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Metab Clin. Exp, 4, 1955

Effect of Carbohydrates on Blood Amino Nitrogen

By Anthony A. Albanese, Louise Orto, Joan Rossy, Rosemarie DiLallo and Aurora Belmont

As EARLY as 1891 Kulz[1] observed that levulose and dextrose were metabolized in different ways in the body. However, it remained for Cori[2] in 1926 to show that fructose is more rapidly and effectively converted to liver glycogen than are dextrose and some other sugars. In recently reported investigations[3] we noted that in terms of blood sugar levels after standardized doses in fasting subjects, the utilization of dextrose decreases markedly with age, whereas the utilization of fructose is only slightly affected by age.

Because of these metabolic differences of dextrose and fructose and the known metabolic interrelationships of proteins and carbohydrate, we were prompted in 1950 to initiate studies on the relative protein-sparing action of commonly occurring dietary sugars. These investigations[4] revealed that diets high in fructose induced a greater nitrogen retention in children than diets high in dextrose or galactose. These findings led us to inquire further into the biochemical mechanism through which this end result was achieved. After unsuccessful experiences with a number of procedures, we finally found that changes induced by different sugars in the blood amino nitrogen levels of fasting subjects might serve as a dynamic index of protein-sparing activity of various carbohydrates. The results of this study are reported here.

Experimental

Procedures and Methods

The subjects (5 to 81 years) employed in these investigations were either normal or in the ultimate phase of their convalescence. They were fasted overnight for 12 hours and then given orally test doses of different quantities of a protein preparation (0 to 0.4 gm. per kilogram) or of different carbohydrates at the rate of 1.0 gm. per kilogram. The calculated amounts of these substances, corrected for hydration and extraneous matter, were dissolved or suspended in 250 cc. of water. In control studies the same quantity of water alone was given. Blood samples were collected prior to the administration of the test substance and exactly one hour later. In most instances comparative data were obtained by serial measurements on the same subjects.

The total blood sugar and fructose were determined colorimetrically by the Folin-Wu and resorcinol methods, respectively, adapted to the Klett-Summer-

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colorimeter.[5] The accuracy of these methods was ascertained previously by us by an ample number of calibration curves and recovery tests. In order to ascertain interference, the relative color indices of glucose and fructose in the methods as employed were determined (Table 1). The corrections indicated by these data have been applied to our experimental results.

Blood amino nitrogen was determined in deproteinized filtrates by the copper method described by one of us.[6] Detailed tests showed that fructose, dextrose and sucrose did not interfere in the measurement of amino nitrogen by this procedure.

Results

As a first approach to the problem, it was necessary to evaluate the blood amino nitrogen as a dynamic index of protein nutrition. This was done by determining the changes in amino nitrogen levels induced by feeding varying amounts of protein under the conditions previously described. The differences in amino nitrogen content observed between the 12-hour fasting sample and that found 60 minutes after administration of the protein (ΔA_{60}) are plotted against dosage in Figure 1. It will be noted therefrom that the fall in blood amino nitrogen which occurs with continuation of the fast to the 13th hour (0 protein intake) can be overcome by a test dose as small as 0.1 gm. of protein per kilogram. However, approximately 0.3 gm. of protein per kilogram (or 21 gm. for a 70-kg. individual) is needed to raise the blood amino nitrogen to average normal levels.[6]

Having thus correlated blood amino nitrogen with protein intake, we then attempted to determine the effect of dextrose, fructose and sucrose on this index of protein nutrition. The results of these measurements are collected in Table 2. Examination of the data obtained for groups 2 and 4 discloses that within 60 minutes of administration, fructose causes an appreciable increase in blood amino nitrogen. Indeed, the effect approximates closely that produced by the administration of 0.3 gm. of protein per kilogram (Fig. 1). In the same time interval, glucose, on the other hand, caused an increase in blood amino nitrogen insignificantly greater than that found on continued fasting of the subjects (Group 1). Tests with sucrose, as might be expected, yielded values intermediate between those obtained for fructose and dextrose. The trials with the maltose-dextrin product (Group 6) disclosed that this carbohydrate preparation (essentially a mixture of glucose polymers) yields amino acid index values similar to those obtained with dextrose.

Table 1. *Specificity of Total Sugar and Fructose Methods*[a]

	Measurement method		
	Total sugars		Fructose
	Picric acid	Folin-Wu	Resorcinol
Body fluids examined	Urine	Blood	Blood and urine
Concentration, mg	0.5	0.4	0.5
Glucose	100	100	1
Fructose	100	100	100

[a] The Klett-Summerson colorimeter was used for the determinations of total sugars and fructose.

[b] The Beckman spectrophotometer was used.

Metab. Clin. Exp. 4, 1955

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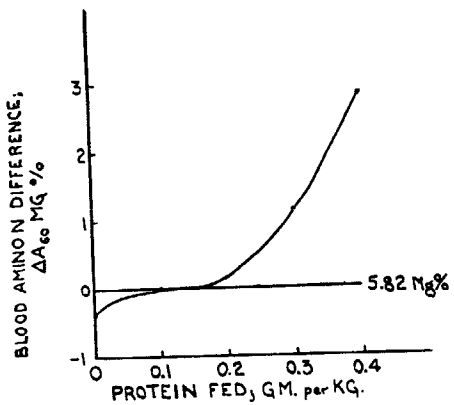

FIG. 1. Effect of oral test doses of a protein product on the blood amino nitrogen.

A statistical analysis of the pertinent ΔA_{60} data was done by determining the standard deviation for the difference in means. In this system a difference of greater than 2.5 standard deviations was considered to be significant, and values between 2.0 and 2.5 of borderline significance. Appropriate calculations showed the difference in mean ΔA_{60} values between fructose and fasting levels and between fructose and glucose tests to be 28 and 6 standard deviations, respectively, which places the observations well beyond the probability of chance occurrence. The difference between the glucose and fasting values was found to be only 2 standard deviations, giving these observations only a borderline significance.

Since the observed greater amino acid index of fructose appears to be real, it seemed worth while to attempt to associate this characteristic with other metabolic patterns of this hexose. Blood sugar measurements disclosed that in normal nourished subjects the amino acid index is inversely related to the level of total sugar induced in the blood of the fasted subject at the end of the 60-minute interval by the test sugar. The data for groups 2, 3 and 4 would seem to support this generalization. The differences in blood sugar levels produced by fructose and dextrose confirm previous observations by us[1] and by others[7].

An apparent contradiction to this surmise is presented by the results obtained with the subjects in Group 5. This group was composed of six college girls who had used a variety of reducing diets of less than 2000 calories/day for some time. The biochemical effects of these regimens were reflected in average fasting blood sugar levels of 78.3 ± 8.2 mg % as contrasted to the norm of 102 ± 11 mg. % and low total sugar increments (ΔS_{60}) following test doses of dextrose and fructose. Further indication of nutritional depletion was evidenced by the low amino nitrogen indices (ΔA_{60}) obtained for both fructose and dextrose. However, the difference between the dextrose and fructose A_{60} values of + 1.1 found for this group is comparable to that found for groups 2 and 4. These observations suggest

TABLE 2.—*Effect of the Oral Administration of Sugars and Proteins on the Blood Sugar, Fructose, and Amino Nitrogen of Fasting Subjects*

Test substance, dosage	Group	No. of subjects	Age range, yrs.	Average differences in 60 min. after administration of test doses, mg.% Total sugars (ΔS) 60	Fructose (ΔF) 60	Amino nitrogen (ΔA) 60
None	1	18	11-80	−2.2 ± 7.8	0.2 ± 0.2	−0.4 ± 0.2
Fructose, 1 gm./kg.	2	40	13-81	24.3 ± 6.5	4.9 ± 0.7	+1.0 ± 0.1
Dextrose, 1 gm./kg.				52.0 ± 11.3	−0.5 ± 0.4	−0.3 ± 0.1
Sucrose, 1 gm./kg.	3	10	22-75	41.0 ± 21.6	4.3 ± 0.6	−0.1 ± 0.1
Dextrose, 1 gm./kg.				56.0 ± 15.2	0.1 ± 0.1	−0.4 ± 0.1
Fructose, 1 gm./kg.	4	8	27-71	26.0 ± 9.2	4.7 ± 1.7	+0.7 ± 0.1
Dextrose, 1 gm./kg.				49.2 ± 21.8	−0.5 ± 0.4	−0.3 ± 0.2
Fructose, 1 gm./kg.	5	6	19-21	9.6 ± 4.1	3.7 ± 1.1	+0.1 ± 0.1
Dextrose, 1 gm./kg.				11.6 ± 10.2	−0.3 ± 0.3	−1.5 ± 0.5
Fructose, 1 gm./kg.	6	4	5-11	9.0 ± 2.2	1.8 ± 0.6	+0.9 ± 0.1
Maltose-dextrin Mixture,* 1 gm./kg.				26.2 ± 6.8	0.5 ± 0.4	−0.3 ± 0.1
Protein† 0.1 gm./kg.	7	3	42-75	−17.5	−1.20	0
0.2 gm./kg.		3	53-71	−7.0	0.0	+0.1
0.3 gm./kg.		10	17-73	6.0 ± 11.7	0.3 ± 0.7	+1.2 ± 0.2
0.4 gm./kg.		2	13-75	10.0	0.0	+2.8
Average fasting levels for all 96 subjects studied				102 ± 11	1.8 ± 0.3	5.82 ± 0.5

* This product (Dextri-maltose No. 1, Mead Johnson) has the following approximate analysis: maltose, 55%; dextrins, 42%; NaCl, 2%; moisture, 1%.

† This test protein was provided in the form of Somagen (Upjohn), which contains 70% protein, 22% carbohydrate and the remainder as vitamin and mineral supplements. This product was kindly supplied to us by Drs. Earl L. Burbidge and Robert W. Heinle.

that the greater nitrogen-sparing effect of fructose as reflected by the amino acid index operates even under conditions of poor nutrition.

Fructose measurements indicate the apparent presence of small amounts of this sugar in the blood (Table 2). This value is small, and it is doubtful at this writing that it represents true fructose. However, the increases in fructose levels (ΔF_{60}) induced by the administration of fructose or sucrose would appear to be real. The ultimate metabolic fate of this circulating fructose and its role in the nitrogen economy of the human remains to be determined.

Consideration of the results obtained with test doses of protein (Group 7) — apart from their providing ΔA_{60} reference values — discloses several nutritionally important facts. These pertain to the finding that the administration of a high protein food in small quantities (0.1 to 0.2 gm. per kilogram) results in a hypoglycemic effect greater than that found with continued fasting. With larger amounts of the protein product (0.3 to 0.4 gm. per kilogram) the glycemia is improved but not at a rate normally commensurate with the increase in amino-acidemia. These observations may provide a biochemical basis for the well-known weight-reducing effect of high protein diets.

Comment

Although at first glance the reported findings would seem to require a revision of the accepted mechanisms for the mammalian metabolism of sugars, a more careful consideration reveals that there is no need for such a revision. Indeed, the observations might well have been forecast, if the now well-established fact that fructose is more rapidly metabolized than glucose were considered in all its possible ramifications. The known high glycogenic[2] and low glycemic[3] activity of fructose bespeaks of its more rapid metabolism by the body than dextrose or other sugars. An inherent, but not generally realized, part of this mechanism is the rapid formation of carbon structures (hydroxy and keto acids) which on combination with labile amino groups that become available by the transamination cycle are converted to amino acids. In this light our measurements of blood amino nitrogen index ($\Delta\Lambda_{60}$) simply suggest that fructose is a readier source of these carbon radicals than dextrose.

This observed difference between fructose and glucose explains in part some of the reported nutritional advantages afforded by fructose or fructose-containing products. These advantages have been found to prevail in the fields of pediatric nutrition,[1] geriatric nutrition[8] and parenteral nutrition.[5] Recent observations in this and other laboratories indicate that fructose or sucrose may provide nutritional benefits for infants not to be found in dextrin-containing products commonly employed in the preparation of infant formulae. These opinions are based partly on considerations of the herein reported carbohydrate-protein relationships and partly on enzymic states prevailing in the infant. This latter factor has been brought into sharp focus by the investigations of Douglass,[9] who has found that pancreatic amylase production remains relatively deficient for some months in early infancy. Ptyalin is present in small amounts in saliva of the newborn, but it takes five to seven weeks for the development of noticeable saliva. These enzymic deficiencies can be expected to interfere seriously with the complete absorption of dextrin and starches.

The far-reaching fundamental and practical implications of this subject have prompted us to undertake a direct detailed investigation of the relative merits of various sugars in infant feeding as they affect protein nutrition. It seems clear now that protein or amino acid needs of the infant should be considered in terms not only of the quality of the protein in the diet but also in reference to quality of the calories provided in the form of sugars and fats.

Summary

Evidence has been presented that test doses of fructose induce a higher blood amino nitrogen index than comparable amounts of dextrose in fasting human subjects. This finding is believed to be associated with the greater protein-sparing activity of fructose observed in previously reported nitrogen balance studies with children and convalescent adults. The metabolic implications of these findings are discussed.

Acknowledgment

We wish to thank Dr. Martin A. Rizack for the statistical analyses of our data and Miss Emita Balaquer for some analyses performed in connection with this work.

References

1. Kelz, E. C.: Ludwig's Festschrift, Marburg, 1891.
2. Cori, C. F.: The rate of glycogen formation in the liver of normal insulinized rats during the absorption of glucose, fructose and galactose, J. Biol. Chem. *70:* 577, 1926.
3. Albanese, A. A., Higgons, R. A., Orto, L., Belmont, A., and DiLallo, R.: Effect of age on the utilization of various carbohydrates by man, Metabolism *3:* 154, 1954.
4. Albanese, A. A., Felch, W. C., Higgons, R. A., Vestal, B. L., and Stephanson, L.: Utilization and protein sparing action of fructose in man, Metabolism *1:* 20, 1952.
5. Snell, F. D., and Snell, C. T.: Colorimetric Methods of Analysis, New York, D. Van Nostrand Co., Inc., 1937, pp. 199, 455 and 480.
6. Albanese, A. A., and Irby, V.: Determination of amino nitrogen of blood filtrates by the copper method, J. Lab. & Clin. Med. *30:* 718, 1945.
7. Weinstein, J. J.: Parenteral therapy with invert sugar, Ann. West. Med. & Surg. *4:* 373, 1950.
8. Albanese, A. A., Arnold, W. H., Hays, D. R., Belmont, A., Orto, L., and DiLallo, R.: Protein sparing action of intravenously administered carbohydrate solutions, Metabolism, *3:* 523, 1954.
9. Douglass, F. H.: Why solid foods for newborn? Northwest Med. *52:* 832, 1953.

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we have found that the degree of
ıg, and the preparation is suitable for
ɔne, acetylsalicylic acid, aminopyrine,
s employed were within the range of

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hysiol. Revs. **40**, 580-646.
E., and WATERHOUSE, P. D. (1952).
some derivatives of salicylamide. *J.*

antipyretic activity and toxicity of
d Pharmacol. **10**, 609-620.
(1957). The comparative antipyretic
ıide in fever-induced rats. *J. Pharm.*

ıation of anti-inflammatory agents, a
ibutazone (Butazolidine T.M.). *Ann.*

influ of fever on the vago-insulin
ology , 141-143.
ncy of pyrogen fever. Appearance of
animals and in plasma incubated with

.tion of small laboratory animals at
Physiol. **129**, 123-139.
rties of an antigenic material isolated
180.
ıethod for pyrogens. *J. Am. Pharm.*

935). The ratio of the toxicity of
J. Pharmacol. Exptl. Therap. **54**,

and ... genic properties of fractions
lla dysenteriae. J. Immunol. **58**, 337-

uantitative assay of pyrogens by the
52.

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The Utilization of Schardinger Dextrins by the Rat

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Schardinger dextrins are a homologous series of cyclic dextrins composed of 6 or more -D-glucopyranose units linked by $1 \rightarrow 4$ bonds as in amylose. The 6- and 7-unit Schardinger dextrins are the most common and are called, respectively, α-dextrin and β-dextrin.

The Schardinger dextrins can be obtained from the breakdown of starch by a few known strains of bacteria and fungi—the most common being *Bacillus macerans*. These dextrins are resistant to hydrolysis by acid, α- and β-amylase, and yeast. They are of interest in food applications because of their ability to form complexes with many organic and inorganic substances. These observations were noted in the work of Schlenk *et al.* (1955). Although relatively insoluble, many of these complexes dissociate in aqueous solutions to release included compounds. This observation resulted in an increased interest in the cyclic dextrins for binding volatile or unstable substances used in foods and attention was directed toward their potential toxicity.

Very little is known about the metabolism of the Schardinger dextrins by animals. French (1957) has summarized the available data in his review of these compounds. He reported that a group of rats fed purified β-dextrin died after 1 week on the diet. Von Hoesslin and Pringsheim (1923) reported that no glycogen could be detected in the livers of fasted rabbits and guinea pigs 3 hours after they were fed the dextrins.

The above references show that experimental animals have been fed diets containing Schardinger dextrins. However, the experiments were

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too limited in the number of animals or duration of tests to yield meaningful data concerning utilization of the cyclic dextrins.

The Schardinger dextrins are very resistant to attack by the common starch-hydrolyzing enzymes. It has been reported that the Schardinger dextrins are completely resistant to α- and β-amylase action. However, French (1957) found that the α-dextrin is essentially completely resistant to salivary amylase, the β-dextrin is very slowly utilized, and the γ-dextrin (8 glucose units) is attacked about 1% as rapidly as starch.

It has been reported by Ben-Gershom (1955) that certain fungal preparations contain enzymes that are capable of hydrolyzing Schardinger dextrins.

Green and Stumpf (1942), in their work on purified potato phosphorylase, showed that this enzyme is competitively inhibited in its action on starch by α- or β-dextrins. They theorized that the Schardinger dextrins and starch were competing for the same active group in the enzyme.

Cramer (1953) showed that β-dextrin retards the cleavage of indican by emulsin or by acid hydrolysis. The formation of including compounds may have been involved in the above inhibition.

Thoma and Koshland (1960) demonstrated a competitive inhibition of sweet potato β-amylase with α- and β-Schardinger dextrins. The results were interpreted as added support for the induced-fit theory of specificity and suggested that this new type of competitive inhibition by substrate exists in other enzyme systems.

McCloskey and Porter (1945) reported that of 18 bacterial species and 4 yeasts, only *Bacillus macerans* and *Bacillus polymyxa* were able to utilize Schardinger dextrins.

It is evident that uncertainty exists in the literature regarding the metabolism of Schardinger dextrins. The studies reported here were undertaken to clarify this situation by comparing the utilization by rats of radioactive α- and β-dextrins with radioactive potato starch.

METHODS

The Biosynthetic Preparation of Uniformly Labeled Carbon-14 Potato Starch

Potted potato plants, in which the tubers were just beginning to form, were used to prepare the C^{14}-labeled starch. The plants were maintained in the apparatus described by Weinstein *et al.* (1959) differing only in that the apparatus used in this work to enclose the plants was a 43-liter chamber. A bank of ten 40-watt fluorescent bulbs was arranged over the

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Metabolism Studies

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chamber, and six incandescent spot lights were focused on it to supply light to the growing plants. A timing circuit was set up to turn the lights on and off so that the plants would have light for 11 hours and darkness for 13 hours each day. The plants were fed a total of 3 mc of $C^{14}O_2$ in 3 doses of about 1 mc per dose. Two days after the initial millicurie was given, 91% of $C^{14}O_2$ given the plants was fixed by them. At that time, another portion of about 1 mc was given; a third 1 mc portion 3 days later. The second dose resulted in 89% fixation and the third 93%. These figures were obtained by sampling the atmosphere at the beginning and end of each fixation period as described by Weinstein *et al.* (1959).

Two days after the last feeding period, the plants were opened to air for a week to allow the fixed $C^{14}O_2$ to be converted into starch in the tubers. At the end of this period, the tubers were separated from the plant. The total net weight of the potatoes was 46 g. The potatoes were peeled and homogenized in a Waring blendor three times with cold water. The filtrate was discarded and the cold water-insoluble crude starch was freeze-dried was washed with alcohol. The yield was 3.5 g of labeled potato starch of specific activity 214 μc/g.

The Synthesis of Radioactive Schardinger Dextrins

Radioactive α- and β-Schardinger dextrins were prepared by enzymatic digestion of a 2.5% dispersion of potato starch by *B. macerans* amylase at 40° C for 4 hours, using essentially the method described by Tilden and Hudson (1939). The digestion mixture contained 25 ml of enzyme solution equivalent to 288 units of enzyme (Tilden and Hudson, 1942). The starch consisted of 3.1 g of uniformly labeled C^{14} potato starch and 8.9 g of Maine potato starch giving a specific activity of 55.3 μc/g. The resulting α- and β-dextrins were isolated as trichloroethylene and bromobenzene complexes as described by French (1957). The yield of α-dextrin was 9.4% and β-dextrin 14.7%, based on the weight of starch used. The cyclic dextrins had the same specific activity; 56 μc/g. The specific rotations of the α- and β-dextrins in water were 150.6 ± 1.0° and 161.0 ± 1.6°, respectively. The specific rotations reported by French (1957) are 150.5 ± 0.5° and 162.5 ± 0.5°.

Metabolism Studies

Charles River rats, Wistar strain, weighing 185 ± 50 g were starved 18 hours prior to use. Six rats were used in the metabolic study; of these rats, two were fed α-dextrin, two β-dextrin, and two potato starch.

The Schardinger dextrin solutions used for these studies were prepared by dissolving the solid materials in warm water. The starch was suspended in boiling water and homogenized in a Virtis homogenizer. Approximately 2.5 ml of material (2.5%) were used for intubation. The quantitative amount delivered to each rat was determined by weight difference of the syringe before and after intubation. Using the same material, weighed amounts were immediately placed in weighed platinum boats for assay.

After intubation, the rats were placed in a metabolic chamber for 16 to 23 hours and respiratory CO_2 was drawn through a continuous radioactive gas analyzer, as described by Long *et al.* (1958), which automatically recorded the rate and total $C^{14}O_2$ respired. The radioactive carbon dioxide was collected in 2 *N* NaOH traps, which were assayed for total carbon-14. At the end of the experiment, the animals were sacrificed by a blow on the head and the organs were removed for assay. The urine, feces, and carcasses were also assayed for radioactivity as described below and a carbon-14 material balance obtained.

Radioactive Determination

Measurement of carbon dioxide excretion. The continuous measurement of total $C^{14}O_2$ respired was accomplished by a method similar to that reported by Long *et al.* (1958).

Combustion. All samples for radioactive assay were handled in the manner described by Domingues *et al.* (1960). Correction for sample absorption was that described by Calvin *et al.* (1949).

RESULTS

The utilization of Schardinger dextrins by the rat has been evaluated using radioactive C^{14} substrates. The amount of $C^{14}O_2$ respired by the rats after oral administration of α-dextrin was 58–62% of the dose in 17–23 hours. The β-dextrin was utilized to a similar extent as shown by $C^{14}O_2$ excretions of 49–69% in a similar time interval (Table 1).

The $C^{14}O_2$ respiratory patterns from the α- and β-dextrins were similar, but they differed greatly from the pattern obtained from radioactive starch (Figs. 1 and 2). The breath pattern from the animal that received the α- or β-dextrin indicated a very slow utilization of the cyclic compound. Since each spike on the recorder chart indicates a 5-minute time interval, it is evident from Fig. 1 that very small amounts of $C^{14}O_2$ were initially respired, using 10 units on the chart as background level. The slope of the respiratory curve showed a definite change after the third

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DISTRIBUTION

Tissue	α-Dextrin (% of dose)	α-D (% dose)
Breath	58.2	6
Cecum content	1.7	
Cecum	0.7	
GI tract	7.0	
Urine	3.6	
Feces	14.4	No
Liver	1.3	3
Heart	0.1	0
Lungs	0.3	Los
Kidneys	0.3	0
Sex organs	0.3	0
Spleen	0.1	0
Carcass	16.7	16
Recovery	104.6	103
Time on test (hours)	17	22
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urve showed a definite change after the third

hour postintubation and reached a broad maximum output between 8 and 9 hours. Small amounts of radioactive $C^{14}O_2$ persisted even after 20 hours postintubation.

