsäure, katalysiert.

Zur Beantwortung dieser Frage scheinen uns die Versuche von Braunstein und Kritzmann[21]) über „Umaminierung" in vorzüglicher Weise geeignet zu sein. Diese Autoren haben gefunden, daß in tierischen Geweben Glutaminsäure bzw. Asparaginsäure sich mit α-Ketosäuren derart umsetzen, daß Ketoglutarsäure bzw. Oxalessigsäure einerseits und die der α-Ketosäure entsprechende Aminosäure andererseits entstehen; die „Umaminierung" ist reversibel.

Durch diese Entdeckung wird die spezifische Fixierung des Ammoniaks an Ketoglutarsäure in ein neues Licht gerückt: Die Fixierung des Ammoniaks in Form von Glutaminsäure eröffnet ja die Möglichkeit, von hier aus durch Umaminierung alle anderen Aminosäuren aufzubauen, soweit die entsprechenden Ketosäuren verfügbar sind. In einer vorläufigen Mitteilung[33a]) wurde diese Auffassung in folgender Weise zum Ausdruck gebracht: „Ketoglutarsäure dürfte als normales Zwischenprodukt des Kohlehydratabbaus in der Zelle stets vorhanden sein. Die Begrenzung der Synthesefähigkeit des Tierkörpers für Aminosäuren wird also lediglich durch den Mangel an gewissen Ketosäuren gegeben sein. Die solchen Ketosäuren entsprechenden Aminosäuren müssen dann fertig mit der Nahrung zugeführt werden (z. B. Leucin, Tryptophan)".

Das System Ketoglutarsäure $\rightleftharpoons$ Glutaminsäure erhält damit eine Schlüsselstellung; Ketoglutarsäure ist die Eintrittspforte für das freie NH_3, Glutaminsäure die Stelle, von der aus die NH_2-Gruppen auf verschiedene Ketosäuren verteilt werden (Schema 12):

$$(12)\quad NH_3 \xrightarrow[+\,CoH_2]{+\,\text{Ketoglutarsäure}} \text{Glutaminsäure} \xrightarrow{+\,\alpha\text{-Ketosäuren}} \text{Aminosäuren}$$

Braunstein und Kritzmann fanden das umaminierende Enzym

[33a]) E. Adler, Sv. Vetensk. Akad. Ark. f. Kemi 12B, Nr. 42 (1937).

in allen Geweben, mit Ausnahme der kernhaltigen Erythrocyten und bösartigen Tumoren*).

Wir haben im Anschluß an die eben mitgeteilten Vorstellungen einige orientierende Versuche über das System der Umaminierung unternommen, wollen aber betonen, daß wir damit keineswegs in das Arbeitsgebiet von Braunstein und Kritzmann eingreifen möchten. Wir prüften nur einige uns im obigen Zusammenhang unmittelbar interessierende Fragen und fanden

a) daß die Umaminierung nicht nur in Gewebebrei, sondern auch in zellfreien Extrakten, sogar noch z. B. in ziemlich weitgehend gereinigten Apodehydrasepräparaten aus Leber stattfindet,

b) daß Umaminierung auch in Enzymlösungen aus höheren Pflanzen, aus Hefe sowie Bacterium coli erfolgt,

c) daß die Umaminierung nicht der Cozymase und wahrscheinlich überhaupt nicht eines bei neutraler Reaktion dissoziierbaren Coenzymes bedarf,

d) daß Iminoglutarsäure-hydrierende und umaminierende Wirkung nicht parallel gehen, d. h., daß die beiden Vorgänge nicht durch das gleiche Enzym katalysiert werden.

Die offenbar sehr weite Verbreitung der beiden Reaktionen — reduktive Aminierung der Ketoglutarsäure und Umaminierung der Glutaminsäure mit anderen Ketosäuren — verleiht dem oben angegebenen Schema (12) einen hohen Grad von Wahrscheinlichkeit und läßt vermuten, daß dieser Weg der Synthese von Aminosäuren aus NH_3 und Ketosäuren quantitativ bedeutungsvoll ist.

Andere Möglichkeiten der Aminosäuresynthese. Außer dem durch Schema (12) gegebenen Weg besitzt der Organismus sicher noch andere Möglichkeiten zum Aufbau von Aminosäuren aus NH_3 und Ketosäure.

a) Eine dieser Möglichkeiten dürfte die sogenannte „acetylierende Aminierung" sein, die schon in der Einleitung (S. 63) besprochen worden ist. Der einfachste Fall ist die Bildung von Alanin aus zwei Molekülen Brenztraubensäure und NH_3, aber es ist schon von Knoop und neuerdings von Du Vigneaud und Irish[10]) gezeigt worden, daß die Reaktion auch zwischen 1 Mol. Brenztraubensäure, NH_3 und einer zweiten Ketosäure stattfindet. Es erscheint durchaus denkbar, daß bei den Aminosäuresynthesen,

*) Im hiesigen Institut (Versuche von Fräulein U. Heyman) wurde gefunden, daß auch Jensen-Sarkom-Gewebe die Fähigkeit zur Umaminierung, wenn auch in geringerem Maße als Muskel, besitzt.

die von Embden und Schmitz[4]), sowie von Dakin und Dudley[6]) beim Durchströmen von Leber mit Ketosäuren + NH_3 nachgewiesen wurden, die Reduktion durch die in der Leber selbst gebildete Brenztraubensäure im Sinne der acetylierenden Aminierung erfolgte.

b) Eine noch ungeklärte Frage ist es, ob die durch die Krebssche Aminosäure-dehydrase („Oxydo-desaminase") katalysierte Reaktion reversibel ist, d. h. ob dieses Enzym in seiner reduzierten Form imstande ist, die reduktive Aminierung von Ketosäuren zu vollziehen.

Orientierende Versuche sprechen für die Reversibilität. Sie wurden so ausgeführt, daß im evakuierten System Brenztraubensäure + NH_4Cl + Nieren-Enzym [Extrakt aus Acetonniere nach Krebs[34])] mit Leukoneutralrot in Gegenwart eines kleinen Überschusses von Hydrosulfit gemischt und das Auftreten der Neutralrotfarbe beobachtet wurde. Für die Versuche bewährte sich der von N. B. Das[35]) angegebene Apparat. Es zeigte sich stets, daß die Dehydrierung des Leukofarbstoffs im vollständigen System am raschesten erfolgt und daß die Weglassung einer Komponente eine Verlängerung der Wiederfärbungszeit auf das Vielfache zur Folge hatte. Erhitztes Enzym war ohne Wirkung und das Enzym konnte nicht durch SH-Verbindungen wie Glutathion und Cystein ersetzt werden.

Die Weiterentwicklung der Kenntnisse über die Aminosäuredehydrase, welche durch die Isolierung ihres Co-Enzyms angebahnt worden ist, wird über die Frage der direkten Umkehrung der Desaminierung wohl Aufklärung bringen.

Im folgenden Schema soll versucht werden, die Stoffwechselwege des Aminosäureumsatzes im Tierkörper zusammenzufassen. Wir stellen dabei die Hintereinanderschaltung von hydrierender Aminierung der Ketoglutarsäure und Umaminierung der gebildeten Glutaminsäure in den Mittelpunkt der synthetischen Vorgänge, weil dieser Weg der gegenwärtig am besten bekannte ist. Das Schema soll andeuten, auf welche Weise die Komponenten dieses synthetisierenden Systems mit anderen Zellreaktionen gekoppelt sind.

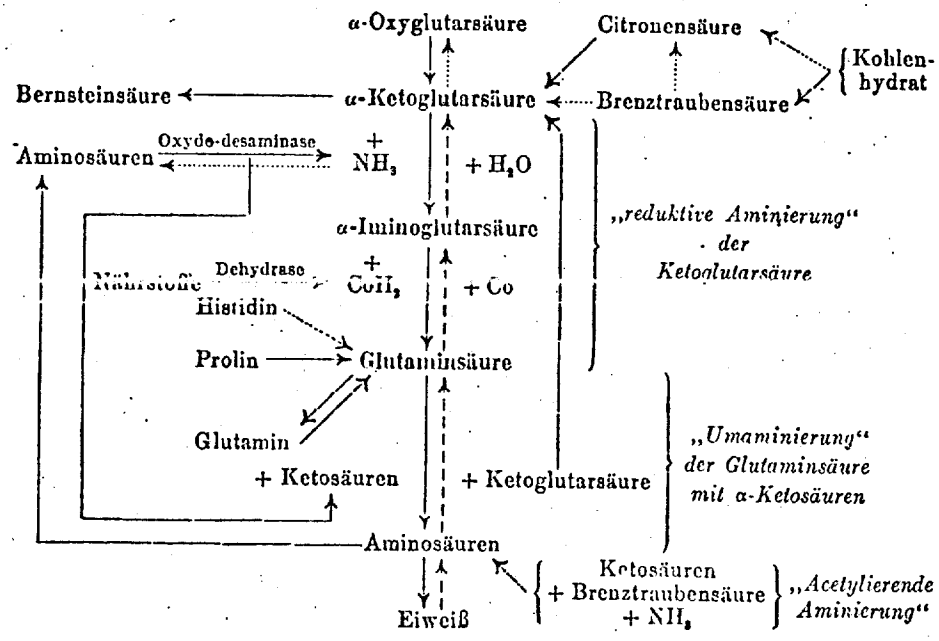
Wie das Schema zeigt, entsteht die Schlüsselsubstanz des NH_3-bindenden Systems, die Ketoglutarsäure, aus Citronensäure [Martius[36])] und steht auch mit dem Brenztraubensäureumsatz, also mit dem Kohlenhydratabbau, in enger Beziehung. Es würde

---

[34]) Biochemic. J. 29, 1620 (1935).
[35]) Biochemic. J. 31, 1124 (1937).
[36]) Martius u. Knoop, Diese Z. 246, I (1937); Martius, Diese Z. 247, 104 (1937).

zu weit führen, hierauf näher einzugehen und es sei statt dessen auf die Arbeiten von Weil-Malherbe[37]) sowie insbesondere auf die Krebssche Theorie vom Citronensäure-Cyklus[38]), welche sich zum Teil auf die Ergebnisse von Martius und Knoop[36]) aufbaut, hingewiesen. Die Beziehung zur Oxyglutarsäure ist nach Thunberg[39]) und Weil-Malherbe[40]) durch die Oxyglutarsäuredehydrase gegeben, deren Reversibilität von dem letztgenannten Autor angenommen wird.

Schema des Aminosäureumsatzes im Tierkörper.



Glutaminsäure steht in engster Beziehung zum Prolin [Weil-Malherbe und Krebs[41]), Neber[42])] und zum Glutamin [Krebs[43]),

[37]) Biochemic. J. 31, 2202 (1937).
[38]) Krebs u. Johnson, Enzymologia (Nd.) 4, 148 (1937); Krebs, Salvin u. Johnson, Biochemic. J. 32, 113 (1938).
[39]) Skand. Arch. Physiol. 40, 1 (1920).
[40]) Biochemic. J. 31, 2080 (1937).
[41]) Biochemic. J. 29, 2077 (1935).
[42]) Diese Z. 240, 70 (1936).
[43]) Biochemic. J. 30, 1968 (1936).

Weil-Malherbe[44])] und kommt nach Edlbacher und Kraus[45]) auch als Abbauprodukt des Histidins in Frage.

Die Funktion der Glutaminase[43]), welche Glutaminsäure reversibel mit NH_3 zu Glutamin verbindet, ist im vorliegenden Zusammenhang von großem Interesse. Es wird von Wichtigkeit sein, zu prüfen, ob das umaminierende Ferment außer Glutaminsäure auch Glutamin als Aminogruppen-Donator verwertet. Wenn dies nicht der Fall ist, so würde dem aus Glutaminsäure aufgebauten Glutamin die Bedeutung eines Aminostickstoff-Speichers zukommen können, aus welchem der Aminostickstoff erst nach Abspaltung der Amidgruppe wieder für die Umaminierung disponibel wird.

NH_3-Fixierung und Aminosäuresynthese bei anderen Organismen. Die weite Verbreitung des spezifischen Ketoglutarsäure-aminierenden sowie des umaminierenden Systems wurde in der vorliegenden Arbeit bereits hervorgehoben. Es ist jedoch wahrscheinlich, daß der quantitative Anteil dieser Systeme an der gesamten Aminosäuresynthese bei verschiedenen Lebewesen sehr variiert und bisweilen vielleicht ganz zurücktritt.

In Bacterium coli fanden wir, wie bereits erwähnt, ebenso wie in Hefe eine CoII-bedingte Glutaminsäure-dehydrase. In Milchsäurebakterien konnte sie jedoch nicht nachgewiesen werden[46]).

Bekanntlich sind nun eine Reihe von Bakterien mit dem Enzym Aspartase ausgerüstet, welches die Anlagerung von NH_3 an Fumarsäure unter Bildung von Asparaginsäure reversibel katalysiert [Quastel[47]), Virtanen[48])]. Die Vorstellung ist naheliegend, daß in solchen Fällen die Synthese der anderen Aminosäuren teilweise oder ganz durch Umaminierung der Asparaginsäure mit Ketosäuren erfolgt. Für das Vorkommen der Aspartase im Tierkörper gibt es nur einen experimentell recht schwach begründeten Hinweis von Jacobson[49]).

Virtanen und Laine[50]) haben kürzlich eine interessante Vorstellung über den Mechanismus der Stickstoff-Fixierung bei

---

[44]) Biochemic. J. 30, 665 (1936).
[45]) Diese Z. 195, 267 (1931).
[46]) Unveröffentlichte Versuche von V. Hellström.
[47]) Quastel u. Woolf, Biochemic. J. 20, 545 (1926); Cook u. Woolf, Biochemic. J. 22, 474 (1928).
[48]) Virtanen, Biochem. Z. 250, 193 (1932).
[49]) Jacobson u. Soares, Enzymologia (Nd.) 1, 183 (1936).
[50]) Nature 141, 748 (1938).

Leguminosen mitgeteilt. Nach dieser Vorstellung reduzieren die Leguminosenbakterien den molekularen Stickstoff zu Hydroxylamin, dieses bildet mit Oxalessigsäure (aus der Wirtspflanze) Oxalessigsäure-oxim, welches schließlich zu Asparaginsäure reduziert wird. Von hier aus kann eine Verteilung der Aminogruppen durch die Umaminierung stattfinden.

E. Zusammenfassung.

1. Aus Leber werden Apodehydrasepräparate gewonnen, die nach Ergänzung durch Cozymase l(+)-Glutaminsäure zu Iminoglutarsäure dehydrieren. Letztere zerfällt spontan durch Hydrolyse in α-Ketoglutarsäure und NH_3.

2. Die Dehydrierungsreaktion ist reversibel, d. h. Dihydrocozymase hydriert in Gegenwart der spezifischen Apodehydrase Iminoglutarsäure (Ketoglutarsäure + NH_3, welche mit Iminoglutarsäure im Gleichgewicht stehen) zu Glutaminsäure. p_H-Abhängigkeit und Gleichgewichtskonstante der reversiblen Dehydrierungsreaktion wurden bestimmt.

An Stelle der Dihydro-cozymase kann auch ein CoH_2-lieferndes Dehydrasesystem als Wasserstoffdonator für die hydrierende Aminierung der Ketoglutarsäure dienen.

Durch diese Versuche ist die enzymatische Synthese einer Aminosäure aus Ketosäure und NH_3 im zellfreien System zum erstenmal durchgeführt.

3. Die Glutaminsäure-apodehydrase aus Leber bildet nicht nur mit Cozymase (Codehydrase I), sondern auch mit Codehydrase II eine Holodehydrase, die in gleicher Weise die reversible Reaktion vollzieht.

4. Die scharfe Substratspezifität der Apodehydrase in bezug auf das Kohlenstoffskelett bzw. die Iminogruppe wird hervorgehoben. Von allen bisher untersuchten Substanzen werden nur l(+)-Glutaminsäure dehydriert, bzw. nur α-Iminoglutarsäure (α-Ketoglutarsäure + NH_3) hydriert.

5. Im tierischen Organismus enthält Leber die höchste Konzentration an Glutaminsäure-apodehydrase. Darauf folgen Niere mit etwa $2^1/_2$ mal kleinerer Konzentration und die übrigen Gewebe in weiterem Abstand.

6. Die physiologische Funktion der Glutaminsäure-dehydrase dürfte weniger in der Dehydrierung von Glutaminsäure, als viel-

mehr vorzugsweise in der Umkehrung dieser Reaktion, der spezifischen reduktiven Fixierung von NH_3 an α-Ketoglutarsäure zu erblicken sein. Der Umstand, daß die Zellen mit einem System zur spezifischen Synthese der Glutaminsäure ausgerüstet sind, erhält eine besondere Bedeutung durch die Tatsache, daß gerade von dieser Aminosäure aus durch Umaminierung (Braunstein und Kritzmann) eine Verteilung des gebundenen Stickstoffs auf andere Ketosäuren, also der Aufbau anderer Aminosäuren, erfolgen kann.

Durch das System Ketoglutarsäure $\rightleftarrows$ Glutaminsäure wird also gleichzeitig freies NH_3 in organische Bindung übergeführt und die NH_2-Gruppe übertragen.

Es wird ein Schema über den Aminosäureumsatz in der tierischen Zelle gegeben, und es werden schließlich die verschiedenen Wege der Aminosäuresynthese im Tierkörper und bei anderen Organismen kurz diskutiert.

THE METABOLISM OF GLUTAMIC ACID *

HEINRICH WAELSCH
M.D., Ph.D. Prague

From the Department of Biochemistry, New York State Psychiatric Institute

DURING the past five years my colleagues and I have been studying the metabolism of glutamic acid with particular attention to its relation to the nervous system. Our interest in this subject was stimulated partly by the beneficial effects of giving this amino-acid to certain types of epileptics and mental defectives (Price et al. 1943, Waelsch and Price 1944, Albert et al. 1946). Since most of the diseases of the nervous system have no known counterpart in the lower animals, the biochemist interested in the fundamental mechanisms responsible for the normal and abnormal reactivity of the nervous system has to rely largely on clinical information and is handicapped by the impossibility of experimenting on animals. The lack of methods for an objective assessment of functional changes in the central nervous system presents a further difficulty. Though the clinical observations on the effect of glutamic acid have served to orient our biochemical studies, it is beyond my intention or ability to discuss glutamic acid as a therapeutic agent.

GLUTAMIC ACID IN EPILEPSY

Five years ago (Price et al. 1943) we suggested a study of the effect of dl-glutamic-acid hydrochloride in patients with petit mal. This suggestion was made partly because investigations with the aid of the tissue-slice technique had shown that glutamic acid is the only amino-acid capable of taking the place of glucose in maintaining the respiration of brain-cortex slices (Weil-Malherbe 1936). It was also known that an enzyme system which converts glutamic acid into the amide—glutamine (Krebs 1935)—is present in the brain.

Of particular interest was the change in mental status observed in epileptics given glutamic-acid hydrochloride by mouth.

> "Universally mental and physical alertness was increased. The degree of improvement cannot be correlated with the incidence of seizures. . . . Usually the patient is noted to be more energetic and happier, mood swings are less pronounced, behaviour mannerisms are ameliorated, and he is more congenial with associates." (Price et al. 1943.)

Later it was shown that the naturally occurring form of glutamic acid was as effective as the racemic dl-glutamic-acid hydrochloride, and it had to be concluded, therefore, that the beneficial effect might be ascribed to that form of glutamic acid which is present in the proteins of the mammalian body and of food (Waelsch and Price 1944). With this form of glutamic acid there was also found the improvement in personality noted before with the dl-glutamic-acid hydrochloride. The amount of glutamic acid given to the patients ranged from 9 to 12 g. a day, whereby the daily intake of dietary glutamic acid was increased by about 30–100%.

From clinical reports it appears that some patients with petit mal are benefited by the administration of glutamic acid by mouth. The average incidence of seizures is cut down, but it should be emphasised that the seizures are rarely eliminated completely. Further, it is noteworthy that seizures of the grand-mal type are not affected.

GLUTAMIC ACID IN MENTAL DEFICIENCY

Since it appeared possible that the removal or improvement of an epileptoid mechanism was the reason for the apparent improvement of the mental status, the effect of glutamic acid was tested in mentally retarded persons without any history of convulsive disorder.

In a first preliminary study 10–12 g. of glutamic acid was given by mouth to 8 mental defectives. Its administration was preceded or followed by a period during which a placebo (lactose) was given (Albert et al. 1946). Each person served as his own control; thus the effect of repeated psychometric tests could be assessed. An attempt was made to select mental defectives suspected of a deficiency of the secondary type. The study of 7 of the 8 patients was completed, and it was found that, when glutamic acid was given, the patients' I.Q. score rose 5–17 points (15–35% of the original base level) and dropped to almost the original level when the glutamic acid was discontinued or placebos were substituted (see also Zimmerman et al. 1946, 1947).

These findings warranted a study of the effect of glutamic acid on a larger group of mental defectives, unselected except for the exclusion of all who had a history of convulsive disorders. Such a group was made available to us by the Bureau for Retarded Children, Board of Education of the City of New York. Table I gives the preliminary results of this study, which is still being continued. The I.Q. score of 30% of the children on glutamic acid rose 5 points or more, whereas none of the children on placebos showed a comparable gain in mental performance. Of 5 children who benefited from the administration of glutamic acid 3 regressed back to the original I.Q. level during a subsequent placebo period. The psychologist who tested the mental performance was not informed whether the children were receiving glutamic acid or placebos.

MODE OF ACTION ON BRAIN

The effects of glutamic acid in epileptics and in mental defectives were observed after the administration of glutamic acid by mouth. Mayer-Gross and Walker (1947) have shown that glutamic acid given intravenously to patients in insulin coma restores consciousness at a considerably lower blood-sugar level than when glucose is used. This effect, however, is not specific for glutamic acid, since it may be obtained with other amino-acids (W. Mayer-Gross, personal communication). At present there is no experimental evidence to indicate a direct effect of glutamic acid on the brain in the intact organism. Though the administration of glutamic acid by mouth produces an increase of its concentration in the blood (see below) it may affect the brain indirectly by an effect on the metabolism of some other organ. It should be emphasised that in all our experiments the free amino-acid was given, and the question arises whether the same effect would be obtained from the same amount of glutamic acid bound in a protein.

On the present experimental evidence we cannot state that the effect of glutamic acid is specific. Many other substances metabolically related or unrelated to it may well have similar effects. On the other hand, there are few metabolites in carbohydrate and amino-acid metabolism which are not linked by some biochemical mechanism with glutamic acid, so it appears justified to concentrate at present on the study of glutamic acid.

If we assume that glutamic acid exerts its effect after its absorption through the intestinal wall and not by modifying the absorption of other compounds or by influencing the intestinal flora, and if we assume further that its calorigenic action is not responsible, we are faced with an interesting biochemical problem. Glutamic acid is one of the amino-acids which can be synthesised in the body; and only under very special conditions, when glutamic acid and all of its metabolic sources, such as ornithine and proline, are excluded, does the absence of glutamic acid affect the growth of rats (Womack and Rose 1947). Such is surely not the

* Based on a lecture presented at the Institute of Psychiatry, Maudsley Hospital, London, on June 15, 1948. The studies reported here were supported by grants from the Rockefeller Foundation, the New York Foundation, and the Williams-Waterman Fund of the Research Corporation.

TABLE I—EFFECT OF GLUTAMIC ACID AND PLACEBOS ON MENTALLY RETARDED PERSONS (I.Q. RANGE 18–58; C.A. RANGE 6–17)

First period (4 months)	Glutamic acid	Placebo
No. of persons	20	27
No. of persons showing increase of 5 points or more	9	0
No. of persons showing decrease of 5 points or more	0	1
Second period (4 months)	**Placebo**	**Glutamic acid**
No. of persons	5	16
No. of persons showing increase of 5 points or more	0	2
No. of persons showing decrease of 5 points or more	3	0

situation in those patients whose mental performance is benefited by the administration of glutamic acid, since, in addition to the amounts available from synthesis in the body, they are receiving a large quantity, probably a multiple of the administered dose, in the protein of the food. It therefore seems that, in this instance, the addition of a body component which is supplied in large amounts by the diet and is also synthesised in the body may exert a beneficial effect if added in relatively small amounts to the diet.

A study of the metabolism of glutamic acid cannot be dissociated from that of its amide, glutamine, which occurs in considerable concentration in proteins and in the non-protein fraction of plant and animal tissues. There are enzyme systems in mammalian tissue which convert glutamic acid into the amide or split the amide to glutamic acid and ammonia (Krebs 1935). The importance of glutamine has been emphasised by the finding that it is an essential nutrient for certain micro-organisms, and in this respect its place cannot be taken by glutamic acid (McIlwain et al. 1939).

ABSORPTION AND DISTRIBUTION

As a first step in the study of the utilisation and metabolism of glutamic acid and glutamine, their absorption and distribution in the tissues were studied. Before reporting this aspect of our study, I wish to discuss the concentrations of glutamic acid and glutamine in the blood-plasma of epileptic and non-epileptic persons (table II; for methods of determination see Prescott and Waelsch 1946, 1947).

For comparative purposes the concentration of an essential amino-acid, phenylalanine, was determined. Neither in the absolute values, nor when the amount of the analysed amino-acids was expressed as a percentage of the total amino-acids, was any difference found between normal persons and epileptics. One point deserves particular attention—the ratio between glutamine and glutamic acid. The amount of glutamine in plasma was about ten times that of glutamic acid, and similar ratios were found in ox, cat, and rat blood. This finding may be of particular significance, since the amide does not take part in many of the metabolic reactions in which glutamic acid is a specific substrate (see below). On the other hand, a varying but large part of glutamic acid in proteins is present as glutamine, and therefore different ratios of the two compounds are offered in the dietary proteins.

The question arose whether any interconversion occurs between glutamic acid and glutamine during their absorption through the intestinal wall (Bessman et al. 1948). Glutamic acid or glutamine was introduced into the small intestine of cats, and the plasma of the portal blood was analysed for its content of glutamic acid and glutamine, 15 and 30 minutes after the introduction of the two compounds (table III). Glutamic acid and glutamine were absorbed independently of each other since immediately after the introduction of either compound there was a sharp rise in the plasma concentration of the administered substance only. When glutamic acid was given, there was a drop in glutamine concentration after 15 minutes, followed by a rise above the baseline after 30 minutes. This decrease in the 15-minute values is interpreted as a shift of glutamine from the plasma into the tissues.

A similar decrease has been found also in peripheral blood after giving glutamic acid by mouth to man, and this appears to be part of a general phenomenon, since an increased concentration of glutamic acid is accompanied by a fall in the levels of glycine and residual amino-acid nitrogen (Christensen et al. 1948).

TABLE III—CONCENTRATION OF FREE GLUTAMIC ACID (ACID) AND GLUTAMINE (AMIDE) IN PLASMA OF PORTAL VEIN (CAT) AFTER INTRA-INTESTINAL ADMINISTRATION OF 100 MG. OF GLUTAMIC ACID OR OF GLUTAMINE

Values expressed as mg. per 100 ml. of plasma *

—	Glutamic acid given				Glutamine given			
Cat no.	1		2		3		4	
Min. after administration	Acid	Amide	Acid	Amide	Acid	Amide	Acid	Amide
0	2·8	10·7	1·7	10·4	2·5	6·4	1·6	5·9
15	12·9	4·2	6·6	8·3	3·9	12·7	1·9	12·3
30	14·8	13·0	1·0	15·5	3·1	25·8	1·6	8·8

* Per cent. blood-cells in cat no. 1 at 0, 15, 30 minutes: 44, 36, 35. In cats no. 2–4: change in blood-cell volume smaller than 3%.

The significance of this finding is still obscure. The increase of the glutamine concentrations at 30 minutes over the values at 0 time is interpreted as the mobilisation of tissue glutamine or the release of glutamine formed in the organs from the administered glutamic acid. We may assume that glutamic acid and glutamine are liberated from the dietary proteins by the enzymes of the digestive tract without extensive interconversions, and our experiments show that the amide and its parent amino-acid may be absorbed without significant changes. This may mean that the administration of 10–15 g. of glutamic acid by mouth, a small amount compared with the potential intake of glutamic acid (glutamic acid plus glutamine), may increase the *relative* amount of glutamic acid considerably. Administration of an additional 10 g. of glutamic acid with 100 g. of food

TABLE II—FREE GLUTAMIC ACID, GLUTAMINE, PHENYLALANINE, AND TOTAL AMINO-NITROGEN IN PROTEIN-FREE FILTRATES OF HUMAN PLASMA

—	Glutamic acid		Glutamine		Phenylalanine	Amino-nitrogen
	NE	E	NE	E		
No. of samples	13	19	12	18	17	17
Range (mg. per 100 ml.)	0·4–1·7	0·3–1·2	4·5–10·6	4·8–10·1	0·6–1·3	3·4–5·2
Mean (mg. per 100 ml.)	0·7	0·7	8·0	7·8	0·95	4·2
Standard error	± 0·07	± 0·05	± 0·5	± 0·4	± 0·06	± 0·1

NE, non-epileptics; E, epileptics.

TABLE IV—CONCENTRATION OF FREE GLUTAMIC ACID (ACID) AND GLUTAMINE (AMIDE) IN PLASMA OF PERIPHERAL BLOOD AFTER ADMINISTRATION OF GLUTAMIC ACID BY MOUTH TO HUMAN SUBJECTS (1 G. PER 10 KG. OF BODY-WEIGHT)

Values expressed as mg. per 100 ml. of plasma

Hours after administration	Subject 1		Subject 2		Subject 3		Subject 4	
	Acid	Amide	Acid	Amide	Acid	Amide	Acid	Amide
0	0·6	8·4	0·6	10·6	1·2	8·4	0·8	10·3
1	1·0	10·3	1·2	14·0	5·0	5·8	9·5	8·3
2	1·0	8·9	0·8	11·3	0·7	6·3	1·0	10·0

protein, containing 10 g. of glutamine and 10 g. of glutamic acid, increases the intake of potential glutamic acid from 20 to 30 g. but doubles the intake of the amino-acid itself.

LEVELS IN BLOOD AND TISSUES

As the next step in tracing the pathway of glutamic acid, the changes in the peripheral blood were determined after the administration of glutamic acid by mouth (Bessman et al. 1948). Glutamic acid 1 g. per 10 kg. of body-weight was given by mouth to four human subjects, and the concentrations of glutamic acid and glutamine in the plasma of the peripheral blood were determined 1 and 2 hours afterwards. The amounts given corresponded to two or three times the dose given to epileptics or to mental defectives.