Figure 2 depicts a respiratory pattern obtained from radioactive starch. Maximum output of $C^{14}O_2$ was observed within an hour of intubation and reached background level approximately 8 hours later. The rat has shown

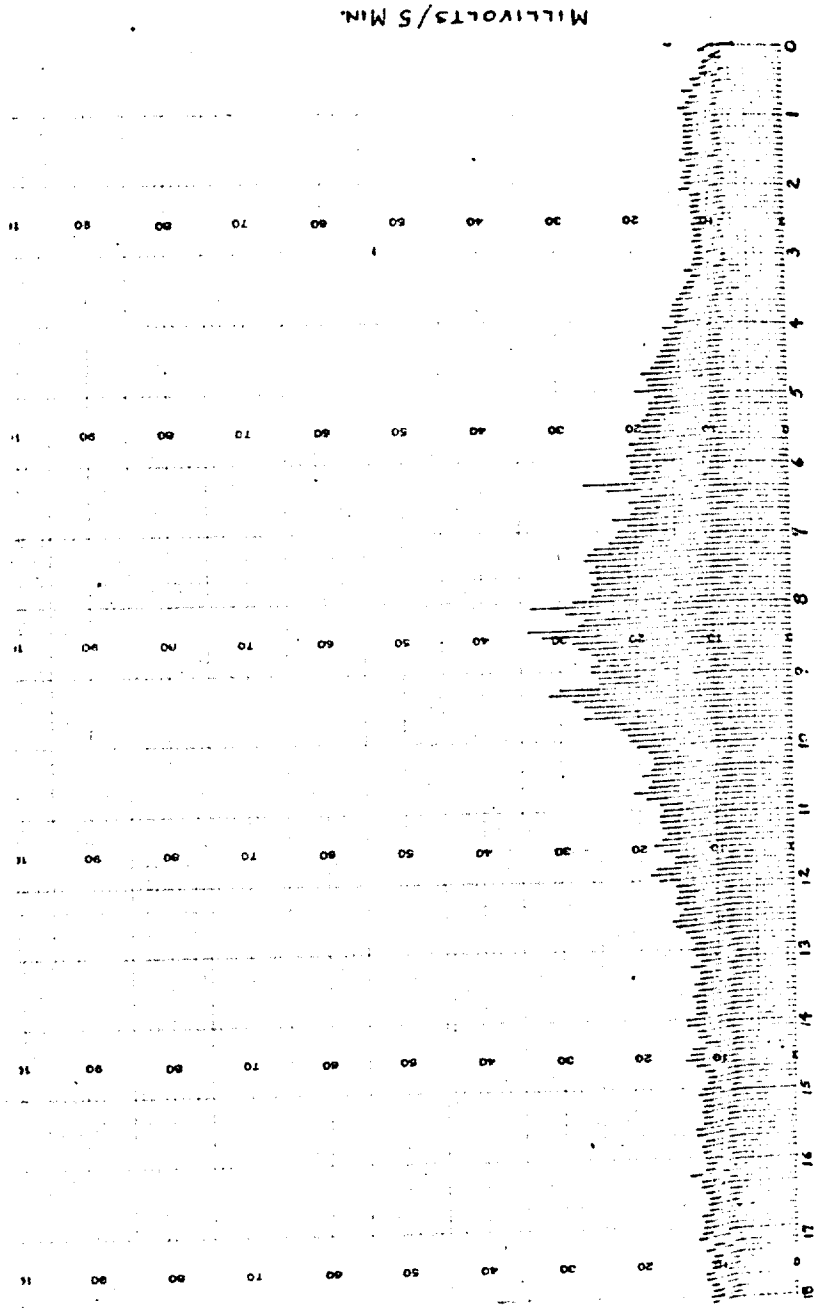
TABLE 1
DISTRIBUTION OF RADIOACTIVITY IN ANIMALS

Tissue	α-Dextrin (% of dose)	α-Dextrin (% of dose)	β-Dextrin (% of dose)	β-Dextrin (% of dose)	Starch control (% of dose)	Starch control (% of dose)
Breath	58.2	62.4	48.6	66.8	64.3	58.6
Cecum content	1.7	3.8	4.0	4.9	1.2	—
Cecum	0.7	0.4	1.6	1.1	0.2	—
GI tract	7.0	8.0	4.0	8.8	2.6	6.1[a]
Urine	3.6	8.0	5.1	3.6	1.8	3.0
Feces	14.4	None	5.4	None	None	Lost
Liver	1.3	3.2	3.0	0.2	0.4	3.1
Heart	0.1	0.1	0.1	0.1	0.1	0.04
Lungs	0.3	Lost	0.1	0.1	0.1	0.04
Kidneys	0.3	0.4	0.4	0.3	0.3	0.1
Sex organs	0.3	0.3	0.2	0.2	0.4	0.02
Spleen	0.1	0.3	0.1	0.1	0.1	0.1
Carcass	16.7	16.2	22.5	14.2	26.0	15.6
Recovery	104.6	103.2	95.1	100.2	97.4	86.7
Time on test (hours)	17	22	17	23	22	22
	Male	Male	Male	Male	Male	Female

[a] The radioactivity in the gastrointestinal tract of this animal includes the cecum and its contents.

the ability to utilize starch quite rapidly since significant amounts of $C^{14}O_2$ were evident in the breath within 15 minutes. Although a difference in the rate of $C^{14}O_2$ production was noted, the total amount respired was essentially the same as the Schardinger dextrins.

The distribution of radioactivity in the various tissues and organs of the rats subjected to the above-mentioned compounds is given in Table 1. It should be noted that the cecum and its contents were analyzed separately from the rest of the gastrointestinal tract. The gastrointestinal tract, as analyzed, contains any contents present at the time of sacrifice of the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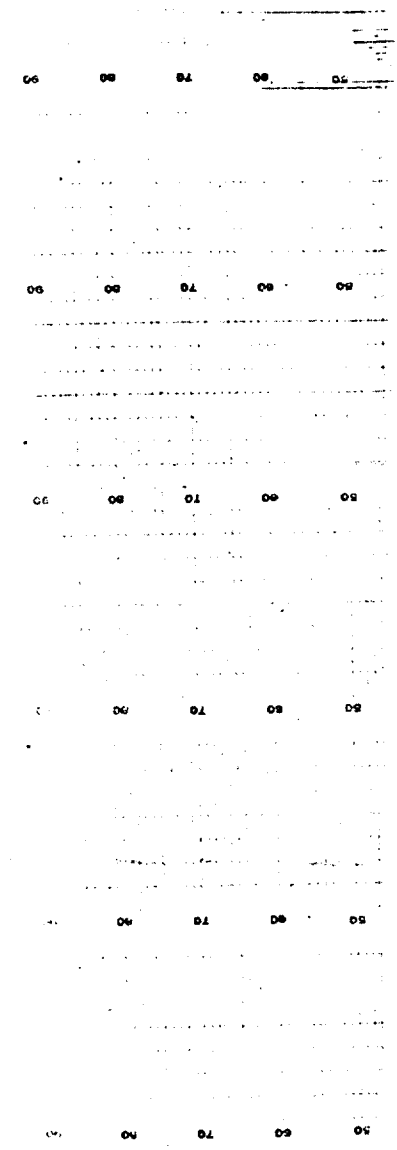
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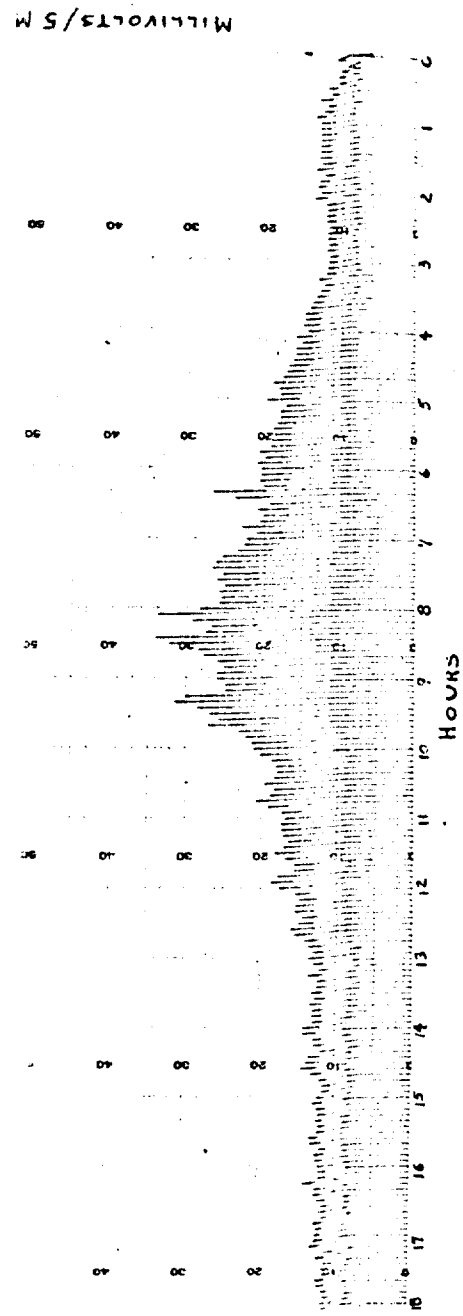

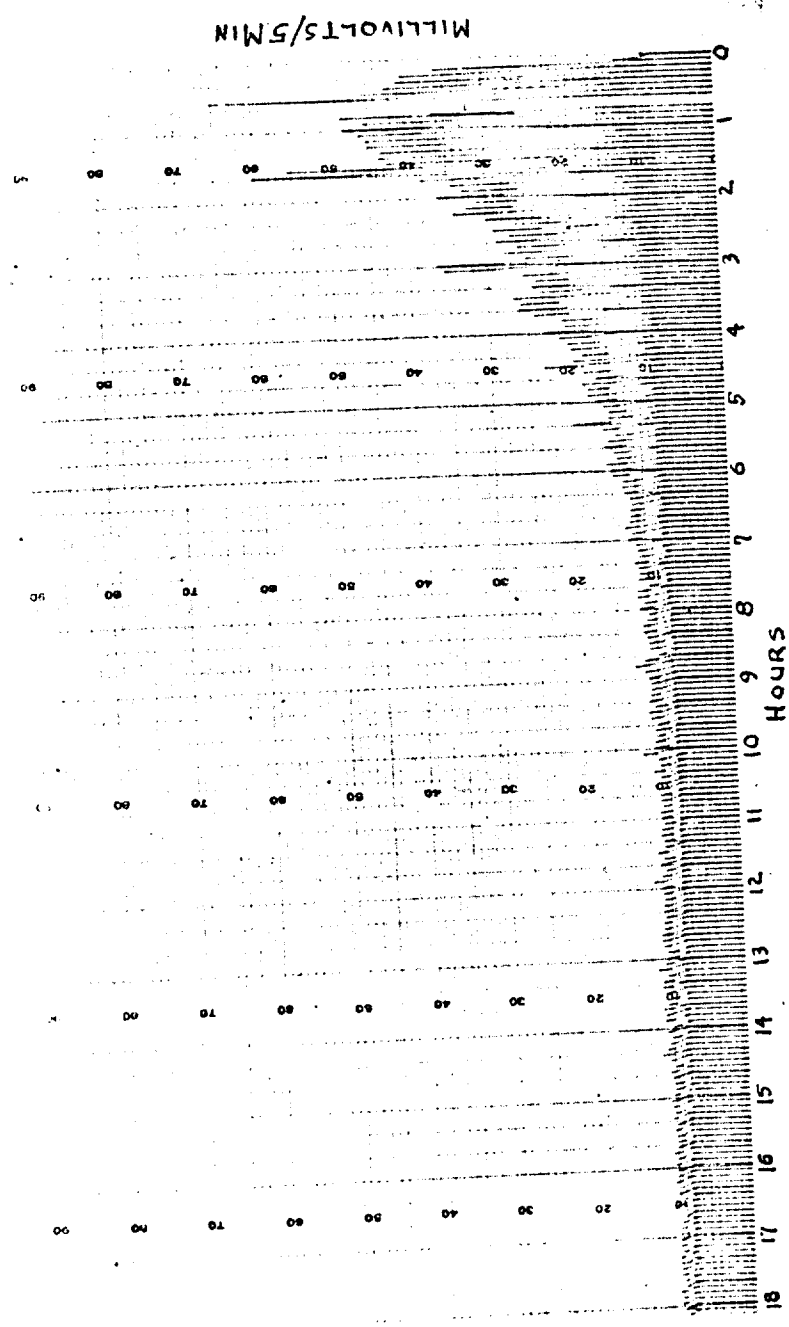

FIG. 2. $C^{14}O_2$ respiratory curve after oral intubation of starch-C^{14} in the rat.

animal. The total radioactivity in the cecum, cecum contents, and the gastrointestinal tract range from 9 to 15% from the α- and β-dextrins and from 4 to 6% from starch. In general, there are no marked differences in the distribution of radioactivity in the other tissues between the rats fed dextrins and those fed starch.

The distribution of radioactivity in the urine and feces is also given in Table 1. No comparison was made of the feces as one of the animals given radioactive starch was void of feces during the experiment and the other animal's feces was lost. Nevertheless, there was a significant amount of radioactive material detected in the feces of the animals fed α- and β-dextrins. This would imply to a certain degree that these compounds were digested slowly. No attempt was made to identify the radioactive components present in any of the urine or feces samples.

DISCUSSION

As one criterion generally used to determine the utilization of a radioactive substrate by the mammalian system is the production of carbon dioxide, it may be stated that the Schardinger dextrins are metabolized by the rat. One may also conjecture that the utilization or partial degradation of the substrate occurred by intestinal flora; followed by absorption of the microbial products and finally metabolic conversion of these absorbed products into a wide variety of compounds. The data presented in this study do not allow for a differentiation between the animal's ability to metabolize the compounds or their prior conversion to digestible products by the intestinal flora preceding absorption.

In animal experiments, von Hoesslin and Pringsheim (1923) reported that they were unable to detect any synthesis of liver glycogen after 3 hours when Schardinger dextrins were fed to fasted rabbits and guinea pigs. Using a 48-hour starved rabbit, they noted 0.14 g of glycogen was recovered from 15 g of the β-dextrin. It is quite possible that the reserves of the animal were depleted to such an extent that glycogen synthesis was not possible. A 24-hour fasted animal given 40 g of glucose resulted in the isolation of 7 g of liver glycogen. It one assumes that the starvation regime above did not completely deplete the reserves of the animal, the data reported here would suggest that von Hoesslin and Pringsheim did not wait a sufficient length of time for absorption of the dextrins or microbial products to take place.

The effect of α- and β-dextrins in the animal diet for long periods of time has been evaluated in this laboratory. These data, to be published

at a later date, indicate that rats containing 1% of the α- or β-dextr efficiencies equal to the control diet

In unpublished data referred to by a diet in which part of the carbohyd β-dextrin refused to eat the test diet maintained on this diet died within 1 because of insufficient food intake r dextrin.

Considering the stability of the fracture by enzymes and the general species, it is quite significant that capable of oxidizing these compound ring must be opened prior to any should result in the formation of an glucose molecules. This being the cas of these cyclic compounds are not which are easily metabolizable.

SUM

Comparative metabolism studies were Schardinger dextrins. This paper describe the isolation of radioactive starch from th α and β-cyclic dextrins.

The radioactive starch and the two cy expired $C^{14}O_2$ was measured continu residual radioactivity in the individual o and Schardinger dextrins was determined results show that the cyclic dextrins are m starch. However, after 24 hours, the tota the distribution of radioactivity within t

ACKNOWL

Grateful acknowledgment is made to D Experimental Station, for supplying potat Boyce Thompson Institute for Plant Rese The authors also wish to acknowledge J. Gildner and Donald J. Rizzo.

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CUSSION

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at a later date, indicate that rats maintained for 3 months on a diet containing 1% of the α- or β-dextrins showed growth curves and food efficiencies equal to the control diet containing no α- or β-dextrins.

In unpublished data referred to by French (1957), rats maintained on a diet in which part of the carbohydrate was supplied by highly purified β-dextrin refused to eat the test diet except in small quantities. The rats maintained on this diet died within 1 week. It is possible that the rats died because of insufficient food intake rather than from toxic effects of the dextrin.

Considering the stability of these cyclic glucose polymers toward fracture by enzymes and the general limited utilization by a few bacterial species, it is quite significant that the rat, by some metabolic route, is capable of oxidizing these compounds to CO_2. It is evident that the cyclic ring must be opened prior to any oxidation. The splitting of the ring should result in the formation of an oligosaccharide containing 6 or more glucose molecules. This being the case, it would appear that the utilization of these cyclic compounds are not different from simple oligosaccharides which are easily metabolizable.

SUMMARY

Comparative metabolism studies were made with radioactive starch and cyclic Schardinger dextrins. This paper describes the growth of radioactive potato tubers, the isolation of radioactive starch from the tubers, and the preparation of radioactive α- and β-cyclic dextrins.

The radioactive starch and the two cyclic dextrins were administered to rats and the expired $C^{14}O_2$ was measured continuously for 17–23 hours. The distribution of residual radioactivity in the individual organs and carcasses of animals fed starch and Schardinger dextrins was determined by standard combustion techniques. The results show that the cyclic dextrins are metabolized, but at a slower initial rate than starch. However, after 24 hours, the total amounts metabolized are about the same. The distribution of radioactivity within the animals is similar to that of starch.

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Grateful acknowledgment is made to Dr. John Campbell, New Jersey Agricultural Experimental Station, for supplying potato plants, and to Dr. Leonard H. Weinstein, Boyce Thompson Institute for Plant Research, Inc., for advice on growing the plants.

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REFERENCES

BEN-GERSHOM, E. (1955). Anomeric inversions by glycosidases. *Nature* **175**, 593.

CALVIN, M., HEIDELBERGER, C., REID, J. C., TOLBERT, B. M., and YANKWICH, B. F. (1949). *Isotopic Carbon*, p. 30. Wiley, New York.

CRAMER, F. (1953). Über einschlusse Verbindungen. IV. Die Hemmung der Gly-kosidspaltung durch Cyclodextrin. *Ann. Chem. Liebig's* **579**, 17-22.

DOMINGUES, F. J., LONG, C. L., ANDERSEN, G. H., BALDWIN, R. R., THIESSEN, R., JR., and ZEITLIN, B. R. (1960). The suitability of β-D-glucose pentaacetate for food use. II. Studies on the absorption and metabolism. *Toxicol. Appl. Pharmacol.* **2** 281-294.

FRENCH, D. (1957). The Schardinger dextrins. *Advances in Carbohydrate Chem* **12**, 189-260.

GREEN, D. E., and STUMPF, P. K. (1942). Starch phosphorylase of potato. *J. Biol Chem.* **142**, 355-366.

HOESSLIN, H. VON, and PRINGSHEIM, H. (1923). Zur Physiologie der Polyamylosen II. Glykogenbildung und Tierische Verbrennung. *Z. Physiol. Chem. Hoppe-Seyler's* **131**, 168-176.

LONG, C. L., DOMINGUES, F. J., STUDER, V., LOWRY, J. R., ZEITLIN, B. R., BALDWIN R. R., and THIESSEN, R., JR. (1958). Studies on absorption and metabolism of propylene glycol distearate. *Arch. Biochem. Biophys.* **77**, 428-439.

MCCLOSKEY, C. M., and PORTER, J. R. (1945). Utilization of certain rare sugars by microorganisms. *Proc. Soc. Exptl. Biol. Med.* **60**, 269-271.

SCHLENK, H., SAND, D. M., and TILLOTSON, J. A. (1955). Stabilization of au toxidizable material by means of inclusion. *J. Am. Chem. Soc.* **77**, 3587-3590.

THOMA, J. A., and KOSHLAND, D. E., JR. (1960). Competitive inhibition by sub strate during enzyme action. Evidence for the induced-fit theory. *J. Am. Chem Soc.* **82**, 3329-3333.

TILDEN, E. B., and HUDSON, C. S. (1939). The conversion of starch to crystalli dextrins by the action of a new type of amylase separated from cultures of *Aero bacillus macerans*. *J. Am. Chem. Soc.* **61**, 2900-2902.

TILDEN, E. B., and HUDSON, C. S. (1942). Preparation and properties of th amylases produced by *Bacillus macerans* and *Bacillus polymyxa*. *J. Bacteriol.* **43** 527-544.

WEINSTEIN, L. H., PORTER, C. A., and LAURENCOB, H. J., JR. (1959). Quinic ac as a precursor in aromatic biosynthesis in the rose. *Contribs. Boyce Thompson Inst.* **20**, 121-134.

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Effects of Sodium Selenite Componen

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Department of Physiology and Pharmacolo State University of South Dakota, V

Received August 5

Acidosis has been reported following th of sodium selenite to dogs (Heinrich and decreased from a control level of 7.43 a occurred 30 minutes after the intravenou sodium selenite. The terminal acidosis onset was delayed, when lower doses of one instance, the venous pH fell to 6.76 be after the intravenous injection of 0.1 mg. tions that selenite produced marked res and MacCanon, 1961) and inconstant dioxide (Heinrich and MacCanon, 1960) of metabolic origin. Consequently, the ef on plasma lactate and pyruvate were stud plasma sodium, potassium, calcium, pho interest because of the hemoconcentration a recorded after selenite administration (Hei

METHODS

Serial heparinized blood samples were intravenous administration of sodium sel tized dogs. Dosage is expressed as milligr gram of body weight (mg/kg) administere ance with common usage. The effects of 5 dogs: 0.1 mg/kg Se was administered 0.25 mg/kg to 2 dogs; 1.0 mg/kg to 2 do

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INHIBITION OF THE ANAPHYLACTOID REACTION IN RATS

BY

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The primary intraperitoneal or intravenous injection of egg-white (Selye, 1937), dextran (Voorhees, Baker & Pulaski, 1951 ; Morrison, Richardson & Bloom, 1951) or dextrin (Rowley, 1963 ; Veilleux, 1963) into rats produces an acute inflammatory response termed the anaphylactoid reaction. Some rats of the Wistar albino strain are genetically resistant to dextran and do not exhibit the reaction. Selective breeding experiments have since shown that the character of " non-reactivity " is associated with an autosomal recessive gene (Harris, Kalmus & West, 1963).

It was recently found that relatively high intravenous doses of egg-white elicit an anaphylactoid reaction in dextran-resistant rats (termed non-reactors) and that dextrin is equally active in both reactor (that is, sensitive) and non-reactor rats (Ankier & West, 1964a, b). In the present work, the effects of some antagonists of histamine and 5-hydroxytryptamine (5-HT) and of some carbohydrates on the anaphylactoid reaction elicited in both types of rat by egg-white and dextrin and in reactor rats by dextran have been studied. A preliminary note of part of this work has already been published (Ankier & West, 1965).

METHODS

Test for reactivity to dextran

Male Wistar albino rats (body weight 120–150 g) obtained from the Agricultural Research Council's Field Station (Compton) were injected with a single intraperitoneal dose of dextran 240 mg/kg (Intradex, Glaxo) once a week on three occasions. Animals showing peripheral oedema were classed as " reactors " and those failing to react to any of three injections were classed as " non-reactors." Rats were used subsequently after a period of time of not less than one week.

Measurement of the anaphylactoid reaction

Azovan blue dye (18 mg/kg) was injected intravenously immediately before the intraperitoneal or intravenous injection of fresh hens' egg-white (12 ml/kg), dextran (240 mg/kg) or dextrin (Astra, 1,250 mg/kg). The extent of oedema formation and colloidal dye accumulation in the extremities were estimated visually every half an hour over a period of 4 hr, using an arbitrary shock-score (with values of 0, 1, 2 or 3) for each of twelve areas (nose and bristle area, eyelids, lips, tongue, ear

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The maximum score therefore was 36 for each observation. The cumulative shock-scores after drug pretreatment have been expressed in Tables 1–3 as percentages of those obtained in untreated rats. In all experiments, differences of 20% or more in percentage inhibitions were found, using the t-test, to be statistically significant ($P = 0.05$).

Intradermal injections

Rats were lightly anaesthetized with ether, and azovan blue dye (8 mg/kg) was injected intravenously. Each animal then was given six intradermal injections (dose-volume 0.1 ml.) into the shaved ventral abdominal skin, one of these always being isotonic neutralized saline (pH 7.4). After 30 min. the rats were killed and the injection sites were carefully excised and pinned, with the outer surface downwards, on to cork boards. The extent of the vascular permeability changes was estimated by measuring the mean diameter of each blue lesion. All drugs were dissolved in isotonic saline and the solutions were adjusted to pH 7.4 before injection.

Estimation of glucose in dextrin

A modification of a method described by Rédei & Nagy (1961) for the simultaneous determination of glucose and dextran was used. Samples of dextrin (5 ml., 25% w/v) were hydrolysed with hydrochloric acid (0.05 ml., 10 N). At different times, aliquots of 0.1 ml. were removed, neutralized and added to 8 ml. of freshly prepared *o*-toluidine reagent (6%, *o*-toluidine in glacial acetic acid). After centrifugation at 3,000 rev/min for 20 min, the clear supernatant liquid was removed and heated in a boiling water bath for 8 min. The extinction value of the resultant green colour was read colorimetrically at 610 mμ and the amount of glucose present was calculated by reference to calibration-curves obtained with glucose standards. Using this method, recovery of added glucose is about 90%.

Drugs used

These included mepyramine maleate (May & Baker, 10 mg/kg), 2-bromo-*d*-lysergic acid diethylamide (Sandoz, BOL 148, 3 mg/kg), promethazine hydrochloride (May & Baker, 8 mg/kg) and cyproheptadine hydrochloride (Merck, Sharp & Dohme, 0.5 mg/kg); they were injected intravenously 30 min before the anaphylactoid agents. In other experiments, 2-deoxyglucose (B.D.H., 500 mg/kg) was injected intraperitoneally 30 min before the active agents, but glucose, galactose, mannose or N-acetylglucosamine (B.D.H.) was injected intraperitoneally (in two doses, each of 1,500 mg/kg) 30 min before the active agents as well as at the same time they were administered.

RESULTS

Effect of antagonists of histamine and 5-hydroxytryptamine

The antihistamine mepyramine did not reduce the egg-white and dextrin reactions in reactor and non-reactor rats or the dextran reaction in reactor rats, but BOL 148, a specific antagonist of 5-HT, was a potent antagonist of most of the responses (Table 1). Promethazine and cyproheptadine were also effective antagonists of most responses ; both these drugs possess antihistamine and anti-5-HT activities (Stone, Wenger, Ludden, Starvorski & Ross, 1961). These results show that 5-HT is an important mediator of the anaphylactoid reaction produced in rats by egg-white, dextrin and dextran.

Inhibition by carbohydrates

Glucose reduced all the responses in reactor rats but was only weakly active against egg-white and completely inactive against dextrin in non-reactor animals (Table 2).

Galactose and maltose were about as effective as glucose as inhibitors. *N*-Acetylglucosamine was more potent in reactor rats and reduced the responses in non-reactor animals, whereas 2-deoxyglucose effectively blocked all the responses.

TABLE 1

EFFECT OF SOME ANTAGONISTS ON THE ANAPHYLACTOID REACTION PRODUCED BY INTRAPERITONEAL DOSES OF EGG-WHITE, DEXTRIN AND DEXTRAN IN REACTOR RATS (R) AND BY INTRAVENOUS DOSES OF EGG-WHITE AND INTRAPERITONEAL DOSES OF DEXTRIN IN NON-REACTOR RATS (NR)

Each value is the mean of results from a group of eight rats, and is expressed as a percentage inhibition of the control responses.

Antagonist	Dose (mg/kg)	Egg-white		Dextrin		Dextran
		R	NR	R	NR	R
Mepyramine	10	3	0	7	15	12
BOL 148	3	70	100	100	90	56
Promethazine	8	83	100	90	93	33
Cyproheptadine	0·5	89	100	97	65	52

TABLE 2

EFFECT OF SOME CARBOHYDRATES ON THE ANAPHYLACTOID REACTION PRODUCED BY INTRAPERITONEAL DOSES OF EGG-WHITE, DEXTRIN AND DEXTRAN IN REACTOR RATS (R) AND BY INTRAVENOUS DOSES OF EGG-WHITE AND INTRAPERITONEAL DOSES OF DEXTRIN IN NON-REACTOR RATS (NR)

Each value is the mean of results from a group of eight rats, and is expressed as a percentage inhibition of the control response.

Carbohydrate	Egg-white		Dextrin		Dextran
	R	NR	R	NR	R
Glucose	72	33	77	6	54
N-Acetylglucosamine	84	73	83	47	79
2-Deoxyglucose	93	99	98	80	98

Effect of hydrolysed dextrin

The anaphylactoid activity of intraperitoneal dextrin (25% w/v, 5 ml./kg) decreased as the time of hydrolysis increased when tests were made in both reactor and non-reactor rats (Fig. 1). However, the rates of loss of activity differed; for example, after 5 hr of hydrolysis no reaction was produced in reactor rats and yet a full response was obtained in non-reactor animals; at this time, 84% of the dextrin in the hydrolysate was present as glucose. Furthermore, after 7 hr of hydrolysis, the hydrolysate was inactive intravenously in reactor rats and yet a full response was obtained in non-reactor animals (Fig. 2); on assay, this sample contained 96% glucose. It is possible that glucose, released from the dextrin during the hydrolysis, inhibited the dextrin response in reactor rats and was ineffective in non-reactor animals (Table 2), but the possibility also exists that hydrolysis of dextrin yields a glucose polymer of low molecular weight which is a potent anaphylactoid agent only in non-reactor rats. Dextrin hydrolysed for 5 hr was therefore dialysed against running saline (flow rate, 2 l./hr) for different times to remove the glucose, and then tested in both types of rat. The concentration of glucose in the non-dialysable fraction as well as its biological activity in non-reactor rats decreased in parallel but reactivation did not occur in reactor rats. The biological activity of unhydrolysed dextrin in both types of rat, on the other hand, was unaffected by dialysis and so the active material in the hydrolysed dextrin eliciting the anaphylactoid reaction in non-reactor animals may be of a relatively low molecular weight. This glucose polymer has not so far been identified.

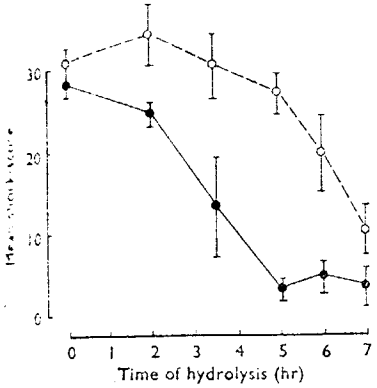

Fig. 1. Anaphylactoid reaction produced by hydrolysed dextrin after intraperitoneal injection into reactor (●——●) and non-reactor (○- - - -○) rats. Shock-scores (±S.E.) are measured on an arbitrary scale (see text).

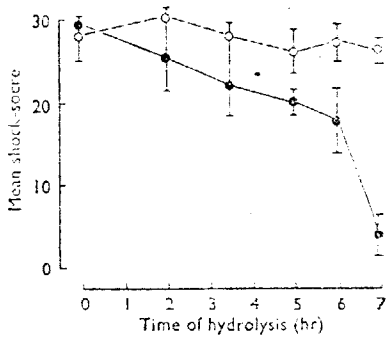

Fig. 2. Anaphylactoid reaction produced by hydrolysed dextrin after intravenous injection into reactor (●——●) and non-reactor (○- - - -○) rats. Shock-scores (±S.E.) are measured on an arbitrary scale (see text).

Intradermal studies

The increase in vascular permeability induced by intradermal dextrin in both reactor and non-reactor rats was similar in threshold and intensity, a dose of 60 μg producing a maximal response. The concomitant intradermal injection of glucose inhibited the reaction equally well in both types of animal, 50% inhibition of the response to 60 μg dextrin being produced by about 500 μg glucose. When egg-white was injected intradermally, the threshold of activity in non-reactors was relatively higher (by a factor of 8–16) than that in reactors and glucose, injected together with the egg-white, was inhibitory at the doses indicated in Table 3; the extent of the inhibition corresponded with the difference in threshold. For example, 360 μg glucose completely inhibited the response of 0.25% egg-white in reactors and 1% in non-reactors, and 720 μg glucose inhibited 0.5% egg-white in reactors and 2% in non-reactors.

TABLE 3

EFFECT OF GLUCOSE ON THE INCREASE IN VASCULAR PERMEABILITY PRODUCED BY THE INTRADERMAL INJECTION OF DIFFERENT CONCENTRATIONS OF EGG-WHITE IN REACTOR (R) AND NON-REACTOR (NR) RATS

Each value is the mean of results from a group of twelve rats, and is expressed as a percentage inhibition of the control response.

Egg-white (%)	Glucose (μg) 180 R	180 NR	360 R	360 NR	720 R	720 NR
0·125	100	—	100	—	100	—
0·25	31	—	100	—	100	—
0·5	18	—	46	—	100	—
1·0	9	70	17	100	48	100
2·0	0	19	8	35	19	100
4·0	0	8	0	16	0	74

Other studies showed that 720 μg glucose did not inhibit the increases in vascular permeability induced by histamine (20 μg), 5-HT (0.4 μg) or bradykinin (0.2 μg) in both reactor and non-reactor rats.

DISCUSSION

The results of the present study using specific antagonists of histamine and 5-HT show that 5-HT has an important role as a mediator of the anaphylactoid reactions elicited by egg-white and dextrin in reactor and non-reactor rats and by dextran in reactor rats. Inhibition of the dextran response by antagonists of 5-HT was more difficult to achieve although higher doses of the antagonists have been shown in other experiments to be completely effective.

Ovomucoid, the active anaphylactoid-inducing portion of egg-white (Léger, Masson & Prado, 1947; Benditt, Bader & Arase, 1953; Ankier & West, 1964b), is a glycoprotein containing galactose, mannose and *N*-acetylglucosamine (Dixon, 1955; Bragg & Hogg, 1961; Hartley & Jevons, 1962; Montgomery & Wu, 1963), whereas both dextrin and dextran are polymers of glucose. When these carbohydrates were used to antagonize the anaphylactoid reaction, a striking difference between the reactivity of reactor and that of non-reactor rats was found. For example, doses of glucose, galactose and mannose which prevent the anaphylactoid reaction produced by dextrin in reactor rats fail to modify the dextrin response in non-reactor rats. This suggests that an initial or intermediary stage in the dextrin reaction is different in the two types of animals. Furthermore, doses of *N*-acetylglucosamine which prevent the dextrin response in reactor rats are only partially effective against dextrin in non-reactor rats. In this respect, several monosaccharides and disaccharides share a similar inhibitory activity in reactor rats as parenteral injections of equimolar concentrations of glucose, galactose, maltose, lactose and sucrose have been shown to produce an analogous inhibition of the generalized anaphylactoid reaction elicited by dextran (Adamkiewicz, 1965; Adamkiewicz & Sacra, 1967). The mechanism by which these carbohydrates inhibit a generalized anaphylactoid reaction is unknown. It seems unlikely that a biochemical carrier system is involved because the configurational specificity of these carbohydrates for known biological transport systems does not parallel their ability to inhibit the [illegible] rats. Moreover, a direct influence on the intracellular metabolism of carbohydrates is not necessarily implicated for non-utilizable, as well as utilizable, carbohydrates are capable of inhibiting the reaction. The reports showing that a correlation exists between glucosuria or hyperglycaemia and an inhibition of the anaphylactoid reaction (Adamkiewicz & Adamkiewicz, 1960; Adamkiewicz & Sacra, 1967) suggest that the extracellular concentration of glucose or a structurally similar carbohydrate is related to antagonism of the reaction.

In the present study, 2-deoxyglucose was the most effective carbohydrate antagonist of the anaphylactoid reaction elicited by egg-white and dextrin in reactor and non-reactor rats and by dextran in reactor rats. 2-Deoxyglucose raises the blood glucose (Landau & Tubs, 1958) and inhibits glucose utilization (Kipnis & Cori, 1959); however, hyperglycaemia does not explain the relative activity of 2-deoxyglucose in inhibiting the egg-white and dextrin reactions in both reactor and non-reactor rats, because glucose is only effective in reactor animals. Görög & Szporny (1965) showed that suppression of glycolysis inhibits the anaphylactoid reaction, and 2-deoxyglucose is active in this respect (Sols & Crane, 1954; Wick, Drury & Morita, 1955; Wick, Drury, Nakado & Wolfe, 1957); such a mechanism may block an energy-dependent process, possibly involving adenosine triphosphate, which normally leads to the release of mediators of the anaphylactoid reaction. The high activity of the amino-sugar *N*-acetylglucosamine as an antagonist of the egg-white and dextrin reaction in non-reactor rats may also depend on such a mechanism, because *N*-acetylglucosamine is a potent inhibitor of glucokinase (Harpur & Quastel, 1949). Recently, 2-deoxyglucose has been found to be a potent diuretic and removal of oedema fluid from the reactive sites represents a possible inhibitory mechanism; this is unlikely, however, because other potent diuretics such as hydrochlorothiazide and theophylline do not inhibit the dextran reaction (Ankier, unpublished results). 2-Deoxyglucose may be potent in inhibiting the anaphylactoid response, as it is an efficient releaser of catecholamines from the adrenal medulla (Brown & Bachrach, 1959; Hökfelt & Bydgeman, 1961), and catecholamines are known to be active in antagonizing the reactions of rats to egg-white (Clark & MacKay, 1949) and dextran (Brod & Halpern, 1952).

Dextrin is equally active in eliciting the anaphylactoid response in both reactor and non-reactor rats (Ankier & West, 1964b) and yet when this glucose polymer is partially hydrolysed anaphylactoid activity remains in non-reactor rats but is lost in reactor rats.

The active component of hydrolysed dextrin is freely dialysable and is therefore of a relatively lower molecular weight than unhydrolysed dextrin (average molecular weight 9,000). This anaphylactoid material may be active only in non-reactor rats, although the possibility exists that hydrolysis of dextrin yields glucose which is an effective inhibitor of the reaction in reactor rats.

Because both dextrin and dextran are branched polymers of glucose and because ovomucoid contains several small carbohydrate side-chains linked to a peptide main-chain (Neuberger & Papkoff, 1963), one of the structural requirements of a molecule for intrinsic anaphylactoid activity may be the presence of side-chain branching. The importance of the molecular weight has been shown with regard to the reactivity of dextran in reactor rats (Harris & West, 1963), and this structural feature may govern distribution

to reactive sites. Dextrin of a molecular weight of 4,000, but not of 10,000 or greater, is active in non-reactor rats (Ankier, Starr & West, 1967) so important restrictions in the distribution of injected polysaccharide to active sites in the extracellular spaces, lymphatic system, cell wall membrane or within the cell, may exist in this type of animal.

As on intraperitoneal injection, dextrin when given intradermally is equally active in the two types of rat but glucose locally applied is also equally active in inhibiting this response. This suggests that a similar mechanism of action of intradermal dextrin is involved in both types of rat, a result which is in contrast with that found when the agents are injected intraperitoneally. Furthermore, galactose on intraperitoneal injection is about as active as glucose as an inhibitor of the dextrin reaction in reactor rats and yet on intradermal injection it is much weaker (Poyser & West, 1965). These results show how the processes involved at the different tissue levels after intraperitoneal and intradermal injection may not be similar. It may, however, be easier to apply data obtained from experiments involving intraperitoneal injections to those obtained after intravenous injections; for example, it has been found that both intravenously and intraperitoneally injected glucose inhibit the intravenous dextrin reaction in reactor rats and have no effect on the reaction in non-reactor rats. The character which originally distinguished non-reactor animals was that they did not react to intraperitoneal or intravenous dextran, and yet recent work (Ankier, Starr & West, 1967; Harris, Luscombe & Poyser, 1967) shows that dextrans of low molecular weight are equally active in both types of rat when injected parenterally as well as topically.

SUMMARY

1. 5-Hydroxytryptamine is an important mediator of the anaphylactoid reactions elicited both by egg-white and dextrin in reactor and non-reactor rats and by dextran in reactor rats.

2. Glucose, galactose and mannose prevent the anaphylactoid reactions elicited by egg-white, dextrin and dextran in reactor rats and reduce that produced by egg-white in non-reactor rats; the reaction produced by dextrin in non-reactor animals, however, is not altered. *N*-acetylglucosamine prevents the reactions except that produced by dextrin in non-reactor rats, whereas 2-deoxyglucose is a potent inhibitor of all these anaphylactoid reactions.

3. When dextrin is hydrolysed under certain conditions, the product produces the anaphylactoid reaction in non-reactor rats but not in reactor rats. A polysaccharide of low molecular weight may be formed during the hydrolysis and this is only active in non-reactor rats.

4. The increase in vascular permeability produced by intradermal injections of dextrin in both types of rat is equally prevented by intradermal glucose.

REFERENCES

ADAMKIEWICZ, V. W. (1965). Humoral specificity of glucose (haptenic-like inhibition of glucose). *Fedn Proc.*, **24**, 251.

ADAMKIEWICZ, V. W. & ADAMKIEWICZ, L. M. (1960). Glucose and the dextran "anaphylactoid" inflammation. *Am. J. Physiol.*, **198**, 51–53.

ADAMKIEWICZ, V. W. & SACRA, P. J. (1967). Histamine and sugars. *Fedn Proc.*, **26**, 224–229.

ANKIER, S. I. & WEST, G. B. (1964a). The anaphylactoid reaction in rats. *J. Pharm. Pharmac.*, **16**, 129–130.

ANKIER, S. I. & WEST, G. B. (1965). Further studies on the inflammatory anaphylactoid reaction in rats. *J. Pharm. Pharmac.*, **25**, 112–126.

ANKIER, S. I. & WEST, G. B. (1966). [illegible] *Immun.*, **28**, 41.

ANKIER, S. I., STARR, M. S. & WEST, G. B. (1967). Is the anti-[illegible]'s defence against injury and [illegible] phenomena linked with resistance to the anaphylactoid reaction? *Int. Arch. [illegible] exp. Immun.*, **32**, 329–341.

BENDITT, E. P., BADER, S. & ARASE, M. (1953). Anaphylaxis versus the anaphylactoid reaction produced by testis extract and ovomucoid in rats. *Fedn Proc.*, **12**, 384–385.

BRAGG, P. D. & HOCGH, L. (1961). An investigation of the egg-white mucoproteins, ovomucoid and ovalbumin. *Biochem. J.*, **78**, 11–23.

BRIGT, M. & HALPERN, B. N. (1952). Inhibiting action of phenergan and adrenaline on production of oedema in the rat by dextran. *C.r. Seanc. Soc. Biol.*, **146**, 21–24.

BROWN, J. & BACHRACH, H. L. (1959). Effects of 2-deoxyglucose on blood glucose levels in the rat. *Proc. Soc. exp. Biol. Med.*, **100**, 641–643.

CLARK, W. G. & MACKAY, E. M. (1949). Effect of l-epinephrine and l-arterenol on egg white edema in the rat. *Proc. Soc. exp. Biol. Med.*, **71**, 85–87.

DIXON, A. ST. J. (1955). Studies on the use of sulphonated cation-exchange resins for the hydrolysis of ovomucoid. *Biochem. J.*, **60**, 165–170.

GOROG, P. & SZPORNY, L. (1965). The effect of compounds inhibiting carbohydrate metabolism on the dextran anaphylactoid inflammation. *Acta physiol. Acad. Sci. Hung.*, **26**, 263–267.

HARPUR, R. P. & QUASTEL, J. H. (1949). Phosphorylation of *d*-glucosamine by brain extracts. *Nature, Lond.*, **164**, 693–694.

HARRIS, J. M. & WEST, G. B. (1963). Rats resistant to the dextran anaphylactoid reaction. *Br. J. Pharmac. Chemother.*, **20**, 550–562.

HARRIS, J. M., KALMUS, H. & WEST, G. B. (1963). Genetical control of the anaphylactoid reaction in rats. *Genet. Res.*, **4**, 346–355.

HARRIS, J. M., LUSCOMBE, D. K. & POYSER, R. H. (1967). The influence of molecular weight and structure on the vascular permeability responses induced by glucose polymers in rat skin. *Br. J. Pharmac. Chemother.*, **29**, 16–24.

HARTLEY, F. K. & JEVONS, F. R. (1962). The attachment of carbohydrate in ovomucoid. *Biochem. J.*, **84**, 134–139.

HOKFELT, B. & BYDGEMAN, S. (1961). Increased adrenaline production following administration of 2-deoxy-D-glucose in the rat. *Proc. Soc. exp. Biol. Med.*, **106**, 537–539.

KIPNIS, D. M. & CORI, C. F. (1959). Studies of tissue permeability. V. The penetration and phosphorylation of 2-deoxyglucose in the rat diaphragm. *J. biol. Chem.*, **234**, 171–177.

LANDAU, B. R. & LUBS, H. A. (1958). Animal responses to 2-deoxy-D-glucose administration. *Proc. Soc. exp. Biol. Med.*, **99**, 124–127.

LÉGER, J., MASSON, G. M. C. & PRADO, J. L. (1947). Factors influencing an anaphylactoid reaction in the rat. *Fedn Proc.*, **6**, 150–151.

MONTGOMERY, R. & WU, Y.-C. (1963). The carbohydrate of ovomucoid. Isolation of glycoprotein and the carbohydrate-protein linkage. *J. biol. Chem.*, **238**, 3547–3554.

MORRISON, J. L., RICHARDSON, A. P. & BLOOM, W. L. (1951). The effects of antihistaminic agents on the reaction of the rat to dextran. *Archs int. Pharmacodyn. Ther.*, **88**, 98–105.

NEUBERGER, A. & PAPKOFF, H. (1963). Carbohydrates in protein. 7. The nature of the carbohydrate in ovomucoid. *Biochem. J.*, **87**, 581–585.

POYSER, R. H. & WEST, G. B. (1965). Changes in vascular permeability produced in rats by dextran, ovomucoid and yeast cell wall polysaccharides. *Br. J. Pharmac. Chemother.*, **25**, 602–609.

RIEGI, A. & NAGY, S. (1961). Simultaneous determination of dextran and glucose in serum. *Nature, Lond.*, **191**, 173–174.

ROWLEY, D. A. (1963). Mast cell damage and vascular injury in the rat: an electron microscopic study of a reaction produced by thorotrast. *Br. J. exp. Path.*, **44**, 284–290.

SELYE, H. (1937). Studies on adaptation. *Endocrinology*, **21**, 169–188.

SOLS, A. & CRANE, R. K. (1954). Substrate specificity of brain hexokinase. *J. biol. Chem.*, **210**, 581–595.

SOME, C. A., WENGER, H. C., LUDDEN, C. T., STAVORSKI, J. M. & ROSS, C. A. (1961). Antiserotonin-antihistaminic properties of cyproheptadine. *J. Pharmac. exp. Ther.*, **131**, 75–84.

VERLEUX, R. (1963). Anaphylactoid oedema produced in rats by certain dextrins. *Br. J. Pharmac. Chemother.*, **21**, 235–237.

VOORHIES, A. B., BAKER, H. J. & PULASKI, E. J. (1951). Reactions of albino rats to injections of dextran. *Proc. Soc. exp. Biol. Med.*, **76**, 254–256.

WICK, A. N., DRURY, D. R. & MORITA, T. N. (1955). 2-Deoxyglucose—a metabolic block for glucose. *Proc. Soc. exp. Biol. Med.*, **89**, 579–582.

WICK, A. N., DRURY, D. R., NAKADO, H. I. & WOLTE, J. B. (1957). Localization of the primary metabolic block produced by 2-deoxyglucose. *J. biol. Chem.*, **224**, 963–969.

Bull. Agricult. Chem. Soc. Japan, 6, 1930

13

ON THE RELATIVE NUTRITIVE VALUE OF VARIOUS CARBOHYDRATES AND RELATED COMPOUNDS.

By

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The authors determined the relative nutritive value of different carbohydrates and related compounds by feeding experiments with albino rats by some different method from that of Ozaki. (Nippon Nogeikwagaku-kwaishi, **2**, 845, 1926). For this purpose the following 29 compounds were used:

Starch, Agar-Agar, Inulin, Dextrin, Glycogen, Funkan, Konjak powder, Saccharose, Maltose, Lactose, d–Glucose, d–Fructose, d–Galactose, d–Mannose, d–Glucosamine, α–Methylglucoside, l–Xylose, l–Arabinose, Dioxyacetone, d–Mannite, Glycerin, Ethyleneglycol, d–Gluconic acid, d–Saccharic acid, Mucic acid, Glyceric acid, Ethylalcohol, d, l–Alanine, l–Phenylalanine,

The basal ration used in this experiment consisted of:

Meat protein	27%
Butter	70%
McCollum salt mixture	3%

(As the source of vitamin B, 0.2 g. of dry yeast was given per day per rat.)

Thus the diet was very poor on carbohydrate, neverthless the albino rats fed on this diet ad. libitum attained nearly normal growth, as shown in Chart I (A). But when it is reduced to 4.2 g. daily the animals grew up to 80∼90 g. but no further. (Chart I (B))

Experiment I.

The carbohydrates tested were:

Starch, Agar–Agar, Inulin, Dextrin, Saccharose, Maltose, Lactose.

Feeding period: April to July 1928.

Average room temperature: 23C.

Young male albino rats of 40 to 45 g. were kept in a wire cage separately and were previously fed with 4.2 g. of the basal ration for about one week. Then each 1.25 g. of the samples to be tested were supplimented to it.

The rate of growth of animals during 70 days was as follows: (Chart II)

Samples	Average gain of the body weight.	
	for 70 days (g.)	per day (g.)
Maltose	114.0	1.63
Starch	95.7	1.37

Dextrin	89.3	1.28
Lactose	89.0	1.27
Saccharose	81.3	1.26
Inulin	79.8	1.14
Agar-Agar	60.0	0.86
Basal ration	62.5	0.89

As the above result shows, the relative value is in the following order: Maltose, Starch, Dextrin, Lactose, Saccharose, Inulin and Agar-Agar; Maltose being the best. The addition of Agar-Agar had almost no effect upon the growth.

Experiment II.

In the second experimint the following substances were tested:

Glucose, Fructose, Galactose, Mannose, α-Methylglucoside, Xylose, Arabinose, Mannite, Glycerin, Ethyleneglycol. (For the control group, Starch and Dextrin were simultaneously tested.)

Feeding period: May to August 1928.

Average room temperature: 27°C.

In this experiment the animals were fed with 4.2 g. of the basal ration, as long as the animals continue to grow and when the growth was stopped 1.25 g. of each sample were added to the basal ration, then the animals began to grow up again.

Thus, the gain of the body weight after the addition of the samples may be taken as the measure of their nutritive value. The average body weight attained with 4.2 g. of the basel ration was 95 g. and the period needed for it was 41 days.

The average gain of the body weight for 40 days after adding the samples was as follows: (Chart III.)