In all four experiments (table IV) the administration of glutamic acid led to an increase in the glutamic-acid content of the peripheral blood, but the extent of the increase varied considerably. In those experiments in which only a small rise in the glutamic-acid level was found a concomitant significant increase of glutamine was observed; but where the glutamic-acid level rose considerably the glutamine values decreased at first and increased later. Here the picture was very similar to that found in the portal blood of cats after the intra-intestinal administration of glutamic acid. The fall in the plasma-glutamine level, which appears to be accompanied by a fall in the levels of other amino-acids, may be a clue to the effect of giving glutamic acid. These findings indicate the discouraging complexity of the

TABLE V—TISSUE DISTRIBUTION OF GLUTAMIC ACID (ACID) AND GLUTAMINE (AMIDE) AFTER INTRAVENOUS INJECTION IN MICE (1·3 MG. PER G. OF BODY-WEIGHT)

Values expressed in mg. per 100 g.

Min. after administration	Glutamic acid given		Glutamine given	
	Acid	Amide	Acid	Amide
		Brain		
0	145	53	145	53
15	153	80	140	116
		Liver		
0	19	35	19	35
15	70	32	300	90

changes resulting from the administration of glutamic acid.

The administration of glutamic acid by mouth may increase both the glutamic acid and glutamine concentrations in the peripheral blood, thereby fulfilling a condition obligatory for an effect of glutamic acid on the brain. A direct influence of glutamic acid or of glutamine on the metabolism of any organ can only be expected after the entry of these compounds into the organ.

The uptake of glutamic acid and its amide by tissues was studied by tissue analyses after the intravenous administration of the two compounds. It should be kept in mind that the intravenous injections of large amounts of a single amino-acid create unphysiological conditions. Different results may be obtained when the amino-acid and its amide are accompanied by all the other protein components, as under physiological conditions. The uptake of glutamic acid and glutamine by several tissues during the first 30 minutes after intravenous administration to mice and rats was determined. Preparatory to this study the normal concentrations of glutamic acid and its amide in several tissues were estimated. Though the glutamine values for the protein-free filtrates of tissues of the dog and of blood-plasma of several species have been reported (Archibald 1945), no information on the concentration of glutamic acid was available.

Our analyses (tables V and VI, values at 0 time) show that some organs (muscle and liver), of the rat and mouse contain an excess of glutamine over glutamic acid, whereas in others (brain and kidney) the concentration of glutamic acid exceeds that of glutamine. In the brains

TABLE VI—TISSUE DISTRIBUTION OF GLUTAMIC ACID (ACID) AND GLUTAMINE (AMIDE) AFTER INTRAVENOUS INJECTION IN RATS (1·3 MG. PER G. OF BODY-WEIGHT)

Values expressed in mg. per 100 g.

Min. after administration	Glutamic acid given		Glutamine given	
	Acid	Amide	Acid	Amide
		Brain		
0	133	62	133	62
10	120	65	120	68
20	140	56	140	84
		Liver		
0	33	55	33	55
10	94	38	210	210
20	72	45	200	73
		Muscle		
0	21	32	21	32
10	40	35	18	68
20	18	42	13	53
		Kidney		
0	98	19	..	..
10	400	29	..	..
20	424	39	..	..

of mice and rats as well as in those of rabbits, calves, and pigeons a twofold to threefold excess of the amino-acid over its amide was found, and the absolute concentration of glutamic acid was about twice that of glucose.

It would be feasible to consider glutamic acid as a respiratory substrate of brain tissue, particularly since a determination of the respiratory quotient by analysis of the arterial-venous difference cannot differentiate between the utilisation of glucose and that of glutamic acid. Recent investigations on brain respiration in progressive hypoglycæmia have led to the conclusion that under such conditions the brain utilises its own carbohydrate stores or some other endogenous substrate (Kety et al. 1948).

ORIGIN OF GLUTAMIC ACID IN THE BRAIN

The high concentration of glutamic acid in brain made it of particular interest to ascertain whether the amino-acid originates from the circulating blood. After the intravenous administration of glutamic acid to mice and rats no significant increase was found in the concentration of glutamic acid in the brain; the length of the experimental period was such that metabolic conversions would probably not significantly distort the changes resulting from uptake into the tissue (tables V and VI). Whereas liver and muscle took up considerable amounts of glutamic acid, the amount of glutamic acid in the brain varied within the range found in the control animals. This led to the conclusion that glutamic acid as such apparently does not enter the brain.

Such a result had been suggested before by studies in which the total amino-acid nitrogen of the brain was measured after the administration of glutamic acid (Friedberg and Greenberg 1947), but only the specific determination of glutamic acid can give an unequivocal answer. A considerable increase in the concentration of glutamic acid occurred in the liver of mice, and in the liver, muscle, and kidney of rats. No indication of conversion of glutamic acid to glutamine was found except in the rat kidney. There may have been a slight increase in the glutamine content of the mouse brain.

After the injection of glutamine there was a considerable increase of that substance in the mouse brain and a slight increase in the rat brain. An enormous increase in the amount of glutamine in the liver was observed, and an immediate liberation of glutamic acid from the accumulated glutamine. The increase in muscle-glutamine was comparable to the increase in glutamic acid observed after the administration of the amino-acid. The results of the injection experiments suggest that glutamine enters the liver, and possibly also the brain, with greater ease than does glutamic acid.

Glutamic acid does not enter the brain of animals under the particular conditions of our experiments. It cannot be concluded that the same holds true for the human brain or in pathological conditions, such as epilepsy and mental deficiency. We cannot state definitely whether the glutamine of the blood is likewise excluded from the brain. since in some of the experiments an increase in the amount of "apparent glutamine" (Prescott and Waelsch 1947) in the brain tissue was noted.

Though glutamic acid and glutamine are possibly derived from the glutamine of the circulating blood, it appeared desirable to find out whether the two amino-acids are synthesised in the brain itself. In preliminary experiments slices of brain cortex from a guineapig were shaken in Krebs-Ringer-phosphate solution under aerobic conditions, and the concentrations of glutamic acid and glutamine were determined in the absence and presence of glucose after different intervals of time. In the absence of glucose there was a rapid decrease in the concentrations of glutamic acid and glutamine in the slices. If glucose was added to the exhausted slices, the content of glutamic acid and glutamine was increased. If glucose was added at the beginning, the depletion of the slices in glutamic acid and glutamine was arrested. These results would indicate that glutamic acid and glutamine are synthesised in the brain slices, were in not for the possibility that the increase in the amount of glutamic acid is the result of autolytic processes by which glutamic acid might be liberated from protein.

The experimental evidence presented so far is compatible with the view that the effect of glutamic-acid administration on the metabolism and function of the central nervous system does not result from a direct action of the amino-acid on the brain. It is doubtful whether glutamine can pass the blood-brain barrier and thereby supply the brain with glutamine and glutamic acid. The alternative is that the brain manufactures the amino-acid and the amide, and that the effect of administered glutamic acid on the brain is indirect through other organs. The finding that an increased glutamic-acid level in the blood leads to a removal of other amino-acids adds another complicating factor, and we cannot exclude the possibility that the effect of glutamic-acid administration is the result of the concentration of substances in the tissues by such a mechanism.

GENERAL CONSIDERATIONS

Glutamine appears to enter the tissues more easily than does the parent amino-acid. The preferential entrance of the amide into the tissues may have considerable significance for the organism. Glutamic acid is metabolically one of the most active of amino-acids. It mediates the entrance of ammonia into the amino-acid pool and it takes part in transamination reactions. It is also the parent substance of ketoglutaric acid, one of the essential members of the tricarboxylic-acid, or Krebs, cycle by which pyruvic acid originating from carbohydrate breakdown is finally burned to carbon dioxide and water. This part of the metabolism of carbohydrates contributes the lion's share of energy during their metabolic degradation. It is assumed today that the tricarboxylic-acid cycle is also the pathway for the oxidative breakdown of fatty acids and the carbon skeleton of many amino-acids. Whereas glutamic acid, or ketoglutaric acid, participates in all these processes, as well as in the synthesis of urea (Cohen and Grisolia 1948) and in the reactivation of the enzyme system synthesising acetylcholine (Nachmansohn et al. 1943), glutamine is inert in all these metabolic reactions. It is a depot from which glutamic acid is easily liberated by enzymatic action but which by itself does not change the rate of the basic metabolic reactions proceeding in the tissue (cf. Chibnall 1939).

This concept of the function of glutamine disregards for the moment other functions of the amide, of which we know very little—e.g., the removal of ammonia, and its rôle in glycolysis and the synthesis of protein.

The concept that glutamine represents a store of metabolically inert glutamic acid and ketoglutaric acid, easily mobilised by enzymatic action—similar to the rôle of glycogen in carbohydrate metabolism—may be extended to explain the presence of a large concentration of free glutamic acid and of glutamine in brain and other organs. The rate of the tricarboxylic-acid cycle determines the amount of energy derived from the combustion of carbohydrates, and a certain, if only catalytic, concentration of each of its components is needed to carry the cycle at a rate compatible with average physiological function.

The physiological rhythm of function and inactivity of an organ may find one of its metabolic expressions in the rate of the tricarboxylic-acid cycle, particularly in organs such as the brain, which derives its energy requirements from the breakdown of glucose. The concentration of the different components fed into the tricarboxylic-acid cycle from many metabolic pathways undoubtedly varies widely and requires some physiological regulation. It is likely that such a basic metabolic cycle will be well buffered to ensure a physiological rate. One of the metabolic buffers of the tricarboxylic-acid cycle may be the system: glutamic acid, glutamine. The presence of glutamine and of glutamic acid in large concentrations may doubly ensure the adequate supply of ketoglutaric acid. It is too early to speculate whether the large amounts of glutamic acid and the smaller amounts of glutamine in brain are the metabolic expressions of its functional state, which has to be kept within carefully guarded limits.

We are still a long way from understanding the effect of glutamic acid on the central nervous system. By trying to reach an understanding of this effect, we may hope to learn something about the metabolism of amino-acids and its relation to the function of the nervous system.

REFERENCES

Albert, K., Hoch, P., Waelsch, H. (1946) *J. nerv. ment. Dis.* **104**, 263.
Archibald, R. M. (1945) *Chem. Rev.* **37**, 161.
Bessman, S. P., Magnes, J., Schwerin, P., Waelsch, H. (1948) *J. biol. Chem.* **175**, 817.
Chibnall, A. C. (1939) Protein Metabolism in the Plant. London.
Christensen, H. N., Streicher, J. A., Elbinger, R. L. (1948) *J. biol. Chem.* **172**, 515.
Cohen, P. P., Grisolia, S. (1948) *Fed. Proc.* **7**, 150.
Friedberg, F., Greenberg, D. M. (1947) *J. biol. Chem.* **168**, 411.
Kety, S. S., Lukens, F. D. W., Woodford, R. B., Harmel, M. H., Freyhan, F. A., Schmidt, C. F. (1948) *Fed. Proc.* **7**, 64.

Continued at foot of next page

5 2-

The American Journal of Clinical Nutrition 26 + 8 Aug. 1973

original communications

Effects of dietary supplementation of monosodium glutamate on infant monkeys, weanling rats, and suckling mice[1,2]

Chi-Pang Wen, M.D., M.P.H., Dr. P.H., Kenneth C. Hayes,[3] D.V.M., Ph.D., and Stanley N. Gershoff,[4] Ph.D.

The observation that large amounts of monosodium glutamate (MSG) could produce brain damage either when injected subcutaneously in mice (1), rats (2), and infant monkeys (3), or when force-fed to monkeys (3), newborn mice (4), and rats (5) has caused some concern over its use as a common food additive. Subsequent reports have not always been consistent (6–9) and the extent of MSG-induced lesions of hypothalamic neuronal degeneration have been suggested to vary with the length of the experimental period (10) and to depend on critical stages of brain development (5).

In the experiments described here, the effects of a wide range of dietary levels of MSG on the growth and development and possible acute and long-term toxicity in infant monkeys, weanling rats, and suckling mice have been studied.

Materials and methods

Five experiments have been done, the first three involving infant monkeys, the fourth, weanling rats, and the fifth, suckling mice.

Experiment 1

Ten newborn male and female squirrel monkeys (*Saimiri sciureus*), born in the breeding colony of this department (11), were taken from their mothers within hours of birth and placed in temperature-controlled incubators. For the first 6 or 7 days, the infants were hand-fed a half-strength (6.8 g/100 ml) liquid formula diet.[5] This was then changed to the full-strength formula until day 11. Details of the hand-rearing techniques used have been reported (12). On the 11th day, each monkey was randomly assigned to one of four dietary groups, groups 1 and 3 having two animals each and groups 2 and 4 having three animals each. Group 1 continued to consume the full-strength formula with no added MSG. The other groups received the same liquid formula with supplements of 5, 10, or 20 g monosodium L-glutamate added to 100 g of the powdered formula diet. Expressed on a dry weight or liquid basis, respectively, the diet fed group 2 contained 4.8 or 0.68% MSG; group 3, 9.1 or 1.36% MSG; and group 4, 16.7 or 2.72% MSG.

At approximately 3 weeks of age, the infants were removed from their incubators and housed individually in steel cages with free access to both formula and water bottles. For 9 to 10 weeks their food intakes were recorded daily and they were weighed twice each week. Measurements of body length and head and chest circumferences were made at 2-week intervals. Clinical observations were made daily. During the 9th or 10th week an electroencephalogram (EEG) was obtained from each animal, both awake and asleep and without anesthesia. Within the following week, the monkeys were anesthetized with an intravenous dose of sodium pentobarbital (20 mg/kg body wt) and electroretinograms (ERG) were recorded. During the 11th week, the monkeys were re-anesthetized, total body radiographs made, and arterial blood pressure measured directly from the abdominal aorta or femoral artery using closed-system manometry and a physiological strain-gauge transducer (13). The

[1] From the Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, Massachusetts 02115.

[2] Supported in part by Public Health Service Research Grant AM-03056 and the Fund for Research and Teaching, Department of Nutrition, Harvard School of Public Health.

[3] Assistant Professor. [4] Associate Professor.

[5] Similac with iron from Ross Laboratories, Columbus, Ohio.

The American Journal of Clinical Nutrition 26: August 1973, pp. 803–813. Printed in U.S.A.

thoracic cavity was then opened and a terminal blood sample (for amino acid analysis described below) drawn from the heart prior to intracardiac perfusion with a formaldehyde-glutaraldehyde fixative. A complete set of tissues representing all major organs was taken for light microscopy and sections of the retina and hypothalamus were prepared for electron microscopy according to a previously published procedure (2).

Experiment 2

One cynomolgus monkey (*Macaca fascicularis*) and one bushbaby (*Galago crassicaudatus*), both Old World monkeys, were fed from 1 month of age the diet used in experiment 1 containing 9.1% MSG on a dry weight basis for a period of 11 months. A control monkey of each species was fed the liquid diet with no MSG addition during the same comparison period. Growth and development of these monkeys were periodically assessed and at the end of the study period, an EEG and ERG were obtained as described in the previous experiment.

Experiment 3

A 3-week-old cynomolgus monkey was injected intramuscularly with 2.7 mg MSG/g body wt in a 20% solution, a dose that reportedly produced hypothalamic lesions in infant rhesus monkeys (3). Blood was drawn at 0, 15, 30, and 60 min after the injection and analyzed for plasma amino acids. This monkey was then kept under observation for 2.5 years.

Experiment 4

In this experiment with rats, a basal semi-purified diet of the following composition was used (in percent): casein, 25; sucrose, 60.7; cod liver oil (vitamin A, 1,800 IU; vitamin D, 180 IU/g), 1.0; corn oil, 9.0; salts IV (14), 4.0; and choline chloride, 0.3. Vitamins were added so that each 100 g of diet contained (in milligrams): thiamin HCl, 0.4; riboflavin, 0.8; pyridoxine HCl, 0.4; niacin, 4.0; biotin, 0.02; calcium D-pantothenate, 2.0; folic acid, 0.1; menadione, 0.1; vitamin B_{12}, 0.005; DL-α-tocopherol, 10.0.

Thirty weanling, male Charles River CD rats were randomly divided into five groups. The groups were fed ad libitum the following diets for 5 weeks: group 1, 100 g basal diet plus 20 g glucose; group 2, 100 g basal diet plus 20 g MSG; group 3, 100 g basal diet plus 40 g MSG; group 4, 100 g basal diet plus 17.4 g L-glutamic acid, the amount of glutamic acid in 20 g MSG; group 5, 100 g of basal diet plus 1.5 g Na_2SO_4, 1.5 g Na_2CO_3, 2 g $NaHCO_3$, 2 g NaCl, 3 g glycine, 2.5 g L-alanine, 2.5 g L-glutamic acid, 1.3 g ammonium citrate, and 2.3 g L-serine. These supplements provided approximately the same quantity of sodium and nonessential amino acid nitrogen found in 20 g MSG.

Weekly body weights, daily water consumption during the last 2 weeks, and the pH of the pooled urine samples from each group were recorded. After a 5-week period, the rats were fasted overnight and then fed ad libitum for 90 minutes prior to decapitation. Postprandial blood samples were collected from the neck for plasma glutamate determination. Organs of interest were weighed and brains and testes were fixed in 10% buffered formalin for histologic examination of hematoxylin- and eosin-stained sections. Serial frozen sections of formalin-fixed brain were also stained with the Fink and Heimer silver stain for degenerating axons (15).

Experiment 5

Fifteen litters of suckling Swiss albino mice were housed with their mothers and randomly assigned to experimental groups. Each mouse received a supplemental oral feeding three times a day for 5 days (from 6 to 10 days of age). Tuberculin syringes were used with a metal cap on the blunt end tip of a 24-gauge needle. Care was observed when passing the needle into the esophagus so that regurgitation did not occur. The total daily supplements given to each mouse, expressed as per gram body weight, were as follows: experiment A: MSG 2 mg; 6 mg; 9 mg; L-lysine HCl 9 mg; 0.9% NaCl with equal volume. Experiment B: MSG 6 mg; NaCl with equal volume and osmolarity as MSG given (1,784 milliosmoles); control (none given).

The mice in experiment A were killed at 11 days of age and perfused with 10% formalin solution through the left ventricles of their hearts after being flushed with normal saline. After formalin fixation, 8-μ thick sections were cut every 80 μ from the preoptic region to the postinfundibular region. These sections were then stained with cresyl violet Nissl stain for light microscopic examinations. One mouse treated with 9 mg MSG/g body wt was prepared for electron microscopy as previously described (2).

Treated mice in experiment B remained with their mothers until 21 days of age when they were weaned and housed in individual cages with free access to Purina Chow for a period of 12 months. During this period, food intake was recorded twice weekly and each mouse was weighed monthly. At 6 and 12 months of age, the physical activity of each mouse was determined twice for 2-hr periods in the morning and evening, using automatically calibrated rotating activity cages.

Amino acid analysis

A Technicon TSM-1 system AutoAnalyzer was used for plasma amino acid analysis, using sulfosalicylic acid for deproteinization (16).

Plasma glutamate determination

A modification of Graham's enzymatic method (17) was used to measure plasma glutamate concentrations.

Serum testosterone determination

Serum testosterone was determined by a competitive protein-binding method using chromatog-

raphy on silica impregnated fiberglass and protein precipitation with ammonium sulfate (18).

Results

Experiment 1

During the course of feeding different levels of MSG to infant squirrel monkeys, one of the monkeys from group 2 died at 3.5 weeks of age and two from group 4 died at 3.5 and 4.5 weeks of age. The monkey from group 2 and one from group 4 apparently died from infection of undetermined etiology. The other monkey in group 4, receiving the highest level of MSG, developed convulsive seizures of a grand mal type at the 4th week of age. These were confirmed by EEG patterns. Seizures persisted for 2 days and then gradually subsided. The monkey accidentally strangulated itself on the 5th day after the initial development of seizures.

The weight gains and food intakes of the monkeys are presented in Table 1 and are compared with a group of 13 infant squirrel monkeys previously raised in the same nursery on a diet similar to that fed group 1. The weight gains of monkeys in groups 1, 2, and 3 fed 0, 4.8, and 9.1% MSG were within expected variation of these 13 control monkeys. However, the food intake and weight gain of monkeys in group 4 fed 16.7% MSG were significantly depressed and their efficiency of food utilization (grams weight gain/100 kcal consumed) was decreased.

Both the crown-rump length and the total length of the sole survivor in group 4 were shorter than similar measurements in the other animals. Head and chest circumferences varied considerably between and within groups and were less revealing.

Fasting plasma amino acids of all four groups showed no remarkable differences, indicating that the large intakes of MSG were cleared from circulation overnight.

Although the surviving group 4 monkey had a daily intake of sodium 10 times greater than the group 1 monkeys, its blood pressure was not increased. In fact, terminal blood pressures of all these anesthetized monkeys were somewhat less than the norms published for adult squirrel monkeys (19).

No abnormalities were found in any of the seven monkeys which had EEG and ERG recordings at the end of the experiment.

TABLE 1

Effects of feeding MSG to infant squirrel monkeys for 9 weeks

Diet	Nursery controls[a] (13)	Group 1 (0% MSG) (2)	Group 2 (4.8% MSG) (3)	Group 3 (9.1% MSG) (2)	Group 4 (16.7% MSG) (3)
	Formula only	Formula only	Formula + 5% MSG	Formula + 10% MSG	Formula + 20% MSG
Body weight[b]					
Initial, g	126 ± 12	95	111	110	108
Gain, g/day	2.92 ± 0.36	2.46	2.75	2.71	1.67
Food intake g wt gain/100 kcal[b]	3.38 ± 0.15	3.80	3.32	3.22	2.41
MSG intake, mg/g body wt/day		0	3.8	7.6	13.8
Total Na^+ intake, mg/day		33.3	147.0	248.1	334.5
Anthropometric measurements, 8-week increase in cm					
1) Crown-rump length	4.5 ± 0.3[c]	5.0	6.2	5.7	4.0
2) Total length		6.8	8.1	7.6	4.7
3) Head circumference	2.5 ± 0.3[d]	1.8	1.3	0.9	2.0
4) Chest circumference		4.0	3.4	2.5	1.3
Terminal blood pressure, mm Hg		124	99	93	101

Number in parentheses represents number of monkeys.

[a] Data obtained from Ausman et al. (12) on 13 monkeys raised under nursery conditions and fed a similar diet as group 1. Values are mean ± SE. [b] Body weight and food intake data for groups 2 and 4 represent mean values during the period when all three animals were alive. Other data for these groups were obtained from the animals that survived the experimental period. [c] Mean ± SE of 9 monkeys. [d] Mean ± SE of 8 monkeys.

Radiological assessment of all skeletons and routine histopathologic examination of tissues were also unremarkable. Electron microscopy of the hypothalamus and retina in the infant squirrel monkeys revealed no differences between groups. Changes related to imperfect fixation of the nervous system (8) such as ballooning of dendrites, mitochondrial fragmentation, and loss of cytoplasmic and nuclear staining affinity, were seen in several blocks of tissue irrespective of treatments. No pyknotic nuclei, lysosomal bodies, phagocytic vacuoles or other evidence of cellular necrosis were observed in the hypothalamus of any animal. In the examination of retinae, particular care was given to the inner plexiform layers and ganglion cells. No dendritic swelling in the former, nor necrosis in the latter, as described in mice (20), was found.

Experiment 2

Neither the cynomologus nor the bushbaby fed the diet containing 9.1% MSG for 11 months showed any adverse effects of the diet. Daily observation revealed no behavioral abnormalities. Their final weight gains, ERG, EEG, and plasma amino acid patterns were similar to controls not consuming MSG. No evidence of gross obesity developed.

Experiment 3

The 3-week-old cynomolgus monkey injected intramuscularly with MSG vomited several times within 10 min. Plasma glutamate at 15 min rose to 3.60 μmoles/ml from a fasting level of 0.04 μmoles/ml. This monkey has been observed for 30 months with no apparent sequelae developing from the MSG insult.

Experiment 4

Table 2 shows the results of experiment 4 in which weanling rats were fed diets for 5 weeks with supplements of MSG, L-glutamic acid, or sodium salts plus nonessential amino acids. It is to be noted that diets for groups 2, 4, and 5 were isonitrogenous. No mortality occurred even though some rats (group 3) consumed 28% of their diet (approximately 30 mg/g body wt) as MSG. Food intake was decreased in the four treatment groups, and the average terminal body and brain weights were less than those of the control groups. This growth depression was greatest in rats receiving MSG (groups 2 and 3). The salt control diet (group 5) but not the glutamic acid control (group 4) depressed growth to the extent obtained with the 20% MSG-added diet when compared with the glucose control

TABLE 2
Effects of feeding MSG to weanling rats for 5 weeks

	Groups					Least significant difference at 5% level[a]
	1	2	3	4	5	
	Basal + 20% glucose	Basal + 20% MSG	Basal + 40% MSG	Basal + 17.4% L-glutamic acid	Basal + Na salt + amino acids	
Body weight, g	267.7 ± 8.0	224.0 ± 8.5	169.3 ± 5.0	245.7 ± 8.6	231.0 ± 11.5	24.9
Brain weight, g	1.89 ± 0.02	1.77 ± 0.04	1.73 ± 0.03	1.88 ± 0.03	1.70 ± 0.08	0.13
Adrenal glands, mg 100 g body wt	16.1 ± 0.6	17.3 ± 0.9	20.0 ± 1.5	17.3 ± 0.1	14.5 ± 0.8	2.7
Testes, g 100 g body wt	1.04 ± 0.03	1.12 ± 0.06	1.36 ± 0.05	1.10 ± 0.04	1.07 ± 0.04	0.14
Kidneys, g 100 g body wt	0.87 ± 0.05	1.21 ± 0.02	1.38 ± 0.05	0.97 ± 0.03	1.19 ± 0.01	0.10
Water consumption, ml week 6 rats	1,130	2,359	3,848	1,731	2,155	
Plasma glutamate, μmoles ml	0.27 ± 0.01	0.54 ± 0.05	0.82 ± 0.06	0.43 ± 0.03	0.34 ± 0.02	0.11
Urinary pH	6.7	8.3	8.4	7.2	7.6	

Values are mean ± SE of groups of six rats.
[a] Difference of any two mean values exceeding LSD is significant ($P < 0.05$) (20).

diet, indicating that the body and brain weight differences in animals fed high levels of MSG were probably associated with their sodium content.

The weights of adrenal glands and testes, expressed per 100 g body wt, were increased by supplements of either MSG or L-glutamic acid but were significantly increased only at the highest MSG level. The sodium concentration of each diet was directly reflected in the relative size of the kidneys.

Water consumption was also roughly proportional to the sodium content of the diets although the group fed L-glutamic acid consumed more water than the control group fed a similar amount of glucose.

Plasma glutamate levels, measured 90 min postprandially, reflected the amount of glutamate added to the diet, with twofold and threefold increases, respectively, in rats fed 20 and 40% MSG-added diets. Rats fed free glutamic acid in group 4 had lower plasma glutamate than rats fed diets containing the same amount of glutamic acid as MSG.

Brain sections through the hypothalamus and its associated nuclei from rats fed the high MSG diets were unremarkable in H and E preparations. Although no estimates of neuronal concentrations were made, no apparent change in density or spacial relationships were observed. There was no observable edema, phagocytosis, necrosis, neuronal swelling, nor chromatin clumping. Silver staining of frozen sections for degenerating axons was also negative, even in the optic tract adjacent to the vestibular nucleus where degenerative change has been demonstrated following injection of MSG (22).

Experiment 5A

Mortality rates observed in infant mice force-fed up to 9 mg/g body wt of MSG and lysine or a control quantity of normal saline are shown in Table 3. Higher mortality was seen with higher doses of MSG. None of the mice receiving the lowest dose of MSG died, but by the end of the 5-day treatment, stunted growth could easily be seen.

Mice that survived the 5-day treatment period were examined histologically and found to have no evidence of damage in the ventromedial nuclei, arcuate nuclei, or median eminence. Neither neuronal necrosis nor gliosis was observed in any section of the hypothalamic regions. Degenerating microglial cells, as seen by Arees and Mayer (2), were absent. Electron microscopy of the mouse examined was also negative.

Experiment 5B

In this experiment in which mice were force-fed either 6 mg MSG/g body wt or an equivalent quantity of NaCl at the age of 6 through 10 days, there were a large number of deaths prior to 30 days of age, as the immediate and carryover effect of treatment. This amounted to 44% (11/25) in the MSG-treated and 28% (8/21) in the NaCl-treated mice. No deaths occurred in an untreated control group of six mice. In the survivors of the treated groups, no statistically significant differences were found in body weight changes or total food intake during the 12-month period, as shown in Table 4. Three male controls were also similar to the treatment groups, whereas growth of three female controls was less than that of the treated female mice. The results of activity testing among these three groups were not significantly different.

Discussion

The amount of MSG added to various diets in this study was excessive when compared with amounts that might conceivably be consumed in human diets. Calculation of the amounts of MSG consumed on a body weight basis by the various animals used in this study indicates that the three groups of

TABLE 3
Mortality rates of infant mice observed during 5-day oral treatment period (experiment 5A)

Treatment	Amount, mg/g body weight/day	No. of mice started at 6 days of age	No. of mice examined at 11 days of age	Mortality rate, %
MSG	2	5	5	0
	6	5	4	20
	9	5	2	60
Lysine HCl	9	5	0	100
Saline	[a]	5	5	0

[a] Normal saline given with equal volume.

TABLE 4
Effects of MSG on weight gain and food intake in mice

Sex	No. of mice[a]	Treatment	Initial weight at weaning, g	Final weight at 1 year of age, g	Increment, g	Total food intake, g
Male	6	MSG[b]	13.9 ± 0.7	37.8 ± 0.7	23.9 ± 0.3	1,924 ± 52
	9	NaCl[c]	15.7 ± 1.1	35.8 ± 0.8	20.1 ± 1.5	1,932 ± 71
	3	Control[d]	15.6 ± 0.7	35.7 ± 1.4	20.1 ± 1.9	1,983 ± 132
Female	8	MSG[b]	14.8 ± 0.7	33.0 ± 0.7	18.2 ± 0.5	1,940 ± 32
	5	NaCl[c]	13.7 ± 1.7	33.1 ± 0.7	19.4 ± 1.4	1,925 ± 50
	3	Control[d]	15.1 ± 0.1	29.7 ± 0.6	14.6 ± 0.6	1,927 ± 18

Values are mean ± SE. Using Student's *t* test, no significant differences exist between MSG and NaCl or control groups, except for weight gain of female control.