Samples	Average gain of the body weight.	
	for 40 days (g.)	per day (g.)
Fructose	55.0	1.38
Glucose	52.5	1.31
Starch	52.0	1.30
Dextrin	51.3	1.28
Glycerin	49.0	1.23
Arabinose	35.7	0.89
Xylose	35.2	0.88
Galactose	34.2	0.86
Mannose	32.8	0.82
Mannite	25.8	0.65
α-Methylglucoside	19.5	0.49

As is shown in the above table, the relative value of the samples tested is in the following order: Fructose, Glucose, Starch, Dextrin, Glycerin, Arabinose, Xylose, Galactose, Mannose, Mannite and α-Methylglucoside, the last named being the lowest. Ethyleneglycol was found to be noxious. All the animals fed with 1.25 g. of this substance died on the next day. Contra-

ry to the statements of some observers, Arabinose and Xylose are obviously nutritive and neither diuretic-nor diarrhetic action were observed when the amount of 1.25 g. of them were administered daily to rats. Mannose is also said to be diarrhetic, but such cases were not observed.

Experiment III.

The substances tested were as follows:

Gluconic acid, Saccharic acid, Mucic acid, Glyceric acid, Glycogen, Funkan, Konjak, Glucosamine, Dioxyacetone, Alanine, Phenylalanine, Ethylalcohol. For the control group, Starch and Glucose were simultaneously tested. (Gluconic-, Saccharic-and Glyceric acid were given as Ca-salts, Mucic acid as Na, K-salt and Glucosamine as HCl-salt.)

Feeding period: January to April 1929.
Average room temperature: 16.5°C.

The experiment was carried out exactly in the same way as in the second experiment. The maximum body weight of the animals fed on 4.2 g. of the basal ration was in average 80 g.

The average gain of the body weight for 40 days after adding the samples was as follows: (Chart IV.)

Samples	Average gain of the body weight. for 40 days (g)	per day (g)
Ethylalcohol	49.0	1.23
Funkan	38.7	0.97
Glucose	35.3	0.88
Starch	34.3	0.86
Glycogen	32.7	0.82
Alanine	21.8	0.55
Konjak	19.5	0.49
Gluconic acid	19.5	0.49
Saccharic acid	19.3	0.48
Dioxyacetone	7.2	0.18

As is shown in the above table, Ethylalcohol secured the best growth, followed by Funkan and Glycogen as well as Glucose and Starch. Alanine, Konjak, Gluconic acid and Saccharic acid are far inferior to Glucose, Dioxyacetone being least effective. Mucic acid, Glyceric acid, Glucosamine and Phenylalanine are found to be noxious. The animals fed with 1.25 g. of them have all died after about 2 to 25 days.

When the rats were fed with 1.25 g. of absolute Alcohol mixed with basal ration, they ate it up within 30 minutes and showed no signs of drunkness moreover, the growth was far better than on Glucose and Starch.

From these results, the albino rat seems to have relatively high resistance toward Ethylalcohol and it can assimilate fair amount of alcohol.

Assimilability of the carbohydrates tested:

At the end of the feeding period the urine of the animals excreted dur-

ing 18 hours after the ingestion of the carbohydrates was collected and it was tested qualitatively for reducing substances with Benedict's reagent.

The result of the test was as follows:

Carbohydrates	Reducing power of the urine	Carbohydrates	Reducing power of the urine
Starch	−	Glucose	−
Dextrin	−	Fructose	−
Inulin	−	Galactose	+++
Agar-Agar	−	Mannose	++
Glycogen	−	α-Methylglucoside	+
Konjak	−	Glucosamine	++
Saccharose	−	Arabinose	+++
Maltose	−	Xylose	+++
Lactose	++	Dioxyacetone	++

The urine of the animals fed with Lactose, Galactose, Mannose, α-Methylglucoside, Glucosamine, Arabinose, Xylose and Dioxyacetone reduced the reagent. Thus the above mentioned carbohydrates were excreted partially in the urine without being utilized by the animals and the low assimilability of these carbohydrates may be regarded as a caure of their relatively poor effect upon the nutritive value. In spite of its low assimilability, Lactose was found much effective on the growth of the rats especially in the early part of the feeding period.

Summary.

(1) The basal ration used in the feeding experiments consisted of 27% Meat protein, 70% Butter and 3% McCollum salt mixture. (As the source of vitamin B, 0.2 g. of dry yeast was given per day per rat.) Male albino rats were fed with 4.2 g. of the basal ration and 1.25 g. of different kinds of carbohydrates and other substances to be tested. Observing the rate of growth of the animals, the nutritive value of the samples were compared.

(2) The order of the nutritive value of the substances tested is as follows: As to Polysaccharide and Disaccharide, Maltose is most effective, followed by Starch, Dextrin, Lactose, Saccharose, Inulin, Glycogen and Funkan, and Konjak is far inferior to them and Agar-Agar is entirely invalid.

About Monosaccharides, Fructose and Glucose are most effective as well as Starch and Galactose, Mannose, Arabinose and Xylose come next, and Dioxyacetone and α-Methylglucoside are least effective; Glucosamine is noxious at the amount of 1.25 g. As to the effectiveness of Alcohols, Ethylalcohol seems to be most nutritive one and Glycerin follows it ranking with Starch, while Mannite is far inferior to the latter. Ethyleneglycol is poisonous, when fed with the amount of 1.25 g. of it.

As to the Acids, the nutritive value of Gluconic acid, Saccharic acid and

Chart I.

The increase of body weight of rats on basal ration.

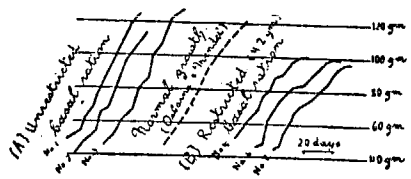


Chart II.

Graphic records of the increase of body weight of rats after adding samples (Experiment I.).

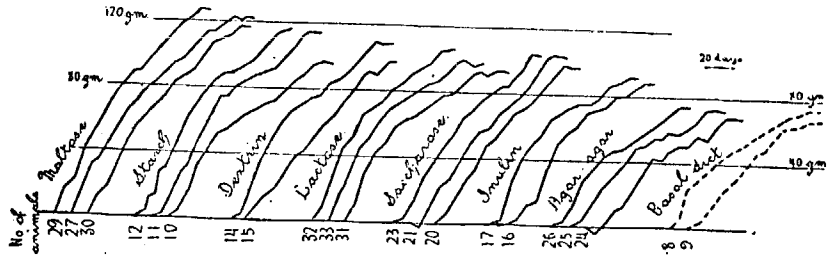


Chart III.

Graphic records of the increase of body weight of rats after adding samples. (Experiment II.).

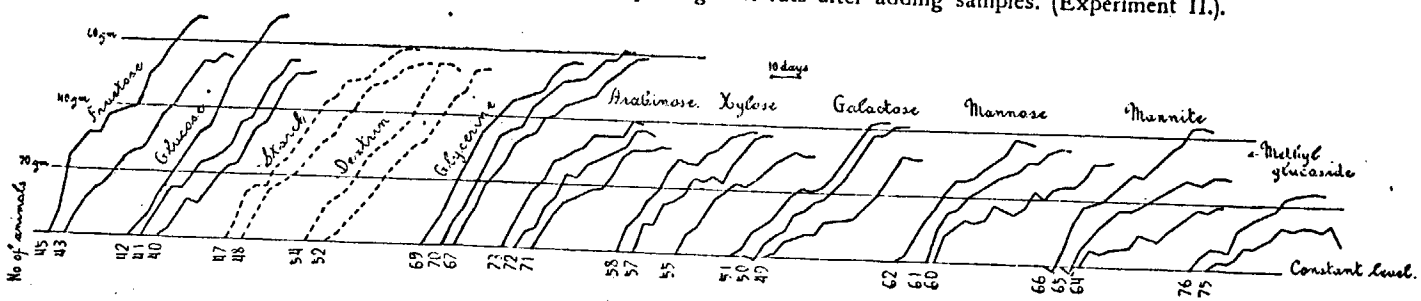
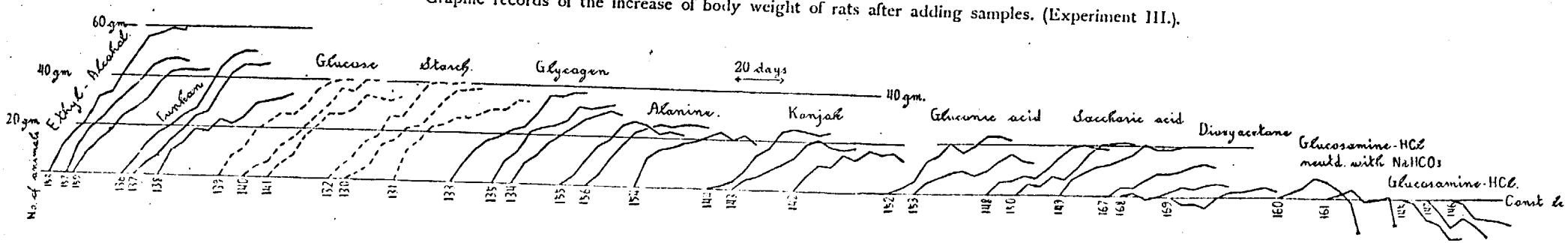


Chart IV.

Graphic records of the increase of body weight of rats after adding samples. (Experiment III.).



Alanine is half of Glucose and Mucic acid, Glyceric acid and Phenylalanine are noxious at the amount of 1.25 g.

(3) Pentoses i. e., Arabinose and Xylose are found to be nutrive under our experimental condition.

(4) At the end of the feeding period, the urine of the animals was tested for reducing substances with Benedict's reagent and with the urine of Lactose, Galactose, Mannose, α-Methylglucoside, Glucosamine, Arabinose, Xylose and Dioxyacetone positive results were obtained.

The relatively low assimilability of these carbohydrates may be regarded as a cause of their low nutritive value. In spite of its low assimilability, Lactose was much effective on the growth of the animals, especially in the early part of the feeding period.

THE ASSIMILATION LIMIT OF CARBOHYDRATE

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As to the assimilability of carbohydrates, various studies have been reported by many investigators but they used mainly man, dog and rabbit as their experimental subjects.

In our previous experiment on the nutritive value of various carbohydrates, we observed that the assimilability of carbohydrates had a close relation to their nutritive value and in the present experiment, using the albino rats as the experimental animals, we measured assimilation limits of 13 kinds of carbohydrates.

Experimental method.

The experiments were carried out chiefly after the method of Mendel and Jones (J. Biol. Chem., **43**, 491, 1920.) who had measured the tolerance of various sugars with rabbits. The ration for the albino rats consisted of meat protein 15%, starch 70%, lard 10% and McCollum salt mixture 5%.

to Wintex barley leaves in experi-
ree sugar. The sedoheptulosan-C^{14}
by boiling with Dowex 50 in the
the two forms were separated by
phenol–water solvent. The barley
the sedoheptulosan-C^{14}. When the
ere was no utilization of the sedo-
a sedoheptulose-C^{14} control was
on. In experiments run in the light,
-C^{14} was converted to four other
se compounds were all unknowns.

RY

he light and in air is rapidly con-
ents in the dark the sedoheptulose
s such as glutamic, aspartic, and

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sphate. The data suggest cleavage
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lized in barley leaves.

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N, M., HALL, A. G., HIRSCH, H. E.,
BL, N. E., *J. Biol. Chem.* **196**, 703

E, C. H., AND JANG, R., *J. Biol. Chem.*

ND SEEGMILLER, J. E., *J. Biol. Chem.*

., *J. Biol. Chem.* **194**, 261 (1952).
, *J. Am. Chem. Soc.* **74**, 2123 (1952).
, *J. Am. Chem. Soc.* **75**, 2021 (1953).
Physiol., in press.
, M., GOODALE, T. C., HAAS, V. A.,
, 1710 (1950).
EIG, G., "Paper Chromatography."
2.
l. Chem. **24**, 1628 (1952).
N, W. H., *J. Chem. Soc.* **1950**, 1702.
iol. Chem. **30**, 61 (1917).

Arch. Biochem. Biophys., 50, 1954

22

Various Carbohydrates in a Semipurified Diet for the Growing Pig

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INTRODUCTION

During the course of studies by Becker *et al.*(1) pertaining to the nutritive value of various carbohydrates in a synthetic milk for the young pig it became evident that the pig failed to react as some other animal species. For instance, a diet containing substantial quantities of lactose yielded excellent results in the young pig; whereas, according to the review of Fischer and Sutton (3), lactose has been observed to produce diarrhea, slow growth, poor general appearance, etc. in the cat, dog, chick, human, and rat. An age difference in the reaction to dietary lactose has been reported in the rat by Ershoff and Deuel (2) the young rats being more susceptible to lactose-induced diarrhea than the more mature individuals.

In addition, sucrose failed to support normal survival rate and body-weight gains in the newborn pig, but this sugar has been noted to produce normal growth responses in the rat by Mitchell (5) and in the the chick by Monson *et al.*(6). The studies reported herein were conducted to ascertain the response of the post-weaning pig to high levels of various carbohydrates in a semipurified diet and to study the dietary level of lactose compatible with optimum performance in the growing pig.

EXPERIMENTAL

Two growth experiments were conducted using pigs produced in the University of Illinois swine herd. In Expt. I, Hampshire and Chester White pigs about 9 weeks of age were used, and in Expt. II 30 Hampshire × Yorkshire pigs approximately 16 weeks of age were employed. In both cases, the pigs had been fed practical rations devoid of milk or milk by-products prior to the test. In the first test the pigs had been completely weaned for at least 1 week prior to the initiation of the

TABLE I

Composition of Diet Fed

Ingredient	Per cent
Carbohydrate	50.0
Soybean oil meal (solvent)	41.2
Mineral mixture #2[a]	4.0
Woodflock	2.0
Corn oil	2.0
Vitamin A and D oil (3000 A, 600 D)	0.5
DL-Methionine	0.3
Vitamin mixture #37[b]	+
Antibiotic mixture[c]	

[a] The composition of mineral mixture No. 2 was as follows: $CaHPO_4$, 65.00%; iodized NaCl, 16.00%; K_2CO_3, 14.00%; $MgCO_3$, 3.43%; $FeSO_4 \cdot 7H_2O$, 1.00%; $MnSO_4 \cdot H_2O$, 0.30%; $CoCl_2 \cdot 6H_2O$, 0.10%; $CuSO_4$, 0.10%; NaF, 0.02%; $ZnCO_3$, 0.04%; and KI, 0.01%.

[b] Additions per 45.36 kg. diet were: thiamine·HCl, 50 mg.; riboflavin, 140 mg.; niacin, 500 mg.; calcium pantothenate, 450 mg.; pyridoxine·HCl, 60 mg.; folic acid, 35 mg.; inositol, 9 mg.; *p*-aminobenzoic acid, 1 g.; biotin, 3 mg.; vitamin B_{12}, 500 μg., and choline chloride, 45.4 g.

[c] In Expt. I a mixture of equal parts by weight of oxytetracycline, bacitracin, and procaine penicillin G was fed at a rate of 11.3 mg./kg. In Expt. II the various lots were fed with and without a mixture of equal parts chlorotetracycline and procaine penicillin G at 22.6 mg./kg.

test. All pigs were considered healthy at the start of the tests. Feeding was conducted twice daily on an individual, full-feeding basis.

The dietary constituents, other than the carbohydrate tested, are presented in Table I. In Expt. I[1] glucose, lactose, sucrose, dextrin, and corn starch were compared as the primary source of carbohydrate for 39 days. In Expt. II lactose was combined with glucose to yield dietary levels of 0.00, 6.25, 12.50, 25.00, and 50.00% lactose. The levels of lactose were fed with and without the antibiotic mixture outlined in Table I. Three pigs were employed in each of the treatment groups. The latter study was continued for 39 days.

Results and Discussion

The results of feeding various carbohydrates to the pig at 9 weeks of age are presented in Table II.

[1] Glucose (cerelose 53) was manufactured by Corn Products Refining Co., New York City. Lactose (97.5% guaranteed minimum) was manufactured by Western Condensing Co., Appleton, Wis. Sucrose (very fine granulated) was manufactured by Godchaux Sugars, Inc., New Orleans, La. Dextrin (stadex #105) and corn starch (sugar grinder's grits) were manufactured by A. E. Staley Mfg. Co., Decatur, Ill.

TABLE II

Response of the Weanling Pig to Various Carbohyd

Carbohydrate fed	Glucose	Lac
No. of pigs	4	4
Av. initial wt., *kg.*	18.8	19
Av. final wt., *kg.*	40.5	30
Av. daily gain, *kg.*	0.56	(
Av. daily feed intake, *kg.*	1.39	]
Feed/kg. gain, *kg.*	2.50	:

On the basis of rate and efficiency of weig that glucose, sucrose, dextrin, and corn starc factory results. Lactose produced a depressed and a moderate diarrhea. Pigs fed lactose 74% as much daily feed as pigs fed sucrose or of feed conversion was equal to the remai variance revealed that the differences in da were statistically significant ($P < 0.01$); h feed required per kilogram of gain were not pressed feed intake is accompanied by a low zation. Perhaps the data presented indicate made body-weight gains of a different comp

In contrast to the newborn pig, the wea efficiently but it has difficulty utilizing lactos diet. In an earlier report by Becker *et al.*(1), born pig failed to utilize sucrose, and it m lactose was the dietary carbohydrate. It was anisms for the utilization of sucrose are freq age—apparently the mechanisms are con weeks of age.

The results of feeding various levels of antibiotics are presented in Table III. Dieta as 25% permitted satisfactory performance of the diet had a pronounced depressing ef growth rate. In addition, a moderate diar served. At 50% lactose a lower efficiency of in the absence of supplementary antibiotics, affected in the presence of antibiotics. Othe biotics tended to depress the severity of th

Analysis of variance showed that the an

TABLE I

omposition of Diet Fed

	Per cent
	50.0
:)	41.2
	4.0
	2.0
	2.0
) A, 600 D)	0.5
	0.3
	+

al mixture No. 2 was as follows: $CaHPO_4$, 65.00%; , 14.00%; $MgCO_3$, 3.43%; $FeSO_4 \cdot 7H_2O$, 1.00%; H_2O, 0.10%; $CuSO_4$, 0.10%; NaF, 0.02%; $ZnCO_3$,

et we.. thiamine·HCl, 50 mg.; riboflavin, 140 mg.; othenate, 450 mg.; pyridoxine·HCl, 60 mg.; folic *p*-aminobenzoic acid, 1 g.; biotin, 3 mg.; vitamin de, 45.4 g.

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ther than the carbohydrate tested, are presented e, lactose, sucrose, dextrin, and corn starch were e of carbohydrate for 39 days. In Expt. II lactose yield dietary levels of 0.00, 6.25, 12.50, 25.00, and lactose were fed with and without the antibiotic hree pigs were employed in each of the treatment continued for 39 days.

LTS AND DISCUSSION

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TABLE II

Response of the Weanling Pig to Various Carbohydrates in a Semipurified Diet

Carbohydrate fed	Glucose	Lactose	Sucrose	Dextrin	Corn starch
No. of pigs	4	4	4	4	4
Av. initial wt., *kg.*	18.8	19.4	19.3	19.3	18.9
Av. final wt., *kg.*	40.5	36.0	42.5	41.4	40.7
Av. daily gain, *kg.*	0.56	0.42	0.60	0.57	0.56
Av. daily feed intake, *kg.*	1.39	1.04	1.42	1.42	1.33
Feed/kg. gain, *kg.*	2.50	2.45	2.38	2.50	2.38

On the basis of rate and efficiency of weight gains it may be noted that glucose, sucrose, dextrin, and corn starch produced equally satisfactory results. Lactose produced a depressed feed intake, slow growth, and a moderate diarrhea. Pigs fed lactose consumed approximately 74% as much daily feed as pigs fed sucrose or dextrin, but the efficiency of feed conversion was equal to the remaining groups. Analysis of variance revealed that the differences in daily gains and feed intake were statistically significant ($P < 0.01$); however, the variations in feed required per kilogram of gain were not significant. Usually a depressed feed intake is accompanied by a lower efficiency of feed utilization. Perhaps the data presented indicate that the lactose-fed pigs made body-weight gains of a different composition.

In contrast to the newborn pig, the weanling pig utilizes sucrose efficiently but it has difficulty utilizing lactose when fed as 50% of the diet. In an earlier report by Becker *et al.*(1), we observed that the newborn pig failed to utilize sucrose, and it made excellent gains when lactose was the dietary carbohydrate. It was indicated that the mechanisms for the utilization of sucrose are frequently active by 1 week of age—apparently the mechanisms are consistently developed by 9 weeks of age.

The results of feeding various levels of lactose with and without antibiotics are presented in Table III. Dietary levels of lactose as high as 25% permitted satisfactory performance; however, lactose at 50% of the diet had a pronounced depressing effect upon feed intake and growth rate. In addition, a moderate diarrhea was occasionally observed. At 50% lactose a lower efficiency of feed conversion was noted in the absence of supplementary antibiotics, but this criterion was unaffected in the presence of antibiotics. Otherwise, the presence of antibiotics tended to depress the severity of the adverse effects.

Analysis of variance showed that the antibiotic mixture exerted a

TABLE III

Response of the Growing Pig to Various Levels of Lactose

Dietary lactose, %	0	6.25	12.50	25.00	50.00
Av. initial wt., *kg.*					
−Antibiotic	56.4	57.5	57.9	57.9	58.3
+Antibiotic	56.9	56.5	55.6	54.9	53.6
Av.	56.6	57.0	56.8	56.4	56.0
Av. daily gain, *kg.*					
−Antibiotic	0.53[a]	0.60	0.52	0.57	0.34
+Antibiotic	0.59	0.58	0.65	0.56	0.51
Av.	0.56	0.59	0.59	0.57	0.42
Av. daily feed intake, *kg.*					
−Antibiotic	1.75	1.99	1.85	1.87	1.29
+Antibiotic	1.92	1.85	2.00	1.69	1.55
Av.	1.84	1.92	1.93	1.78	1.42
Feed/kg. gain, *kg.*					
−Antibiotic	3.27	3.29	3.58	3.25	3.77
+Antibiotic	3.28	3.19	3.07	2.98	3.05
Av.	3.28	3.24	3.29	3.12	3.31

[a] One pig was removed before the termination of the trial, since a leg injury impaired performance.

marked effect ($P < 0.05$) upon daily gains although the experimental design did not permit a precise evaluation of this treatment. In addition, the highest level of lactose was inferior to lower levels in daily gains and feed intake ($P < 0.05$), and there was a significant ($P < 0.05$) interaction of lactose levels and antibiotic upon gains and feed intake. All treatments failed to affect the feed required per unit of gain.

Apparently the post-weaning pig is similar to the rat in response to various levels of dietary lactose. Riggs and Beaty (7) reported that 50 % lactose produced severe and persistent symptoms of lactose feeding in the rat; whereas, 30 % lactose yielded satisfactory results. It was observed that 25 % lactose failed to cause diarrhea in this test, but Krider *et al.*(4) reported that 2.4–4.8 % lactose in dried whey products caused a marked diarrhea in growing pigs. Possibly the purgative powers of lactose are greater as it occurs in dried whey products than in an isolated state.

The mechanisms by which the antibiotic mixture reversed the severity of the symptoms following high lactose consumption are uncertain. Fischer and Sutton (3) have offered the postulation that lactose acts to produce diarrhea indirectly through the alteration of the intestinal flora to an aciduric type. If such an alteration participated to any

extent in the adverse effects noted then it is e
bacterial agents may exert an ameliorative a

A comparison of the results presented here
by Becker *et al.*(1) in the baby pig definitel
tolerance of the pig diminishes with age. In
utilize sucrose increases with age.

Acknowledgments

We are indebted to Western Condensing Company,
Manufacturing Company, Decatur, Ill.; Merck and
Chas. Pfizer Company, Inc. Brooklyn N. Y.; Lederl
N. Y.; Commercial Solvents Corporation, Terre H
Company, Midland, Mich.; Hoffmann-La Roche, Inc
products which made this study possible.

Summary

Various carbohydrates were studied in a se
ing supplementary antibiotics for the pig at 9 w
ent carbohydrates constituted 50 % of the diet
trin, and corn starch produced equally satisfa
lactose-fed pigs exhibited a depressed feed inta
moderate diarrhea, but the efficiency of gains w
fed other carbohydrates. At 16 weeks of age the
25 % lactose in the diet without harmful effect
older pig exhibited the same symptoms noted
of the adverse effects was markedly retarded b
biotics in the diet. Supplementary antibiotics y
daily gains, particularly at the high level of
biotics failed to exert an effect upon the efficie

References

1. Becker, D. E., Ullrey, D. E., and Terrill, S. W
phys. **48**, 178 (1954).
2. Ershoff, B. H., and Deuel, H. J., *J. Nutrition* 28
3. Fischer, J. E., and Sutton, T. S., *J. Dairy Sci.* 32
4. Krider, J. L., Becker, D. E., Curtin, L. V., an
Animal Sci. **8**, 112 (1949).
5. Mitchell, H. S., *Am. J. Physiol.* **79**, 537 (1927).
6. Monson, W. J., Dietrich, L. S., and Elvehjem, C.
Med. **75**, 256 (1950).
7. Riggs, L. K., and Beaty, A., *J. Dairy Sci.* **30**, 939 (

LE III

g to Various Levels of Lactose

0	6.25	12.50	25.00	50.0
56.4	57.5	57.9	57.9	58.3
56.9	56.5	55.6	54.9	53.6
56.6	57.0	56.8	56.4	56.0
0.53[a]	0.60	0.52	0.57	0.34
0.59	0.58	0.65	0.56	0.55
0.56	0.59	0.59	0.57	0.42
1.75	1.99	1.85	1.87	1.29
1.92	1.85	2.00	1.69	1.53
1.84	1.92	1.93	1.78	1.42
3.27	3.29	3.58	3.25	3.77
3.28	3.19	3.07	2.98	3.06
8	3.24	3.29	3.12	3.34

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extent in the adverse effects noted then it is entirely possible that antibacterial agents may exert an ameliorative activity.