[a] Survivors of 12-month experimental period. [b] MSG given 2 mg/g body wt three times a day from 6 to 10 days of age in 20% solution. [c] NaCl given same volume and osmolarity as MSG group. [d] Control, no treatment.

squirrel monkeys (4.8, 9.1, and 16.7% MSG), the rats fed the highest level of MSG (40% supplement), and the mice force-fed 9 mg MSG/g body wt actually received daily 7, 15, 27, 60, and 18 times, respectively, the minimal dose (0.5 mg/g body wt) reported to induce hypothalamic lesions in mice (4). All the treated monkeys in this study were given more than the injected and intubated doses, 1.0, 2.7, and 4.0 mg/g body wt reported to have been pathogenic in infant rhesus monkeys (3). Nevertheless, neither hypothalamic damages, retinal changes, nor obesity reported in other studies were observed in these animals. This was probably due to the differences in study designs. Acute parenteral insults of MSG used in most other studies and in experiment 3 of this study are quite different from the medium to long-term ad libitum feeding of MSG in mixed diets. Thus, although in the long-term feeding experiments of this study, the animals used received more MSG daily and much more MSG during the whole experimental period than that administered to animals in acute studies which produced neural pathology, they were not subjected to the instant massive administration of MSG of the acute studies.

Furthermore, during the consumption of meals containing MSG, a number of factors may modify the utilization of the glutamate. The ability of the intestinal mucosa to transaminate glutamate is of importance. Transamination is not well developed in preweanling animals and may be depressed by vitamin B_6 deficiency (17). In these studies, animals as young or younger than those reported in other studies brain damaged by MSG were used. Decreased ability to transaminate glutamate in the intestinal wall may result in abnormally high plasma glutamate in infant animals. This phenomenon would be accentuated by oral administration of MSG to fasted animals, whereas feeding MSG with food as done in this study would substantially delay gastric emptying time and hence the rate of glutamate absorption. This difference in absorption was demonstrated by the following observation. The plasma glutamate of an 8-week-old rat given 2 mg MSG/g body wt in 20% solution intraduodenally was compared with that of another rat consuming daily 30 mg MSG/g body wt ad libitum in a semipurified diet (40 g MSG added to 100 g basal diet described in experiment 4). The former developed a plasma glutamate concentration of 1.85 μM/ml 60 to 90 min after MSG administration, whereas the highest concentration achieved in the latter was 0.91 μM/ml. Thus, although it received much less total MSG, the injected rat achieved a serum level of glutamate in excess of the amount reported needed to produce brain lesions in monkeys (3), whereas the fed rat did not.

In the present study, rats, mice, and three species of monkeys were used because of the possibility that different effects might occur with different species of animals. Gold thioglucose, used to produce obesity by inducing similar but more extensive hypothalamic le-

sions than MSG, is effective only in some strains of mice and rats, but not goats, sheep, dogs, or many other animals (23, 24).

Based on the comparable initiation and completion of deciduous dentition (25), the 2nd to 11th week study period in infant squirrel monkeys in experiment 1 might be considered to roughly correspond to an age of 6 months to 2 years in humans, the period MSG-containing baby foods used to be consumed. The levels of MSG in the monkey diets were much greater than those formerly added to commercial baby foods that invariably supplied only a part of babies' diets. Neither the growth nor the food intake of these monkeys was significantly affected when they were fed diets containing 4.8 or 9.1% MSG. However, growth and development as well as food intake of the group fed a diet containing 16.7% MSG were found to be depressed.

When MSG is fed, each mole of glutamate is accompanied by one mole of sodium. The sodium ion effect of MSG should not be ignored as intestinal transport (26) and the neurotoxic pathogenicity (27) of MSG have been reported to be sodium dependent. Sodium intake has long been implicated as a causal factor for later development of hypertension (28). In this study, no association could be found between high sodium intake and the terminal blood pressure in monkeys. Salted commercial baby food (containing 202 mg Na/100 kcal) along with milk (containing 86 mg Na/100 kcal), when consumed by a 6-month-old human infant, provide approximately 830 mg sodium daily (29). The infant squirrel monkeys in the three treated groups in this study received 6, 10, and 15 times as much on a body weight basis. The failure in this relatively short-term experiment to demonstrate an effect of these sodium loads on blood pressure does not preclude the possibility of long-term effects in squirrel monkeys.

Although MSG-induced hypothalamic lesions have been reported in newborn rhesus monkeys intubated or injected with MSG (3), investigators in two other laboratories were unable to obtain comparable lesions in similar experiments on infant monkeys (8, 9). Retinopathy caused by MSG has been reported in mice (20), with ERG changes being noted in rats (30). Neither the squirrel monkeys in experiment 1 nor the cynomolgus and bushbaby in experiment 2 showed any changes in their ERG. This was also true for the EEG except for one squirrel monkey of group 4 in experiment 1.

The deaths of three of the squirrel monkeys were upsetting because the availability of newborn monkeys was extremely limited. Two of them died probably of intercurrent infection in a manner seldom seen today but which was not uncommon at the time these studies were done. There is no reason to believe that the feeding of MSG affected resistance to infection. The death of the third monkey fed the highest level of MSG was unusual in that it developed grand mal convulsive seizures. After the administration of a mixture of vitamins, the convulsions disappeared but high amplitude spikes in EEG persisted. Unfortunately, the monkey strangulated. Glutamic acid was, at one time, used therapeutically for treating epileptic patients to decrease the frequency of seizures (31–33). Glutamic acid was claimed to be effective in patients with petit mal but the protective effect of glutamic acid was not fully accepted (34). It is interesting that glutamic acid was reported to aggravate grand mal type seizures (31). Convulsive disorders have recently been reported induced by MSG injection in rats (35). Although one monkey fed the diet with 16.7% MSG survived the experimental period and showed no evidence of central nervous system abnormalities, it would be impossible to conclude that the convulsions observed in the monkey fed the same diet were not related to the MSG content of the diet.

In the other species of monkeys studied, two points were noted. The monkey in experiment 3 recovered fully from acute toxic symptoms induced by parenteral administration of MSG and displayed a normal growth and development pattern for 2.5 years. The same amount of MSG has been reported to cause hypothalamic lesions in infant rhesus monkeys killed within a few hours of treatment (3). Secondly, the long-term feeding of high levels of MSG to cynomolgus and bushbaby monkeys in experiment 2 did not result in any observable harmful effects.

With more rats available than monkeys,

groups large enough for statistical analysis could be used. Considering the large amounts of MSG used in the rat experiment, the absence of mortality and the limited growth depression observed do not support the idea that MSG is toxic. This is of some importance as glutamic acid is present in food proteins in greater quantities than the other amino acids. The feeding of smaller amounts of other amino acids, such as methionine or tryptophan, appeared to be able to produce graver effects than MSG (36). This may be related in part to the fact that glutamic acid has been reported to be cleared from the intestines slower than 17 other amino acids (37). In the groups in which varying degrees of growth stunting occurred, sodium content seemed to be able to explain more of the decrease in body weight and brain weight than the glutamic acid content of the diets, as the sodium-supplemented diet produced effects comparable to those obtained with MSG-supplemented diets. The effects of the dietary sodium levels were also reflected in relative kidney size, in the amount of water consumption, and in urinary pH.

Plasma glutamate levels were slightly higher in the rats fed the diet with 20% MSG added than one with an equivalent amount of glutamic acid added. This may have been due to the higher solubility of MSG than its free acid in water. Pond and Pond (33) have reported that humans also had higher plasma glutamate and excreted more glutamic acid in their urine after the ingestion of MSG than an equivalent amount of free glutamic acid.

The role of glutamate on the endocrine axis has been implicated in many reports (1, 6, 38). The trend observed in this study that glutamate tended to increase adrenal and testicular weights seems provocative and requires further research. Weil-Malherbe (39) observed the classical triad of hypertension, tachycardia, and hyperglycemia, characteristics of catecholamine release, following the administration of L-glutamic acid to hypoglycemic coma patients. He also reported that epinephrine concentrations in blood following intravenous L-glutamic acid administration were increased (40). Recently, Schaumburg (41) has listed burning, flushing, and pressure over the face and chest as the clinical manifestations of the so-called Chinese Restaurant Syndrome, reportedly caused by meals high in MSG content. These signs are all suggestive of transient autonomic imbalance. The observed increase of adrenal weights in this experiment may be related to hyperfunction. Preliminary results of 24-hr urinary catecholamine determinations, however, did not detect any remarkable difference in the groups of rats studied in this investigation.

In view of the increased testicular weights in glutamate-fed rats, the experiment was repeated with six rats fed the basal diet plus 40% MSG and six rats fed the basal diet plus 40% glucose so that serum testosterone and histopathology could be comparably ascertained. In MSG-fed rats, mean serum testosterone levels ± SE were 306.5 ± 112.5 ng/100 ml and in glucose-fed rats were 404.3 ± 90.4 ng/100 ml. Testicular activity appeared not to be increased as the result of MSG feeding. Electron microscopic examination of these perfused testes (3% glutaraldehyde in pH 7.4 collidine buffer and fixed with OsO_4) was not remarkable, both in terms of interstitial cell integrity or activity of spermatogenesis. Serum cholesterol concentrations were determined in these rats (42) in view of the report that feeding large amounts of MSG in man and gerbils lowered serum cholesterol and associated lipoproteins (43, 44). The serum cholesterol of rats fed the 40% MSG-added diet (88 ± 8 mg/100 ml) was not significantly different from that of control rats (84 ± 15 mg/100 ml). However, the fact that both values were low might have masked the cholesterol-lowering ability of MSG.

The primary purpose of the first part of the mouse experiment (experiment 5A) was to determine whether chronic oral dosing of MSG would produce hypothalamic damage during the critical period of development. Although mortality rates were high in most of the MSG groups and in the lysine comparison group, no histopathologic lesions were observed in the survivors. The hypothalamic lesions reported in infant mice within 24 hr after a single MSG treatment (5) were not seen in this study in mice examined 6 days

after daily MSG administration during the vulnerable period. As it is improbable that the amount of MSG able to produce hypothalamic lesions in a single dose would not produce similar lesions when given for 5 days, it seems reasonable to suggest that the availability of food to the treated animals protected them from the neurotoxic effects of MSG.

The negative finding of the second part of the mouse experiment is also of interest. The early mortality in the MSG-treated mice may have involved the combined effects of sodium concentration and osmolarity because similar results were observed in the group that received equivalent amounts of NaCl. This explanation is supported by another experiment similar to experiment 5A in which groups of 6-day-old mice were force-fed daily for 5 days 6 mg MSG/g body wt, an isomolaric, isovolumetric amount of NaCl or 9 mg L-lysine HCl/g body wt. In this study, however, the mice were also injected intraperitoneally at the time of each force-feeding with five times as much by volume of a 2.5% glucose solution to partially correct for the water deficit.

Mortality in the MSG and lysine supplemented animals was 23.8% (5/21) and 25% (4/16) compared with 60 and 100% in experiment 5A; 16.7% (3/18) of the NaCl-fed mice also died.

The adverse effects of hypertonic saline solutions introduced into the gastrointestinal tract have been documented (45). The fact that signs such as obesity, hyperphagia, and hyperactivity (46) did not develop over 1 year in the mice that survived the acute toxicity from MSG administration suggests that important histologic changes were not missed and that there were no long-term effects of MSG in the mice studied.

Summary

The effects of large dietary supplements of MSG were studied in infant monkeys, weanling rats, and preweanling mice for varying periods of time. Ten infant squirrel monkeys were fed either a 0, 4.8, 9.1, or 16.7%-MSG formula diet for 9 weeks and evaluated clinically and histopathologically. None developed hypothalamic or retinal lesions. Three of the ten squirrel monkeys receiving MSG died, although two deaths appeared unrelated to treatment. The growth of the surviving monkey receiving the largest supplement of MSG was retarded, but a battery of clinical examinations was unremarkable in any of the surviving monkeys. Feeding the 9.1% MSG formula for 1 year to an infant cynomolgus and another infant bushbaby monkey had no effect when compared with two control monkeys. The intramuscular injection of 2.7 mg MSG/g body wt into a 3-week-old cynomolgus monkey produced marked elevation in circulating glutamate and vomiting but has had no demonstrable deleterious effect during the subsequent 2.5 years.

Groups of weanling rats were fed high levels of MSG or isocaloric, isonitrogenous, or isonatrogenous control diets. The growth depression observed in rats fed MSG diets appeared to reflect sodium intake more than glutamic acid consumption. Dietary sodium content was also a factor in kidney size, water consumption, urinary pH, and plasma glutamate concentration. No conclusive endocrine effect was found.

Infant mice dosed with varying levels of MSG from 6 through 10 days of age did not have hypothalamic lesions when killed on the 11th day. In a similar experiment in which the mice were allowed to live, approximately one-third died within 30 days. The high mortality was thought to be a function of the sodium content or osmolarity of the MSG administered. The surviving mice were raised for 1 year without developing hyperphagia, obesity, or hyperactivity.

The relative lack of toxicity of MSG in these studies when compared with previous reports is believed to be related to the route of administration, as MSG was neither injected nor force-fed to fasted animals but offered as a supplement to an otherwise normal diet.

The authors wish to thank Drs. Eliot L. Berson, Frank H. Duffy, and Edward A. Arees for their help in evaluating the ERG, EEG, and electron microscopic materials as well as special staining of rat brains, respectively. We should also like to thank Dr. Wen-Feng Jan who did the serum testos-

terone determinations, Dr. Nelson Westmoreland who did the electron microscopy of the testes, and Mr. Thomas Faherty who prepared some of the histologic sections.

References

1. OLNEY, J. W. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. *Science* 164: 719, 1969.
2. ARLES, E. A., AND J. MAYER. Monosodium glutamate-induced brain lesions: electron microscopic examination. *Science* 170: 549, 1970.
3. OLNEY, J. W., L. G. SHARPE AND R. D. FEIGIN. Glutamate-induced brain damage in infant primates. *J. Neuropathol. Exptl. Neurol.* 31: 464, 1972.
4. OLNEY, J. W., AND O. L. HO. Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature* 227: 609, 1970.
5. BURDE, R. M., B. SCHAINKER AND J. KAYES. Acute effect of oral and subcutaneous administration of monosodium glutamate on the arcuate nucleus of the hypothalamus in mice and rats. *Nature* 233: 58, 1971.
6. ADAMO, N. J., AND A. RATNER. Monosodium glutamate: lack of effects on brain and reproductive function in rats. *Science* 169: 673, 1970.
7. OSER, B. L., S. CARSON, E. E. VOGEN AND G. E. COX. Oral and subcutaneous administration of monosodium glutamate to infant rodents and dogs. *Nature* 229: 411, 1970.
8. REYNOLDS, W. A., N. LEMKEY-JOHNSTON, L. J. FILER, JR. AND R. M. PITKIN. Monosodium glutamate: absence of hypothalamic lesions after ingestion by newborn primates. *Science* 172: 1342, 1971.
9. ABRAHAM, R., W. DOUGHERTY, L. GOLBERG AND F. COULSTON. The response of the hypothalamus to high doses of monosodium glutamate in mice and monkeys. Cytochemistry and ultrastructural study of lysosomal changes. *Exptl. Mol. Pathol.* 15: 43, 1971.
10. OLNEY, J. W. Glutamate-induced neuronal necrosis in the infant mouse hypothalamus: an electron microscopic study. *J. Neuropathol. Exptl. Neurol.* 30: 75, 1971.
11. HAYES, K. C., G. FAY, A. ROACH AND F. J. STARE. Breeding New World monkeys in a laboratory environment. In: *International Symposium on Breeding Primates for Laboratory Use*, edited by W. I. B. Beveridge. Basel, Switzerland: S. Karger, 1972.
12. AUSMAN, L. M., K. C. HAYES, A. LAGE AND D. M. HEGSTED. Nursery care and growth of Old and New World infant monkeys. *Lab. Animal Care* 20: 907, 1970.
13. HAYES, K. C., AND J. COREY. Measurement of cerebrospinal fluid pressure in the rat. *J. Appl. Physiol.* 28: 872, 1970.
14. HEGSTED, D. M., C. R. MILLS, C. A. ELVEHJEM AND E. B. HART. Choline in the nutrition of chicks. *J. Biol. Chem.* 138: 459, 1941.
15. FINK, R. P., AND L. HEIMER. Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. *Brain Res.* 4: 369, 1967.
16. Technicon Instruments Corporation. Operating Instruction Manual for the Technicon TSM-1 System. *Technical Publication* No. TA 1-0233-00. Tarrytown, N. Y.: Technicon Instruments Corporation, 1971.
17. WEN, C. P., AND S. N. GERSHOFF. The effects of dietary vitamin B_6 on the utilization of monosodium glutamate by rats. *J. Nutr.* 102: 835, 1972.
18. JAN, W., AND N. I. GOLD. Serum testosterone analysis by competitive protein binding: chromatography on silica-impregnated fiberglass and protein precipitation with ammonium sulfate. *Biochem. Med.* 6: 7, 1972.
19. HERD, J. A., W. H. MORSE, R. T. KELLEHER AND L. G. JONES. Arterial hypertension in the squirrel monkey during behavioral experiments. *Am. J. Physiol.* 217: 24, 1969.
20. OLNEY, J. W. Glutamate-induced retinal degeneration in neonatal mice. Electron microscopy of the acutely evolving lesion. *J. Neuropathol. Exptl. Neurol.* 28: 455, 1969.
21. SNEDECOR, G. W., AND W. G. COCHRAN. *Statistical Methods* (6th ed.). Ames, Iowa: Iowa State Univ. Press, 1967, p. 272.
22. ARLES, E. A., B. SANDREW AND J. MAYER. Monosodium glutamate-induced optic tract pathway lesions in infant mice following subcutaneous injection. *Federation Proc.* 30: 287, 1971 (abstr.).
23. BAILE, C. A., J. MAYER, B. R. BAUMGARDT AND A. PETERSON. Comparative gold thioglucose effects on goats, sheep, dogs, rats and mice. *J. Dairy Sci.* 53: 801, 1970.
24. LIEBELT, R. A., AND J. H. PERRY. Action of gold thioglucose on the central nervous system. In: *Handbook of Physiology, Alimentary Canal.* Washington, D. C.: Am. Physiol. Soc., 1967, Sect. 6, vol. I, chapt. 21, p. 271–285.
25. LONG, J. O., AND R. W. COOPER. Physical growth and dental eruption in captive-bred squirrel monkeys, *Saimiri sciureus* (Leticia, Columbia). In: *The Squirrel Monkey*, edited by L. A. Rosenblum and R. W. Cooper. New York: Academic, 1968, p. 193.
26. SCHULTZ, S. G., L. YU-TU, O. O. ALVAREZ AND P. F. CURRAN. Dicarboxylic amino acid influx across brush border of rabbit ileum. *J. Gen. Physiol.* 56: 621, 1970.
27. KUHAR, M. J. A possible mechanism for the pathogenic action of monosodium glutamate. *Res. Commun. Chem. Pathol. Pharm.* 2: 95, 1971.
28. MENEELY, G. R., AND L. K. DAHL. Electrolytes in hypertension: the effects of sodium chloride. The evidence from animal and human studies. *Med. Clin. N. Am.* 45: 271, 1961.
29. FOMON, S. J., L. N. THOMAS AND L. J. FILER, JR. Acceptance of unsalted strained foods by normal infants. *J. Pediat.* 76: 242, 1970.

30. BUCKSER, S., AND C. BUCKSER. Effect of sodium glutamate on the electroretinogram. *Am. J. Optom.* 48: 504, 1971.
31. WAELSCH, H., AND J. C. PRICE. Biochemical aspects of glutamic acid therapy for epilepsy. *Arch. Neurol. Psychiat.* 51: 393, 1944.
32. PRICE, J. C., H. WAELSCH AND T. J. PUTNAM. DL-Glutamic acid hydrochloride in treatment of petit mal and psychomotor seizures. *J. Am. Med. Assoc.* 122: 1153, 1943.
33. POND, D. A., AND M. H. POND. Glutamic acid and its salts in petit mal epilepsy. *J. Mental Sci.* 97: 663, 1951.
34. GOODMAN, L. S., E. A. SWINYARD AND J. E. P. TOMAN. Effects of L-glutamic acid and other agents on experimental seizures. *Arch. Neurol. Psychiat.* 56: 20, 1946.
35. BHAGAVAN, H. N., D. B. COURSIN AND C. N. STEWARD. Monosodium glutamate induces convulsive disorders in rats. *Nature* 232: 275, 1971.
36. Harper, A. E., N. J. BENEVENGA AND R. W. WOHLHEUTER. Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev.* 50: 428, 1970.
37. DELHUMEAU, G., G. V. PRATT AND C. GITLER. The absorption of amino acid mixtures from the small intestine of the rat. *J. Nutr.* 77: 52, 1962.
38. REDDING, T. W., A. V. SCHALLY, A. ARIMURA AND I. WAKABAYASHI. Effect of monosodium glutamate on some endocrine functions. *Neuroendocrinol.* 8: 245, 1971.
39. WEIL-MALHERBE, H. The action of glutamic acid in hypoglycaemic coma. *J. Mental Sci.* 95: 930, 1949.
40. WEIL-MALHERBE, H. Glutamic acid and its relation to the nervous system. In: *Biochemical Society Symposia No. 8: Metabolism and Function in Nervous Tissue.* London: Cambridge Univ. Press, 1952, p. 16.
41. SCHAUMBURG, H. H., R. BYCK, R. GERSTL AND J. H. MASHMAN. Monosodium L-glutamate: its pharmacology and role in the Chinese Restaurant Syndrome. *Science* 163: 836, 1969.
42. CARPENTER, K. J., A. GOTSIS AND D. M. HEGSTED. Estimation of total cholesterol by a micro-method. *Clin. Chem.* 3: 233, 1957.
43. OLSON, R. E., G. BAZZANO AND J. A. DELIA. The effects of large amounts of glutamic acid upon serum lipids and sterol metabolism in man. *Trans. Assoc. Am. Physicians* 83: 196, 1970.
44. BAZZANO, G., J. A. DELIA AND R. E. OLSON. Monosodium glutamate: feeding of large amounts in man and gerbils. *Science* 169: 1208, 1970.
45. DENTON, D. A. Salt appetite. In: *Handbook of Physiology, Alimentary Canal.* Washington, D. C.: Am. Physiol. Soc. 1967, Sect. 6, vol. I, chapt. 31, p. 433–459.
46. CZOK, G., AND K. LANG. Über einige wirkungen von L-glutaminsäure, D-glutaminsäure and γ-aminobuttersäure. *Arch. Exptl. Pathol. Pharmakol.* 227: 214, 1955.

lactose tolerance in the families is compatible with a single autosomal gene as the cause of intolerance. If genetic in origin, lactose tolerance in this population is likely to be an expression of heterozygosity for a gene causing the formation of intestinal brush border lactase, while lactose intolerant individuals would be homozygous for a gene preventing the full activity of this enzyme.

Davis and Bolin[1] pointed out the difficulty of explaining the high prevalence of lactose intolerance in some populations on a genetic basis. If a single gene is responsible for lactose intolerance, its frequency must be very near to 1 in most populations of south-east Asia. It is very unlikely that this frequency could have been attained by recurrent mutation. This difficulty, however, arises only if the hypothetical gene causing lactose intolerance in the homozygous state is assumed to be a "deficiency gene". An alternative explanation would be that the gene responsible for the formation of intestinal lactase in most Europeans is replaced in most Asians by an allele governing the formation of a different gene product with some useful function; for example, an enzyme with altered specificity. This allele may convey a selective advantage in an environment lacking the facilities for the production of animal milk for human consumption. From this point of view the question of adaptive versus genetic origin becomes a question of whether it is a matter of a physiological adaptation of the individual or a genetic adaptation of the population. The lack of substrate induction and the results of the survey of the families seem to favour the latter possibility.

Increased tolerance is often observed a certain time after lactose intolerant individuals start drinking milk. Is this the consequence of an adjustment in the amount of milk consumed at any one time, and in the frequency with which it is drunk, in line with the inability to digest lactose, rather than the consequence of enzyme induction? Such an adjustment could be the factor that makes it possible for subjects with lactose intolerance to consume large amounts of milk each day without discomfort. Consequently, our findings do not provide an argument against giving aid in the form of milk for developing countries.

This work was supported by Stiftung Volkswagenwerk, Hanover, Germany. We thank Aroon Ridthimat for his encouragement and Miss C. Heinemann, Miss A. Heisig and Sukree Inthanagool for technical assistance.

G. FLATZ
CH. SAENGUDOM

Human Genetics Laboratory and
Division of Clinical Pathology,
Department of Pathology,
Faculty of Medicine, Chiang Mai University,
Chiang Mai, Thailand.

Received August 19, 1969.

[1] Davis, A. E., and Bolin, T. D., *Nature*, 216, 1244 (1967).
[2] Bolin, T. D., and Davis, A. E., *Nature*, 222, 382 (1969).
[3] Flatz, G., Saengudom, Ch., and Sanguanbhokhai, T., *Nature*, 221, 758 (1969).
[4] Keusch, G. T., Troncale, F. J., Thavaramara, B., Prinyanont, P., Anderson, P. R., and Bhamarapravathi, N., *Amer. J. Clin. Nutrit.*, 22, 638 (1969).
[5] Hultmann, E., *Nature*, 183, 108 (1959).

Glutamic Acid Decarboxylase and Gamma-aminobutyric Acid in Mammalian Kidney

THE discovery of glutamic acid decarboxylase (GAD) in mammalian brain was first reported in 1950[1,2]. The product of this enzyme reaction is gamma-aminobutyric acid (GABA), an important neuroinhibitor[3]. Because GABA occurs in large amounts in the central nervous system, subsequent interest in GAD emphasized its importance in brain to the exclusion of other tissues. But GABA is also present in significant amounts in human kidney[4,5] and, because it is normally absent from blood, enzymatic synthesis *in situ* is the most likely explanation for its presence here. We report that GAD is in fact present in mammalian kidney and to a lesser extent in other tissues outside the central nervous system.

Homogenates of brain and kidney from several mammalian species and of rat liver and spleen were prepared in ice-cold potassium phosphate buffer (0·026 M, *p*H 6·8) with a Potter–Elvehjem glass homogenizer. Aliquots were deproteinized with 1 per cent picric acid (w/v) or 3 per cent sulphosalicylic acid (w/v) and analysed for GABA by partition chromatography or high voltage electrophoresis on filter paper, and by elution chromatography with a Beckman–Spinco amino-acid analyser[6]. Fresh homogenate (100 mg) was also incubated in conditions slightly modified from those described by Roberts and Simonsen[7], using L-glutamic acid-U-^{14}C (specific activity, 186 mCi/mmole, New England Nuclear Corp.) or L-glutamic acid-1-^{14}C (20 mCi/mmole, California Biochemical Corp.), both of confirmed radiochemical purity. GAD activity was assayed by measuring $^{14}CO_2$ released from L-(1-^{14}C)-glutamate.

Table 1. GABA CONCENTRATION AND GAD ACTIVITY IN MAMMALIAN KIDNEY

Species*	GABA† (μmoles g)	GAD activity‡ (μmoles CO_2/100 mg homogenate/120 min)	Relative GAD activity $\frac{\text{Kidney}}{\text{Brain}} \times 100$
Man (1)	0·440	0·076	—
Rat (10)	0·037	0·144	17·1
Mouse (4)	0·033	0·096	7·4
Guinea-pig (2)	0·078	0·056	5·3

* Numbers in parentheses indicate the number of kidneys examined; results are the mean. The human tissue was obtained at necropsy 10 h after death; its GABA concentration is comparable with values cited in refs. 4 and 5.

† Measured in deproteinized homogenate by elution chromatography on 22 cm column of Beckman–Spinco amino-acid analyser[6].

‡ Conditions of reaction. Concentration of materials in final volume: L-^{14}C-glutamate, 3·1 mM; L-(1-^{14}C)-glutamate, 0·00065 mM; pyridoxal-5′-phosphate, 0·236 mM, 0·1 M potassium phosphate buffer *p*H 6·8. Reaction started by adding 0·6 ml. of kidney homogenate in phosphate buffer, equivalent to about 50 mg wet weight, to 0·2 ml. of reaction mixture. Incubation performed at 37° C for 120 min in cross-arm flasks[7] and terminated by addition of 0·2 ml. 8 N H_2SO_4. $^{14}CO_2$ was collected in a scintillation flask containing 0·4 ml. hyamine. After addition of scintillation mixture (0·1 g POPOP, 4·0 g PPO in 1 l. of toluene), the radioactivity was measured in a Nuclear Chicago Unilux II counter operating at 80 per cent efficiency. Values are the mean of at least three observations.

GABA is present in appreciable amounts in homogenates of human, rat, guinea-pig and mouse kidney (Table 1). The identity of GABA was confirmed by partition and elution chromatography and by two electrophoretic methods[6]. Kidney homogenates incubated with uniformly ^{14}C-labelled L-glutamic acid formed a radioactive ninhydrin-positive compound which had the chromatographic properties of GABA[6]. Homogenates incubated with 1-^{14}C labelled glutamate produced 0·153 μmoles of $^{14}CO_2$/100 mg homogenate/120 min from 2·97 μmoles of endogenous and exogenous substrate; the net synthesis of GABA in paired samples, analysed by ion exchange chromatography, was 0·168 μmoles/100 mg homogenate/120 min. The GAD activity in kidney relative to brain in each of the species studied is shown in Table 1. The apparent GAD activity in rat liver and spleen relative to brain was also evaluated and found to be 16 per cent and 2 per cent respectively.

We investigated some of the characteristics of GAD in rat kidney homogenates. Activity is directly proportional to the amount of homogenate used in the reaction (Fig. 1*a*). First-order kinetics are observed when the substrate concentration is varied between 0·38 mM and 3·1 mM; the first approximation of the Km obtained from the Lineweaver–Burk plot is 4 mM (Fig. 1*b*), a value which is close to 3·0 mM, cited for GAD in acetone extracts of mouse brain and measured by the same assay technique[6].

Additional characteristics of GAD in brain and kidney of the rat were compared. The *p*H optimum is 6·8 in both

tissues, a value corresponding to earlier work in rat brain[1]. The temperature optimum is 37° C in both tissues; however, activity at 37° C compared with 27° C is proportionately higher in brain than in kidney. Removal of pyridoxal-5-phosphate from the incubation medium inhibits brain GAD 81 per cent and kidney GAD 48 per cent; the difference in tissue response may reflect either variation in the amount of endogenous coenzyme present in the two types of homogenate, or possibly an isozymic characteristic for the apoenzyme.

GAD activity is more or less confined to the renal cortex; the activity, in relation to wet weight, is less than 1 per cent in renal papilla compared with cortical activity. Activity in the cell is found predominantly in the supernatant fraction in brain and kidney after centrifugation of the homogenate at 100,000*g* for 1 h at 0° C.

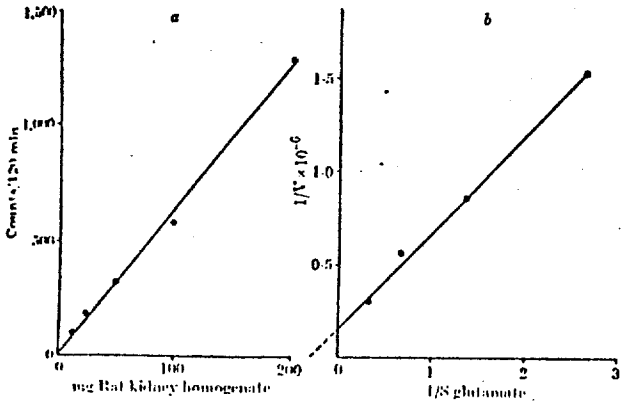


Fig. 1. Rat kidney homogenates were incubated in conditions for GAD assay (see Table 1). *a*, Activity is directly proportional to amount of homogenate (wet weight) added to reaction mixture. *b*, Lineweaver–Burk plot depicting Michaelis–Menten kinetics for GAD activity. The apparent K_m is 4 mM.

Mammalian brain synthesizes GABA by decarboxylation of glutamic acid, rather than by reverse transamination of succinic acid semialdehyde[10]. The quantities of GABA in kidney and the conditions in which it is formed thus indicate that GAD activity is important also in this organ. Because GAD is not found in the renal papilla where anaerobic metabolism prevails, it is likely that the GABA pathway in kidney functions as an oxidative shunt in cortical metabolism[11]; there is no neuroinhibitory role for GABA in this organ. The GABA pathway could provide an efficient route for glutamate oxidation in conditions of renal acid-base metabolism, where the amide group of glutamine is the principal source of urinary ammonia[12].

The presence of GAD in kidney is also relevant to an autosomal recessive convulsive disorder in man known as vitamin B_6 dependency[6,13]; the trait is believed to result from a mutation which impairs the binding of pyridoxal-5-phosphate by the GAD apoenzyme. The presence of GAD in kidney may offer an opportunity, through renal biopsy material, to investigate this hypothesis.

This work was supported in part by a grant from the Medical Research Council of Canada. D. T. W. is a Queen Elizabeth II Canadian Fund research fellow.

DONALD T. WHELAN
CHARLES R. SCRIVER
FAZL MOHYUDDIN

deBelle Laboratory for Biochemical Genetics,
The McGill University–Montreal Children's Hospital
Research Institute,
2300 Tupper Street,
Montreal 108, Canada.

Received July 2; revised August 14, 1969.

[1] Roberts, E., and Frankel, S., *J. Biol. Chem.*, 187, 55 (1950).
[2] Wingo, W. J., and Awapara, J., *J. Biol. Chem.*, 187, 207 (1950).
[3] Roberts, E. (edit. by), *Proc. Internat. Confer., New York* (Pergamon, London, 1960).
[4] Scriver, C. R., Pueschel, S., and Davies, E., *New Engl. J. Med.*, 274, 636 (1966).
[5] Zachman, M., Tocci, P., and Nyhan, W. L., *J. Biol. Chem.*, 241, 1355 (1966).
[6] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, 30, 1190 (1958).
[7] Roberts, E., and Simonsen, D., *Biochem. Pharmacol.*, 12, 113 (1963).
[8] Scriver, C. R., and Whelan, D. T., *Ann. NY Acad. Sci.*, 166, 83 (1969).
[9] Suss, J. P., Haber, B., and Roberts, E., *Biochemistry*, 8, 2870 (1969).
[10] Roberts, E., Wein, J., and Simonsen, D. G., *Vitamins and Hormones*, 22, 503 (1964).
[11] McKhann, G. M., Albers, R. W., Sokoloff, L., Mickelsen, O., and Tower, D. B., in *Inhibitions of the Nervous System and γ-Aminobutyric Acid* (edit. by Roberts, E., *et al.*), 169 (Pergamon Press, London, 1960).
[12] Pitts, R. F., and Stone, W. J., *Proc. Third Intern. Cong. Nephrology*, 1, 123 (Karger, New York, 1967).
[13] Scriver, C. R., *Pediatrics*, 26, 62 (1960).

Detoxification of Diazinon by Subcellular Fractions of Diazinon-resistant and Susceptible Houseflies

HOUSEFLIES metabolize dialkyl phosphorothionate insecticides, such as diazinon and parathion, to the corresponding dialkyl phosphorothioic acids[1,2]. This reaction is thought to be controlled by gene *a*, on chromosome II (ref. 3), associated with low aliesterase activity[4]. The cleavage mechanism is reported to be in the microsomes[5,6,8]. I have found three detoxification mechanisms in subcellular fractions of six strains (Table 1) of housefly and related these to the genetics of diazinon-resistance[7,8] of the flies.

Table 1. FACTORS OF DIAZINON-RESISTANCE IN STRAINS OF HOUSEFLY

Strain*	Degree of resistance	Type of resistance	Located on chromosome (ref. 3)	Detoxification mechanism
ocra SRS	1	None (susceptible)	—	*A*
ac; *ar*; *bwb*; *ocra* SRS	1	None (susceptible)	—	*A*
29	10–15	Gene *a* (low aliesterase)	II	*A* + *B*
393	10–15	Gene *a* (low aliesterase)	II	*A* + *B*
466·500	10	Sesamex-inhibited	V	*A* + *C*.
SKA	400	Gene *a*	II	*A* + *B* + *C*
		and penetration factor	III	
		and sesamex-inhibited	V	

* Strains 29, 393 and 466·500 were derived from the SKA strain.

The subcellular fractions were prepared by differential centrifugation of whole-fly homogenates in 0·15 M phosphate buffer (*p*H 7·3). The supernatant fluid from centrifuging at 10,000*g* for 10 min was recentrifuged at 100,000*g* for 60 min, to give a soluble fraction (fluid) and a microsomal fraction (precipitate). Material from ten flies was incubated with insecticide, and any other reactants, in 2 ml. buffer and shaken in air or nitrogen at 30° C. Decomposition products of [^{14}C-ethoxy]diazinon and [^{32}P]diazoxon in the reaction mixture were identified by paper chromatography, using two solvent systems: (I) acetonitrile/water/ammonia (40 : 9 : 1), and (II) propan-2-ol/ammonia (75 : 25). Autoradiography was also used. The products were measured by solid scintillation counting of the dissected areas of the chromatograms[9]. Desethyl diazinon (R_F 0·85 and 0·95 in solvent systems I and II respectively) and desethyl diazoxon (R_F 0·60 and 0·80 in systems I and II respectively) were identified by co-chromatography with materials synthesized by methods described for the preparation of analogous derivatives of other insecticides[10].

The first mechanism (*A*), cleavage of diazinon and diazoxon to diethyl phosphorothioic acid (DEPTA) and diethyl phosphoric acid (DEPA), respectively, occurred entirely in the microsomal fraction and required oxygen. NADPH and reduced glutathione (GSH) separately

J. Physiol. Soc. Japan (1961) 23, 383-385)

DL-Metionin, DL-Sulfoximin の毒性について

612.825:612.014.469

Study on the toxicity of DL-metionin and DL-sulfoximin

柳沢一夫・中村俊郎・宮田慶三郎・
亀田務・北村重晴・伊藤一夫
(YANAGISAWA-Kazuo・NAKAMURA-Toshiro・MIYATA-Keisaburo・
KAMEDA-Tutomu・KITAMURA-Shigeharu・ITO-Kazuo)*

The substance, DL-metionin, DL-sulfoximin which was identified by Sir e. Mellandy in 1943, have differing from other convulsant, a long latent period from a few hours to more than ten.

The auihours determined the lethal doses and established that 50% lethal doses (LD_{50}) in case of abdominal injection was 0.132 mg/g. Their toxicity was calculated 53 times as that of the sodium glutamate, 60 times as NaCl solution and 40 times as glycocoll.

I. 緒　　論

過去十数年にわたる，林並びにその門下研究員によってなされた，塩縮の研究の結果，遂に前に於ける興奮催起物質（R物質）はCarnosin，抑制物質（X物質）はCarnitinであることを決定した．その後の研究により，中枢神経においてもこれに類似する拮抗作用のあることが判明し，1957年に至り，林，永井等[2]は中枢神経における抑制物質はγ-Amino-β-hydroxy酪酸(GABOB)であることを実験生理学的立場より証明するに至った．

これに対し中枢神経におけるR物質は鋭意研究中であるが未だその本体は明らかでない．

翻って1943年Mellambyは犬の痙攣病が小麦粉の精白にクロール窒素を用いるが，そのパンを多食することによって起るということを発見した．

その後Mellamby及びその門下は小麦粉の蛋白質がクロール窒素の作用で変性して，その毒性の原因となる物質が生ずることを証明した．

その後，英国及び米国でその物質の本体を研究し，Metioninが変化してDL-Metionin，DL-Sulfoximinとなると強い痙攣作用があることを発見すると同時に，他の3種の類似物質の合成にも成功した．

しかも，このDL-Metionin，DL Sulfoximinを犬の小脳延髄槽及び側脳室，皮質運動領にある濃度で投与すると，他の間接刺激物質と異なり，数時間乃至十数時間という長い潜時を以って痙攣を発動し，しかもその痙攣は反復するという特長をもっている．

著者等は，これらの特長をもつDL-Metionin，DL-Sulfoximinの致死量を決定すべく実験を試みたので報告する．

II. 実　験　方　法

実験動物にはDD系マウス10g内外のものを用い，1群を10匹として各群を作り実験を行ないLD_{50}は

$$LD_{50}=b_m-\frac{\Sigma(z\cdot d)}{m}$$

の式より求めた．

III. 実　験　成　績

試薬はDL-Metionin，DL-Sulfoximinの濃度0.01 *mol*のものをそれぞれ0.5 cc，0.6 cc，0.7 cc，0.8 cc，1.0 cc，1.5 ccの6種に分けて，腹腔内注射を行ない，その結果を観察した．

その実験結果を表示すると次の如くである．

* 日本大学歯学部理化学研究室
Dept. of Physical Chemistry, Nihon Univ. School of Dentistry
(昭35年9月26日受付)

Table 1.　0.01 *mol* 0.5 cc

No.1 3.55.35. Injection Spontaneous motion 8.30.00. Inactivity	No. 6 3.59.10. Injection Spontaneous 4.10.00. Excite 10.20.00. Inactivity
No. 2 3.56.00. Injection Spontaneous movement 4.30.00. Inactivity	No. 7 3.59.50. Injection 8.20.00. Inactivity
No. 3 3.57.50. Injection Spontaneous motion 9.00.00. Inactivity 9.40.00. Spontaneous	No. 8 4.01.00. Injection 9.00.00. Inactivity 9.26.00. K.K.
No. 4 3.58.25. Injection 9.20.00. Inactivity	No. 9 4.02.10. Injection Spontaneous 8.40.00. Inactivity 9.25.40. K.K.
No. 5 3.58.50. Injection Spontaneous motion 5.10.00. Exciting state 9.00.00. Inactivity	No. 10 4.02.50. Injection Spontaneous 9.10.00. Inactivity 9.25.30. K.K. 9.30.00. Dead

0.01 *mol* 0.5 cc（之はマウスの体重 1 g に対して 0.09 mmg の割）を注射した第1群においては，実験数10例の中，18時間の観察によって死亡数は1例であった．

Table 2.　0.01 *mol* 0.6 cc

No. 1 10.51.30. Injection Spontaneous motion 12.10.00. Excite 16.30.00. Inactivity	No. 6 10.54.10. Injection Spontaneous motion 16.50.00. Inactivity
No.2 10.51.50. Injection Spontaneous motion 12.00.00. Excite 14.30.00. Inactivity 18.00.00. Lie down 22.00.00. Dead	No. 7 10.55.00. Injection 14.30.00. Inactivity 16.00.00. Excite
No. 3 10.52.50. Injection 12.00.00. Excite 16.00.00. Inactivity 20.30.00. Spontaneous motion 20.40.00. Dead	No. 8. 10.55.30. Injection 14.30.00. Excite 16.50.00. Inactivity
No. 4 10.52.50. Injection Spontaneous motion 12.30.00. Inactivity 17.30.00. Excite	No. 9 10.56.00. Injection Spontaneous motion 11.50.00. Inactivity
No. 5 10.53.20. Injection 13.50.00. Inactivity	No. 10 10.56.30. Injection 14.00.00. Excite 16.30.00. Inactivity

0.01 *mol* 0.6 cc を注射した第2群においては，実験数10例中，18時間の視察によって死亡数は2例であった．

Table 3.　0.01 *mol* 0.7 cc

No. 1 9.10.05. Injection 9.10.06. Spontaneous motion 9.25.00. Inactivity 23.00.00. Dead	No. 6 9.13.00. Injection Spontaneous motion 9.30.00. Inactivity 23.00.00. Dead
No. 2 9.10.30. Injection Spontaneous motion 9.20.00. Inactivity 23.50.00. Dead	No. 7 9.14.30. Injection 9.20.00. Excite 9.40.00. Inactivity
No. 3 9.11.30. Injection Spontaneous motion 9.20.30. Inactivity	No. 9 9.15.00. Injection 9.30.00. Inactivity 20.00.00. Dead
No. 4 9.11.50. Injection 9.20.00. Inactivity 23.10.00. Dead	No. 9 9.15.30. Injection Spontaneous motion 9.30.00. Inactivity 12.00.00. Walk but awkward 23.50.00. Dead
No. 5 9.12.15. Injection Spontaneous motion 9.30.00. Inactivity	No. 10. 9.16.00. Injection Spontaneous motion 9.40.00. Inactivity

0.01 *mol* 0.7 cc を注射した第3群においては，実験数10例中，18時間の視察によって死亡数は6例であった．

Table 4.　0.01 *mol* 0.8 cc

No. 1 10.57.10. Injection Spontaneous motion 13.10.00. Excite 17.30.00. Inactivity 18.30.00. Lie down 20.40.00. Dead	No. 6 11.00.00. Injection 14.20.00. Excite 16.40.00. Inactivity 18.30.00. Walk but awkward 20.25.00. A little excite 23.30.00. Dead
No. 2 10.57.50. Injection Spontaneous motion 13.00.00. Excite 16.20.00. Inactivity 18.00.00. Lie down 22.10.00. Dead	No. 7 11.00.40. Injection 12.00.00. Excite 16.50.00. Inactivity 19.20.00. Walk but awkward
No. 3 10.58.30. Injection Spontaneous motion 16.30.00. Inactivity 18.30.00. Lie down 22.10.00. Dead	No. 8 11.01.50. Injection 13.25.00. Excite 16.10.00. Inactivity 17.30.00. Walk but awkward 18.00.00. Lie down 22.10.00. Dead
No. 4 10.59.00. Injection 14.50.00. Excite 17.10.00. Inactivity 18.00.00. Lie down 19.30.00. Dead	No. 9 11.02.30. Injection 13.20.00. Excite 16.00.00. Inactivity 17.50.00. Layed down
No 5 10.56.30. Injection 16.00.00. Excite 18.10.00. Inactivity 18.20.00. Lie down	No. 10 11.03.50. Injection Spontaneous motion 16.00.00. Excite 17.10.00. Inactivity

0.01 *mol* 0.8 cc を注射した第4群においては，実験数10例中，18時間の視察によって死亡数は6例であった．

Table 5.　0.01 *mol* 1.0 cc

No. 1 3.49.50. Injection Spontaneous motion 4.15.00. Inactivity 4.30.30. Walk but awkward 5.30.20. Asphyxia 4.50.00. Dead	No. 6 3.52.30. Injection Spontaneous motion 5.10.00. Inactivity 6.10.00. Walk but awkward 9.30.00. Asphyxia 10.00.00. Dead
No. 2 3.50.20. Injection Spontaneous motion 4.15.00. Inactivity 4.25.00. Walk but awkward 3.50.00. Dead	No. 7 3.53.15. Injection Spontaneous motion 4.25.30. Walk but awkward 9.10.00. Asphyxia 10.50.00. Dead
No. 3 3.50.50. Injection 4.20.00. Inactivity 4.35.00. Walk but awkward 5.30.00. Lie down 9.10.00. Dead	No. 8 3.53.50. Injection Spontaneous motion 4.05.10. Walk but awkward 10.00.00. Spontaneous motion 10.15.00. Dead
No. 4 3.51.15. Injection 4.20.00. Inactivity 4.35.00. Walk but awkward 9.50.00 Dead	No. 9 3.54.15. Injection 4.30.00. Inactivity 8.20.00. Walk but awkward 10.10.00. Dead
No. 5 3.51.50. Injection Spontaneous motion 4.30.00. Inactivity 4.40.00. Walk but awkward	No. 10 3.55.10. Injection Spontaneous motion 4.30.00. Inactivity 5.10.00. One-sided walk 6.25.30. Lie down 9.10.00. Dead

0.01 *mol* 1 cc を注射した第5群においては，実験数10例中，18時間の観察によって死亡数は9例であった．

Table 6.　0.01 *mol* 1.5 cc

No. 1 3.45.40. Injection Spontaneous motion 4.05.00. Inactivity 6.30.00. Walk but awkward 9.10.30. Lie down 9.50.00. Dead	No. 6 3.41.00. Injection 4.10.00. Inactivity 5.10.30. Walk but awkward 9.00.00. Dead
No. 2 3.46.40. Injection Spontaneous motion 4.10.00. Inactivity 4.35.00. Lie down 4.50.00. Closing of eye-lids 4.52.10. Rotation 5.10.00. Spontaneous motion 9.10.00. Spontaneous motion 10.00.00. Dead	No. 7 3.42.50. Injection Spontaneous motion 4.10.00. Inactivity 6.30.00. Spontaneous motion 9.50.00. Dead
No. 3 3.47.10. Injection 4.15.00. Inactivity 4.40.00. Walk but awkward 4.45.00. Lie down 10.10.00. Dead	No. 8 3.43.15. Injection 3.43.30. Shock 4.05.00. Inactivity 9.00.00. Dead
No. 4 3.48.00. Injection 4.10.00. Inactivity 5.00.00. Walk but awkward 10.20.00. Dead	No. 9 3.43. 45. Injection Spontaneous motion 4.50.00. Walk but awkward 5.4 .00. Lie down 9.40.00. Dead
No. 5. 3.48.50. Injection Sponteneous motion 4.15.00. Walk but awkward	No. 10 3.45.00. Injection 4.10.00. Inactivity 4.15.00. Walk butawkward 4.35.10. Lie down 9.00.00. Asphyxiation 9.30.00. Dead

0.01 *mol* 1.5 cc を注射した第6群においては，実験数10例中，18時間の観察によって死亡数は9例であった．

Ⅳ．総括及び結論

以上の実験成績を総括すると第7表の如くである．

これらの実験成績を前述の式にあてはめると，

$LD_{50}=0.132$ mg/g

となる．

なお対照として行なった実験では，

グルタミン酸ソーダは　$LD_{50}=6.9$ mg/g　で 2% NaClは$LD_{50}=7.8$ mg/gで 0.2 *mol* Glycocollは $LD_{50}=5.3$ mg/g であった．

以上を比較検討すると DL-Metionin, DL-Sulfoximin の LD_{50} はグルタミン酸ソーダの約53倍で，2% NaCl の約60倍 0.2 *mol* Glycocoll の約40倍で，上記3種の比較実験薬物よりも相当に強烈な毒性を有する．

Table 7.　Toxicity of DL-Metionin DL-Sulfoximin.

Injected quantity of 0.01 mol	mg/g	Number of experiment	Cases of death	Observation
0.5 cc	0.090	10	1	18 hours
0.6 cc	0.108	10	2	18 hours
0.7 cc	0.126	10	6	18 hours
0.8 cc	0.144	10	6	18 hours
1.0 cc	0.190	10	9	18 hours
1.5 cc	0.270	10	9	18 hours

注射後の経過は，その注射量及び，濃度によって異るが，概ね注射直後に自発現象を見，その後運動不活発となり，濃度，量の少ない時は間もなく恢復するが，多量の時は，運動不活発のまま，横転し仮死状態となる．

稿を終るに際し御指導御鞭撻を賜り，且つ御校閲の労を煩られた，生理学教室林　髞教授並びに理化学教室永井一夫教授に感謝の意を表する．なお実験に際し御助力を戴いた理化学教室大橋正敬講師に感謝する．

文　献

1) 森島庫太・張生見垣夫：薬理学
2) 青木義久・永井甲子四郎・宇佐見八郎・逸見一郎・伊藤裕之：γ-Amino-β-Oxy 酪酸の毒性に就いて

Effects of Monosodium Glutamate on Rabbit Fetuses

Shinobu Yonetani

Central Research Laboratories
Ajinomoto Co., Inc., Kawasaki

Introduction

It is only a few years ago that thalidomide, a derivative of glutamic acid, was found to induce malformation in human fetuses, which became a social problem. Tugrul reported recently that glutamidine, one of glutamic acid drugs, is teratogenic in rabbits[17]. Today glutamic acid drugs are being used clinically for treatment of hypoacidity[16], epilepsy[19], concussion of the brain[2], organic neurosis[3], various types of psychosis, amyosthenia[14], and hypoglycemic coma[12].

Sodium glutamate is being used widely as flavor enhancer. Absence of toxicity of glutamic acid (L-and D-forms) in mice[14] and that of sodium glutamate in rats, rabbits, dogs and cats[6] have been confirmed. However, their effects on fetuses, as reported by Tugrul, have not as yet been studied.

Since glutamidine, a drug based on the salt of glutamic acid hydrochloride(referred to as Glu-HcI), and monosodium glutamate (referred to as Na-Glu) are both ~~related to~~ salts of glutamic acid, it seems necessary to examine whether or not Na-Glu might be as teratogenic ~~as glutamidine.~~ We have studied teratogenic effects of Glu-Hcl on rabbit fetuses to reconfirm the results of Tugrul. The same type of experiments has been done with Na-Glu.

Materials and Methods

1. Animals and experimental groups.

Healthy female rabbits (2.5 - 3.5kg) over 7 months old were purchased for experiments. Several male rabbits were prepared for

mating. These animals were kept in a temperature - and humidity-controled room (23 ± 3°C, 60 ± 10%, respectively), and were fed freely on compressed diet (ORIENTAL GC5, for breeding) and water.

The rabbits were allowed for free mating. Copulation was confirmed by observation, and sometimes by detection of sperm spermatozoa in vagina.

After mating female rabbits were divided into three groups; the one to which salt of L-glutamic acid hydrochloride was administered (referred to as Glu-Hcl Group), the one sodium L-glutamate administered, (referred to as Na-Glu Group), and a control group (consisting of two groups; saline-administered and none administered or intact).

Each male rabbit was used for mating every second day at most.

2. Method of drug administration

Day of copulation was regarded as day 0 of pregnancy. Administration of the drugs was started on day 1 and continued for 15 days (Fig.2).

The drug (Glu-Hcl or Na-Glu) was dissolved in distilled water (0.83g/dl). The appropriate quantity of the solution was administered to give each rabbit 25mg of the drug per kg body weight with a syringe equipped with a narrow rubber catheter.

At a certain time of the day when rabbits were hungry, their body weights were determined. Then the animal was fixed in a frame

(Fig.1a) for indroduction of the catheter (Fig.1b). The procedure of administration is as follows.

The operator stood on the right side of the rabbit and held the animal on the head covering from the back of the head to the forehead by the palm of left hand. He placed his middle, ring, and little fingers of the left hand under the jaw of the rabbit to fix its head and forced to open the right corner of its mouth with his index finger and thumb to expose the inter-alveolar margin. By his right hand he forced to insert the catheter between the dorsum of the tongue and the palate. Introduction of the catheter through the oesophagus to the stomach was facilitated by deglutition movement of the animal. When the head of the rabbit was held too tight, the animal was irritated and introduction of the catheter was unsuccessful. When it was held too loose, the animal chewed off the catheter.

After the first insertion of the catheter, the operator concentrated his efforts to holding the upper and the lower jaws of the rabbit tightly with his index finger and thumb to prevent movement of the jaw.

Holding the head of the rabbit vertical to extend the cervical region, he palpated the oesophagus of that region by his right hand to confirm introduction of the catheter. After this confermation, the appropriate amount of the solution was injected by right hand.

The present method of introduction appears to be superior to the conventional method by which the mouth of an animal is forced to open to take a solution, in that it requires only one operator

and scarcely gives damage to the tongue or to the wall of the oral cavity, and in that the method itself is more natural and more successful.

3. Diagnosis of pregnancy and examination of abnormal gestation.

During the drug-administration period the status of pregnancy, such as formation and development of placenta, growth of fetus, and resorption of fetus, was examined. Besides, pseudopregnancy was examined by palpation, copulation test, or laparotomy.

Figure 2 shows the time schedule for these examinations during pregnancy

Diagnosis of gestation was done three times, on days 10, 13, and 16. The subsequent propress of pregnancy was examined three times, every four days. All examinations were performed when the animals were hungry.

In a normal progress of pregnancy, brood bean-sized and thumb-sized embryos can be detected by palpation on days 10 and 15, respectively(9)

At the first diagnosis on day 10, the rabbit was palpated carefully and briefly not to receive excessive pressure on the uterus. On days 13 and 16, palpation was performed in details. If pregnancy was unclear on day 16, the rabbit was subjected to copulation test.

When mating was successful, the rabbit was considered not to be pregnant. When mating was unsuccessful, pseudopregnancy or

abnormal pregnancy was suspected and the animal was examined by laparotomy.

The rabbits finally diagnosed on day 16 to be pregnant were subsequently palpated every fourth day. If any abnormality was detected, the rabbit was immediately laparotomized or autopsied for further detailed examinations. The examinations were directed foward the following points.

Condition of the uterus; size of the organ, thickness and color of the endometrium, condition of implanted region, number of implantation, and conditions of embryo and placenta.

Condition of the ovary; degree of congestion, regression of corpus luteum, condition of ovarian follicle, its size and its weight.

Macroscopic observation of other organs, and the weights of main visceral organs.

We minimized contact to the rabbits during the puerperal period not to induce premature delivery. The cage was covered to keep the animal calm.

4. Examination after delivery.

As Figure 3 shows, the rabbits of each experimental group were further divided into two groups, non-nursed and nursed.

i. Non-nursed and nursed groups.

Mothers and youngs of the non-nursed group were sacrificed on day 3 after delivery. Mothers were examined for the condition of the uterus (remaining or resorbed fetuses, delay

of afterbirth, and number of implanted embryos) and youngs, for body weight, weight of visceral organs, and malformation. On the other hand, the youngs of the nursed group were nursed for 30 days. During that time they were observed for growth rate and possible appearance of malformation. Nursing rate (or death rate) was also calculated. On day 30, only youngs were sacrificed for examination of body weights, weights of organs and malformation as done with the youngs of the non-nursed group.

ii. Autopsy and skeletal examination of youngs.

For autopsy mothers and 30-day-old youngs were sacrificed by bleeding, and 3-day-old youngs, with ether. In the case of mothers, heart, lung, liver, kidney, spleen, pancreas, digestive organs, body fat, adrenal gland, and genital organs were mainly examined macroscopically. Especially, heart, liver, kidney, spleen, adrenal gland and genital organs were weighed. Adrenal gland, genital organs and those showing pathological alterations were fixed with 10% formaldehyde solution for further histological examination.

At autopsy of youngs, we examined macroscopically appearance, skeletal system, body fat and main visceral organs. Adrenal gland, testis and ovary were fixed with 10% formaldehyde solution after they were weighted. Examination of appearance to detect possible malformation was directed especially toward nose, eye, and ear; that of skeletal system, toward cranial bone, cervix, vertebral, limb, digital, and tail.

The skeletal system was stained with alizarin red[15] (described below) for ~~histological~~ morphological observation.

Results

1. Conception rate

Table 1 indicates the conception rate of each group.

In Glu-HCl group 73% of rabbits gave the highest conception rate (1/1). This value was higher than that of control (64%). The percentage of the animals that gave the highest conception rate was the lowest in Na-Glu group.

The percentage of rabbits that showed lower conception rates (1/4, 1/3) in the control group was found to be lower than that in Na-Glu group and higher than that in Glu-Hcl group.

Conception rate was calculated with animals for which copulation and pregnancy were confirmed. Since all the female rabbits used in the experiment had been confirmed to be fertile, it is unlikely that infertility affected the results.

As described above, the conception rate appears to differ considerably among diffe rent groups. This difference may be ascribable to the different time of mating or different potency of the individual male rabbit. Statistical analysis of the results demonstrated absence of significant variations among the groups.

2. Observation of pregnant rabbit (for abnormal case of pregnancy)

Progress of pregnancy was normal in most of the cases in each group. But there were a few abnormal cases in each group.

In Glu-HCl group consisting of 15 rabbits, there were 6 abnormal cases; in one case one premature young was delivered on day 28 of pregnancy (referred to as a case of partial premature

delivery), in one case all embryos died and were resorbed (referred to as a case of resorption of all embryos), in two cases the number of youngs delivered was less than that of implantation, and degenerating fetuses and placenta were found in the uterus (referred to as a case of resorption of some portion of fetuses), and in two cases, pregnant animals died. The one rabbit died through an acute course and its visceral organs and uterus were found to be macroscopically normal. The other died of gastrophrenic pericarditis.

The Na-Glu group (8 rabbits) had two cases of abnormal pregnancy. The one aborted all the fetuses on day 26 of pregnancy. The other, which had a coccigenic disease, died on day 13.

In the control group (11 cases) there were three cases of abnormal pregnancy; a case of partial premature delivery, a case of resorption of all fetuses, and a case of unclear implantation.

These observations were considered in view of the drugs administered. Premature delivery and abortion occurred not only in the drug-administered groups but in the control. No malformation was observed with any fetuses obtained. Therefore it seems unlikely that the drugs induce abnormal pregnancy and embryonic malformation.