A comparison of the results presented here with the results obtained by Becker *et al.*(1) in the baby pig definitely shows that the lactose tolerance of the pig diminishes with age. In addition, the ability to utilize sucrose increases with age.

Acknowledgments

We are indebted to Western Condensing Company, Appleton, Wis.; A. E. Staley Manufacturing Company, Decatur, Ill.; Merck and Company, Rahway, N. J.; Chas. Pfizer Company, Inc. Brooklyn N. Y.; Lederle Laboratories, Pearl River, N. Y.; Commercial Solvents Corporation, Terre Haute, Ind.; Dow Chemical Company, Midland, Mich.; Hoffmann-La Roche, Inc., Nutley, N. J. for funds or products which made this study possible.

Summary

Various carbohydrates were studied in a semipurified diet containing supplementary antibiotics for the pig at 9 weeks of age. The different carbohydrates constituted 50 % of the diet. Glucose, sucrose, dextrin, and corn starch produced equally satisfactory results. However, lactose-fed pigs exhibited a depressed feed intake, slow growth, and a moderate diarrhea, but the efficiency of gains was equal to that of pigs fed other carbohydrates. At 16 weeks of age the pig was able to tolerate 25 % lactose in the diet without harmful effect. At a level of 50 % the older pig exhibited the same symptoms noted above, and the severity of the adverse effects was markedly retarded by the presence of antibiotics in the diet. Supplementary antibiotics yielded an increment in daily gains, particularly at the high level of lactose; however, antibiotics failed to exert an effect upon the efficiency of feed utilization.

References

1. Becker, D. E., Ullrey, D. E., and Terrill, S. W., *Arch. Biochem. and Biophys.* **48**, 178 (1954).
2. Ershoff, B. H., and Deuel, H. J., *J. Nutrition* **28**, 225 (1944).
3. Fischer, J. E., and Sutton, T. S., *J. Dairy Sci.* **32**, 139 (1949).
4. Krider, J. L., Becker, D. E., Curtin, L. V., and Van Poucke, R. F., *J. Animal Sci.* **8**, 112 (1949).
5. Mitchell, H. S., *Am. J. Physiol.* **79**, 537 (1927).
6. Monson, W. J., Dietrich, L. S., and Elvehjem, C. A., *Proc. Soc. Exptl. Biol. Med.* **75**, 256 (1950).
7. Riggs, L. K., and Beaty, A., *J. Dairy Sci.* **30**, 939 (1947).

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A Comparison of Carbohydrates in a Synthetic Milk Diet for the Baby Pig

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Introduction

Nutritional variations among the carbohydrates have been reported in different species by many investigators. Elvehjem (2, 3) has thoroughly reviewed the studies of nutritional differences in dietary carbohydrates.

In the pig there is relatively little data pertaining to the effect of various carbohydrates in the diet. With the pig at 8 weeks of age, Becker *et al.*[1] have found that dextrose, sucrose, dextrin, and corn starch produced equal growth rates; whereas, lactose yielded a slower rate of gain. In 1949, Johnson (7) reported that dextrose and lactose were satisfactory, and sucrose and levulose were unsatisfactory as the carbohydrates in a purified synthetic diet for baby pigs. With the very young pig he found that when sucrose was the dietary carbohydrate the pigs quickly developed acute diarrhea which was followed by death within 48 hr. Newton and Sampson (8) have reported that the intravenous administration of sucrose, fructose, and lactose failed to resuscitate hypoglycemic comatose pigs, and mannose, galactose, and maltose yielded slower responses than glucose in the comatose pig.

The objective of the studies reported herein was to determine the nutritive value of dextrose, lactose, sucrose, dextrin, and corn starch as the carbohydrate source in a synthetic milk diet for the baby pig.

Experimental

The pigs used were of various breeds, and they had previously been nursed by their dams from birth until the initiation of the tests. In every case the dam were fed adequate diets prior to and during farrowing. Except for Expt. IV, all tests were conducted in the baby pig laboratory at the University Swine Farm and

[1] Becker, D. E., and Terrill, S. W., unpublished data.

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the pigs were individually fed *ad libitum* in wi
were group fed *ad libitum* on the floor of a shel
by pigs previously.

The feedings were made three times daily i
placed in the pens. The milk was heated to app
10 days of the test and thereafter it was fed col

The ingredients of the basal diet are present

TABLE I

Composition of Die

Ingredient[a]
Carbohydrate
Casein, vitamin-free
Corn oil
Methyl cellulose, 1500 cps.
Potassium phosphate, dibasic
Sodium bicarbonate
Mineral mixture 12[b]
Vitamin mixture 16[c]
Antibiotic mixture[d]

[a] Ingredients were incorporated into a milk

[b] Mineral additions per 45.36 kg. of dry m
$CaCO_3$, 622.3 g.; NaCl, 322.9 g.; $MgSO_4$, 96
$MnSO_4 \cdot H_2O$, 2.7 g.; KI, 1.5 g.; $CuSO_4 \cdot 5H_2O$, 0.
and $CoCl_2 \cdot 6H_2O$, 0.2 g.

[c] Vitamin additions (in a 20% ethanol soluti
were: aqueous vitamin A, 340,909 U.S.P. units
U.S.P. units; α-tocopherol acetate, 340.9 m
acid, 1.36 g.; thiamine-HCl, 222.7 mg.; riboflav
222.7 mg.; calcium pantothenate, 2.66 g.; inosito
; *p*-aminobenzoic acid, 886.4 mg.; biotin, 3.40
7.7 mg.; and vitamin B_{12}, 1.36 mg.

[d] A mixture of equal parts terramycin-HCl, pr
to supply 396.8 mg./kg. of dry matter.

ined 20% total solids was prepared by a modific
Wiese *et al.* (9). The ingredients were mixed
prevent the corn starch from increasing the vi
was not considered necessary to homogenize th
formed a rather stable emulsion upon mixing
s employed in the diet at a level of 1.0% of
servations had indicated that it might decrea
redients were added at the time of mixing, in
tamins. The mixed diet was refrigerated until
In all tests the pigs were weighed on alternat
re made at the time of feeding and weighing.

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Introduction

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Experimental

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. S. unpublished data.

the pigs were individually fed *ad libitum* in wire-bottom cages. Pigs of Expt. IV were group fed *ad libitum* on the floor of a shelter which had never been occupied by pigs previously.

The feedings were made three times daily in small metal troughs which were placed in the pens. The milk was heated to approximately 38°C. during the initial days of the test and thereafter it was fed cold.

The ingredients of the basal diet are presented in Table I. The diet which con-

TABLE I

Composition of Diet Fed

Ingredient[a]	Per cent
Carbohydrate	56.58
Casein, vitamin-free	30.00
Corn oil	5.00
Methyl cellulose, 1500 cps.	1.00
Potassium phosphate, dibasic	1.55
Sodium bicarbonate	1.42
Mineral mixture 12[b]	4.45
Vitamin mixture 16[c]	+
Antibiotic mixture[d]	+

[a] Ingredients were incorporated into a milk containing 20% solids.

[b] Mineral additions per 45.36 kg. of dry matter were: $CaHPO_4$, 919.9 g.; $CaCO_3$, 622.3 g.; NaCl, 322.9 g.; $MgSO_4$, 96.2 g.; $FeC_6H_5O_7 \cdot 6H_2O$, 52.6 g.; $MnSO_4 \cdot H_2O$, 2.7 g.; KI, 1.5 g.; $CuSO_4 \cdot 5H_2O$, 0.5 g.; $ZnCl_2$, 0.4 g.; NaF, 0.3 g.; and $CoCl_2 \cdot 6H_2O$, 0.2 g.

[c] Vitamin additions (in a 20% ethanol solution) per 45.36 kg. of dry matter were: aqueous vitamin A, 340,909 U.S.P. units; aqueous irradiated ergosterol, 34,091 U.S.P. units; α-tocopherol acetate, 340.9 mg.; vitamin K, 88.6 mg.; ascorbic acid, 1.36 g.; thiamine-HCl, 222.7 mg.; riboflavin, 443.2 mg.; pyridoxine-HCl, 222.7 mg.; calcium pantothenate, 2.66 g.; inositol, 8.86 g.; choline chloride, 68.17 g.; *p*-aminobenzoic acid, 886.4 mg.; biotin, 3.40 mg.; niacin, 1.80 g.; folic acid, 17.7 mg.; and vitamin B_{12}, 1.36 mg.

[d] A mixture of equal parts terramycin-HCl, procaine penicillin, and bacitracin fed to supply 396.8 mg./kg. of dry matter.

tained 20% total solids was prepared by a modification of the procedure described by Wiese *et al.* (9). The ingredients were mixed at approximately 38°C. in order to prevent the corn starch from increasing the viscosity of the milk. Furthermore, it was not considered necessary to homogenize the final preparation since the corn oil formed a rather stable emulsion upon mixing. A methylated cellulose product was employed in the diet at a level of 1.0% of the dry matter, as preliminary observations had indicated that it might decrease the incidence of diarrhea. All ingredients were added at the time of mixing, including the alcoholic solution of vitamins. The mixed diet was refrigerated until fed.

In all tests the pigs were weighed on alternate days, and general observations were made at the time of feeding and weighing.

Results

The results of feeding various carbohydrates alone or as a combination of lactose and corn starch to the baby pig from 7 to 35 days of age are presented in Table II.

In Expt. I all surviving pigs gained satisfactorily on the various carbohydrates. According to Student's "t" test for unpaired data, the differences obtained in rate of gain were not statistically significant. However, it may be noted that lactose and dextrin tended to produce the most rapid gains. In the group fed corn starch it was observed that a slow growth rate occurred during the initial half of the test, but it was compensated for by rapid gains during the latter half of the test.

TABLE II

Survival, Growth and Feed Intake of Baby Pigs Fed Various Carbohydrates

Age and dietary carbohydrate	No. of pigs started	No. of pigs[a] surviving	Average initial wt.	Average final wt.	Average daily gain	Dry matter intake per pig	Dry matter per lb of gain
			lb.	*lb.*	*lb.*	*lb.*	*lb.*
Experiment I:							
7–35 days of age							
1. Dextrose	7	6	6.17	19.93	0.49	25.60	1.86
2. Lactose	6	6	6.10	22.25	0.58	25.34	1.57
3. Sucrose	8	5	6.40	20.78	0.51	23.42	1.63
4. Dextrin	6	6	6.08	22.27	0.58	28.31	1.75
5. Corn starch	6	6	6.05	21.48	0.55	26.72	1.73
Experiment II:							
7–35 days of age							
1. Lactose	5	4[b]	5.58	19.30	0.49	22.41	1.63
2. Corn starch	5	4[b]	5.22	17.58	0.44	22.94	1.86
3. 50% lactose 50% corn starch	5	5	5.04	19.54	0.52	22.64	1.56

[a] Data are for surviving pigs.

[b] One pig fed lactose was removed as it showed chronic bloat, and one pig fed corn starch died suddenly: post-mortem decomposition prevented an autopsy.

With respect to efficiency of feed conversion the statistical tests revealed significant differences. In particular, lactose produced more efficient body-weight gains than glucose ($P < 0.01$) and than dextrin or corn starch ($P < 0.05$), but the variation between lactose and sucrose

was not of statistical significance. The
diet gained more efficiently than pigs
All pigs survived on lactose, dextri
hand, one pig succumbed on the dextro
sucrose diet. The dextrose-fed pig show
to death or lesions at autopsy, but the
a marked diarrhea which became incre
at an average of 3.6 days after treatme
Lactose was never observed to yield

TABLE
Survival, Growth and Feed Intake

Age and dietary carbohydrate	No. of pigs started	No. o pigs survivi
Experiment III:		
Initial age, 2 days		
Sucrose	6	0
Experiment IV:		
Initial age, 1 day		
1. Dextrose	4	4
2. Sucrose	4	0

[a] All pigs sacrificed for tissue studies at
were removed from test in an extremely mo

and it was further noted that corn star
well-formed, black stool.

In the succeeding study the results p
lactose and corn starch substantiated t
definite advantage in favor of equal po
in the synthetic milk as compared to
combination was only slightly superior
of gain and efficiency of feed conversio

Two additional tests of the nutritive
the pig at 1–2 days of age are reported
sucrose was fed to six pigs starting at 2
treatment the pigs uniformly exhibited
appetites remained satisfactory. The diarr

RESULTS

:arious carbohydrates alone or as a combinati
:: to the baby pig from 7 to 35 days of age an

: pigs gained satisfactorily on the various carb
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TABLE II

:nd Feed Intake of Baby Pigs Fed Various Carbohydrates

No. of pigs started	No. of pigs[a] surviving	Average initial wt.	Average final wt.	Average daily gain	Dry matter intake per pig	Dry matter per lb. of gain
		lb.	*lb.*	*lb.*	*lb.*	*lb.*
7	6	6.17	19.93	0.49	25.60	1.86
	6	6.10	22.25	0.58	25.34	1.57
	5	6.40	20.78	0.51	23.42	1.63
6	6	6.08	22.27	0.58	28.31	1.75
6	6	6.05	21.48	0.55	26.72	1.73
5	4[b]	5.58	19.30	0.49	22.41	1.63
5	4[b]	5.22	17.58	0.44	22.94	1.86
5	5	5.04	19.54	0.52	22.64	1.56

ns.

moved as it showed chronic bloat, and one pig fed st-mortem decomposition prevented an autopsy.

in were not statistically significant. However, se and dextrin tended to produce the most ted corn starch it was observed that a slow g the initial half of the test, but it was com- during the latter half of the test.

v of feed conversion the statistical tests re- s. In particular, lactose produced more effi- an glucose (P <0.01) and than dextrin or the variation between lactose and sucrose

was not of statistical significance. The pigs that survived on the sucrose diet gained more efficiently than pigs fed glucose (P <0.01).

All pigs survived on lactose, dextrin, and corn starch. On the other hand, one pig succumbed on the dextrose diet, and three pigs died on the sucrose diet. The dextrose-fed pig showed no particular symptoms prior to death or lesions at autopsy, but the three pigs fed sucrose exhibited a marked diarrhea which became increasingly severe until they perished at an average of 3.6 days after treatment began.

Lactose was never observed to yield an unusual incidence of diarrhea,

TABLE III

Survival, Growth and Feed Intake of Baby Pigs Fed Sucrose

Age and dietary carbohydrate	No. of pigs started	No. of pigs surviving	Average time until death	Average initial wt.	Mean gain during test	Dry intake per pig
			days	*lb.*	*lb.*	*lb.*
Experiment III:						
Initial age, 2 days						
Sucrose	6	0	4.2	3.42	−0.67	0.91
Experiment IV:						
Initial age, 1 day						
1. Dextrose	4	4	[a]	3.22	+0.95	1.22
2. Sucrose	4	0	3.8[a]	3.22	−0.50	1.07

[a] All pigs sacrificed for tissue studies at the time the pair mates fed sucrose were removed from test in an extremely moribund state.

and it was further noted that corn starch-fed pigs usually voided a firm, well-formed, black stool.

In the succeeding study the results pertaining to the relative value of lactose and corn starch substantiated the previous results. There was a definite advantage in favor of equal portions of lactose and corn starch in the synthetic milk as compared to corn starch alone, although the combination was only slightly superior to lactose alone in regard to rate of gain and efficiency of feed conversion.

Two additional tests of the nutritive value of sucrose in the diet of the pig at 1–2 days of age are reported in Table III. In the initial test sucrose was fed to six pigs starting at 2 days of age. Following 18 hr. of treatment the pigs uniformly exhibited a severe diarrhea, although appetites remained satisfactory. The diarrhea, which was characterized by

the excretion of thin, watery, bilious fecal matter, progressed in severity until by the third day the pigs were rapidly losing weight, and were unthrifty, weak, and thin. Just prior to death, appetite failure was noted and the pigs shivered and trembled. The pigs died after slightly more than 96 hr. on the diet, and during this time they consumed 0.91 lb. of dry matter.

In order to establish further the influence of dietary sucrose upon the newborn pig, Expt. IV was conducted in rather "disease-free" quarters using dextrose-fed pigs as control animals. The results showed that with pigs 1 day of age sucrose produced the same response as noted in the preceding study. Likewise, the pigs were all sacrificed in an extremely moribund condition 3.8 days after initiating treatment and after 1.07 lb. of dry matter had been consumed. On the contrary, control pigs fed dextrose during the same period consistently exhibited positive body-weight gains, diarrhea was absent, and a normal appearance was maintained.

Discussion

The sucrose syndrome briefly described by Johnson (7) in the baby pig (unspecified age) has been observed to occur consistently in the pig at 1–2 days of age. Bustad *et al.* (1) were also unable to formulate a successful synthetic diet for the newborn pig when they used sucrose as the carbohydrate source. However, the data reported here indicate that approximately 60% of the pigs develop a capacity to utilize sucrose by 7 days of age. It is noted that Johnson (7) reported that baby pigs fed cow's milk could be gradually changed to a sucrose diet without harmful effects, although he does not indicate at what age this transition may be made.

The difficulty involved in the utilization of sucrose by the newborn pig is not readily explained. It seems entirely possible that the enzymatic activity is insufficient in the newborn pig to enable it to readily absorb sucrose. In such case the sucrose would remain within the lumen of the gut and possibly function as a hydragogue. In this regard Jeans and Marriot (6) have called attention to the fact that lactose or sucrose solutions of 8% or greater concentration are hypertonic and may act as hydragogue cathartics in the human infant. Further studies on the metabolism of sucrose are under way at the Illinois Station.

The results of Flipse and co-workers (4, 5) obtained with the young calf parallel the results presented here in many respects. In particular it was noteworthy that lactose fed as the sole carbohydrate produced eff-

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rate and efficiency of growth when ad
it appears that the young calf cannot
young pig.

Acknowled

We are indebted to Western Condensing
Staley Manufacturing Company, Decatur, I
New Jersey; Chas. Pfizer Company, Inc.,
ories, Pearl River, New York; Commerci
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Richmond Hill, New York, and Hoffmann-
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1. Bustad, L. K., Ham, W. E., and Cun
2. Elvehjem, C. A., *J. Am. Dietet. Assoc.*
3. Elvehjem, C. A., *Federation Proc.* 7,
4. Flipse, R. J., Huffman, C. F., Webst
Sci. 33; 548 (1950).
5. Flipse, R. J., Huffman, C. F., Dunca
Sci. 33, 557 (1950).
6. Jeans, P. C., and Marriot, W. M., I
Company, St. Louis, 1947.
7. Johnson, S. R., *Federation Proc.* 8,
8. Newton, W. C., and Sampson, J., (
Wiese, A. C., Johnson, B. C., Mitch
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ent and rapid weight gains, and it was also capable of improving the rate and efficiency of growth when added to a starch diet. In contrast, it appears that the young calf cannot utilize starch as efficiently as the young pig.

Acknowledgments

We are indebted to Western Condensing Company, Appleton, Wisconsin; A. E. Staley Manufacturing Company, Decatur, Illinois; Merck and Company, Rahway, New Jersey; Chas. Pfizer Company, Inc., Brooklyn, New York; Lederle Laboratories, Pearl River, New York; Commercial Solvents Corporation, Terre Haute, Indiana; Dow Chemical Company, Midland, Michigan; Endo Products, Inc., Richmond Hill, New York, and Hoffmann-LaRoche, Inc., Nutley, New Jersey; for funds or products which made this investigation possible.

Summary

The nutritive value of dextrose, lactose, sucrose, dextrin, and corn starch for the newborn pig was studied using a synthetic milk diet. From 7 to 35 days of age dextrose, lactose, dextrin, and corn starch produced equal body-weight gains and survival. Likewise, the efficiency of feed conversion was similar, except for lactose which was superior. Lactose did not cause diarrhea, and the feeding of corn starch was characterized by a low incidence of diarrhea. About 40 % of pigs fed sucrose perished from severe diarrhea, although the surviving animals gained efficiently and rapidly. A combination of lactose and corn starch yielded superior weight gains and efficiency of feed utilization to either one fed alone.

The pig at 1–2 days of age responded to sucrose with severe diarrhea, rapid weight loss, unthriftiness, thinness, and death. Death usually followed 4 days of sucrose feeding. The evidence indicated that some pigs develop a marked ability to utilize sucrose by 7 days of age.

References

1. Bustad, L. K., Ham, W. E., and Cunha, T. J., *Arch. Biochem.* **17**, 249 (1948).
2. Elvehjem, C. A., *J. Am. Dietet. Assoc.* **22**, 959 (1946).
3. Elvehjem, C. A., *Federation Proc.* **7**, 410 (1948).
4. Flipse, R. J., Huffman, C. F., Webster, H. D., and Duncan, C. W., *J. Dairy Sci.* **33**; 548 (1950).
5. Flipse, R. J., Huffman, C. F., Duncan, C. W., and Webster, H. D., *J. Dairy Sci.* **33**, 557 (1950).
6. Jeans, P. C., and Marriot, W. M., Infant Nutrition, 4th Ed. The C. V. Mosby Company, St. Louis, 1947.
7. Johnson, S. R., *Federation Proc.* **8**, 387 (1949).
8. Newton, W. C., and Sampson, J., *Cornell Vet.* **41**, 377 (1951).
9. Wiese, A. C., Johnson, B. C., Mitchell, H. H., and Nevens, W. B., *J. Dairy Sci.* **30**, 87 (1947).

23a

Dextrins

TOXICOLOGY AND APPLIED PHARMACOLOGY 23, 537–540 (1972)

The Influence of Pregnancy on the Acute Toxicity of Various Compounds in Mice

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The Influence of Pregnancy on the Acute Toxicity of Various Compounds in Mice. BELILES, R. P. (1972). *Toxicol. Appl. Pharmacol.* 23, 537–540. LD50 values were determined in pregnant (day 13) and nonpregnant mice. Iron dextran, iron dextrin, folic acid, colchicine and chlorpromazine hydrochloride were more toxic in the pregnant mouse than in the nonpregnant mouse following iv administration. Aspirin administered po appeared less toxic in the pregnant mouse. No remarkable differences between pregnant and nonpregnant animals in respect to the LD50 values were noted (a) following iv administration of atropine sulfate, caffeine, chlorpheniramine maleate, chloramphenicol, diphenhydramine hydrochloride, ethanol, ergonovine maleate, ferrous sulfate, hexobarbital sodium, isoproterenol hydrochloride, metyrapone ditartrate, pentobarbital sodium and trypan blue, and (b) following po administration of ferrous sulfate, folic acid and naladixic acid.

The Food and Drug Administration Advisory Committee on Protocols for Safety Evaluations (1970) indicated that the maternal organism was particularly vulnerable during pregnancy because of altered pathways of metabolism, variations in dietary intake, endocrine changes and the possibility of initiating spontaneous labor. No specific illustrations of altered toxicity were presented, although the biochemical data reviewed by Guarino *et al.* (1966) suggest that it is only logical to believe that such alterations do exist. Review of the literature did not suggest that this aspect of toxicity has been systematically considered with any materials.

In this investigation, acute toxicity was used as a criterion to evaluate possible changes in the sensitivity of female mice to various chemicals due to pregnancy.

METHOD

Pregnant adult female Charles River CD-1 mice mated at the Charles River Mouse Farms, Inc. were received by this laboratory. The day sperm or a vaginal plug was observed was considered day 0 of gestation. Nonpregnant adult female mice of the same age and strain were also supplied by The Charles River Mouse Farms, Inc. Upon receipt, the mice were group-housed in suspended wire-mesh cages and provided water and food (Rockland Mouse Breeder Diet) ad libitum.

On day 13 of gestation the pregnant mice were administered graded doses of test materials. A minimum of 4 dose levels and 5 mice per dose level were used. On day 17

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of gestation, a cesarean section was performed, and the contents of the uterus were inspected. The mice that died were also necropsied, and the pregnancy was confirmed. Implantation sites, resorption sites, dead and live fetuses were recorded. Any pregnant mouse in which total resorption of the litter was judged to have occurred before the day of compound administration was discarded and replaced with a suitable pregnant mouse. After the same length of time in our laboratory, nonpregnant mice received graded doses of the test materials. These mice were treated in a manner similar to the pregnant mice, except that nonpregnant mice were sacrificed after 7 days. Body weights at the time of treatment were recorded. All mice were observed daily. According to the method described by Horn (1956), the LD50 values were obtained in both pregnant and nonpregnant mice after 4 and 7 days, respectively. If the surviving pregnant females had resorbed or dead fetuses, the fetal LD50 value based on the dose given to the dam was calculated using the method of Litchfield and Wilcoxon (1949).

Ergonovine maleate,[1] chloramphenicol,[2] chlorpheniramine maleate,[3] diphenhydramine hydrochloride,[4] iron dextran,[5] iron dextrin[6] and metyrapone ditartrate[7] were obtained as injectable formulations, reconstituted as directed, if so required, and administered iv. Solutions of atropine sulfate, caffeine, folic acid, trypan blue, isoproterenol hydrochloride, hexobarbital sodium, ethanol, colchicine, ferrous sulfate, chlorpromazine hydrochloride and pentobarbital sodium were prepared using distilled water. Aspirin and naladixic acid[8] were suspended in 0.5% gum tragacanth.