As for the change of fetuses, there were cases of resorption of embryos. From the time of detection and the condition of embryos, the degeneration might have started before differentiation of various organs. At this embryonic stage, fetuses are said to be the most susceptible to teratogenic agents. (7) Since teratogenic

change may be fatal to fetuses (5), their death and resorption observed in the present experiment may possibly be due to the teratogenic effects. However, it is unlikely that death of all embryos results from malformation. Generally, rabbits are highly susceptible to teratogenic agents (7) and the frequency of their spontaneous malformation is high.(13) Even if death and resorption of fetuses in the present experiment were ascribable to malformation, their incidence was still low. Moreover, such cases were also observed in the control group. Then it can be said that occurrance of such cases is not specific to the drugs but spontaneous.

The case of resorption of all fetuses in Glu-Hcl group was considered to be ascribable to abnormal pregnancy due to the mother's disease.

In Glu-Hcl group, two pregnant rabbits were found to be abnormal on day 20 of pregnancy. They died through an acute clinical course. The one rabbit was infected with coccidium. Similar autopsy findings, such as parenchymatous hemorrhage of the liver, and bleedings under intestinal mucous membrane and in the pelvic canal, were obtained with the two cases.

Infection of the mature rabbit with coccidium is not serious nor fatal. In the present experiment, pregnancy might have deteriorated the infection. But the case was suspected as an acute septicemic disease because the clinical course was acute and hemorrhagic inflammation was found in the tissues of various organs.

It was clear that the one rabbit in Na-Glu group died of gastrophrenic pericarditis.

3. Litter-size.

The litter-size of each rabbit is shown in Table 2. Table 3 shows the average litter-size of each group.

The mean litter-size was 8.1 ± 2,21 S.D. for Glu-Hcl group, 7.2 ± 2.23 S.D. for Na-Glu group, and 7.5 ± 2.27 S.D. for the control. There was no significant difference among the three groups ($P < 0.05$).

4. Nursing

Figure 4 shows the change in average number of youngs in the nursed group of each experimental group during the nursing period (the data obtained with the non-nursed group were included until day 3). Nursing of each mother rabbit was shown in "observation schedule of youngs after delivery" of Tables 2a, b and c.

Since the litter-size varies with the individula case, we normalized it assuming the mean litter-size as 100. The average of the normalized value in each group was taken as a mean nursing index or rate.

As can be seen from Figure 4, the nursing rates of three groups continued to decrease markedly until day 7 and did not change after then. The pattern of change during nursing period did not differ much among the three groups. Whereas the nursing rate just after delivery was 100% for Na-Glu group and the saline control, it was as low as 91% for Glu-HCl group and 82% for the

intact control. This fact means that youngs in the latter two groups died or were killed more frequently than those in the former two groups did.

Between days 0 and 4 the nursing rates of Glu-HCl, Na-Glu, and saline control groups decreased similarly. But after then the rate of saline control was always higher by 10% than those of the other two groups.

The nursing rate of the intact control was always lower than those of the other groups.

When the rates of Glu-HCl and Na-Glu groups were compared with the average of the two control groups, there was no difference among the groups. The rates of three groups all reached a level of about 40% after day 15.

The nursing rate: 40% corresponds to a litter-size consisting of three youngs.

Under normal conditions one rabbit is generally said to nurse 4 - 6 youngs after some selection. Then the average of the present experiment is slightly lower than the normal level. This is probably due to the fact that the cages used in the present study were not large enough.

5. External, skeletal, and other autopsy findings of youngs.

All the youngs of each experimental group were examined on days 3 and 30 chiefly for mouth, nose, eye, ear, and other parts and for general form of the animal. Body fat, expecially subcutaneous and subserous storage fat, and skeletal characteristics

were inspected at autopsy.

The results were indicated in Tables 2a, b, c, and d. Plates 1 - 12 illustrate representative cases of each group.

In each experimental group, none of the youngs were found to be abnormal. In Tables 2a, b, c, and d, a mark (-) means that nothing abnormal was found.

6. Body weight of youngs and weights of main visceral organs of youngs and mother rabbits.

Table 3 shows the mean body weight of youngs in each group and also summarizes the mean relative weights of organs (relative to body weight) of youngs and mothers.

The difference among the groups was studied statistically. The results are as follows.

i. Body weight of 3-day-old youngs (Fig. 5a)

The average body weight of each group was 69.5±11.2 S.D. g for Glu-HCl group, 61.7±12.1 g for Na-Glu group and 77.7±13.5g for the control. The values of two drug-administered group are significatly smaller than that of the control ($P < 0.01$).

ii. Body weight of 30-day-old youngs (Fig. 5b)

The average body weight of each group was 352.0±92.1 S.D. g for Glu-HCl group, 340.0±32.7 g and 463.0±68.9g for the control. The difference between Glu-HCl group and the control is not significant ($P < 0.05$). On the other hand,

the value of Na-Glu group is significantly smaller than that of the control ($P < 0.01$).

iii. Average specific weights ($X10^{-5}$) of various organs from 3-day-old youngs.

1) Testis (Fig. 6a).

The values obtained were 6.72± 0.8 S.D. for Glu-HCl group, 6.20±0.75 for Na-Glu group, and 7.08±0.5 for the control. There was no significant difference among the three ($P < 0.05$).

2) Ovary (Fig. 6b)

The values obtained were 3.40± 1.13 for Glu-HCl group, 2.98±0.58 for Na-Glu group, and 4.08±0.23 for the control. They did not differ significantly each other ($P < 0.05$).

3) Adrenal gland (Fig. 6c)

The specific values were 4.5±1.34 for Glu-HCl group, 4.04±0.51 for Na-Glu group, and 5.3±1.13 for the control. The difference between Glu-HCl group and the control was not significant ($P < 0.05$), but the value for Na-Glu group was significantly smaller than that of the control ($P < 0.01$).

iv. Average specific weights ($x10^{-5}$) of organs from 30-day-old youngs.

1) Testis (Fig. 7a).

The values were 7.9±1.67 for Glu-HCl group, 7.9±0.71 for Na-Glu, and 7.2±1.03 for the control. The differ-

ences among the groups were not significant ($P < 0.05$).

2) Ovary (Fig. 7b)

The specific values were 2.08±0.38 for Glu-HCl group, 2.36±0.29 for Na-Glu group, and 2.09±0.49 for the control. These values did not differ significantly each other (P 0.05).

3) Adrenal gland (Fig. 7c)

The values obtained were 5.03±0.92 for Glu-HCl group, 4.67±0.61 for Na-Glu group and 4.71±1.72 for the control. The differences were not significant (P 0.05).

v. Average specific weights of main organs from mothers (Fig. 8)

Specific values for ovary, liver, and spleen did not differ significantly from group so group. But the specific weights of adrenal gland for Na-Glu group and of kidney for Glu-HCl group were smaller than those of the control.

Discussion

We studied whether or not glutamic acid hydrochloride (Glu-HCl) and sodium glutamate (Na-Glu) have teratogenic effects on rabbit embryos chiefly by morphological observation of youngs after delivery. We also recorded growth of the youngs and determined conception and nursing rates to study some effects of the drugs on breeding and nursing.

The results of our experiments did not agree with those of Tugrul who reported that Glutamidine (a drug based on Glu-HCl) induces similar malformation of rabbit embryos to that observed with thalidomide administration. In the present experiment, oral administration of Glu-HCl at a dose of 25 mg/kg. body weight/day did not give rise to any morphological abnomality of rabbit fetuses.

Furuya et al [8] obtained similar results to ours, using rats. Oral administration of Glu-HCl at two doses, 25 mg and 125 mg/kg body weight/day did not induce any abnormal morphological alteration in fetuses. Body weight and feeding of mature male, mature female, and pregnant rats were not influenced by the drug administration. The drug did not affect sexual cycle of female rats.

Clinically, Glu-HCl has been used mainly for the treatment of diseases. Influence of the drug has naturally been discussed concerning its therapeutic effects. Few studies on effects of the drug on pregnancy and malformation in human has been reported. Therefore the results obtained with clinical use of the drug may not rule out completely the following possibilities. The method of administration and the

quantities of the drug might have been inappropriate for detection of possible intrinsic teratogenic ability of the drug. Effects of the drug on pregnancy and malformation might had been overlooked.

There had been no reports on experimental animals until 1965 when Tugrul reported that glutamidine induced malformation of rabbit fetuses. He suspected that glutamic acid might be also teratogenic.

Our results and those of Furuya et al with rats did not demonstrate induction of malformation by Glu-HCl and Na-Glu each at an equivalent dose to that of glutamidine in Tugrul's experiments. Furthermore, Glu-HCl at five times the dose of the glutamidine induced no malformation at all in rats.

The drug dose used in the present experiments, 25 mg/kg body weight/day roughly corresponds to the estimated daily uptake of monosodium glutamate for an adult in Japan (1), 1.5 (-2.0)g/60 kg body weight. Generally, it is desirable to test the teratogenic effects of a certain new drug at ten times the estimated ordinary dose. In view of this point, the amount of the drugs used in this study may be insufficient. They the present results may not rule out completely the possible intrinsic teratogenicity of the drugs.

Since the use of glutamic acid as flavor enhancer and medicine may be increasing in future, it would be necessary to test the drugs at ten times the ordinary dose (at least several times) or at the maximum safety dose (at least the maximum ordinary dose).

On the other hand, the amount of the drug used in the present study is unlikely to be excessive. Excessive administration readily causes

resorption of embryos. Pregnancy progressed normally and death or resorption of fetuses did not occur in most of the cases in the present experiments.

However, resorption of embryos was found in some rabbits. Most of these cases may be ascribable to the diseases of mother rabbits, not to excessive administration of the drug.

Whether or not the two drugs tested are intrinsically teratogenic remains to be studied further. It may at least be concluded from the present results that Glu-HCl and Na-Glu are not teratogenic each at an equivalent dose to that of glutamidine which was reported by Tugrul to induce malformation. The fact that rabbits are highly susceptible to teratogenic agents further supports the above conclusion.

The standard procedure (11) to test effects of a new medicine on fetuses requires two species of the test animals. Our results with rabbits and those of Furuya et al with rats appear to satisfy the above condition.

It is clear from the present results that neither Glu-HCl nor Na-Glu affects significantly the growth of youngs, and the breeding and the nursing abilities of mother rabbits.

The average body weight of 3-day-old youngs varied with the experimental group. These values appeared to be influenced by the selection (death) of youngs just after delivery, but not by the drug administration. The average body weights of 30-day-old youngs did not differ among the groups. The drug administration did not affect conception rate and litter-size. The nursing rate after day 7 did not vary with the group. The observed difference of nursing rate among the groups

before day 7 would be ascribable to the method of feeding and the unstable condition of individual mother rabbit just after delivery.

The organs of fetuses in all groups were found to be macroscopically normal.

The specific weights of embryonic organs did not differ among the groups except that of adrenal gland. The specific value for adrenal gland was smaller in Na-Glu group than in the control. This observation held for the mother rabbits. They also had the kidneys smaller than those of the control. These results may not imply immediately that administration of the drug influenced development or growth of the organs. Detailed histological examination would be needed for the study on effects of the drug on the organs.

Effects of Monosodium Glutamate on Rabbit Fetuses

Shinobu Yonetani

Central Research Laboratories
Ajinomoto Co., Inc., Kawasaki

Introduction

It is only a few years ago that thalidomide, a derivative of glutamic acid, was found to induce malformation in human fetuses, which became a social problem. Tugrul reported recently that glutamidine, one of glutamic acid drugs, is teratogenic in rabbits(17). Today glutamic acid drugs are being used clinically for treatment of hypoacidity(16), epilepsy(19), concussion of the brain(2), organic neurosis(3), various types of psychosis, amyosthenia(14), and hypoglycemic coma(12).

Sodium glutamate is being used widely as flavor enhancer. Absence of toxicity of glutamic acid (L-and D-forms) in mice(14) and that of sodium glutamate in rats, rabbits, dogs and cats(6) have been confirmed. However, their effects on fetuses, as reported by Tugrul, have not as yet been studied.

Since glutamidine, a drug based on the salt of glutamic acid hydrochloride(referred to as Glu-Hcl), and monosodium glutamate (referred to as Na-Glu) are both ~~related to~~ salts of glutamic acid, it seems necessary to examine whether or not Na-Glu might be as teratogenic ~~as glutamidine~~. We have studied teratogenic effects of Glu-Hcl on rabbit fetuses to reconfirm the results of Tugrul. The same type of experiments has been done with Na-Glu.

Materials and Methods

1. Animals and experimental groups.

Nealthy female rabbits (2.5 - 3.5kg) over 7 months old were purchased for experiments. Several male rabbits were prepared for

mating. These animals were kept in a temperature - and humidity-controled room (23 ± 3°C, 60 ± 10%, respectively), and were fed freely on compressed diet (ORIENTAL GC5, for breeding) and water.

The rabbits were allowed for free mating. Copulation was confirmed by observation, and sometimes by detection of sperm spermatozoa in vagina.

After mating female rabbits were divided into three groups; the one to which salt of L-glutamic acid hydrochloride was administered (referred to as Glu-Hcl Group), the one sodium L-glutamate administered, (referred to as Na-Glu Group), and a control group (consisting of two groups; saline-administered and none administered or intact).

Each male rabbit was used for mating every second day at most.

2. Method of drug administration

Day of copulation was regarded as day 0 of pregnancy. Administration of the drugs was started on day 1 and continued for 15 days (Fig.2).

The drug (Glu-Hcl or Na-Glu) was dissolved in distilled water (0.83g/dl). The appropriate quantity of the solution was administered to give each rabbit 25mg of the drug per kg body weight with a syringe equipped with a narrow rubber catheter.

At a certain time of the day when rabbits were hungry, their body weights were determined. Then the animal was fixed in a frame

(Fig.1a) for indroduction of the catheter (Fig.1b). The procedure of administration is as follows.

The operator stood on the right side of the rabbit and held the animal on the head covering from the back of the head to the forehead by the palm of left hand. He placed his middle, ring, and little fingers of the left hand under the jaw of the rabbit to fix its head and forced to open the right corner of its mouth with his index finger and thumb to expose the inter-alveolar margin. By his right hand he forced to insert the catheter between the dorsum of the tongue and the palate. Introduction of the catheter through the oesophagus to the stomach was facilitated by deglutition movement of the animal. When the head of the rabbit was held too tight, the animal was irritated and introduction of the catheter was unsuccessful. When it was held too loose, the animal chewed off the catheter.

After the first insertion of the catheter, the operator concentrated his efforts to holding the upper and the lower jaws of the rabbit tightly with his index finger and thumb to prevent movement of the jaw.

Holding the head of the rabbit vertical to extend the cervical region, he palpated the oesophagus of that region by his right hand to confirm introduction of the catheter. After this conformation, the appropriate amount of the solution was injected by right hand.

The present method of introduction appears to be superior to the conventional method by which the mouth of an animal is forced to open to take a solution, in that it requires only one operator

and scarcely gives damage to the tongue or to the wall of the oral cavity, and in that the method itself is more natural and more successful.

3. Diagnosis of pregnancy and examination of abnormal gestation.

During the drug-administration period the status of pregnancy, such as formation and development of placenta, growth of fetus, and resorption of fetus, was examined. Besides, pseudopregnancy was examined by palpation, copulation test, or laparotomy.

Figure 2 shows the time schedule for these examinations during pregnancy.

Diagnosis of gestation was done three times, on days 10, 13, and 16. The subsequent propress of pregnancy was examined three times, every four days. All examinations were performed when the animals were hungry.

In a normal progress of pregnancy, brood bean-sized and thumb-sized embryos can be detected by palpation on days 10 and 15, respectively[9]

At the first diagnosis on day 10, the rabbit was palpated carefully and briefly not to receive excessive pressure on the uterus. On days 13 and 16, palpation was performed in details. If pregnancy was unclear on day 16, the rabbit was subjected to copulation test.

When mating was successful, the rabbit was considered not to be pregnant. When mating was unsuccessful, pseudopregnancy or

abnormal pregnancy was suspected and the animal was examined by laparotomy.

The rabbits finally diagnosed on day 16 to be pregnant were subsequently palpated every fourth day. If any abnormality was detected, the rabbit was immediately laparotomized or autopsied for further detailed examinations. The examinations were directed foward the following points.

Condition of the uterus; size of the organ, thickness and color of the endometrium, condition of implanted region, number of implantation, and conditions of embryo and placenta.

Condition of the ovary; degree of congestion, regression of corpus luteum, condition of ovarian follicle, its size and its weight.

Macroscopic observation of other organs, and the weights of main visceral organs.

We minimized contact to the rabbits during the puerperal period not to induce premature delivery. The cage was covered to keep the animal calm.

4. Examination after delivery.

As Figure 3 shows, the rabbits of each experimental group were further divided into two groups, non-nursed and nursed.

i. Non-nursed and nursed groups.

Mothers and youngs of the non-nursed group were sacrificed on day 3 after delivery. Mothers were examined for the condition of the uterus (remaining or resorbed fetuses, delay

of afterbirth, and number of implanted embryos) and youngs, for body weight, weight of visceral organs, and malformation. On the other hand, the youngs of the nursed group were nursed for 30 days. During that time they were observed for growth rate and possible appearance of malformation. Nursing rate (or death rate) was also calculated. On day 30, only youngs were sacrificed for examination of body weights, weights of organs and malformation as done with the youngs of the non-nursed group.

ii. Autopsy and skeletal examination of youngs.

For autopsy mothers and 30-day-old youngs were sacrificed by bleeding, and 3-day-old youngs, with ether. In the case of mothers, heart, lung, liver, kidney, spleen, pancreas, digestive organs, body fat, adrenal gland, and genital organs were mainly examined macroscopically. Especially, heart, liver, kidney, spleen, adrenal gland and genital organs were weighed. Adrenal gland, genital organs and those showing pothological alterations were fixed with 10% formaldehyde solution for further histological examination.

At autopsy of youngs, we examined macroscopically appearance, skeletal system, body fat and main visceral organs. Adrenal gland, testis and ovary were fixed with 10% formaldehyde solution after they were weighted. Examination of appearance to detect possible malformation was directed especially toward nose, eye, and ear; that of skeletal system, toward cranial bone, cervix, vertebral, limb, digital, and tail.

The skeletal system was stained with alizarin red[15] (described below) for ~~histological~~ morfological observation.

Results

1. Conception rate

Table 1 indicates the conception rate of each group.

In Glu-HCl group 73% of rabbits gave the highest conception rate (1/1). This value was higher than that of control (64%). The percentage of the animals that gave the highest conception rate was the lowest in Na-Glu group.

The percentage of rabbits that showed lower conception rates (1/4, 1/3) in the control group was found to be lower than that in Na-Glu group and higher than that in Glu-Hcl group.

Conception rate was calculated with animals for which copulation and pregnancy were confirmed. Since all the female rabbits used in the experiment had been confirmed to be fertile, it is unlikely that infertility affected the results.

As described above, the conception rate appears to differ considerably among diffe rent groups. This difference may be ascribable to the different time of mating or different potency of the individual male rabbit. Statistical analysis of the results demonstrated absence of significant variations among the groups.

2. Observation of pregnant rabbit (for abnormal case of pregnancy)

Progress of pregnancy was normal in most of the cases in each group. But there were a few abnormal cases in each group.

In Glu-HCl group consisting of 15 rabbits, there were 6 abnormal cases; in one case one premature young was delivered on day 28 of pregnancy (referred to as a case of partial premature

delivery), in one case all embryos died and were resorbed (referred to as a case of resorption of all embryos), in two cases the number of youngs delivered was less than that of implantation, and degenerating fetuses and placenta were found in the uterus (referred to as a case of resorption of some portion of fetuses), and in two cases, pregnant animals died. The one rabbit died through an acute course and its visceral organs and uterus were found to be macroscopically normal. The other died of gastrophrenic pericarditis.

The Na-Glu group (8 rabbits) had two cases of abnormal pregnancy. The one aborted all the fetuses on day 26 of pregnancy. The other, which had a coccigenic disease, died on day 13.

In the control group (11 cases) there were three cases of abnormal pregnancy; a case of partial premature delivery, a case of resorption of all fetuses, and a case of unclear implantation.

These observations were considered in view of the drugs administered. Premature delivery and abortion occurred not only in the drug-administered groups but in the control. No malformation was observed with any fetuses obtained. Therefore it seems unlikely that the drugs induce abnormal pregnancy and embryonic malformation.

As for the change of fetuses, there were cases of resorption of embryos. From the time of detection and the condition of embryos, the degeneration might have started before differentiation of various organs. At this embryonic stage, fetuses are said to be the most susceptible to teratogenic agents. (7) Since teratogenic

change may be fatal to fetuses (5), their death and resorption observed in the present experiment may possibly be due to the teratogenic effects. However, it is unlikely that death of all embryos results from malformation. Generally, rabbits are highly susceptible to teratogenic agents (7) and the frequency of their spontaneous malformation is high.(13) Even if death and resorption of fetuses in the present experiment were ascribable to malformation, their incidence was still low. Moreover, such cases were also observed in the control group. Then it can be said that occurrance of such cases is not specific to the drugs but spontaneous.

The case of resorption of all fetuses in Glu-Hcl group was considered to be ascribable to abnormal pregnancy due to the mother's disease.

In Glu-Hcl group, two pregnant rabbits were found to be abnormal on day 20 of pregnancy. They died through an acute clinical course. The one rabbit was infected with coccidium. Similar autopsy findings, such as parenchymatous hemorrhage of the liver, and bleedings under intestinal mucous membrane and in the pelvic canal, were obtained with the two cases.

Infection of the mature rabbit with coccidium is not serious nor fatal. In the present experiment, pregnancy might have deteriorated the infection. But the case was suspected as an acute septicemic disease because the clinical course was acute and hemorrhagic inflammation was found in the tissues of various organs.

It was clear that the one rabbit in Na-Glu group died of gastrophrenic pericarditis.

3. Litter-size.

The litter-size of each rabbit is shown in Table 2. Table 3 shows the average litter-size of each group.

The mean litter-size was 8.1 ± 2.21 S.D. for Glu-Hcl group, 7.2 ± 2.23 S.D. for Na-Glu group, and 7.5 ± 2.27 S.D. for the control. There was no significant difference among the three groups ($P < 0.05$).

4. Nursing

Figure 4 shows the change in average number of youngs in the nursed group of each experimental group during the nursing period (the data obtained with the non-nursed group were included until day 3). Nursing of each mother rabbit was shown in "observation schedule of youngs after delivery" of Tables 2a, b and c.

Since the litter-size varies with the individual case, we normalized it assuming the mean litter-size as 100. The average of the normalized value in each group was taken as a mean nursing index or rate.

As can be seen from Figure 4, the nursing rates of three groups continued to decrease markedly until day 7 and did not change after then. The pattern of change during nursing period did not differ much among the three groups. Whereas the nursing rate just after delivery was 100% for Na-Glu group and the saline control, it was as low as 91% for Glu-HCl group and 82% for the

intact control. This fact means that youngs in the latter two groups died or were killed more frequently than those in the former two groups did..

Between days 0 and 4 the nursing rates of Glu-HCl, Na-Glu, and saline control groups decreased similarly. But after then the rate of saline control was always higher by 10% than those of the other two groups.

The nursing rate of the intact control was always lower than those of the other groups.

When the rates of Glu-HCl and Na-Glu groups were compared with the average of the two control groups, there was no difference among the groups. The rates of three groups all reached a level of about 40% after day 15.

The nursing rate, 40% corresponds to a litter-size consisting of three youngs.

Under normal conditions one rabbit is generally said to nurse 4 - 6 youngs after some selection. Then the average of the present experiment is slightly lower than the normal level. This is probably due to the fact that the cages used in the present study were not large enough.

5. External, skeletal, and other autopsy findings of youngs.

All the youngs of each experimental group were examined on days 3 and 30 chiefly for mouth, nose, eye, ear, and other parts and for general form of the animal. Body fat, expecially subcutaneous and subserous storage fat, and skeletal characteristics

were inspected at autopsy.

The results were indicated in Tables 2a, b, c, and d. Plates 1 - 12 illustrate representative cases of each group.

In each experimental group, none of the youngs were found to be abnormal. In Tables 2a, b, c, and d, a mark (-) means that nothing abnormal was found.

6. Body weight of youngs and weights of main visceral organs of youngs and mother rabbits.

Table 3 shows the mean body weight of youngs in each group and also summarizes the mean relative weights of organs (relative to body weight) of youngs and mothers.

The difference among the groups was studied statistically. The results are as follows.

i. Body weight of 3-day-old youngs (Fig. 5a)

The average body weight of each group was 69.5±11.2 S.D. g for Glu-HCl group, 61.7±12.1 g for Na-Glu group and 77.7±13.5g for the control. The values of two drug-administered group are significatly smaller than that of the control ($P < 0.01$).

ii. Body weight of 30-day-old youngs (Fig. 5b)

The average body weight of each group was 352.0±92.1 S.D. g for Glu-HCl group, 340.0±32.7 g and 463.0±68.9g for the control. The difference between Glu-HCl group and the control is not significant ($P < 0.05$). On the other hand,

the value of Na-Glu group is significantly smaller than that of the control ($P < 0.01$).

iii. Average specific weights ($X10^{-5}$) of various organs from 3-day-old youngs.

1) Testis (Fig. 6a).

The values obtained were 6.72± 0.8 S.D. for Glu-HCl group, 6.20±0.75 for Na-Glu group, and 7.08±0.5 for the control. There was no significant difference among the three ($P < 0.05$).

2) Ovary (Fig. 6b)

The values obtained were 3.40± 1.13 for Glu-HCl group, 2.98±0.53 for Na-Glu group, and 4.08±0.25 for the control. They did not differ significantly each other ($P < 0.05$).

3) Adrenal gland (Fig. 6c)

The specific values were 4.5±1.34 for Glu-HCl group, 4.04±0.51 for Na-Glu group, and 5.3±1.13 for the control. The difference between Glu-HCl group and the control was not significant ($P < 0.05$), but the value for Na-Glu group was significantly smaller than that of the control ($P < 0.01$).

iv. Average specific weights ($x10^{-5}$) of organs from 30-day-old youngs.

1) Testis (Fig. 7a).

The values were 7.9±1.67 for Glu-HCl group, 7.9±0.71 for Na-Glu, and 7.2±1.03 for the control. The differ-

ences among the groups were not significant ($P < 0.05$).

2) Ovary (Fig. 7b)

The specific values were 2.08±0.38 for Glu-HCl group, 2.36±0.29 for Na-Glu group, and 2.09±0.49 for the control. These values did not differ significantly each other (P 0.05).

3) Adrenal gland (Fig. 7c)

The values obtained were 5.03±0.92 for Glu-HCl group, 4.67±0.61 for Na-Glu group and 4.71±1.72 for the control. The differences were not significant (P 0.05).

V Average specific weights of main organs from mothers (Fig. 8)

Specific values for ovary, liver, and spleen did not differ significantly from group so group. But the specific weights of adrenal gland for Na-Glu group and of kidney for Glu-HCl group were smaller than those of the control.

Discussion

We studied whether or not glutamic acid hydrochloride (Glu-HCl) and sodium glutamate (Na-Glu) have teratogenic effects on rabbit embryos chiefly by morphological observation of youngs after delivery. We also recorded growth of the youngs and determined conception and nursing rates to study some effects of the drugs on breeding and nursing.

The results of our experiments did not agree with those of Tugrul who reported that Glutamidine (a drug based on Glu-HCl) induces similar malformation of rabbit embryos to that observed with thalidomide administration. In the present experiment, oral administration of Glu-HCl at a dose of 25 mg/kg. body weight/day did not give rise to any morphological abnomality of rabbit fetuses.

Furuya et al [8] obtained similar results to ours, using rats. Oral administration of Glu-HCl at two doses, 25 mg and 125 mg/kg body weight/day did not induce any abnormal morphological alteration in fetuses. Body weight and feeding of mature male, mature female, and pregnant rats were not influenced by the drug administration. The drug did not affect sexual cycle of female rats.

Clinically, Glu-HCl has been used mainly for the treatment of diseases. Influence of the drug has naturally been discussed concerning its therapeutic effects. Few studies on effects of the drug on pregnancy and malformation in human has been reported. Therefore the results obtained with clinical use of the drug may not rule out completely the following possibilities. The method of administration and the

quantities of the drug might have been inappropriate for detection of possible intrinsic teratogenic ability of the drug. Effects of the drug on pregnancy and malformation might had been overlooked.

There had been no reports on experimental animals until 1965 when Tugrul reported that glutamidine induced malformation of rabbit fetuses. He suspected that glutamic acid might be also teratogenic.

Our results and those of Furuya et al with rats did not demonstrate induction of malformation by Glu-HCl and Na-Glu each at an equivalent dose to that of glutamidine in Tugrul's experiments. Furthermore, Glu-HCl at five times the dose of the glutamidine induced no malformation at all in rats.

The drug dose used in the present experiments, 25 mg/kg body weight/day roughly corresponds to the estimated daily uptake of monosodium glutamate for an adult in Japan [1], 1.5 (-2.0)g/60 kg body weight. Generally, it is desirable to test the teratogenic effects of a certain new drug at ten times the estimated ordinary dose. In view of this point, the amount of the drugs used in this study may be insufficient. They the present results may not rule out completely the possible intrinsic teratogenicity of the drugs.

Since the use of glutamic acid as flavor enhancer and medicine may be increasing in future, it would be necessary to test the drugs at ten times the ordinary dose (at least several times) or at the maximum safety dose (at least the maximum ordinary dose).

On the other hand, the amount of the drug used in the present study is unlikely to be excessive. Excessive administration readily causes

resorption of embryos. Pregnancy progressed normally and death or resorption of fetuses did not occur in most of the cases in the present experiments.

However, resorption of embryos was found in some rabbits. Most of these cases may be ascribable to the diseases of mother rabbits, not to excessive administration of the drug.

Whether or not the two drugs tested are intrinsically teratogenic remains to be studied further. It may at least be concluded from the present results that Glu-HCl and Na-Glu are not teratogenic each at an equivalent dose to that of glutamidine which was reported by Tugrul to induce malformation. The fact that rabbits are highly susceptible to teratogenic agents further supports the above conclusion.

The standard procedure (11) to test effects of a new medicine on fetuses requires two species of the test animals. Our results with rabbits and those of Furuya et al with rats appear to satisfy the above condition.

It is clear from the present results that neither Glu-HCl nor Na-Glu affects significantly the growth of youngs, and the breeding and the nursing abilities of mother rabbits.

The average body weight of 3-day-old youngs varied with the experimental group. These values appeared to be influenced by the selection (death) of youngs just after delivery, but not by the drug administration. The average body weights of 30-day-old youngs did not differ among the groups. The drug administration did not affect conception rate and litter-size. The nursing rate after day 7 did not vary with the group. The observed difference of nursing rate among the groups

before day 7 would be ascribable to the method of feeding and the unstable condition of individual mother rabbit just after delivery.

The organs of fetuses in all groups were found to be macroscopically normal.

The specific weights of embryonic organs did not differ among the groups except that of adrenal gland. The specific value for adrenal gland was smaller in Na-Glu group than in the control. This observation held for the mother rabbits. They also had the kidneys smaller than those of the control. These results may not imply immediately that administration of the drug influenced development or growth of the organs. Detailed histological examination would be needed for the study on effects of the drug on the organs.

The Effect of Daily Peroral Administration of Monosodium Glutamate on Somatic Growth and Reproductive Functions in Mice, with Pathological Observations

Shinobu Yonetani

Hiroyuki Ishii

Jiro Kirimura

(Central Research Laboratories, Ajinomoto Co., Inc.)

The Effect of Daily Peroral Administration of Monosodium Glutamate on Somatic Growth and Reproductive Functions in Mice, with Pathological Observations

---

Introduction

It was reported that when 0.5 - 4.0g/kg of Monosodium Glutamate (MSG) was administered by subcutaneous injection to newborn mice, they grew up into obese animals and sterility developed in case of females as a result of neuronal necrosis in several regions of developing brain including hypothalamus.[1] Also there is a preliminary report saying that peroral administration of MSG by tube feeding to 10 days-old mice caused necrosis in hypothalamic neurons.[2] Hypothalamic necrosis was also reported to occur in a rhesus monkey which was injected subcutaneously with 2.7g/kg of MSG eight hours after birth.[3] It was observed that parenteral administration of MSG (4-8g/kg) to newborn mice caused acute lesions in the inner retina.[4][5]

On the contrary, a sodium salt of glutamic acid which is contained in large amounts in natural foods as a component of protein or in free form, has never been observed to have any undesirable effect during long-term studies and teratogenic studies etc. Therefore, it is important to make clear whether or not MSG induces any adverse effects as reported in the ordinary use as a flavor enhancer.

To clarify these points, the authors carried out experiments on the daily peroral administration of MSG at a large dose using two strains of mice. The mice were subjected to investigations of somatic growth and such reproductive functions as estrous cycle, sexual maturity and

progress of gestation as well as histopathological examinations of main organs. Brain (especially hypothalamic region) and retina were carefully examined.

Experimental Methods

About sixty-days-old mice of 4CS strain (weighing 30-35g in males, 25-30g in females) and Swiss white strain of about sixty days (weighing 25-30g in both sexes) were fed on a basal diet for 2-4 weeks preliminarily and those proved to be normal in growth and to exhibit regular 4 day estrous cycles were served as the experimental animals. Both strains of animals were then divided into 6 groups, 3 female and 3 male, respectively, as follows:

Group	Strain	Sex	Diet	No. of Animals
1	4CS	♀	diet containing 4% MSG	5
2	"	"	diet containing 2% MSG	3
3	"	"	basal diet (Control)	3
4	"	♂	diet containing 4% MSG	5
5	"	"	diet containing 2% MSG	4
6	"	"	basal diet (Control)	2
7	Swiss white	♀	diet containing 4% MSG	3
8	"	"	diet containing 2% MSG	3
9	"	"	basal diet (Control).	2
10	"	♂	diet containing 4% MSG	3
11	"	"	diet containing 2% MSG	3
12	"	"	basal diet (Control)	3
			Total	38

The animals thus grouped were fed on different three kinds of diet, respectively, throughout the period of experiments: Groups 1, 4, 7 and 10 on a diet containing 4% of MSG, Groups 2, 5, 8 and 11 on a 2% MSG diet and Groups 3, 6, 9 and 12 on a basal diet. After about two weeks' feeding on the MSG containing diets, males and females of the same strain on the same diet were mated inter se to obtain offspring. The parental animals were sacrificed about 100 days after the delivery and dissected to weigh main internal organs and to examine them histopathologically.

The young mice (F_1) were weaned at 25 days after birth, and both sexes were separately fed on the diets which were the same in MSG content as those for the parents. They were reared under observation and sacrificed at the age of 130 days for dissection and histopathological examinations, except a litter of mice from each group which were selected to obtain offspring (F_2) by sib-mating at 90 days after birth.

One or two of the mice taken from each of the groups 1 to 12 inclusive, both of parents and their offspring F_1, were served for pathological examination. After being weighed, animals were killed with ether and autopsied. Then the macroscopic changes of all the organs were carefully observed. Especially, brain, heart, lung, liver, spleen, kidney, adrenal gland, testis, seminal vesicle, ovary, and uterus were weighed after blotting off the adherent blood with filter paper. All of the organs were fixed in 10% neutral buffered formalin, embeded in paraffin, sectioned to 6μ in thickness and then stained with hemotoxylin-eosin for histological examination. The brain and the eye were also examined on toluidin-blue stained sections.

The young mice of the F_2-generation were subjected to external observation and roentgenographic inspection of skeleton about 20 days after birth.

All the experimental animals were kept in constant temperature ($23 \pm 2^{\circ}C$) and relative humidity ($50 \pm 10\%$) under the condition of 14 hour light and 10 hour dark. They were allowed to take feed and water *ad libitum*. CE-2 (for general growth) of CLEA JAPAN CO. was used as a basal diet throughout the period of experiments including the preliminary rearing but during the gestation of the females was used CA-1 (breeding chow) obtained from the same company. The feed intake was recorded everyday.

Items of observations

Parents:

(1) Body weight and feed intake

(2) Estrous cycle of females

(3) Litter size and body weight of offspring (F_1)

(4) Weight of each internal organ after dissection

(5) Histophathological examinations of main organs

F_1:

(1) Body weight and feed intake after weaning

(2) Estrous cycle of females

(3) Day of vaginal opening in females

(4) Day of descent of testis in males

(5) Litter size and body weight of offspring (F_2)

(6) Weight of each internal organ after dissection

(7) Histopathological examinations of main organs

F_2:

(1) Day of eye opening

(2) External observation and skeletal inspection

Experimental Results

I Somatic Growth and Reproductive Functions

1. Parents

1) Effects of MSG-administration on the change of body weight and feed intake

The mean value of body weight and the feed intake per day in each group are shown in Figs.1-4. There was no difference observed between the two groups fed on MSG containing diet and the control group in the result of examinations conducted before pregnancy, during pregnancy, during nursing and after weaning in both of 4CS (Fig.1) and Swiss white (Fig.3) females. Both Figures 1 and 3 indicate the significant increase of feed intake during 15-25 days after the delivery. But the increase can not be attributed only to mothers, because it was observed that sucklings living in the same cage with mothers began to take feed in this period.

As shown is Tables 2 and 4 there was no difference in the body weight and feed intake of males between the two MSG containing diet groups and the control group in each of 4CS and Swiss white strains.

The occurrence of obesity was observed in neither of these MSG administered groups.

To make the actual intake amount of MSG at 8g/kg/day and 4g/kg/day, the MSG content in the diet was designed to 4% and 2%, respectively. As shown in Figs.1-4, this our intention was satisfactorily achieved.

2) Effects of MSG-administration on the gestation period and the estrous cycle

Females of both 4CS and Swiss white strains had newborn mice normally 20 days after mating, and the litter size was 7-9 (Table 1). Some of the

newborn mice were taken from each group to measure their weight. The results show that there was no difference in the body weight between the offspring of MSG containing diet groups and those of the control group (Table 2).

The estrous cycles of mothers before mating and about 60 days after weaning are indicated in Fig. 5. The females of 4CS strain rutted soon after the weaning, and estrus recurred in regular 4 day cycles from 10 days thereafter. There observed no difference in regularity of estrous cycles among all the groups. Swiss white strain females tended to be the same in regularity of the cycle as 4CS strain so far as observations were conducted until the time of mating, showing no abnormality.

From the facts that males and females fed on a diet of the same MSG content were mated inter se and both delivery and the offspring were normal, it is presumed that the administration of MSG had no influence on the copulative potency and fertilizing ability of the males.

2. Mice in the F_1-generation

Six individuals were taken at random from each litter of the F_1-generation (indicated in Table 1) to make 6 groups (3 female and 3 male) again in each strain as shown below and they were fed on the same MSG containing diet as the parents.

Group	Strain	Sex	MSG content (%) of diet fed to parents	MSG content (%) of diet fed to offspring (F_1)	No. of animals
1	4CS	♀	4	4	11
2	"	"	2	2	9
3	"	"	0	0	7
4	Swiss white	"	4	4	10

Group	Strain	Sex	MSG content (%) of diet fed to parents	MSG content (%) of diet fed to offspring (F_1)	No. of animals
5	Swiss white	"	2	2	7
6	"	"	0	0	6
7	4CS	♂	4	4	10
8	"	"	2	2	8
9	"	"	0	0	7
10	Swiss white	"	4	4	4
11	"	"	2	2	11
12	"	"	0	0	5
				Total	95

The results of observations on these animals are as follows.

1) Effect of MSG-administration on the change of body weight and feed intake

The mean value of body weight and feed intake per day are shown in Fig. 6 (4CS strain) and Fig. 7 (Swiss white strain). There was no difference in these points among the three groups and also no abnormal growth was found during suckling, after weaning and after reaching maturity. Especially, it is noteworthy that no abnormal growth (obesity) was found not only from the growing curves but also from the external appearance in 70 cases administered MSG for a considerably long-term (about 120 days in 4CS strain and about 110 days in Swiss white).

2) Effects of MSG-administration on the sexual maturity and the estrous cycle

The day of vaginal opening in young females and that of the descent

of testis in young males were observed as shown in Figures 8 and 9. The day of vaginal opening in MSG given groups was different from that of the control in neither of 4CS strain nor Swiss white strain. (4CS strain: 32-35 days after birth in MSG-8g/kg/day Group, 34-36 days in MSG-4g/kg/day Group and 33-36 days in the control. SW strain: 33-37 days in MSG-8g/kg/day Group, 35-41 days in MSG-4g/kg/day Group and 35-39 days in the control.) The descent of testis occurred at nearly the same day in all the animals. No effect of MSG was observed.

The estrous cycles observed are shown in Figures 10 and 11.

4CS strain: In nearly all the F_1-females of both the MSG given groups and the control group estrus recurred in normal 4 day cycles as the mother. But there were a few exceptional individuals which showed unstable estrous cycles in the control and also in the MSG given groups. To clarify the difference among the groups, the incidence of estrus (% estrus) was calculated by the method of Wurtman et al. The results are 41.09±5.75% in MSG-8g/kg/day group, 42.58±6.10% in 4g/kg/day group and 44.73±7.48% in the control (Table 3), and no significant difference is noticed. The incidence of estrus is the percentage of the total day exhibited proestrous, estrous and metaestrous smears to whole estrous smears.

Swiss white strain: The estrous cycle was considerably irregular in both the control and MSG given groups, and it was rather difficult to find minute difference between them. Accordingly to clarify this point, incidence of estrus was also calculated and no difference was found among the 3 groups (Table 3). In case of parents, individuals showing normal cyclicity were selected from many females and served for the experiments. But in offspring (F_1) on the other hand, such selection is practically impossible. Accordingly, the fact that some irregularity

of estrous cycle was observed in all three groups of F_1 of Swiss white strain seems to be due to the strain property. It is known that females of 4CS strain recurs a more regular estrous cycles than those of Swiss white strain.

When brothers and sisters on the same diet were mated inter se to obtain offspring, the gestation period was 20 days in all the groups, showing no difference among the groups. There was no difference among the groups in the litter size (Table 4), the weight (Table 5) and the day of eye opening (Fig.12) of offspring (F_2). Also no abnormality was found by external observations and roentgenographic skeletal inspection of these offspring (See photographs of MSG-8g/kg/day-group animals in Fig.13).

II Pathological Examination

1. Macroscopic findings and organ weight

Among the 4CS male F_1 mice, each 1 from the two MSG administered groups and 2 from the control group were found to have a kidney enlarged one and a half times as normal, containing a large amount of urine in their renal pelvis. In few cases including control animals, one or more of the pulmonary lobes were atelectatic, like that of fetus. Such changes have been seen quite often. No macroscopic change was observed in any other organs.

In Table 6, organ weights are shown. There was no remarkable difference between the treated and control groups, although no statistical analysis was made because of few cases.

2. Microscopic findings

1) Brain

No change was found in the brains of every groups of animals by

careful observation on all of the areas. No difference in morphology, number and arrangement of nervus cells of each area of the brain, including bypothalamus as speciffically indicated in Olney's article, was noticed between experimental and control groups (Fig.14).

2) Eye

No difference in cellular morphology and arrangement and the thickness of the layers consisting the retina was found between experimental and control groups (Fig.14).

3) Other organs

Atelectatic pulumonary lobes showed no inflamatory findings. Kidneys, enlargement being observed macroscopically, were found to be hydronephrosis. Interstitial nephritis, which appeared to be ascending, was recognized in the kidneys of over half of the autopsied animals including control group. There were, especially in males, many cells of the reticular zone of the adrenal cortex which contained greyish-yellow pigment in cytoplasm. Such findings have been noticed quite often and are not ascribable to the effect of the administration of MSG.

The observations described above proved that the present experiments of MSG administration gave no pathological change to the mice.

Discussion

Peroral administrations of large dose of MSG were studied over a period of 2 generations to investigate the influence on two strains of mice. In this paper, observations whether or not the administration induced any adverse effect on the growth rate, causation of any disturbance in reproductive functions, were described together with histopathological examinations mainly of the hypothalamus and the retina. Taking the

preceeding reports on long-term feeding by Hara[6], and Arthur D. Little Inc.[7] in consideration, the authors' experiments were planned to cover the gestation and nursing periods of the parental generation and the embryonic and suckling periods of the filial generation. Also test animals were allowed to take MSG containing diet ad libitum to bring the form of intake close to usual using way.

In the parental generation, the administration was continued over 110-120 days including the gestation and nursing periods, but no difference was observed between the control and MSG given groups in the somatic growth, reproductive functions as well as histological findings of hypothalamus and retina. Especially the fact, that no effect was caused by MSG administration on pregnant females, which are in a physiologically unstable condition, shows that MSG does not induce any adverse effects on the physiology of pregnancy.

In the filial generation, young mice born and bred from the mothers taking MSG at a dose of 8g/kg/day or 4g/kg/day, continued to take MSG by themselves from diet after the end of the suckling period. But no case of obesity and sterility in females, pointed out by Olney, was observed among them, and histopathological examinations revealed no abnormality in hypothalamus and retina. Therefore, it may be confidently said that the effect of MSG-intake in the gestation period is out of the question even from a viewpoint of teratogenesis.

In our experiments, a large amount of MSG was administered to mothers in the gestation and nursing periods when F_1-mice were under the influence of them; the F_1-animals were allowed to take MSG containing diet after age of fifteen days when they began to take diet by themselves together with suckling, and they were further fed wholly on MSG containing diet after 25 days of weaning. Such a method of administration appears to

prepare a condition closest to the actual way of MSG intake which the authors aimed at in the planning.

Experimental conditions of Olney should be checked from this point of view, as 1) the subcutaneous injection of MSG is quite unpractical, and 2) tube feeding of MSG solution to too young mice of 10 days of age, when MSG is taken only through mothers' milk.

Morphological change in hypothalamus is always accompanied with some functional disturbances such as abnormal increase in body weight and abnormal reproductive functions, but a functional disturbance is not necessarily accompanied with any visible morphological change in the hypothalamus. From this point of view, changes of the estrous cycle and some physiological functions were taken up as indication for the detection of slight disturbances caused by the peroral administration of MSG. But any abnormal changes in physiological functions which seems to be due to some lesion in the hypothalamus could not be observed throughout our experiments.

In conclusion, it was confirmed both morphologically and physiologically that MSG administration induced no adverse effects on mice in a condition corresponding to actual way of intake.

Summary

The peroral administration of MSG at the doses of 8g/kg/day and 4g/kg/day was conducted over 2 generations in 2 strains of mice to examine the effect on the growth and such reproductive functions as estrous cycle, sexual maturity and gestation period. Histopathological examinations especially brain and retina were also conducted after the autopsy of the mice. The results obtained showed that there was no case of obesity

and no functional disterbance, not to speak of morphological abnormality.

From these results, it was confirmed that the administration of MSG did not induced any adverse effects on the mice in a condition corresponding to the actual form of intake.

:::::::

References

1) J. W. Olney, Science 164, 719 (1969)

2) J. W. Olney and L. G. Sharpe, Science 167, 1018 (1970)

3) J. W. Olney and L. G. Sharpe, Science 166, 386 (1969)

4) D. R. Lucas and J. P. Newhouse, Amer. Med. Ass. Arch. Ophthalmol. 58, 193 (1957)

5) A. I. Cohen, Amer. J. Anat. 120, 319 (1967)

6) S. Hara et. al., Tokyo Ikadaigaku Zasshi (Journal of Tokyo Medical College) 23, 383 (1961)

7) Arthur D. Little, Unpublished Report (1953)

Table 1. Litter Size of Parents. (Number of F_1)

Group	4CS strain		Swiss white strain	
	Number of mothers	Litter size (mean value)	Number of mothers	Litter size (mean value)
MSG 8g/kg/day	4	8.0	3	7.3
MSG 4g/kg/day	3	8.3	3	7.6
Control	4	9.0	3	8.6

Table 2. Mean Body Weight of Newborn Mice (F_1)

4CS Strain, measured at 2 days old

Group	No. of mothers	number of female	mean weight(g)	number of male	mean weight(g)
MSG 8g/kg/day	3	4	1.55	5	1.82
	5	3	1.26	4	1.39
	Total	7	1.43	9	1.63
MSG 4g/kg/day	1	4	1.72	4	1.77
	2	4	1.60	2	1.75
	Total	8	1.68	6	1.76
Control	1	7	1.69	3	1.66

Swiss white strain, measured at 3 days old

Group	No. of mothers	number of female	mean weight(g)	number of male	mean weight(g)
MSG 8g/kg/day	1	6	2.31	3	2.64
MSG 4g/kg/day	2	3	2.22	5	2.58
Control	1	3	2.41	4	2.44

Table 3. Incidence of Estrus (% Estrus) in Female F_1

4CS Strain

Group	Number of animals	Duration		% Estrus (M± S.D.)
		days	number of cycles	
MSG 8g/kg/day	8-11	61-136	19	41.09±5.75
MSG 4g/kg/day	6-9	61-136	19	42.58±6.10
Control	5	61-136	19	44.73±7.48

Swiss white strain

Group	Number of animals	Duration		% Estrus (M± S.D.)
		days	number of cycles	
MSG 8g/kg/day	7-10	48-95	12	37.21±5.55
MSG 4g/kg/day	4-7	48-95	12	33.94±8.69
Control	6	48-95	12	36.69±6.04

Table 4. Litter Size of F_1 (Number of F_2)

Group	4CS Strain		Swiss white strain	
	number of mothers	litter size (mean value)	number of mothers	litter size (mean value)
MSG 8g/kg/day	2	8.0	1	8.0
MSG 4g/kg/day	3	8.0	3	4.3
Control	4	9.0	3	8.6

Table 5. Mean Body Weight of Newborn Mice (F_2)

4CS Strain, measured at 2 days old

Group	No. of mothers	Number of female	mean weight(g)	Number of male	mean weight(g)
MSG 8g/kg/day	1	5	1.66	5	1.90
MSG 4g/kg/day	3	11	1.93	13	2.02
Control	1	7	1.69	3	1.66

Swiss white strain, measured at 3 days old

Group	No. of mothers	Number of female	mean weight(g)	Number of male	mean weight(g)
MSG 8g/kg/day	1	3	1.50	5	1.68
MSG 4g/kg/day	3	6	2.03	7	2.28
Control	1	4	1.85	5	1.94

Table 6-1. Body weight and some organ's weight at autopsy (4CS strain, male)

(unit : g)

Group		Body weight	Brain	Heart	Liver	Spleen	Kidney		Adrenal gland		Testis		Epididymis		Seminal vesicle
							left	right	left	right	left	right	left	right	
Parent	MSG-8g/kg/day*	32.6	0.46	0.16	1.99	0.10	0.31	0.35	0.002	0.002	0.069	0.080	0.061	0.078	0.283
Parent	MSG-4g/kg/day*	36.1	0.47	0.16	2.30	0.146	0.31	0.35	0.003	0.002	0.123	0.151	0.047	0.055	0.179
Parent	Control	31.9	0.47	0.17	1.50	0.132	0.28	0.33	0.004	0.004	0.110	0.110	0.032	0.045	0.155
F_1	MSG-8g/kg/day*	32.4	0.44	0.19	2.36	0.10	0.31	0.60	0.003	0.004	0.120	0.135	0.041	0.067	0.267
F_1	MSG-4g/kg/day*	32.8	0.43	0.16	2.00	0.12	0.28	0.40	0.002	0.001	0.110	0.125	0.020	0.033	0.256
F_1	Control*	32.9	0.45	0.20	2.01	0.09	0.29	0.45	0.001	0.002	0.122	0.125	0.088	0.031	0.264

* = Mean weight of two mice
No mark is weight of one mouse

Table 6-2. Body weight and some organ's weight at autopsy (4CS strain, female)

(unit : g)

Group		Body weight	Brain	Heart	Liver	Spleen	Kidney		Adrenal gland		Ovary		Uterus
							left	right	left	right	left	right	
Parent	MSG-8g/kg/day*	30.3	0.49	0.16	1.82	0.14	0.28	0.32	0.013	0.013	0.020	0.020	0.22
Parent	MSG-4g/kg/day*	32.3	0.47	0.13	2.12	0.15	0.22	0.23	0.005	0.005	0.009	0.010	0.19
Parent	Control	30.7	0.47	0.15	1.83	0.13	0.25	0.27	0.005	0.006	0.009	0.010	0.22
F_1	MSG-8g/kg/day*	24.5	0.44	0.15	1.68	0.13	0.23	0.20	0.010	0.010	0.008	0.006	0.098
F_1	MSG-4g/kg/day*	25.5	0.44	0.15	1.64	0.13	0.23	0.24	0.009	0.009	0.008	0.009	0.125
F_1	Control*	25.2	0.46	0.16	1.55	0.11	0.23	0.23	0.008	0.008	0.007	0.006	0.11

* = mean weight of two mice

No mark is weight of one mouse

Table 6-3. Body weight and some organ's weight at autopsy (Swiss white strain, male)

(unit : g)

Group		Body weight	Brain	Heart	Liver	Spleen	Kidney		Adrenal gland		Testis		Epididymis		Seminal vesicle
							left	right	left	right	left	right	left	right	
Parent	MSG-8g/kg/day														
	MSG-4g/kg/day	34.7	0.44	0.14	1.97	0.10	0.23	0.23	0.002	0.002	0.11	0.11	0.05	0.05	0.36
	Control*	31.9	0.41	0.14	1.70	0.11	0.24	0.25	0.003	0.002	0.10	0.09	0.03	0.03	0.190
F_1	MSG-8g/kg/day*	34.2	0.42	0.16	2.02	0.19	0.24	0.27	0.004	0.003	0.11	0.11	0.037	0.035	0.220
	MSG-4g/kg/day*	33.6	0.45	0.17	1.90	0.17	0.27	0.27	0.003	0.003	0.10	0.11	0.035	0.035	0.270
	Control	30.5	0.43	0.16	1.61	0.14	0.22	0.25	0.005	0.001	0.11	0.12	0.03	0.04	0.320

* = mean weight of two mice

No mark is weight of one mouse

Table 6-4. Body weight and some organ's weight at autopsy (Swiss white strain, female)

(unit : g)

Group		Body weight	Brain	Heart	Liver	Spleen	Kidney		Adrenal gland		Ovary		Uterus
							left	right	left	right	left	right	
Parent	MSG-8g/kg/day	30.7	0.44	0.12	1.63	0.12	0.19	0.195	0.003	0.004	0.009	0.009	0.13
	MSG-4g/kg/day	30.8	0.42	0.12	1.53	0.13	0.19	0.23	0.005	0.004	0.009	0.009	0.21
	Control	31.2	0.45	0.17	1.79	0.14	0.21	0.20	0.007	0.005	0.010	0.008	0.16
F_1	MSG-8g/kg/day*	26.4	0.46	0.13	1.60	0.20	0.18	0.27	0.006	0.005	0.015	0.007	0.15
	MSG-4g/kg/day*	28.4	0.44	0.15	2.05	0.23	0.20	0.21	0.005	0.005	0.007	0.009	0.12
	Control*	29.8	0.41	0.12	1.53	0.16	0.14	0.15	0.007	0.005	0.005	0.008	0.12

* = mean weight of two mice

No mark is weight of one mouse

Fig. 1 Effect of MSG Administration on Body Weight and Feed Intake
(ACS Strain, Parents, ♀)

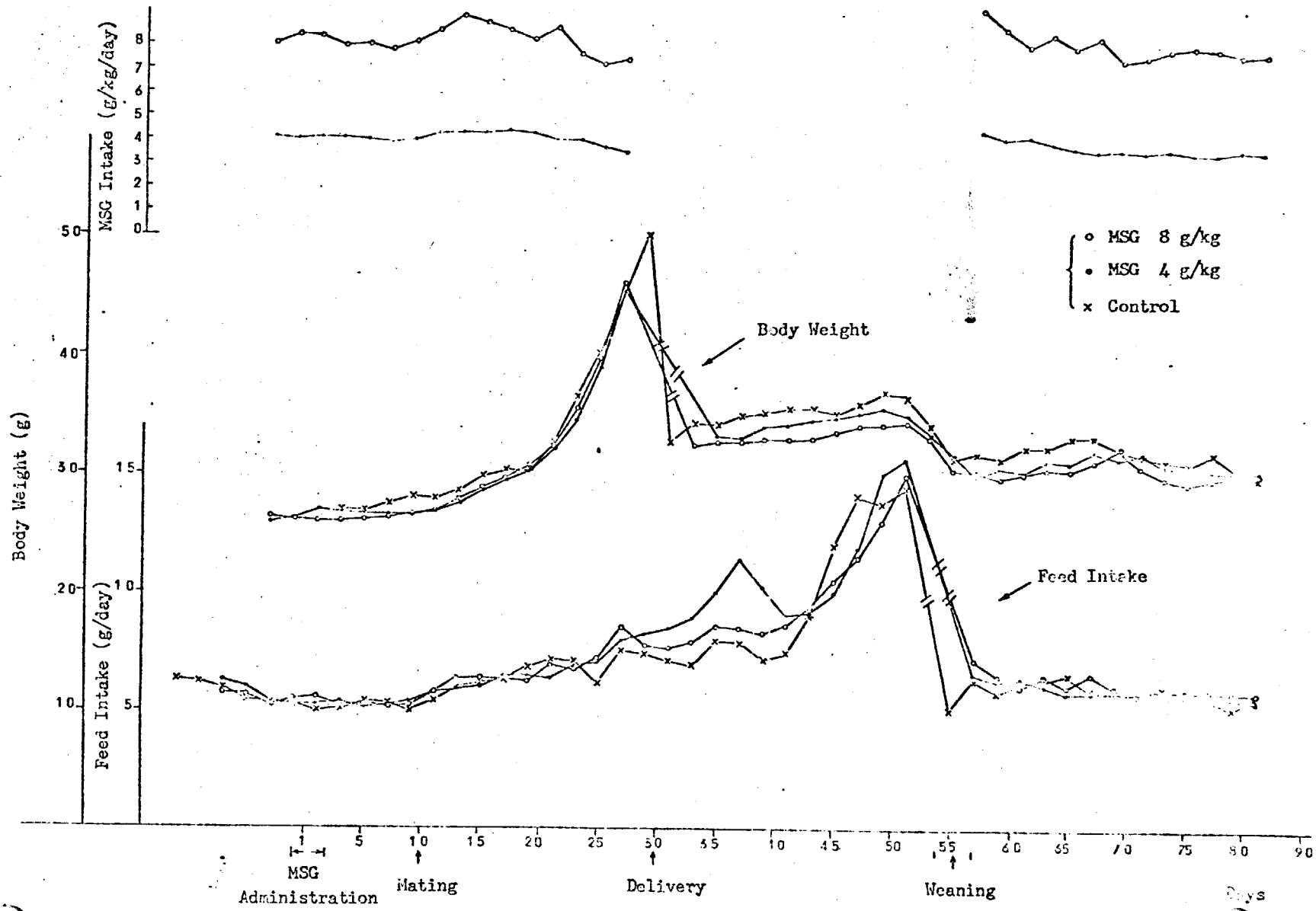


Fig. 2. Effect of MSG Administration on Body Weight and Feed Intake
(4 CS Strain, Parents, ♂)