RESULTS AND DISCUSSION

The LD50 values based on the mortalities observed in the 4 days after dosing and fetal LD50 values of compounds which produced sufficient fetal deaths to permit calculation have been tabulated in Table 1. The LD50 values obtained after 7 days observation period in the nonpregnant mice were identical except for folic acid (iv), iron dextran and iron dextrin. These values and 95% confidence limits were 282 (216–368) mg/kg for folic acid, 2050 (1510–2780) mg Fe/kg for iron dextran and 383 (308–476) mg Fe/kg for iron dextrin.

Iron dextran, iron dextrin, folic acid, colchicine and chlorpromazine HCl were more toxic in the pregnant mouse following iv injection. Aspirin administered po was less toxic. No remarkable differences were noticed between LD50 values following iv administration of atropine sulfate, caffeine, chlorpheniramine maleate, chloramphenicol, diphenhydramine HCl, ethanol, ergonovine maleate, ferrous sulfate, hexobarbital sodium, isoproterenol HCl, metyrapone ditartrate, pentobarbital sodium and trypan blue. There was no difference observed following po administration of ferrous sulfate, folic acid and naladixic acid. Fetal LD50 values suggest that only aspirin and chloramphenicol were significantly more toxic to the fetus than to the dam.

[1] Ergotrate maleate; Eli Lilly and Co., Indianapolis, Indiana.
[2] Chloromycetin sodium succinate; Parke-Davis and Co., Detroit, Michigan.
[3] Chlor-Trimeton; Schering Corp., Bloomfield, New Jersey.
[4] Benadryl; Parke-Davis and Co., Detroit, Michigan.
[5] Imferon; Lakeside Laboratories, Inc., Milwaukee, Wisconsin.
[6] Astrafer; Astra Pharmaceuti[illegible]roducts, Inc., Worchester, Massachusetts.
[7] Metopirone; Ciba Pharmac[illegible] Co., Summit, New Jersey.
[8] NegGram; Winthrop Labor[illegible]es, New York, New York.

TABLE 1

LD50 (95% CONFIDENCE LIMITS) VALUES (mg/kg) FOR NONPREGNANT, PREGNANT AND FETAL MICE AFTER [illegible] OF VARIOUS COMPOUNDS

TABLE 1

LD50 (95% CONFIDENCE LIMITS) VALUES (mg/kg) FOR NONPREGNANT, PREGNANT AND FETAL MICE AFTER ADMINISTRATION OF VARIOUS COMPOUNDS

Compound	Route	Nonpregnant	Pregnant[c]	Fetal[d]	LD50 ratio Nonpreg/Preg.
Aspirin	po	1040 (815–1320)	1400 (1080–1800)	760 (692–835)	0.74
Atropine sulfate	iv	96.3 (76.5–123)	82.6		1.16
Caffeine	iv	89.2 (68.4–116)	96.3 (79.8–116)		0.92
Colchicine	iv	4.13 (3.55–4.82)	1.54 (1.14–2.09)		2.68
Chlorpheniramine maleate	iv	26.1	26.1		1.25
Chlorpromazine HCl	iv	35.5 (30.4–41.3)	24.2 (19.0–30.8)		1.46
Chloramphenicol	iv	1530 (1260–1840)	1210	679 (643–716)	1.26
Diphenhydramine HCl	iv	35.5 (30.4–41.3)	28.2 (24.2–32.9)		1.25
Ethanol (50%)	iv	6.08[a] (5.22–7.08)	5.90 (4.38–7.94)		1.03
Ergonovine maleate	iv	8.26	8.26		1.00
Ferrous sulfate	po	1410 (1170–1700)	1310 (1120–1520)		1.03
Ferrous sulfate	iv	115 (81.0–164)	105 (74.1–148)	75.0 (64.8–87.0)	1.15
Folic acid	po	>10000	>10000		—
Folic acid	iv	305 (239–388)	178		1.71
Hexobarbital sodium	iv	165 (145–192)	178		0.92
Isoproterenol HCl	iv	96.3 (79.8–116)	89.2 (76.5–104)		1.08
Iron dextran	iv	2240[b] (1460–2700)	154 (109–218)		14.55
Iron dextrin	iv	446[b] (350–569)	127 (94.4–171)		3.51
Metyrapone ditartrate	iv	261	305 (252–368)		0.85
Naladixic acid	po	1120 (962–1310)	891 (699–1140)	658 (604–716)	1.25
Pentobarbital sodium	iv	112 (96.2–131)	121		0.92
Trypan blue	iv	328	261		1.25

[a] Values of ethanol (50%) given in ml/kg.
[b] Values given in terms of mg Fe/kg.
[c] Mice dosed on day 13 and examined by cesarean section on day 17 (4 day LD50).
[d] The fetal LD50 based on maternal dosage.

While the difference in LD50 values was greater with iron dextran, this material in terms of iron was the least toxic of all iron compounds that were administered iv. Additional acute studies in rats and rabbits have not revealed the marked effect of pregnancy on the acute toxicity of iron dextran in these species. For example, in female rats the LD50 values in the nonpregnant and pregnant rat obtained following iv administration on day 14 of gestation were both greater than 2000 mg Fe/kg.

During the course of this investigation the difference in body weights of pregnant and nonpregnant mice drew our attention. The mean body weight and standard deviation for the pregnant mice were 39.0 ± 3.5 g at the time of treatment. Analysis of the body weight data from the nonpregnant mice of the same age gave a mean value of 26.5 ± 2.8 g. Thus, the changes during conception caused an increase in body weight of approximately 12.5 g. This increase is composed in part, of the placenta, fetuses and increased size of the uterus. Based on the possibility that some differences in toxicity could be due to compartmentalization of the test material in the maternal organism, a theoretical upper limit of 1.40 was calculated for LD50 ratio (nonpregnant:pregnant) from the body weight data to account for this.

REFERENCES

FDA Advisory Committee on Protocols for Safety Evaluations. (1970). Food and Drug Administration advisory committee on protocols for safety evaluations: panel on reproduction report on reproduction studies in the safety evaluation of food additives and pesticide residues. *Toxicol. Appl. Pharmacol.* **16**, 265–296.

Guarino, A. M., Gram, T. E., Schroeder, D. H., Call, J. B. and Gilette, J. R. (1966). Alternations in kinetic constants for hepatic microsomal aniline hydroxylase and ethylmorphine *N*-dimethylase associated with pregnancy in rats. *J. Pharmacol. Exp. Ther.* **168**, 224–228.

Horn, H. J. (1956). Simplified LD50 (or ED50) calculations. *Biometrics* **12**, 311–322.

Litchfield, J. T., Jr. and Wilcoxon, F. (1949). A simplified method of evaluation dose-effect experiments. *J. Pharmacol. Exp. Ther.* **96**, 99–113.

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24

THE INFLUENCE OF DEXTRIN AND SUCROSE ON GROWTH AND DERMATITIS

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FIVE FIGURES

(Received for publication January 6, 1936)

In the study of the water-soluble vitamins of the B-complex the carbohydrate constituent of the basal ration appears to be a factor influencing both the incidence of dermatitis and rate of growth. The observations of Hogan and Richardson ('32 and '34), who report a high incidence of dermatitis in rats when using sucrose, and the more recent studies by Guerrant and Dutcher ('34) who observed a marked difference in rate of growth when using different carbohydrates, are particularly significant.

For comparative studies concerning the vitamin B-complex in milk and its derivatives and in rice polish, dextrin, as prepared in these laboratories, has been used as the carbohydrate of the basal rations. This product was made from a refined cornstarch, moistened with a 0.2 per cent citric acid solution, autoclaved for 5 hours at 120°C., and subsequently dried and ground. Various rations containing this dextrin have been used successfully with 2000 or 3000 white rats in comparative studies involving growth rate and polyneuritis. The 'pellagra-like condition' as described by Goldberger and Lillie ('26), the 'characteristic dermatitis' as observed by Hogan and Richardson ('32), the '(a) type of dermatis' as recorded by Chick, et al. ('35), and the 'pellagra-like dermatitis' as described by György ('35) has rarely if ever been noted.

However, in numerous cases a non-specific skin or fur condition, possibly indicative of an atypical dermatitis, has been observed especially in those animals the period of survival of which has been extended several weeks with test substances known to contain vitamin B(B_1) and possibly small amounts of the other factors of the vitamin B-complex.

The availability of pure vitamin B(B_1) (Merck) and pure lactoflavin as prepared in these laboratories (Supplee, et al., '36) will undoubtedly facilitate study of the vitamin B factors. The fruitfulness of such investigations however, may be predicated upon the carbohydrate employed in the basal ration. The influence of this constituent on the incidence of dermatitis and growth of white rats is shown by the following data wherein pure vitamin B and lactoflavin were used as the primary supplements.

EXPERIMENTAL

As a basis of departure, a basal ration was used containing dextrin, 62 parts; hydrogenated vegetable oil,[1] 10 parts; vitamin-free casein (Labco),[2] 20 parts; salt mixture 40,[3] 4 parts; powdered agar-agar, 2 parts; and cod liver oil, 2 parts. As the primary modification, the dextrin constituent of this ration was entirely replaced by sucrose; as a further modification the hydrogenated vegetable oil content was reduced from 10 to 3 parts and the sucrose content increased to 69 parts.

White rats, 22 to 26 days old and weighing 40 to 50 gm. when placed on the sucrose rations unsupplemented with other dietary factors showed little or no gain and survived for a period of only 2 to 5 weeks. Those on the dextrin rations gained from 2 to 5 gm. per week for a period of 5 to 8 weeks. No evidence of dermatitis has been observed from any of the unsupplemented rations, death occurring before the development of the

[1] Crisco.

[2] The Labco vitamin-free casein is distributed by The Casein Company of America, Inc., New York.

[3] H. Steenbock and E. M. Nelson. 1923. Fat-soluble vitamine. XIII. Light in its relation to ophthalmia and growth. J. Biol. Chem., vol. 56, p. 355.

symptoms. The survival period of the animals receiving the rations containing 10 parts of hydrogenated vegetable oil averaged from 1 to 2 weeks longer than those receiving the unsupplemented rations with the lower fat content.

Obviously a basal ration entirely devoid of the vitamins of the B-complex must be supplemented with one or more of the factors of this complex if a comprehensive experimental plan is to be carried out. To supply the antineuritic factor 2.5 γ or 12.5 γ of the crystalline vitamin B(B_1) (Merck) was fed as a daily supplement. When 12.5 γ supplemented the dextrin

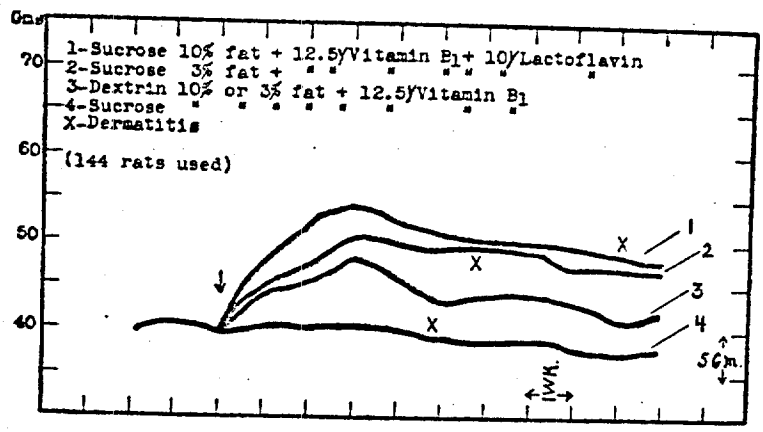

Fig. 1 Growth response of white rats on dextrin and sucrose rations supplemented with crystalline vitamin B_1 and crystalline lactoflavin.

rations some growth resulted for a short period of time, after which the weight remained stationary (fig. 1, curve 3); the survival period was extended over several months. No dermatitis of the character described by the investigators previously mentioned was observed. When the same amount of vitamin B supplement was fed with the sucrose rations containing either 10 or 3 parts of hydrogenated vegetable oil, the growth response was practically nil (fig. 1, curve 4). When 2.5 γ of vitamin B supplemented the sucrose rations the average survival period was shorter than for those animals receiving the same rations supplemented with 12.5 γ.

The significant reaction however, of almost all of the animals receiving the sucrose rations was the development of a dermatitis which is believed to be identical with that described by the various authors previously noted. This manifestation, illustrated in accompanying figure 4, was observed usually as early as the fifth week after starting the vitamin B supplement. The dermatitis also resulted from the sucrose rations with the higher amount of fat, but with less regularity than with the low fat sucrose ration; its appearance with the former was delayed on an average from 3 to 4 weeks.

From these comparisons it would seem that there is a difference in the character of the carbohydrates used, since irrespective of the prolonged survival period of the animals receiving vitamin B and the dextrin ration, no evidence of dermatitis whatsoever was noted. However, when sucrose was employed, irrespective of the level at which vitamin B was fed and irrespective of the fat content of the basal ration, dermatitis developed with marked consistency. As high as 85 per cent of the animals on the sucrose ration containing 3 parts of hydrogenated vegetable oil showed a severe form of dermatitis after 6 to 8 weeks.

In order further to determine whether the sucrose rations would show consistent results in causing dermatitis, they were supplemented with lactoflavin as well as with the pure vitamin B. The response of the animals receiving the low fat sucrose ration supplemented with 12.5 γ vitamin B and 10 γ of pure lactoflavin is shown in figure 1, curve 2, from which it will be noted that the incorporation of 10 γ of lactoflavin caused an immediate, although slight, growth response for a period of 2 to 3 weeks after which the weight remained practically stationary. The dermatitis developed after about 5 to 6 weeks with the same degree of regularity as when the same ration was supplemented with vitamin B only. When the same supplements were fed with the sucrose ration containing the higher amount of fat (fig. 1, curve 1) the incidence of the dermatitis was again delayed for a period of 3 to 4 weeks and its occurrence was not as regular as with the low fat ration.

A further comprehensive comparison of the dextrin and sucrose rations was made involving supplementation with the pure vitamin B, lactoflavin, and a lactoflavin-free material derived from a crude milk vitamin concentrate (Supplee, et al., '31). After an initial depletion period of about 2 weeks all

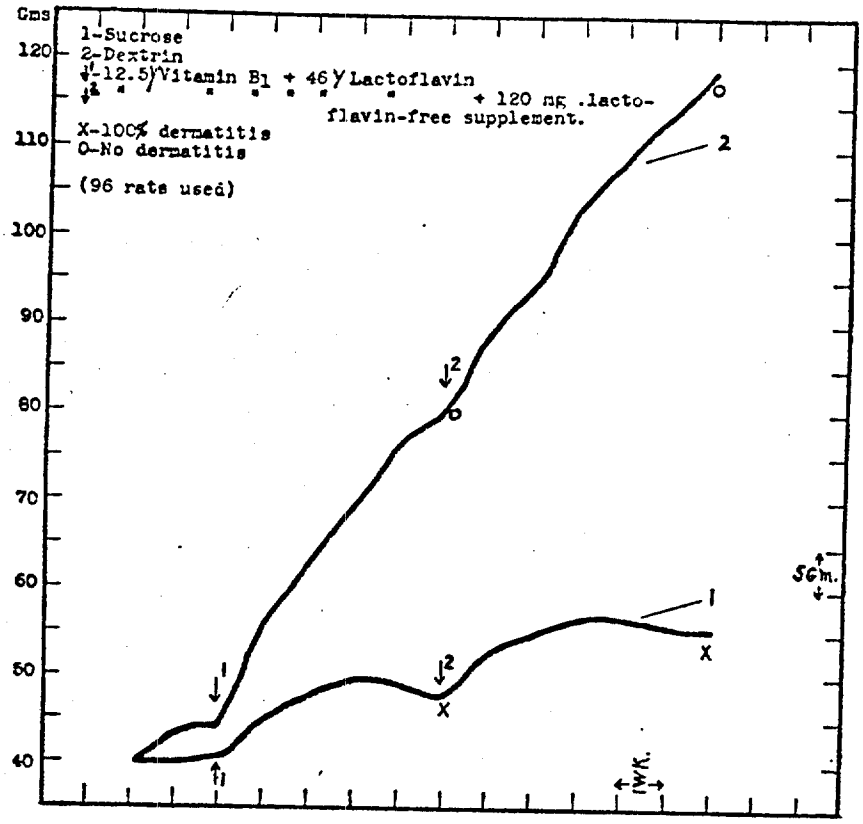

Fig. 2 Influence of dextrin and sucrose on growth and incidence of dermatitis in white rats receiving vitamin B_1 and lactoflavin supplements.

animals on the dextrin and low fat sucrose rations were fed a daily supplement consisting of 12.5 γ of pure crystalline vitamin B and 46 γ of lactoflavin contained in an impure concentrate (2-550) (Supplee, et al., '35). After a 5-week period of feeding these supplements each group was fed the crude milk vitamin concentrate previously freed from lactoflavin,

as an additional supplement in an amount equivalent to 120 mg. of the concentrate.

The animals receiving the sucrose ration (fig. 2, curve 1) supplemented with vitamin B and lactoflavin showed a definite growth response as a result of such supplementation, but declined in weight after about 3 to 4 weeks. At the end of the fifth week 100 per cent of these animals showed the typical dermatitis symptoms. The animals receiving the dextrin ration (fig. 2, curve 2) similarly supplemented, continued to grow at a very satisfactory rate and showed no evidence of

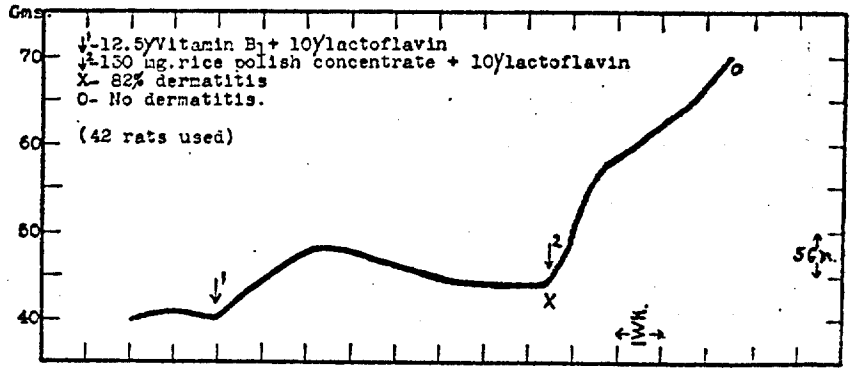

Fig. 3 Influence of vitamin B_1, lactoflavin and rice polish concentrate on growth and incidence of dermatitis in white rats receiving sucrose.

dermatitis. After 5 weeks, when all animals on the sucrose ration showed dermatitis, the lactoflavin-free supplement was added. After feeding this additional supplement for 6 weeks (curve 1) the dermatitis still persisted without apparent diminution in severity. The animals fed this additional supplement with the dextrin ration (curve 2) continued to grow and no evidence of dermatitis whatsoever was noted throughout the entire period of observation. These data seem to indicate that neither the vitamin B nor the lactoflavin, nor the lactoflavin-free material in the form and at the levels at which they were fed, had any influence on the occurrence of the dermatitis.

In order to determine whether the difference in reaction which resulted from the dextrin and sucrose rations may be due to a factor of vitamin character, or a condition in the ceca of the experimental animals (Guerrant, et al., '35) the following experiments were made.

The sucrose ration containing 3 parts of hydrogenated vegetable oil was employed. After the initial depletion period, the animals received vitamin B, lactoflavin and the lactoflavin-free material, all at the same levels as in the previous experiment. These supplements were fed for 7½ weeks with results as shown in figure 3; the curve is the composite average of forty-two individuals. The reaction of the animals both with respect to growth and incidence of dermatitis was substantially the same as previously determined (fig. 2, curve 1). At the 7½-week period 21 per cent of the animals were dead and 82 per cent of the survivors showed the typical dermatitis. At this juncture the supplement of 12.5 γ of pure vitamin B was discontinued and in its place 130 mg. of an especially prepared rice polish concentrate (Supplee, et al., '34) was fed. This amount of the concentrate carried approximately the equivalent of 12.5 γ of vitamin B, and also about 1 mg. of lipid matter and about 30 mg. of non-reducing sugars.

The response of the animals receiving this amount of rice polish concentrate and 10 γ of pure lactoflavin as supplements to the sucrose ration, is shown in the continuation of the curve in figure 3, from which it will be noted that a substantial rate of growth resulted and that the dermatitis began to improve immediately. The accompanying figure 5 illustrates the condition of the animals after about 1 week of feeding of these supplements. Definite signs of healing are clearly evident. After 4 weeks the dermatitis was cured and all the animals appeared to be normal in this respect.

It is evident that these results indicate the presence of an antidermatitis factor in the rice polish concentrate. Supplementary data showed that the small amounts of lipid matter and of non-reducing sugars carried by the concentrate are not the cause of the healing of the dermatitis observed in these

Fig. 4 Characteristic dermatitis resulting from the unsupplemented basal ration containing sucrose.

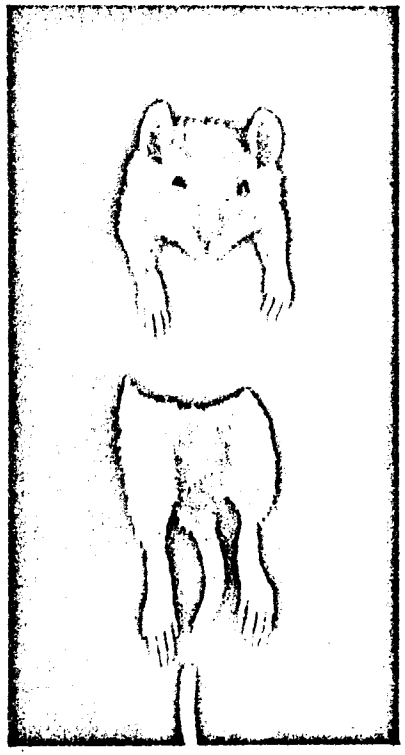

Fig. 5 Characteristic dermatitis resulting from the unsupplemented basal ration containing sucrose, cured by an aqueous extract of rice polish, 120 mg. daily.

experiments. Furthermore in view of the other experiments reported herein, it may be assumed that dextrin carries some of the antidermatitis factor, or that the favorable reaction of the dextrin fed rats is due to cecal fermentation as indicated by Guerrant, et al. ('35). The data are considered to be conclusive in showing that two of the entities of the vitamin B-complex, namely vitamin B and lactoflavin have no antidermatitis effect whatsoever when employed as supplements to the sucrose rations. Neither do these factors used singly or together permit normal or continued growth unless they are supplemented with a third factor or group of factors.

SUMMARY

1. Comparative data from basal rations containing dextrin and sucrose, respectively, show that no dermatitis resulted when the former was used, whereas a high incidence of dermatitis resulted when sucrose served as the basal carbohydrate.

2. Vitamin B and lactoflavin supplementing the sucrose ration did not prevent the development of dermatitis, nor did these supplements permit normal and continued growth; such supplements fed with dextrin promoted a substantial rate of growth.

3. A concentrate prepared from rice polish cured the dermatitis occurring in the sucrose fed animals and at the same time promoted a substantial rate of growth, provided adequate amounts of vitamin B and lactoflavin were fed simultaneously.

4. The occurrence of dermatitis was delayed and not as regular with a sucrose ration containing 10 per cent hydrogenated vegetable oil as with one containing 3 per cent of the same oil.

5. The data as a whole would seem to indicate that the basal ration containing 69 parts of sucrose and 3 parts of hydrogenated vegetable oil is well suited for the determination of the growth-promoting properties of lactoflavin, provided that it is supplemented with adequate amounts of vitamin B and the vitamin factor or group of factors contained in the rice polish concentrate.

ACKNOWLEDGMENT

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LITERATURE CITED

CHICK, H., A. M. COPPING AND C. E. EDGAR 1935 The water-soluble B-vitamins. IV. The components of vitamin B_2. Biochem. J., vol. 29, p. 722.

GOLDBERGER, J. AND R. D. LILLIE 1926 A note on an experimental pellagralike condition in the albino rat. Pub. Health. Rep., U. S. Pub. Health Service, vol. 41, p. 1025.

GUERRANT, N. B. AND R. A. DUTCHER 1934 Some effects of the composition of the diet on the vitamin B and the vitamin G requirement of the growing rat. J. Nutrition, vol. 8, p. 397.

GUERRANT, N. B., R. A. DUTCHER AND L. F. TOMEY 1935 The effect of the type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. J. Biol. Chem., vol. 110, p. 233.

GYÖRGY, P. 1935 Investigations on the vitamin B_2 complex. I. The differentiation of lactoflavin and the 'rat antipellagra' factor. Biochem. J., vol. 29, p. 741.

HOGAN, A. G. AND L. R. RICHARDSON 1932 The effect of ultra-violet rays on the dermatitis preventing vitamin. Missouri Agric. Exp. Station, Research Bull., vol. 178, p. 3.

——— 1934 Irradiated vitamin B complex and dermatitis. J. Nutrition, vol. 8, p. 385.

SUPPLEE, G. C., O. J. KAHLENBERG AND G. E. FLANIGAN 1931 The growth-promoting properties (vitamin B complex) of the concentrated water-soluble portion of milk. J. Biol. Chem., vol. 93, p. 705.

SUPPLEE, G. C., R. C. BENDER AND G. E. FLANIGAN 1934 The vitamin B supplementation of milk. J. Nutrition, vol. 8, p. 365.

SUPPLEE, G. C., S. ANSBACHER AND R. C. BENDER 1935 Photochemical phenomena involved in vitamin G (B_2) studies. J. Biol. Chem., vol. 110, p. 365.

SUPPLEE, G. C., S. ANSBACHER, G. E. FLANIGAN AND Z. M. HANFORD 1936 The fluorometric estimation of lactoflavin. J. Dairy Sci., vol. 19, p. 215.

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Lipotropic Effect of Dextrin Versus Sucrose in Choline-deficient Rats [1]

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ABSTRACT We determined the effect of the type of dietary carbohydrate on the amount of triglycerides accumulating in the livers of rats during the first 8 days of choline deficiency. Animals fed a choline-deficient diet with sucrose as the major carbohydrate accumulated about twice as much triglycerides in the liver as animals fed a diet in which dextrin replaced sucrose. This difference in lipid accumulation between the two groups was maintained even though one group was fed sucrose to which enough choline was added to simulate a small degree of contamination of dextrin with choline while another group of animals was fed dextrin from which most of the choline had been extracted by repeated washings with alcohol. In the choline-deficient rats fed dextrin the ceca were larger and the color of the stools lighter than in the animals fed sucrose. We believe that the partially protective effect of dextrin against fatty livers of choline deficiency is mediated through a change in intestinal bacterial flora making choline, or some related lipotropes, available to the choline-deficient rat.

The amount of lipids accumulating in fatty livers due to choline deficiency can be altered by varying the composition of the diets fed to the experimental animals. This topic was extensively reviewed recently by Lucas and Ridout (1).

To determine what effect, if any, the type of dietary carbohydrate might have on hepatic accumulation of triglycerides in early choline deficiency, we fed rats choline-deficient diets in which the major carbohydrate was either dextrin (partially hydrolyzed cornstarch) or sucrose.

In the present study, we confirmed the work of Artom and Fishman who reported that choline-deficient rats fed dextrin accumulated less fat in the liver than those fed sucrose (2). In addition, we noted that ceca in the dextrin-fed rats were larger than in the sucrose-fed animals, suggesting a difference in intestinal contents between these two groups. Finally, we demonstrated that the partial lipotropic effect of dextrin could not be accounted for by trace contamination of dextrin by choline.

These findings emphasize once more the important role that one type of dietary carbohydrate might play in modifying the response of experimental animals to nutritional challenges.

MATERIALS AND METHODS

Forty male albino rats of the Wistar strain (86 to 104 g) were divided into two groups. Each group was fed ad libitum for 12 days a semisynthetic diet, containing either sucrose (diet A) or dextrin (diet B) as the major source of dietary carbohydrate (table 1). At the end of this adjustment period each group of rats was further divided into two subgroups. The four subgroups were then fed diets either deficient or supplemented with choline (table 1) containing in each case the same carbohydrate as that received during the adjustment period. The major carbohydrate was sucrose in diets A-1 (choline-deficient) and A-2 (choline-supplemented) while it was dextrin in diets B-1 (choline-deficient) and B-2 (choline-supplemented). Groups of animals were fed these diets for either 3 days or 8 days. On days 4 and 9 the food was removed at 8 AM and the animals were killed at 10 AM. The rats were anesthetized with ether, blood was taken from the inferior vena cava and the livers were removed, weighed and frozen immediately. Livers and sera were stored at −20° until analyzed.

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methanol (2:1 v/v). Aliquots of this extract were evaporated and redissolved in chloroform–hexane–ether (2:1:1 v/v/v) and the polar lipids adsorbed on activated silicic acid (5); triglyceride glycerol was then determined on the phospholipid-free supernatants by the method of Galletti (6).

Serum was extracted with chloroform-methanol (2:1 v/v), washed with 0.04% magnesium chloride (7) and phospholipids were determined by the method of Bartlett (8).

Body weights were recorded at each stage of the experiment for the purpose of making comparisons between groups. Means and standard errors of means were calculated for each group and Student's *t* test was applied to determine if differences between groups were significant.

RESULTS

After 12 days of feeding diets A and B, there was no difference in either the average body weights (A, 138 g; B, 138 g) or average rates of weight gain (A, 3.8 g/day; B, 3.7 g/day) between the two groups of rats. Since only 40 experimental animals were used for pairing, according to body weight and food ingested, the least well-paired animals were in the group receiving diet B-2, and this fact accounts for the average weight of this group at the end of the experiment being less than in the other three groups (table 2).

After the rats had been fed the choline-deficient and supplemented diets for either 3 days or 8 days, the livers of the sucrose-fed choline-deficient group were heavier and fatter than those of their dextrin-fed counterparts (table 2). This difference was found despite the fact that the latter group had consumed more food than the former at the end of 8 days.

Serum phospholipids were significantly lower in the sucrose-fed choline-deficient animals as compared to their choline-supplemented controls at the end of 8 days (table 2).

When sucrose supplemented with 0.012% choline chloride was substituted for commercial sucrose in the diet, slightly less fat accumulated in the livers of the choline-deficient rats (table 3). This decrease was not significant and may have been partly due to the fact that the group fed the supplemented sucrose had a slightly lower food intake than its counterpart. Similarly, washing dextrin free of choline did not increase the level of hepatic triglycerides (table 3). As in the first experiment, the livers of the sucrose-fed choline-deficient groups were heavier and fatter than those of the dextrin-fed rats.

In the second experiment, changes in the gastrointestinal tract were sought for specifically: the ceca of the dextrin-fed groups were noted to be about 2.5 times larger than those of the sucrose-fed animals (table 3) and the fecal contents were looser and lighter in color in the former than in the latter groups (fig. 1). No animal, except one in the dextrin group, developed overt diarrhea during the experiment.

DISCUSSION

By manipulating the composition of the diets and the environment to which choline-deficient rats are subjected, it is possible to alter the level of lipids that accumulate within the hepatic cells. But, as Lucas and Ridout have pointed out (1), mere restriction of food intake or decrease in growth rate will cause a decrease in the levels of hepatic lipids: a so-called pseudolipotropic effect. In our experiment the 60% reduction in hepatic triglycerides upon substitution of sucrose by dextrin in the choline-deficient diet cannot be ascribed to a pseudolipotropic effect, in that sense, since the rats fed dextrin ate and grew as well as the animals fed sucrose. The fact that they grew as well excludes any significant loss of energy in the feces of the dextrin-fed animals even though their stools appeared less well formed. Our results confirm those of Artom and Fishman (2) who obtained a 50% reduction in hepatic lipids by changing the carbohydrate in the choline-deficient diet from sucrose to dextrin. On the other hand, Handler (9) could not demonstrate any difference in the levels of hepatic lipids between choline-deficient rats fed either sucrose or starch; in this case, however, 1% cholesterol was included in the diets fed to the animals, which might have raised the choline requirement of the rats.

TABLE 1

Composition of diets

	A	B	A-1	B-1	A-2	B-2
			%			
C_1 assay protein [1]	16	16	16	16	16	16
Sucrose	68.8	0.0	68.8	0.0	68.3	0.0
Dextrin [2]	0.0	68.8	0.0	68.8	0.0	68.3
Salt mix W [3]	4	4	4	4	4	4
Fortified vitamins [4] (complete)	4	4	0	0	0	0
Fortified vitamins [4] (without choline or B_{12})	0	0	4	4	4	4
Alphacel	2	2	2	2	2	2
Crisco	5	5	5	5	5	5
L-Cystine	0.2	0.2	0.2	0.2	0.2	0.2
Choline chloride	0.0	0.0	0.0	0.0	0.5	0.5
Total	100	100	100	100	100	100

[1] Skidmore Enterprises, Cincinnati, Ohio.
[2] Dextrin (White, Tech.) Nutritional Biochemicals Corporation, Cleveland, Ohio.
[3] Wesson's modification of Osborne and Mendel's salt mixture (Nutritional Biochemicals Corporation).
[4] Nutritional Biochemicals Vitamin diet fortification mixture in dextrose.

To determine the level of choline, free and bound, in dextrin, 20 g of dextrin were refluxed with 100 ml of methanol for 2.5 hours. After filtration the methanol was evaporated to dryness under vacuum and the residue suspended in 12 ml of 2 N HCl and digested overnight in a sealed tube at 120°. After filtration the clear solution was evaporated in a rotary evaporator to near dryness and redissolved in 0.1 N HCl. Choline was precipitated with phosphomolybdic acid and the phosphorus content of the precipitate determined (3). To check the specificity of the determination, the extract was spotted on thin-layer plates of silicic acid. The developing solution was acetone–methanol–hydrochloric acid (90:10:10 v/v/v) (4). After development and exposure to iodine vapor a spot was visualized which co-chromatographed with pure choline chloride. This spot could not be seen on chromatography of the supernatant after addition of phosphomolybdic acid to the extract. These determinations revealed that about 12 mg of choline (as choline chloride) were present in 100 g dextrin. Therefore an equivalent amount of choline chloride was dissolved in methanol and added to sucrose. The methanol was then evaporated off in a flash evaporator. To remove the choline contaminant from dextrin, 1 kg of dextrin was extracted consecutively, for 1 hour each time, with 4 liters of hot 50% ethanol once, and 4 liters of hot 95% ethanol three times. This step was followed by extraction with hot methanol for 2.5 hours. At the end of this treatment, the content of choline (as the chloride) in dextrin was reduced to 1.5 mg per 100 g of dextrin. To assess the biological effect of the contamination of dextrin by choline, two groups of 16 rats were fed during the adjustment period, choline-supplemented diets containing, respectively, sucrose (diet A) and dextrin (diet B). Each group was then further subdivided into two groups. Eight rats from the sucrose-fed group were fed diet A-1 (choline-deficient) while the remaining eight were fed the same diet supplemented with 0.012% choline chloride. Similarly, eight rats from the dextrin-fed group were fed diet B-1 (choline-deficient) while the remaining eight animals were fed the same diet with commercial dextrin replaced by the alcohol-extracted dextrin. On day 5, the animals were anesthetized with ether, exsanguinated, and the left lateral lobe of the liver was stored for analysis. The volume of the cecum was determined by excising the cecum at the level of the ileocecal valve, immersing it in a graduated cylinder filled with water and observing the volume of the displaced water. Samples from the left lateral lobe of the livers were lyophilized overnight and extracted with chloroform-

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TABLE 2

Effect of dietary sucrose and dextrin on hepatic triglycerides in rats fed choline-deficient (CD) diets for 3 and 8 days

Duration of exp.	Diet	Number of animals	Body wt at start of CD diet	Body wt at death	Weight gain	Food intake	Hepatic weight	Hepatic triglycerides	Serum phospholipids
days			*g*	*g*	*g/day*	*g/day*	*g/100 g body wt*	*μmoles/100 g body wt*	*μmoles/ml serum*
3	A-1	7	139 ± 3.7 [1]	159 ± 3.9	6.6 ± 0.8	17.6 ± 0.6	5.21 ± 0.12 [2]	278.4 ± 55.9 [2,3]	1.80 ± 0.11
	B-1	7	141 ± 4.5	162 ± 4.5	6.8 ± 0.7	18.6 ± 0.7	4.25 ± 0.11	109.8 ± 13.8 [3]	2.30 ± 0.21
	A-2	3	141 ± 4.4	160 ± 3.5	6.4 ± 1.7	18.9 ± 2.0	5.41 ± 0.31 [2]	49.4 ± 10.1	1.98 ± 0.22
	B-2	3	146 ± 6.0	168 ± 8.0	7.4 ± 0.7	18.5 ± 2.7	4.27 ± 0.18	23.3 ± 4.1	1.85 ± 0.10
8	A-1	7	137 ± 4.7	170 ± 4.6	4.0 ± 0.2 [2]	17.8 ± 0.7 [2,3]	5.53 ± 0.16 [2]	963.2 ± 138.6 [2,3]	1.52 ± 0.26 [3]
	B-1	7	135 ± 3.5	180 ± 4.8	5.8 ± 0.4 [3]	21.0 ± 1.0 [2]	4.41 ± 0.17	372.0 ± 41.8 [3]	1.81 ± 0.16
	A-2	3	138 ± 4.6	170 ± 1.5	4.1 ± 0.5 [2]	16.0 ± 0.6	5.40 ± 0.22 [2]	37.2 ± 5.7	2.52 ± 0.22
	B-2	3	129 ± 12.3	151 ± 14.6	2.7 ± 0.4	14.2 ± 2.2	3.84 ± 0.12	22.2 ± 6.9	1.92 ± 0.25

[1] All values represent means ± SEM.
[2] Mean value significantly different ($P < 0.05$) from corresponding dextrin-fed group (i.e., the B diet in the next row).
[3] Mean value significantly different ($P < 0.05$) from corresponding choline-supplemented group (same letter, but with numeral 2 instead of 1).

TABLE 3

Effect of choline contamination of dextrin on hepatic triglycerides in rats fed a choline-deficient (CD) diet for 4 days

Diet	Number of animals	Body wt at start of CD diet	Body wt at death	Weight gain	Food intake	Hepatic weight	Hepatic triglycerides	Volume of cecum
		g	*g*	*g/day*	*g/day*	*g/100 g body wt*	*μmoles/100 g body wt*	*ml*
A-1	8	139 ± 3 [1]	159 ± 3	4.9 ± 0.2	15.4 ± 0.6	4.95 ± 0.11 [2]	758 ± 113 [2]	2.50 ± 0.20 [2,3]
A-3 [4]	8	140 ± 3	156 ± 3	4.0 ± 0.5	14.2 ± 0.7	4.84 ± 0.18 [2]	639 ± 67 [2]	2.40 ± 0.24 [2,3]
B-1	8	143 ± 2	159 ± 4	4.0 ± 0.6	14.7 ± 0.7	4.32 ± 0.10	376 ± 80	6.70 ± 0.94 [5]
B-3 [6]	8	142 ± 2	161 ± 2	4.5 ± 0.2	15.3 ± 0.4	4.07 ± 0.16	369 ± 63	6.80 ± 1.08 [5]

[1] All values represent means ± SEM.
[2] Mean value significantly different ($P < 0.025$) from dextrin-fed group (i.e., the B-diet with the same numeral).
[3] Mean of 4 animals.
[4] Main carbohydrate component of diet was sucrose supplemented with 0.012% (w/w) choline chloride.
[5] Mean of five animals.
[6] Main carbohydrate component of diet was dextrin extracted with ethanol and methanol.

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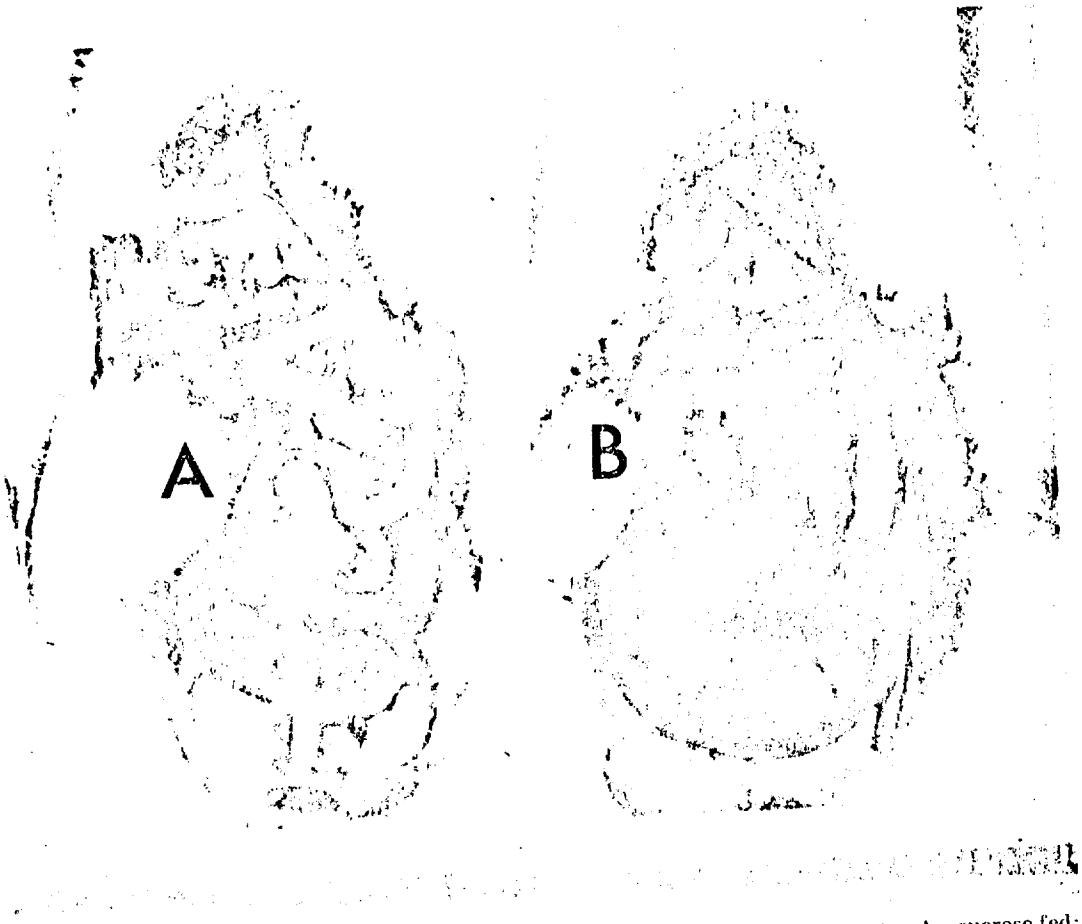

Fig. 1 Effect of dietary carbohydrate on size of cecum in choline-deficient rats. A: sucrose-fed; B: dextrin-fed. The scale is in centimeters.

Several lipotropic effects have been reported recently with a wide range of reduction in liver fat accumulation resulting from choline deficiency. Porta et al. (10) showed a lipotropic effect of orotic acid and obtained a 50% reduction in hepatic triglycerides by including 1% orotic acid in choline-deficient diets fed to rats for 8 days. Their work was confirmed subsequently by Simon et al. (11). Platner and Shields (12) reported a 27% reduction in total liver fatty acids with a diet high in magnesium, and a 51% reduction with a low-magnesium diet in choline-deficient rats. If the rats were cold-acclimated, the liver fatty acids were reduced further by 80% on both high- and low-magnesium diets. A lipotropic role was also assigned recently to zinc (13). Ethionine and methionine were found to have some protective effects; Sidransky and Verney (14) reported a 60 to 67% reduction in liver lipids with a 0.25% DL-ethionine dietary supplement and a 30% reduction with a 0.25% DL-methionine supplement.

By changing only the kind of carbohydrate in a choline-deficient diet, from a disaccharide of glucose and fructose to a glucose polymer (dextrin), we have been able to produce a reduction in liver triglycerides (40 to 60%) of similar proportion.

At this time, we have no definite explanation for the partial lipotropic effect of dextrin as compared to sucrose. The following possibilities have to be considered in arriving at a reasonable explanation:

1) There may be sufficient choline contaminating dextrin to protect the animals

1 All values represent means ± S.E.M.
2 Mean value significantly different ($P < 0.025$) from dextrin-fed group (i.e., the D-diet with the same numeral).
3 Mean of 4 animals.
4 Main carbohydrate component of diet was sucrose supplemented with 0.012% w) choline chloride.
5 Mean of five animals.
6 Main carbohydrate component of diet was dextrin extracted with ethanol and methanol.

partially against the development of fatty livers. Our second experiment demonstrates that the amount of choline present in dextrin is not significant biologically in protecting the liver against fatty metamorphosis.

2) It has been known for some time that simple and complex sugars cannot be readily interchanged in many nutritional experiments without affecting the outcome of the experiments (15). Cornstarch supported better growth in rats than sucrose when included as the major carbohydrate in rations containing 9% casein (16). Rats, made to grow at similar rates by adjustment of the level of dietary protein, retained more fat in the carcass when they were fed dextrin as compared to sucrose (17). There is an increased activity of hepatic enzymes related to de novo fatty acid synthesis in rats fed fructose as compared to glucose (18, 19). The increased hepatic lipogenesis has been ascribed to a greater rate of formation of acetyl CoA from fructose than glucose (20). There is a change in activity of some hepatic enzymes (21, 22) on changing the type of dietary carbohydrate from simple to complex sugars. The activity of hepatic glucose-6-phosphate dehydrogenase increases in rats fed sucrose as compared to cornstarch.[2] This increase in dehydrogenase activity may reflect an increased hepatic lipogenesis (23) in the sucrose-fed rats. That this mechanism is operative in vivo was demonstrated by Cohen and Teitelbaum (24) who suggested that the increased hepatic lipogenesis found in sucrose-fed rats as compared to the animals fed starch was the result of a more rapid hydrolysis of the disaccharide as compared to the polysaccharide.

In early choline deficiency, there is an inconstant and temporary increase in activity of hepatic enzymes related to fatty acid synthesis (25). However, there is no correlation between the activity of hepatic enzymes and the levels of accumulated triglycerides in the livers (25, 26). Therefore, could the differences in the degree of fatty livers between our dextrin- and sucrose-fed choline-deficient rats be ascribed to differences in hepatic lipogenesis? Not entirely. Although in our experiments the level of dietary fat (5%) may not have been high enough to inhibit lipogenesis, Artom and Fishman (2) obtained results similar to ours even though their diets were high in fat (30%) and accordingly must have greatly inhibited hepatic lipogenesis (27). It is conceivable that, because of the slower rate of hydrolysis of dextrin in the intestinal tract as compared to sucrose, a more prolonged influx of carbohydrate into the blood stream is maintained causing a more prolonged decrease in mobilization of fatty acids from adipose tissue to the liver (28). This last hypothesis again minimizes the contribution of dietary fat in the production of choline-deficient fatty livers (29) and thus by itself is not an entirely adequate explanation.

3) Dextrin may be changing the intestinal flora of the rats, thus sparing choline as it spares other vitamins (30). At this time, we favor this last hypothesis because of the observed marked difference in size of the ceca and the change in the character of the stools in the animals fed dextrin as compared to those fed sucrose. It is possible that the presence of dextrin in the diet induces the emergence of some bacterial strains in the intestines which either are able to make available choline or related compounds (e.g., through synthesis from dietary precursors) or, less likely, do not utilize the minimal amounts of choline or methionine (or both) present in the diet. The fact that the dietary requirement of choline is very much decreased in germ-free rats and in rats in which coprophagy has been prevented, as compared to animals living in open animal quarters (31), emphasizes the importance of the intestinal flora in determining the severity of lesions in choline deficiency.

ACKNOWLEDGMENTS

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[2] Wu Chang, M. L., and M. Adams 1965 Influence of heredity and of dietary carbohydrate on some of the carbohydrate-metabolizing enzymes in the tissue of the young rat. Federation Proc. 24: 220 (abstract).