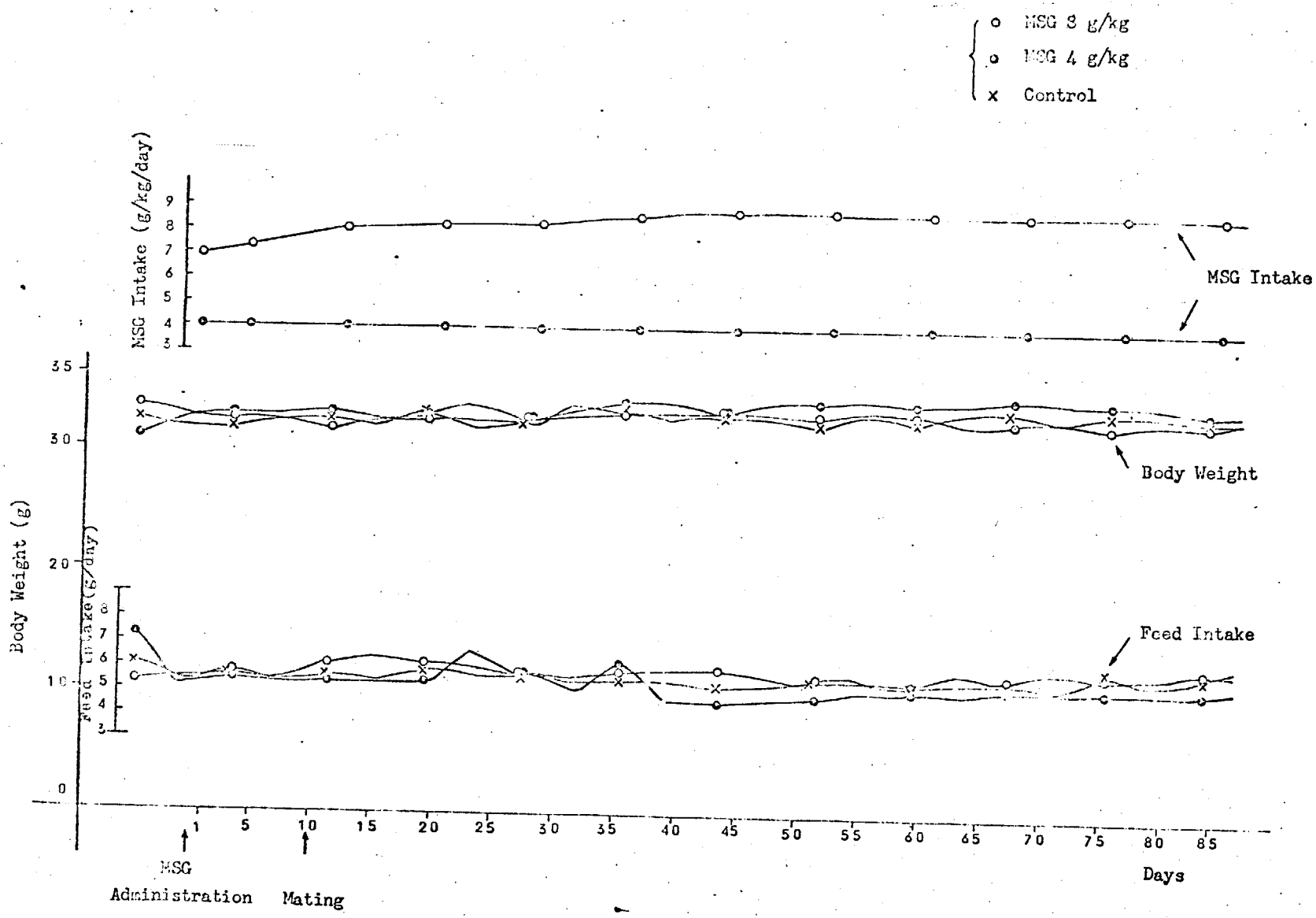


Fig. 3 Effect of MSG Administration on Body Weight and Feed Intake (Swiss White St[illegible])

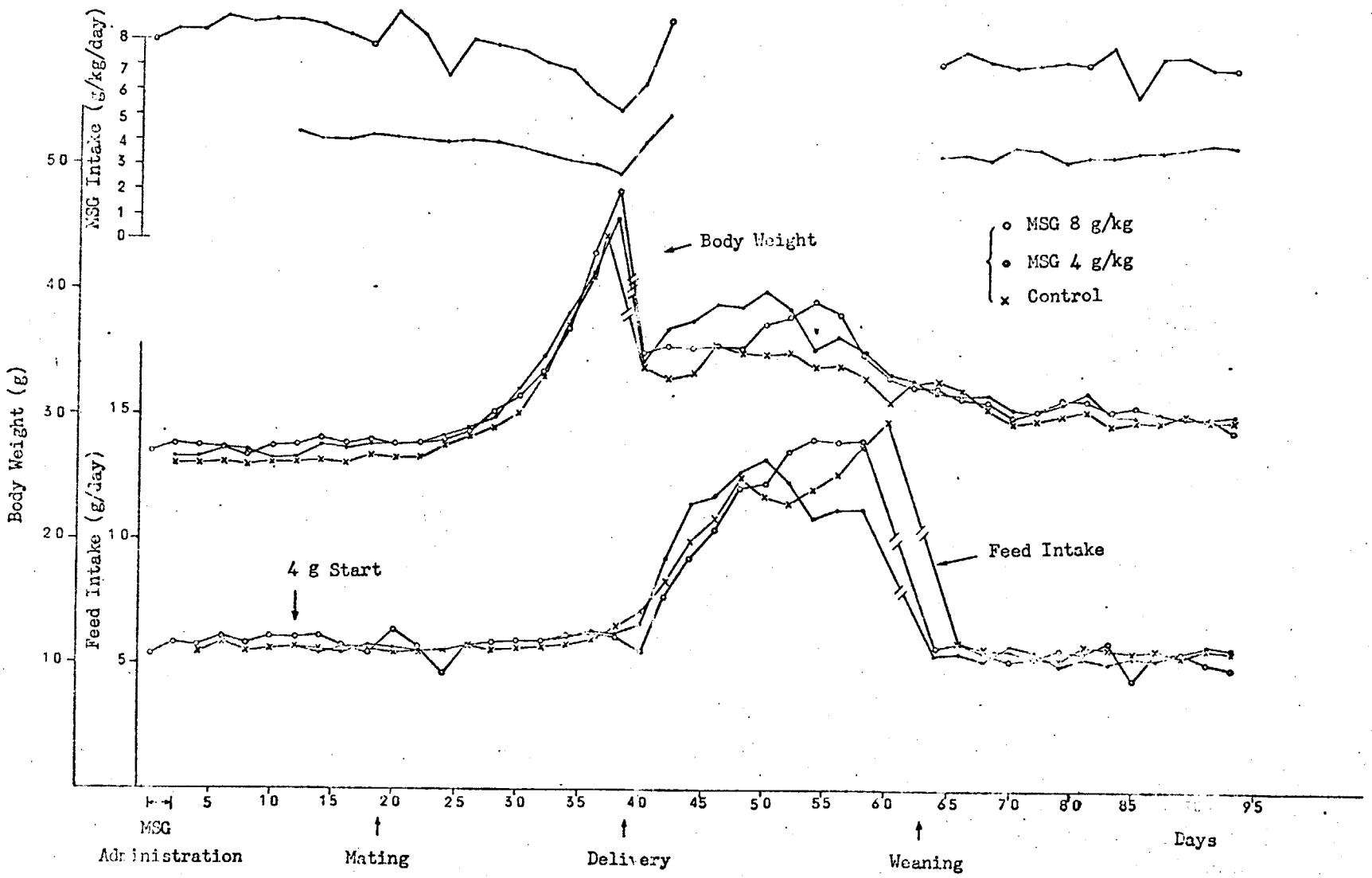


Fig. 4. Effect of MSG Administration on Body Weight and Feed Intake

(Swiss White Strain, Parents, ♂)

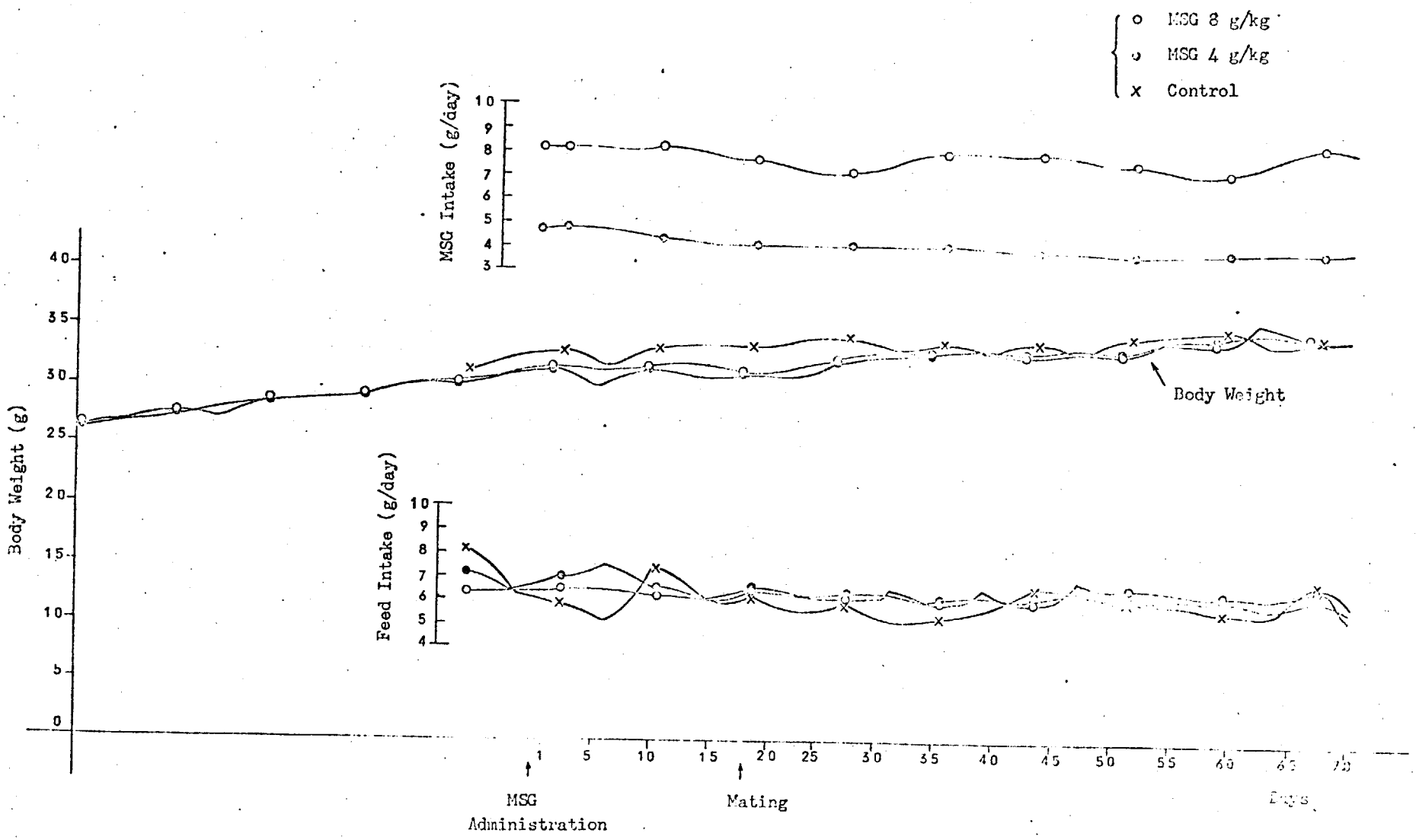


Fig. 5. Effect of MSG Administration on Estrous Cycle in Female Parents.

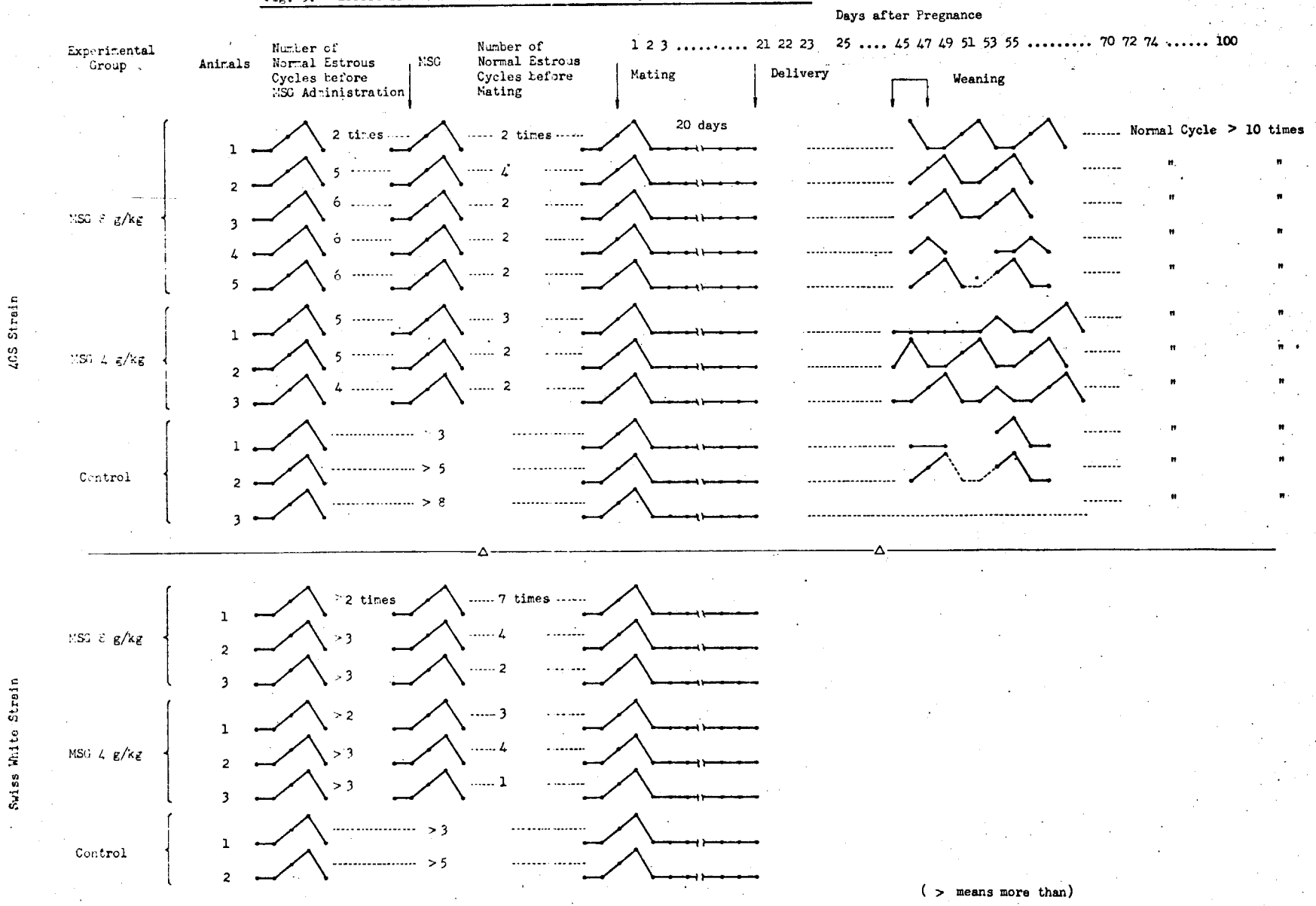


Fig. 6 Effect of MSG Administration on Body Weight and Feed Intake (4CS Strain, F_1)

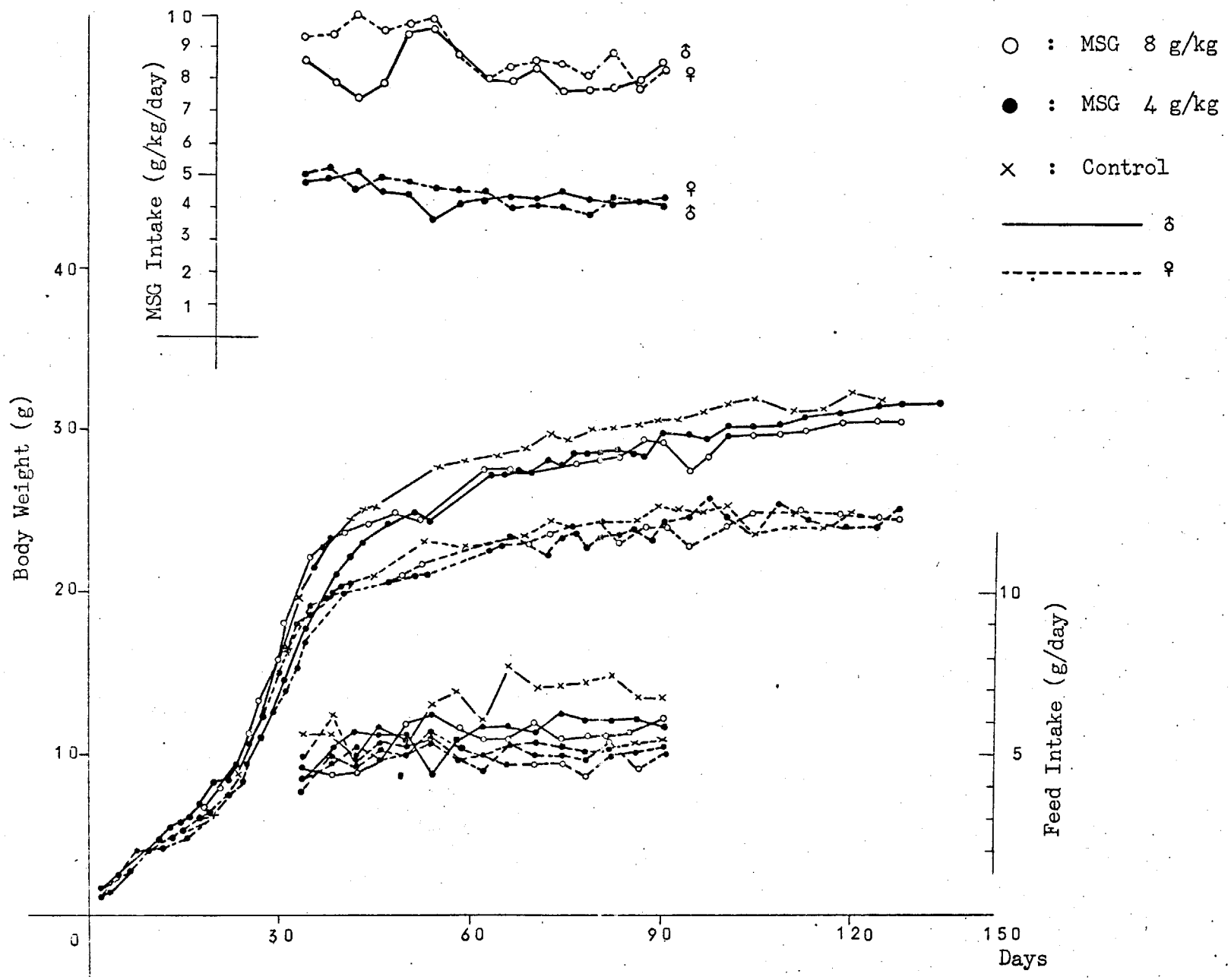


Fig. 7 Effect of MSG Administration on Body Weight and Feed Intake (Swiss White, F)

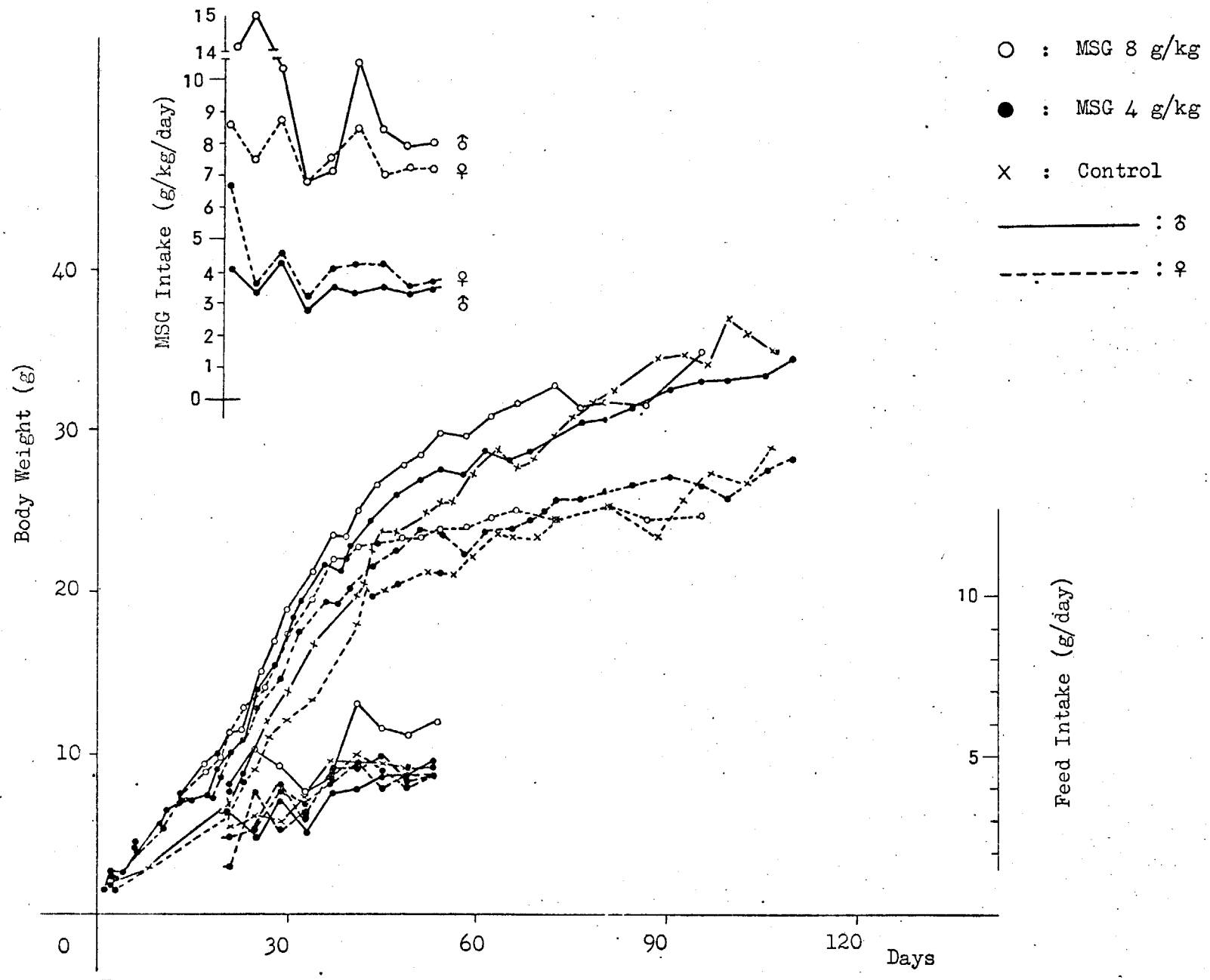


Fig. 8 Vaginal Opening Day of Female F_1

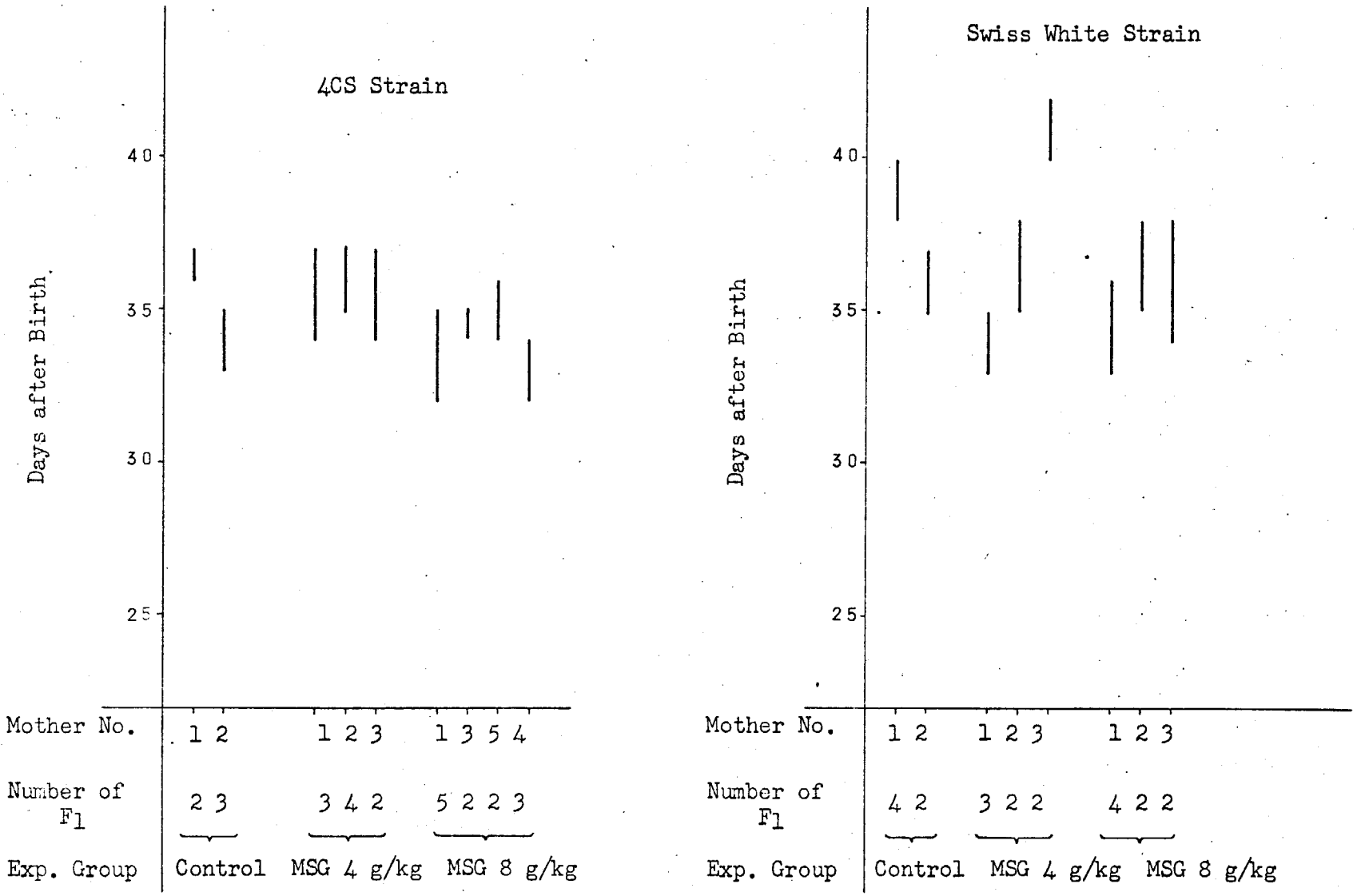
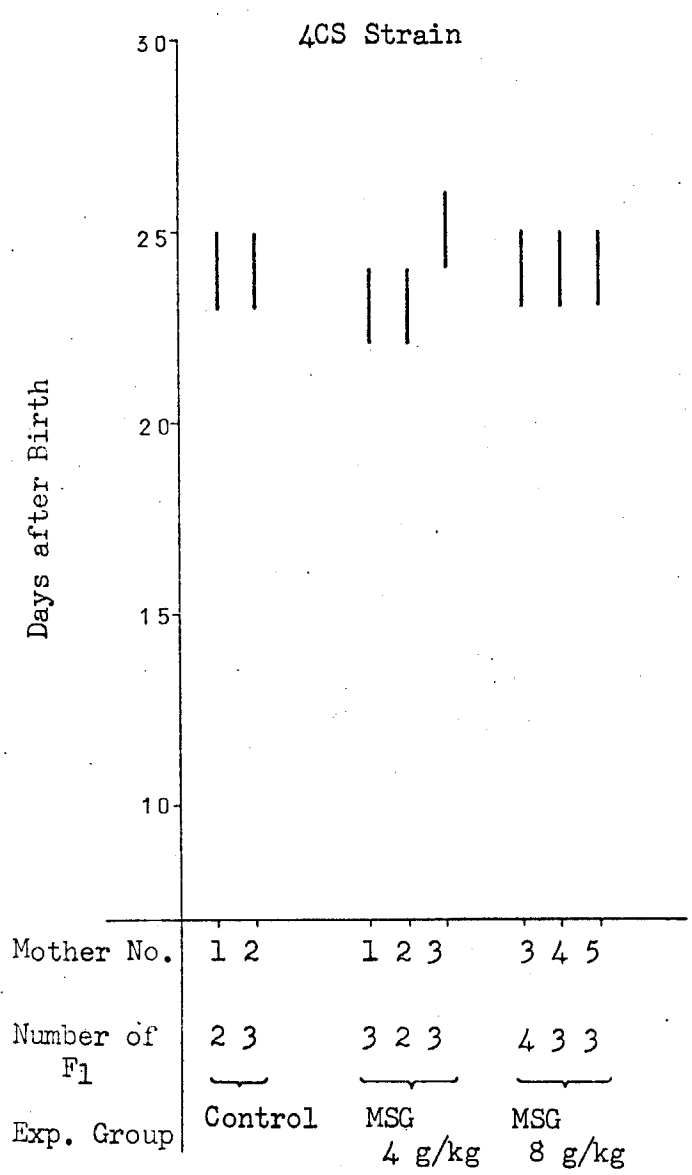


Fig. 9 The Day of Descent of Testis in Male F_1



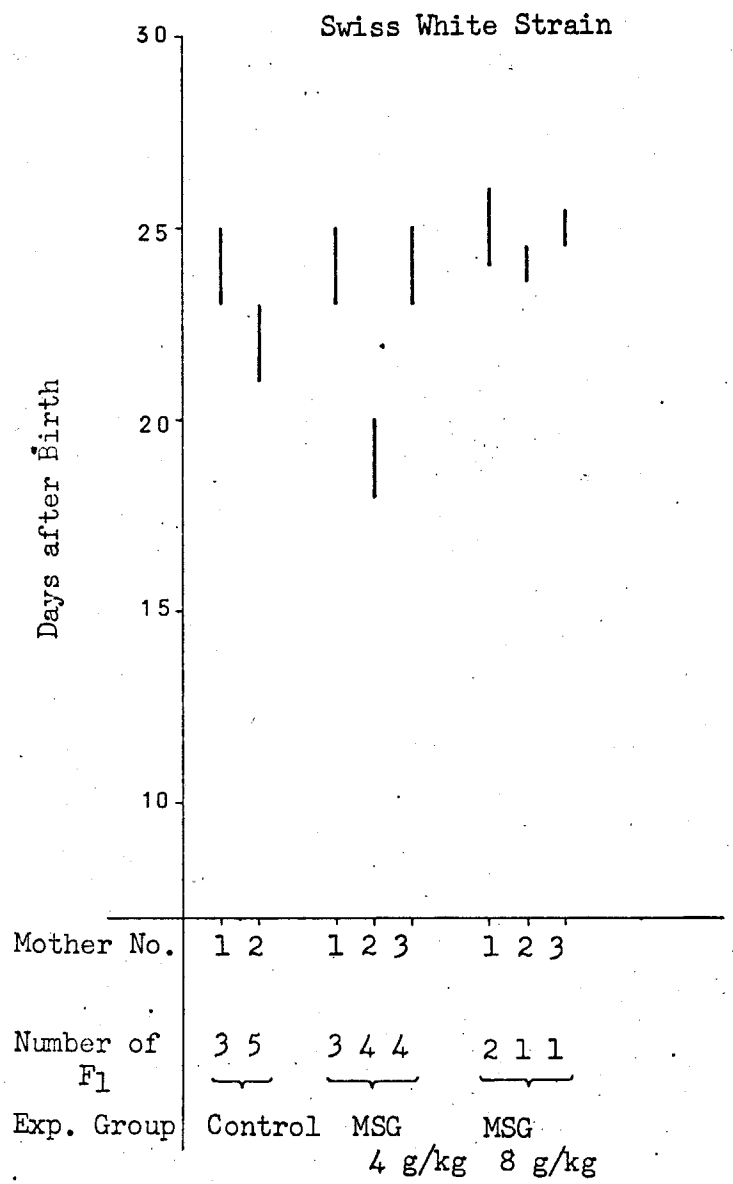


Fig. 10 Effect of MSCT Administration on Estrous Cycle in Female Offspring (F_1) 4CS Strain

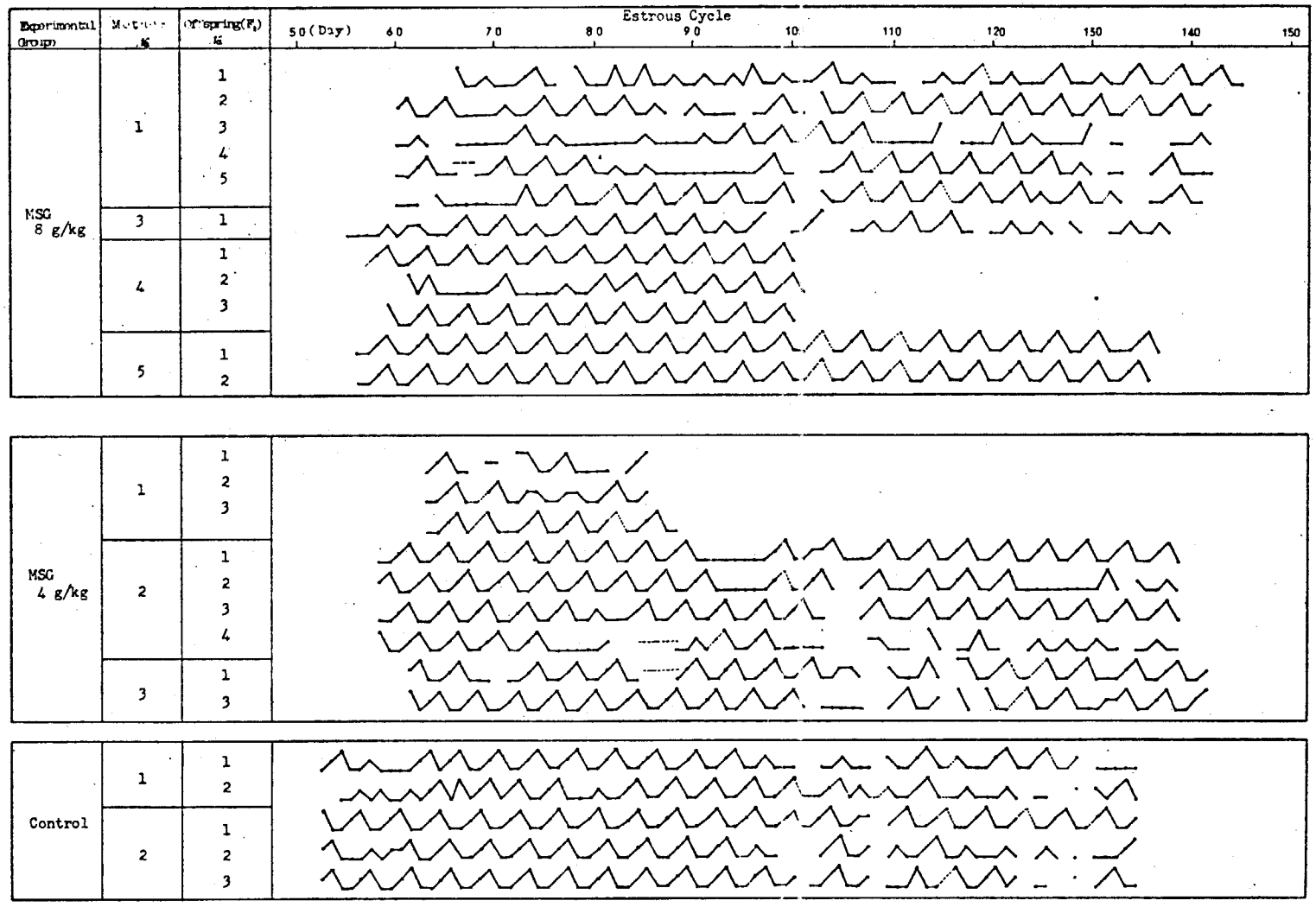


Fig. 11 Effect of MSG Administration on Estrous Cycle in Female Offspring (F_1)

Swiss White Strain

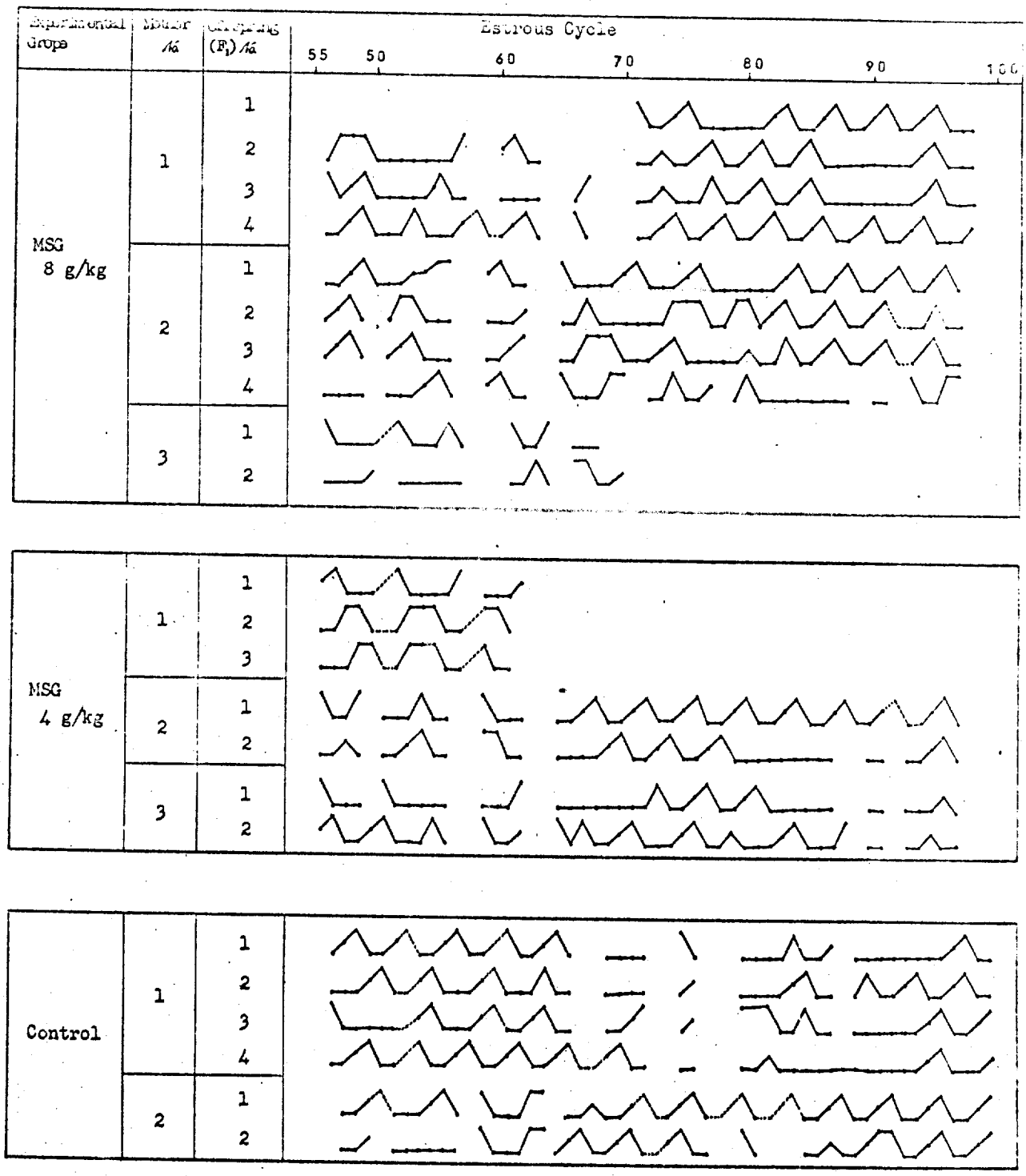


Fig. 12 Eye Opening Day in Offspring (F_2)

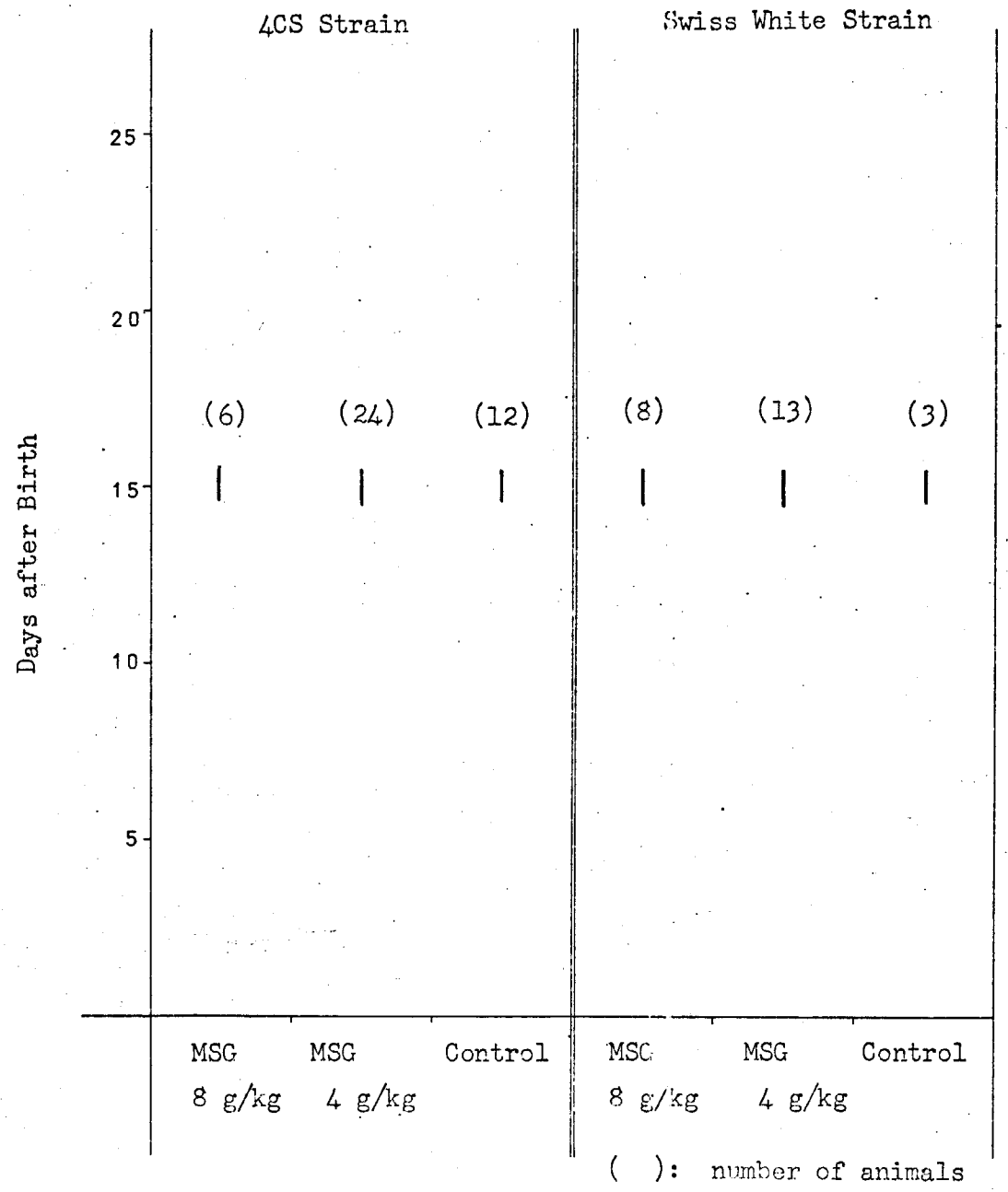
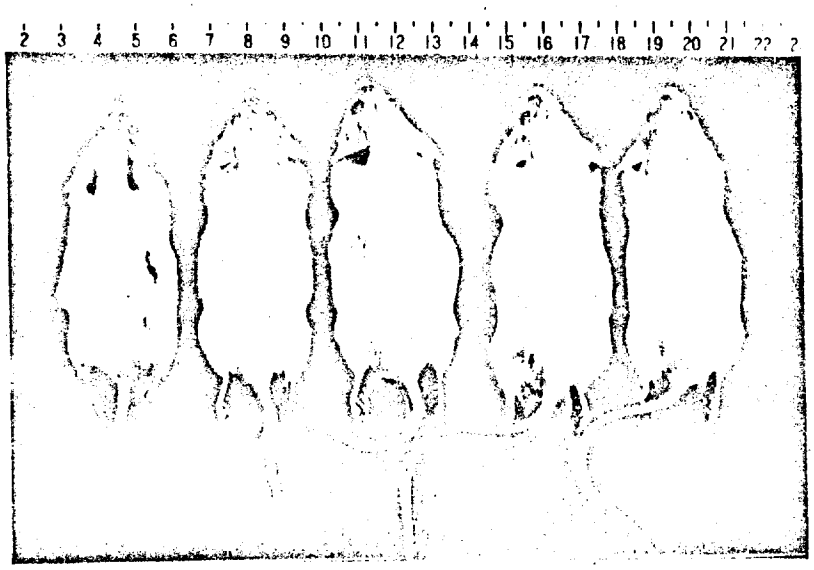
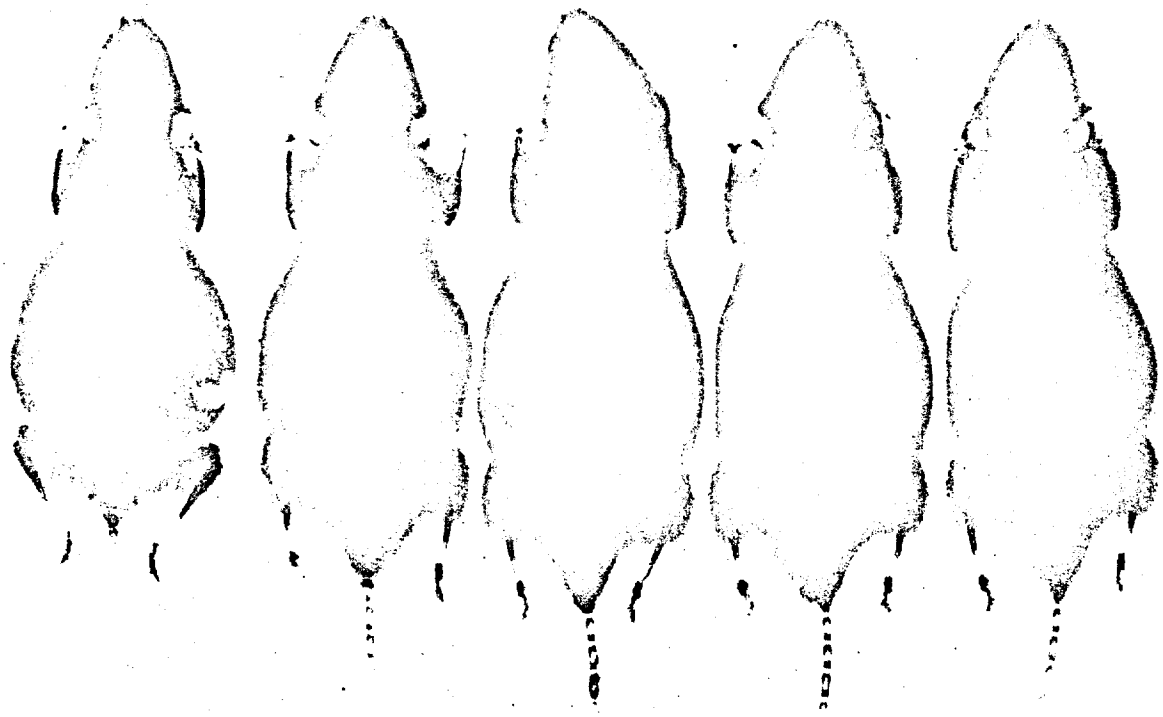
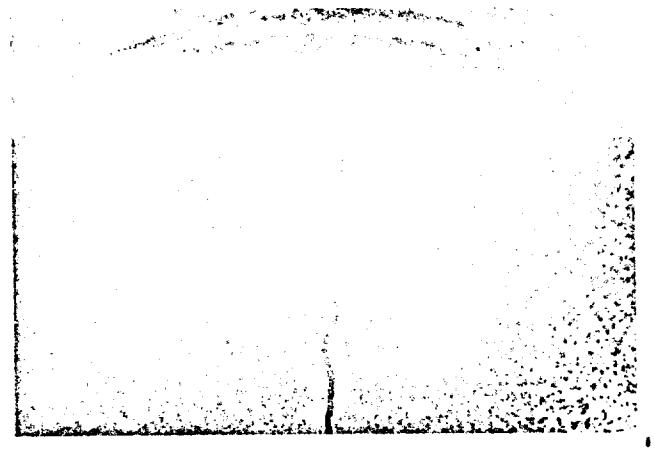


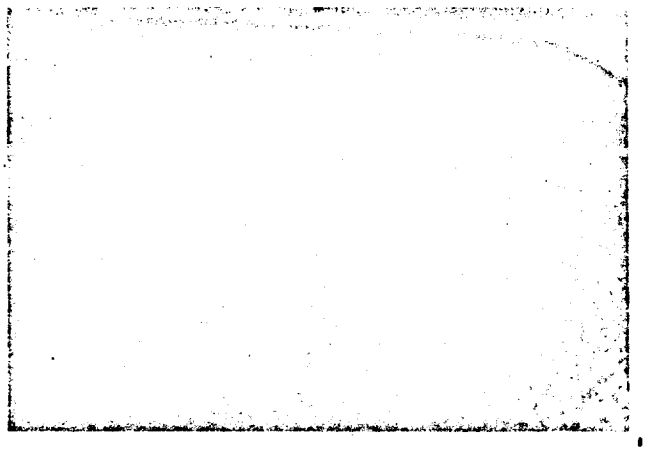
Fig. 13 External Observation and Roentgenographic Skeltal Inspection, (F_2 born from F_1 of MSG 8g/kg/day Group)





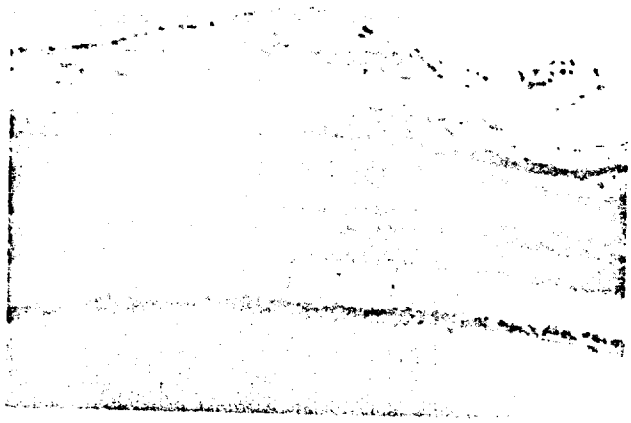


A



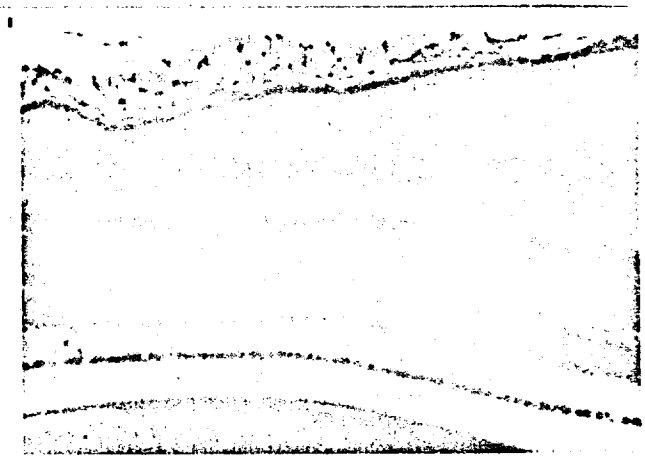
B

C



A

B



C

Fig. 14

Hypothalamus of adult 4CS mouse.(F)A, Fed on a diet containing 4% of MSG. B, Fed on a diet containing 2% of MSG. C, Control.; Toluidin blue stain. x 120.

Retina of adult 4CS mouse.(H)A, Fed on a diet containing 4% of MSG. B, Fed on a diet containing 2% of MSG. C, Control.; Toluidin blue stain. x 300.

BIOMEDICINE 1973, 19, 202-204

A DOUBLE BLIND STUDY ON THE EFFECTS OF MONOSODIUM GLUTAMATE IN MAN

by G. ZANDA, P. FRANCIOSI, G. TOGNONI, M. RIZZO, S. M. STANDEN, P. L. MORSELLI and S. GARATTINI

*(Istituto di Ricerche Farmacologiche « Mario Negri », Milan)**

SUMMARY

Monosodium glutamate was administered at lunch time to 73 healthy subjects using a double blind design with each subject acting as his own control. Care was taken to balance the sexes, and to avoid any psychological effect of grouping. Both subjective and objective measurements were made, and at the dose employed (3 g/subject) no differences in symptomatology were found between the control and MSG treated groups.

RÉSUMÉ

Du glutamate monosodique fut administré au déjeuner à 73 sujets bien portants selon un protocole en double aveugle, chaque sujet étant son propre témoin. Des précautions furent prises pour équilibrer la répartition selon le sexe, et pour éviter tout effet psychologique de groupe. Des mesures à la fois subjectives et objectives furent appliquées, et, à la dose employée (3 g/sujet), aucune différence ne fut trouvée entre les groupes témoin et traité au glutamate monosodique.

INTRODUCTION

Monosodium glutamate (MSG) has been held responsible (1, 4, 7, 8) for the Chinese Restaurant Syndrome (CRS), a burning sensation in the back of the neck, spreading to the forearm and to the anterior thorax accompained by a feeling of infra-orbital pressure, tightness and substernal discomfort.

These previous studies did not use a double blind technique and the experimental conditions were not suitable for assessing the relevance of subjective reactions.

In an earlier work, run as a double blind trial, we could not demonstrate any effect of MSG (5). The results indicated that MSG administration does not provoke any sympatology which differs from the normal postprandial digestive state. Recent study (3, 6) confirmed our observations suggesting that MSG is not « per se » the effective agent of the CRS.

* Via Eritrea, 62-20157 Milan, Italy.

Reprints : Dr P. L. Morselli.

In our previous experiment, subjects were grouped in a single room for the whole observation period and a grouping effect could have masked possible differences. In addition, the sexes were not equal.

This second study has been performed in order to increase the number of observations, to avoid psychological effects of grouping and to balance the sexes.

METHODS

The study was performed on 38 males and 35 females healthy volunteers from 17 to 76 years of age (mean 25 ; S.D. 10). The mean body weight was 60.7 kg (S.D. 11.7).

The amount of MSG administered was 3 g/subject in 150 ml of beef bouillon. The bouillon was given at lunch time (1.00 p.m.) and followed by other dishes.

Subjective and objective evaluations were carried out 20, 40, 60, 90 and 120 minutes after the bouillon was drunk. The subjects received at random MSG bouillon in one session and bouillon without MSG in a second one. Two days elapsed between the two sessions. The subjects were asked to specify if they liked the flavour of the bouillon and to report, at the stated times, if they experienced the following symptoms

headache, flushing facial pressure, chest pressure, gastric distress, nausea, perspiration, prostration or any other symptom or feeling. (The term «burning feeling» was omitted on purpose from the form). These symptoms were considered as *subjective symptoms*.

In addition, four doctors, not informed of the order of MSG administration, recorded, at the stated times, the arterial blood pressure (Max and Min) and the pulse rate. These parameters were considered as *objective symptoms*.

In contrast to the previous experiment, the subjects were not grouped in one room, but were left free to do whatever they wished in the two experimental hours. Observations were made on 8-12 people a day.

RESULTS

The data regarding the subjective symptoms are summarized in table I. As each subject formed part of both the control and the MSG treated groups has to be taken into account when analysing the data for the total sample, for males only and for females only.

There were four categories into which a subject could fall :

1) no symptoms at either session,
2) symptoms at both session,
3) symptoms at control session only,
4) symptoms at MSG session only.

The number of subjects in each of the categories can be denoted by N_1, N_2, N_3 and N_4 respectively where $N_1 + N_2 + N_3 + N_4 = N$ is the total number of subjects.

Clearly only the last two groups are giving information about the possible effect of MSG. Therefore a comparison was made between N_3 and N_4 which can be considered a comparison of N_4 with zero, taking into account the fact that N_3 subjects will be reporting the symptoms after an ordinary meal. The comparison was made by treating the data as estimates of a multinomial distribution and using a normal distribution approximation.

A comparison between males and females within the control or the MSG group was made using a χ^2 test with continuity correction, following the recommendation of Cochran (2).

It can be seen from table I that no significant differences exist between the control and the MSG group with the exception of headache, whose incidence was significantly higher in the females given MSG.

The comparison between the control, and MSG treated groups for the males, and the total sample (i.e. males and females combined) showed no significant difference. Although females tended to report more symptoms than males, the χ^2 analysis showed no significant difference when considering subjects who had symptoms at one session only.

When considering all subjects reporting symptoms at a particular session, there was no difference between males and females for any individual symptoms but for the number of subjects reporting

TABLE I

Incidence of symptoms

	At one treatment session only						At both session		
	Control Session			MSG Session					
	♂	♀	Total	♂	♀	Total	♂	♀	Total
No. of Subjects	38	35	73	38	35	73	38	35	73
Symptom									
Headache	1	1	2	1	*6	7	2	2	4
Flushing	0	2	2	2	3	5	1	1	2
Facial Pressure	0	1	1	0	3	3	0	0	0
Chest Pressure	0	0	0	0	1	1	0	0	0
Fainting Feeling	0	2	2	2	6	8	0	0	0
Gastric Distress	2	1	3	1	1	2	0	0	0
Nausea	0	2	2	0	0	0	0	0	0
Burning Feeling	0	2	2	0	1	1	0	0	0
Perspiration	0	0	0	0	0	0	0	0	0
Others	0	2	2	0	3	3	0	1	1
At least 1 symptom	3	4	7	3	9	12	3	6	9

* Significantly different from female controls ($p < 0.05$)

at least one symptom it was found that there were significantly more females than males ($p < 0.05$).

It is interesting to note that the burning sensation in the back of the neck, considered one of the main symptoms of the CRS was only reported by 2 females during the control session and by 1 female during the MSG session. With regard to the objective parameters, no clinically meaningful differences were observed between the two groups (table II), and therefore it was not considered worthwhile carrying out a statistical test of significance.

TABLE II

Mean values (and S.D.*) of arterial pressure and pulse rate in 75 subjects after a normal bouillon (Controls) and a bouillon containing 3 g MSG

Time (min)	Controls			M S G		
	Pmax	Pmin	Pulse	Pmax	Pmin	Pulse
0	122.94 (16.82)	79.17 (11.08)	76.83 (12.98)	122.19 (14.23)	78.97 (10.94)	77.39 (12.04)
20	123.42 (17.27)	78.08 (11.10)	79.67 (12.38)	124.24 (14.99)	77.75 (12.47)	81.05 (12.50)
40	122.73 (16.54)	75.13 (10.83)	82.76 (12.92)	122.73 (17.34)	75.20 (10.71)	85.32 (12.92)
60	124.79 (17.44)	75.89 (11.45)	83.34 (12.51)	121.71 (16.83)	73.85 (10.45)	84.49 (12.84)
90	120.20 (15.35)	74.79 (12.65)	81.04 (11.43)	121.43 (14.91)	75.41 (11.56)	83.64 (12.29)
120	120.95 (15.35)	75.20 (11.28)	79.36 (11.25)	118.76 (15.91)	73.90 (11.67)	81.24 (12.68)

* S. D. = Standard Deviation

DISCUSSION

There is no doubt that the Chinese Restaurant Syndrome (CRS) does exist, but its real cause is still undetected, and from the reported data it cannot be related to the current use of MSG.

As shown in our previous study (5), many of the subjective symptoms considered to be part of the CRS may also be present in a normal digestive process, and enhanced by «grouping effect», or environmental conditions. With respect to this, it should be noted that with the procedure followed during this experimental study the incidence of symptoms was much lower than in the previous one (5) where most of the subjects complained of one or more symptoms.

No clear differences were observed between males and females. Since each person acted as their control, the tendency of the females to report more symptoms does not affect the results of the trial. In fact, only for the headache symptom, so often present in the female, did the MSG treated females have a significantly higher incidence than the control females, and with the level of significance at only 5 %, it is of doubtful clinical importance.

REFERENCES

1. Ambos M., Leavitt N. R., Marmorek L. & Wolschina S. E. Sin cib-syn : accent on glutamate. *New Engl. J. Med.*, 1968, *279*, 105.
2. Cochran W. G. Some methods of strengthening the common chi-squared test. *Biometrics*, 1954, *10*, 417.
3. Kenney R. A. & Tidball C. S. Human susceptibility to oral monosodium L-glutamate. *Amer. J. clin. Nutr.*, 1972, *25*, 140.
4. Kwok R. H. M. The chinese restaurant syndrome. *New Engl. J. Med.*, 1968, *278*, 796.
5. Morselli P. L. & Garattini S. Monosodium glutamate and the chinese restaurant syndrome. *Nature (Lond.)*, 1970, *227*, 611.
6. Rosemblum I., Bradley J. D. & Coulston F. Single and double blind studies with oral monosodium glutamate in man. *Toxicol. appl. Pharmacol.*, 1971, *18*, 367.
7. Schaumburg H. H. & Byck R. Sin cib-syn : accent on glutamate. *New Engl. J. Med.*, 1968, *279*, 105.
8. Schaumburg H. H., Byck R., Gerstl R. & Mashman J. H. Monosodium L-glutamate : its pharmacology and role in the chinese restaurant syndrome. *Science*, 1969, *163*, 826.