LITERAT

1. Lucas, C. A., and livers and lipot Chem. Fats Other
2. Artom, C., and W relation of the d tissue phospholipi
3. De Koning, A. J. for the microdeter Chim. Acta 29: 5
4. Eneroth, P., and layer chromatogr compounds relate chem. 10: 479.
5. Hornstein, I., P. 1967 Separation classes by nonch Anal. Chem. 39:
6. Galletti, F. 1967 ric micromethod serum glycerides.
7. Folch, J., M. Lee 1957 A simple m purification of to sues. J. Biol. Che
8. Bartlett, G. R. column chromato 466.
9. Handler, P. 194 currence of her choline deficiency
10. Porta, E. A., C. M 1958 The lipotr Arch. Pathol. 86:
11. Simon, J. B., R. S Protection by or necrosis and fatty Proc. Soc. Exp. B
12. Platner, W. S., Pseudolipotropic dietary magnesiu Federation Proc.
13. Saldeen, T. 196 of zinc against due to choline-d chloride. Z. Gesa
14. Sidransky, H., a ence of ethionine liver. J. Nutr. 97
15. Harper, A. E., A review of the drates on vitami ments. J. Agr. F
16. Harper, A. E., The influence o the utilization of white rat. II. C cornstarch in 9% 261.
17. Romberg, B., an fect of type of c bolism and body protein diets. J.
18. Fitch, W. M., an tent and patterns

LITERATURE CITED

1. Lucas, C. A., and J. H. Ridout 1967 Fatty livers and lipotropic phenomena. Progr. Chem. Fats Other Lipids *10:* 1.
2. Artom, C., and W. H. Fishman 1947 The relation of the diet to the composition of tissue phospholipids. J. Biol. Chem. *170:* 587.
3. De Koning, A. J. 1963 A sensitive method for the microdetermination of choline. Anal. Chim. Acta *29:* 510.
4. Eneroth, P., and G. Linstedt 1965 Thin-layer chromatography of betaines and other compounds related to carnitine. Anal. Biochem. *10:* 479.
5. Hornstein, I., P. F. Crose and J. B. Ruck 1967 Separation of muscle lipids into classes by nonchromatographic techniques. Anal. Chem. *39:* 352.
6. Galletti, F. 1967 An improved colorimetric micromethod for the determination of serum glycerides. Clin. Chim. Acta *15:* 184.
7. Folch, J., M. Lees and G. H. Sloane Stanley 1957 A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. *226:* 497.
8. Bartlett, G. R. 1959 Phosphorus assay in column chromatography. J. Biol. Chem. *234:* 466.
9. Handler, P. 1946 Factors affecting the occurrence of hemorrhagic kidneys due to choline deficiency. J. Nutr. *31:* 621.
10. Porta, E. A., C. Manning and W. S. Hartroft 1958 The lipotropic action of orotic acid. Arch. Pathol. *86:* 217.
11. Simon, J. B., R. Scheig and G. Klatskin 1968 Protection by orotic acid against the renal necrosis and fatty liver of choline deficiency. Proc. Soc. Exp. Biol. Med. *129:* 874.
12. Platner, W. S., and J. L. Shields 1969 Pseudolipotropic effect of cold exposure and dietary magnesium on choline-deficient rats. Federation Proc. *28:* 978.
13. Saldeen, T. 1969 On the protective action of zinc against experimental liver damage due to choline-free diet or carbon tetrachloride. Z. Gesamte Exp. Med. *150:* 251.
14. Sidransky, H., and E. Verney 1969 Influence of ethionine on choline-deficiency fatty liver. J. Nutr. *97:* 419.
15. Harper, A. E., and C. A. Elvehjem 1957 A review of the effects of different carbohydrates on vitamin and amino acid requirements. J. Agr. Food Chem. *5:* 754.
16. Harper, A. E., and M. C. Katayama 1953 The influence of various carbohydrates on the utilization of low protein rations by the white rat. II. Comparison of sucrose and cornstarch in 9% casein rations. J. Nutr. *49:* 261.
17. Romberg, B., and D. A. Benton 1965 Effect of type of carbohydrate on energy metabolism and body composition of rats fed low protein diets. J. Nutr. *86:* 289.
18. Fitch, W. M., and I. L. Chaikoff 1960 Extent and patterns of adaptation of enzyme activities in livers of normal rats fed diets high in glucose and fructose. J. Biol. Chem. *235:* 554.
19. Kornacker, M. S., and J. M. Lowenstein 1965 Citrate and the conversion of carbohydrate into fat. Activities of citrate-cleavage enzyme and acetate thiokinase in livers of normal and diabetic rats. Biochem. J. *95:* 832.
20. Zakim, D., R. S. Pardini, R. H. Herman and H. E. Sauberlich 1967 Mechanism for the differential effects of high carbohydrate diets on lipogenesis in rat liver. Biochim. Biophys. Acta *144:* 242.
21. Freedland, R. A., and A. E. Harper 1959 Metabolic adaptation in higher animals. V. The study of metabolic pathways by means of metabolic adaptations. J. Biol. Chem. *234:* 1350.
22. Carroll, C. 1964 Influences of dietary carbohydrate-fat combinations on various functions associated with glycolysis and lipogenesis in rats. II. Glucose vs. sucrose with corn oil and two hydrogenated oils. J. Nutr. *82:* 163.
23. Tepperman, J., and H. M. Tepperman 1958 Effects of antecedent food intake pattern on hepatic lipogenesis. Amer. J. Physiol. *193:* 55.
24. Cohen, A. M., and A. Teitelbaum 1968 Effect of glucose, fructose, sucrose and starch on lipogenesis in rats. Life Sci. *7:* 23.
25. Chalvardjian, A. 1969 Mode of action of choline. IV. Activity of the enzymes related to fatty acid synthesis and the levels of metabolic intermediates in choline-deficient rats. Can. J. Biochem. *47:* 917.
26. Methfessel, A. H., S. Mudambi, A. E. Harper and A. B. Falcone 1964 Biochemical changes in fatty liver induced by choline or threonine deficiency. II. Various hepatic enzymic activities during the development of fatty livers in rats. Arch. Biochem. Biophys. *104:* 360.
27. Hill, R., J. M. Linazoro, F. Chevallier and I. L. Chaikoff 1958 Regulation of hepatic lipogenesis: The influence of dietary fats. J. Biol. Chem. *233:* 305.
28. Baker, N., A. S. Garfinkel and M. C. Schotz 1968 Hepatic triglyceride secretion in relation to lipogenesis and free fatty acid mobilization in fasted and glucose-refed rats. J. Lipid Res. *9:* 1.
29. Chalvardjian, A. 1966 Mode of action of choline. I. Fatty acid composition of liver, serum and adipose tissue of choline-deficient rats. Can. J. Biochem. *44:* 713.
30. Peterson, G. E., E. C. Dick and K. R. Johansson 1953 Influence of dietary aureomycin and carbohydrate on growth, intestinal microflora and vitamin B_{12} synthesis of the rat. J. Nutr. *51:* 171.
31. Kwong, E., G. Fialo, R. H. Barnes, D. Kan and S. M. Levenson 1968 Choline biosynthesis in germ-free rats. J. Nutr. *96:* 10.

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43

Further Studies on the Partial Lipotropic Effect of Dextrin in Choline-deficient Rats [1]

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ABSTRACT Choline-deficient rats fed dextrin as the major dietary carbohydrate accumulated less hepatic triglyceride than those fed sucrose. This effect could not be accounted for by a pseudolipotropic effect of dextrin since the rats fed dextrin grew as well as those fed sucrose. Further, increasing the nonnutritive bulk of the sucrose diets did not reduce the liver fat. Rats fed other simple sugars, viz. glucose, fructose and maltose also accumulated more fat than the dextrin-fed group. Increasing the fat content of the diets to 30% did not eliminate the differences in hepatic triglycerides between the sucrose- and dextrin-fed groups, although the activities of lipogenic enzymes were similar in the two groups. Synchronization of feeding schedules also had no effect. Therefore, a hyperlipogenic effect of sucrose cannot be responsible for the increase in choline requirement in these rats. Supplementation of the diets with neomycin sulfate and sulfaguanidine did not abolish the differences between the sucrose- and dextrin-fed rats. A partially protective role of the intestinal bacterial flora in the dextrin-fed rats against the fatty liver of choline deficiency can still not be ruled out. J. Nutr. *101:* 193–196, 1971.

Rats fed a choline-deficient diet for 8 days accumulate almost twice as much hepatic triglyceride when sucrose replaces dextrin as the major dietary carbohydrate (1). The cause of this phenomenon is not known, but it certainly is not due to trace contamination of dextrin by choline. Also, the dextrin-fed rats have larger ceca than their sucrose-fed counterparts, indicating a difference in intestinal contents between the two groups (1).

To further define the mechanism(s) underlying the partial protective effect of dextrin against the fatty liver of choline deficiency, experiments were performed in which some dietary constituents were altered. These experiments have not ruled out a protective role of bacterial flora in the dextrin-fed rats, but have indicated that a pseudolipotropic effect of dextrin or hyperlipogenic effect of sucrose cannot account entirely for the differences observed between the two groups of rats.

MATERIALS AND METHODS

Male albino rats (170 to 220 g) of the Wistar strain were used throughout the experiment. The diets used have been described (1), except that the vitamin mixture [2] was complete but for its lack of choline chloride and contained vitamin B_{12}. The rats were adjusted to the choline-supplemented diets for 12 days and then were fed the choline-deficient diets for 4 days. In all experiments, some rats continued to be fed the choline-supplemented diets and served as controls. The animals were killed on day 5 between 10 AM and 12 noon. In those experiments in which some dietary constituents were altered, additions to the diets were made replacing an equivalent weight of the major carbohydrate, either sucrose or dextrin. Body weight, food intake, cecal volume and hepatic triglycerides were measured and recorded as described previously (1).

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[2] Nutritional Biochemicals Vitamin diet fortification mixture in dextrose without choline chloride. The diet (100 g) contained the following vitamins: vitamin A, 3600 IU; vitamin D, 400 IU; and (in mg) alpha-tocopherol, 20, ascorbic acid, 225; inositol, 20; menadione, 9; para aminobenzoic acid, 20; niacin, 18; riboflavin, 4; pyridoxine·HCl, 4; thiamin·HCl, 4; calcium pantothenate, 12; and (in μg) biotin, 80; folic acid, 360; and vitamin B_{12}, 5.4.

The activity of hepatic enzymes was determined on the 100,000 × *g* supernatant of hepatic homogenates in 0.25 M sucrose (2).

RESULTS

The data recorded for the control rats fell within the range of those reported previously (1) and no significant differences were noted between the choline-supplemented controls fed either sucrose or dextrin.

Effect of increased nonnutritive bulk in the diets. To rule out a pseudolipotropic effect of dextrin, due possibly to poor digestibility and absorption, the ratio of the amount of food ingested to the gain in body weight was calculated. The difference in ratios between the sucrose-fed and dextrin-fed rats (5.4 mean ± 0.3 SE and 4.7 ± 0.3, respectively) was not significant although the dextrin-fed group showed a slightly more efficient utilization of the food energy.

When the amount of nonnutritive bulk, cellulose,[3] in the sucrose-fed group was increased from the standard 2% to 5% and 10% (experiment 1, table 1), the fecal wet weight increased from 6% of the weight of ingested food to 10% and 15%, respectively, exceeding that in the dextrin-fed rats (9%). Even though the rats fed the higher levels of cellulose did not gain as much weight as those fed the diets containing 2% cellulose, they accumulated as much hepatic triglyceride as the rats fed the standard sucrose diet and significantly more than the animals fed dextrin ($P < 0.01$). All the rats fed the sucrose diets,

[3] Alphacel, Nutritional Biochemicals Corp., Cleveland, Ohio.

TABLE 1

Effect of alterations in the dietary constituents on hepatic triglycerides and cecal volumes of choline-deficient rats fed sucrose or dextrin (mean ± SEM) [1]

Experiment no.	Diet	Weight gain	Food intake	Hepatic triglycerides	Cecal volume
		g/day		*μmoles/100 g b. wt*	*ml*
1	CDS [2]	3.2 ± 0.4	17.3 ± 0.6	471 ± 67	3.2 ± 0.3
	CDD [2]	4.5 ± 0.6	18.8 ± 0.5	215 ± 34	8.0 ± 0.6
	CDS–5% cellulose [3]	2.2 ± 0.5	16.3 ± 0.4	471 ± 63	2.8 ± 0.3
	CDS–10% cellulose [3]	1.9 ± 0.5	18.0 ± 0.4	460 ± 64	3.0 ± 0.3
2	CDS	3.4 ± 0.1	15.7 ± 0.7	378 ± 56	2.7 ± 0.1
	CDD	3.2 ± 0.4	15.7 ± 0.9	199 ± 28	7.9 ± 1.1
	CD–glucose [4]	4.0 ± 0.6	16.7 ± 1.1	323 ± 45	3.1 ± 0.2
	CD–fructose [4]	3.5 ± 0.5	15.9 ± 0.6	416 ± 40	2.6 ± 0.2
	CD–maltose [4]	5.4 ± 0.8	18.3 ± 1.4	389 ± 48	2.5 ± 0.2
3	CDS–30% fat [5]	3.9 ± 0.8	13.4 ± 0.8	580 ± 65	2.2 ± 0.2
	CDD–30% fat [5]	4.8 ± 0.6	14.7 ± 0.4	298 ± 53	4.9 ± 0.3
4	CDS–restricted [6]	3.7 ± 0.3	4.7 ± 0.6 [7]	258 ± 46	2.2 ± 0.2
	CDD–restricted [6]	4.6 ± 0.6	5.3 ± 0.3 [7]	102 ± 17	6.4 ± 0.3
5	CDS	4.2 ± 0.4	16.4 ± 0.6	475 ± 60	2.3 ± 0.2
	CDD	4.7 ± 0.2	16.8 ± 0.9	305 ± 36	6.0 ± 0.6
	CDS–0.3% neomycin [8]	4.7 ± 0.7	16.8 ± 0.9	482 ± 93	6.6 ± 0.5
	CDD–0.3% neomycin [8]	5.4 ± 0.8	14.8 ± 0.9	205 ± 29	18.0 ± 1.7
6	CDS	3.2 ± 0.2	15.2 ± 0.5	352 ± 75	2.8 ± 0.2
	CDD	3.8 ± 0.6	17.6 ± 0.8	128 ± 36	7.7 ± 1.8
	CDS–0.3% sulfa [9]	3.6 ± 0.3	16.1 ± 0.3	367 ± 76	3.0 ± 0.2
	CDD–0.3% sulfa [9]	3.4 ± 0.5	16.9 ± 0.5	147 ± 23	15.1 ± 2.2
7	CDS–1% sulfa [9,10]	1.3 ± 0.4	13.0 ± 0.7	262 ± 41	3.0 ± 0.2
	CDD–1% sulfa [9,10]	0.8 ± 0.7	14.9 ± 0.9	159 ± 28	9.5 ± 1.1
	CDS–1% sulfa [9]	1.4 ± 0.5	13.7 ± 0.6	257 ± 41	2.8 ± 0.1
	CDD–1% sulfa [9]	0.1 ± 0.7	14.6 ± 0.6	158 ± 23	11.4 ± 1.1

[1] Each individual group is comprised of six to eight rats.
[2] CDS = basal choline-deficient diet with sucrose as the major carbohydrate; CDD = diet with dextrin as the major carbohydrate.
[3] Total nonnutritive bulk made up to 5% or 10% cellulose (alphacel) as indicated.
[4] Major dietary carbohydrate as indicated.
[5] Total dietary fat made up to 30%, using hydrogenated vegetable fat (Crisco, Procter and Gamble).
[6] Rats trained to eat daily food ration in 4 hours.
[7] Amount of food ingested on day 5 for 2 hours after starving for 18 hours.
[8] Diet supplemented with 0.3% neomycin sulfate.
[9] Diet supplemented with sulfaguanidine at levels indicated.
[10] Sulfaguanidine not given during the 12-day adjustment period but subsequently included in the choline-deficient diet.

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irrespective of the cellulose contents of the diet fed, had significantly smaller ceca than the dextrin-fed group ($P < 0.01$).

Effect of the nature of dietary carbohydrate. When the major carbohydrate in the diet was replaced by glucose, fructose or maltose (experiment 2, table 1), dextrin still exhibited a greater lipotropic effect than either the monosaccharides or disaccharides ($P < 0.05$). The level of hepatic triglycerides was slightly lower in the rats fed glucose as compared to the other simple carbohydrates, but the differences were not statistically significant. The ceca were still larger in the group fed dextrin than in the other animals.

Effect of increasing the level of dietary fat. In a preliminary experiment, an increase in the level of dietary fat from the standard 5% to 15 or 30% still maintained the differences in levels of hepatic triglycerides between rats fed sucrose and dextrin. The experiment was repeated with the higher fat level (30%) and the state of lipogenesis was assessed by measuring the activity of two hepatic lipogenic enzymes, glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and malic enzyme (EC 1.1.1.40). In confirmation of the preliminary experiment, the differences in levels of hepatic triglyceride were maintained between the sucrose-fed and dextrin-fed rats (experiment 3, table 1). There was no difference in activity, expressed as μmoles of NADP reduced/milligram protein/hour, of either glucose-6-phosphate dehydrogenase (6.26 mean ± 0.72 SE sucrose-fed vs. 5.34 ± 0.93 dextrin-fed) or of malic enzyme (2.66 ± 0.47 vs. 3.63 ± 0.88, respectively) between the two groups of rats.

Effect of restricted daily feeding. Since the periodicity of feeding will affect the hepatic metabolism of fatty acids (3), the feeding schedule of the rats was synchronized by training the animals to eat their respective daily food ration between 8 AM and 12 noon. This adjustment-training period lasted for 12 days. The rats were then fed the choline-deficient diets for 4 hours daily for 4 days. On day 5, the animals received the diets from 6 AM to 8 AM and were killed at 12 noon. There was no significant difference in the amount of food ingested during the 2 hours of feeding between the two groups of animals (experiment 4, table 1). The difference in hepatic triglyceride levels was still maintained ($P < 0.01$).

Effect of supplementation of the diets with neomycin sulfate and sulfaguanidine. Neomycin sulfate fed as 0.3% of the diet caused an increase in the cecal volume of both the sucrose- and dextrin-fed groups compared with the animals on the basal diets (experiment 5, table 1). Neomycin sulfate did not affect the hepatic level of triglycerides in the sucrose-fed rats but caused a significant decrease ($P < 0.05$) in the group fed dextrin.

Sulfaguanidine fed as 0.3% of the diet did not affect significantly the hepatic level of triglycerides or the cecal volume in the sucrose-fed rats (experiment 6, table 1). Although the cecal volume in the dextrin-fed group increased, the difference in hepatic triglyceride levels between the sucrose- and dextrin-fed rats was still maintained. Subsequently the level of dietary sulfaguanidine was increased to 1% and either fed throughout the experiment or withheld during the 12-day adjustment period and given only for the last 4 days with the choline-deficient diets (experiment 7, table 1). In this experiment, the inclusion of 1% sulfaguanidine in the diets decreased the growth rate of the rats. The differences in hepatic triglyceride levels between the sucrose- and dextrin-fed groups were not statistically significant ($0.10 > P > 0.05$) although the sucrose-fed rats still had higher hepatic triglycerides than the dextrin-fed group.

DISCUSSION

The reason for the decrease in the choline requirement of the rat when dextrin is substituted for sucrose, as the major dietary carbohydrate, is not known. In the present series of experiments, the difference in the levels of hepatic triglycerides between the sucrose- and dextrin-fed choline-deficient animals could not be abolished entirely. The difference persisted in spite of various modifications (table 1) introduced in the diets and experimental designs. In one set of experiments, when the diets were supplemented with 1% sulfaguanidine (experiment 7), the difference between the two groups of animals was not statistically significant, although the su-

crose-fed rats still had higher levels of hepatic triglycerides than the dextrin-fed counterparts. This lack of statistical difference between the two groups could be attributed to a decrease in growth rate and might have been demonstrated with an experimental period longer than 4 days.

Four possible mechanisms to account for the partial lipotropic effect of dextrin could be postulated: 1) Dextrin, a polysaccharide, may not be digested and absorbed as efficiently as sucrose and may act partially as nonnutritive bulk. Such a pseudolipotropic effect could not be reproduced by merely increasing the cellulose content of the diet and decreasing the growth rate of the rats. 2) Simple carbohydrates are more lipogenic than the complex ones (4, 5). Decreasing hepatic lipogenesis by feeding the animals a high fat diet (6) did not eliminate the difference in hepatic triglyceride levels between the sucrose- and dextrin-fed groups, in confirmation of previous work (7). 3) The periodicity of feeding may influence the outcome of a nutritional experiment.[4] Synchronization of the feeding schedules of the sucrose- and dextrin-fed groups did not abolish the difference in hepatic triglyceride levels between the two groups of animals. 4) The intestinal bacterial flora in rats fed dextrin is different from the flora in rats fed sucrose (8). It is also known that the choline requirement of germfree rats is reduced as compared to nongermfree animals when fed a moderately hypolipotropic diet (9, 10). Dextrin may possibly decrease the choline requirement of rats by altering the intestinal bacterial flora. Supplementation of the diets with neomycin sulfate or sulfaguanidine did not eliminate the difference in hepatic triglyceride levels between the sucrose and dextrin-fed groups. No definite conclusion may be drawn from this lack of effect, since the bacteria responsible for a change in the choline requirement may not have been sensitive to the antibacterial agents used. One approach to the solution of this problem would therefore involve the use of germfree rats.

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LITERATURE CITED

1. Chalvardjian, A., and S. Stephens 1970 Lipotropic effect of dextrin versus sucrose in choline-deficient rats. J. Nutr. *100:* 397.
2. Chalvardjian, A. 1969 Mode of action of choline. IV. Activity of the enzymes related to fatty acid synthesis and the levels of metabolic intermediates in choline-deficient rats. Can. J. Biochem. *47:* 917.
3. Tepperman, J., and H. M. Tepperman 1958 Effects of antecedent food intake pattern on hepatic lipogenesis. Amer. J. Physiol. *193:* 55.
4. Cohen, A. M., and A. Teitelbaum 1968 Effect of glucose, fructose, sucrose and starch on lipogenesis in rats. Life Sci. *7:* 23.
5. Zakim, D., R. S. Pardini, R. H. Herman and H. E. Sauberlich 1967 Mechanism for the differential effects of high carbohydrate diets on lipogenesis in rat liver. Biochim. Biophys. Acta *144:* 242.
6. Hill, R., J. M. Linazoro, F. Chevallier and I. L. Chaikoff 1958 Regulation of hepatic lipogenesis: The influence of dietary fats. J. Biol. Chem. *233:* 305.
7. Artom, C., and W. H. Fishman 1947 The relation of the diet to the composition of tissue phospholipids. J. Biol. Chem. *170:* 587.
8. Peterson, G. E., E. C. Dick and K. R. Johansson 1953 Influence of dietary aureomycin and carbohydrate on growth, intestinal microflora and vitamin B_{12} synthesis of the rat. J. Nutr. *51:* 171.
9. Levenson, S. M., A. L. Nagler, E. F. Geever and E. Seifter 1968 Acute choline deficiency in germfree, conventionalized and open-animal-room rats: Effects of neomycin, chlortetracycline, vitamin B_{12} and coprophagy prevention. J. Nutr. *95:* 247.
10. Kwong, E., G. Fiala, R. H. Barnes, D. Kan and S. M. Levenson 1968 Choline biosynthesis in germfree rats. J. Nutr. *96:* 10.

[4] Benevenga, N. J. 1970 Enzyme activity and metabolite levels: Limited indicators of the impact of nutrition on metabolism. 11th Annual Ruminant Nutrition Conference, Atlantic City, New Jersey.

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J. Nutr., 78, 1962

Effect of Carbohydrates on Utilization of Protein and Lysine by Rats [1,2]

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Many investigators have demonstrated that the nature and amount of carbohydrate in the diet influence the rate of protein utilization. Monson et al. ('50) reported that chicks fed a dextrin diet grew markedly faster than those supplied with a sucrose diet. Yoshida and Morimoto ('55) found that feeding of raw-potato starch to rats decreased the digestibility of dietary protein. Harper et al. ('53) studied the influence of various carbohydrates on utilization of protein by rats and found that dextrin supported better growth of rats than sucrose. Womack and Marshall ('55) reported that rats fed extra threonine, in diets containing corn, rice or wheat starch, or corn dextrin, had a lower negative nitrogen balance than did rats fed diets containing sucrose and a low level of amino acids. Harper and Katayama ('53) found that rats fed diets containing 9% of casein grew faster when cornstarch replaced sucrose as the dietary carbohydrate.

Lysine is commonly low in protein of vegetable origin. Supplementation with lysine to improve the quality of plant protein has been intensively investigated. Guthneck et al. ('53) found greater utilization of lysine from dried eggs and skim milk (91%) and from fresh meats (84%) than from cereal and legume food (71%). Gupta et al. ('58) reported that the availability of the lysine in beef was 76%, from gelatin 74%, and in the other purified protein listed was 90 to 104%. Deshpande et al. ('57) reported that the presence of amino acid in cereal protein, although in sufficient quantity, does not adequately determine its nutritional quality. Heller et al. ('61) studied the effect of heat treatment on lysine in meat and found that the percentage of available lysine in pork, beef, and lamb muscle ranged from 74 to 92% in either raw samples or after standard cooking procedures. Little is known concerning the effect of various carbohydrates on utilization of lysine. The comparative effects of several carbohydrates on utilization of lysine in wheat gluten by rats have been investigated. The results are reported in this paper.

EXPERIMENTAL

Diets. Twelve diets were prepared and divided into two series of 6 each. The composition of the diets is shown in table 1. The 6 diets in series 1 included the following carbohydrates: potato starch (raw), cornstarch, sucrose, dextrin, glucose, and potato starch (cooked). The 6 diets in series 2 contained the same sources of carbohydrates as in series 1 except that 0.7% of L-lysine·HCl was added to each diet. Wheat gluten was used as the source of protein at an 18% protein level in all diets. The cooked-potato starch was prepared according to the method of Womack and Marshall ('55). Vitamins A and D were provided by cod liver oil (USP). The other vitamins known to be essential for rats were added (mg/kg) to the ration: thiamine, 5; pyridoxine, 5; menadione, 5; cobalamin concentrate (3 mg/gm), 5; riboflavin, 10; *p*-aminobenzoic acid, 10; nicotinic acid, 20; Ca pantothenate, 20; D-α-tocopheryl acid succinate, 75; folic acid, 5.5; biotin, 0.3; inositol, 400; and choline chloride, 1,000. The protein-free ration had the same general composition as the basal ration except that the different carbohydrates tested were used respectively to replace the pro-

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TABLE 1

Composition of diets

Series 1 (without lysine)	Series 2 (with 0.7% L-lysine)
% diet	*% diet*
Wheat gluten[1] 24	24
Carbohydrate[2] 61	60.3
Cottonseed oil, hydrogenated[3] 8	8
Salt mixture USP, 14 4	4
Cod liver oil, USP 2	2
Vitamin mix[4] 1	1
L-Lysine —	0.7

[1] Wheat gluten contained 75% of protein (24 × 0.75 = 18% of protein in the diet).
[2] Carbohydrates and dietary groups:
A-Potato starch (raw)
B-Cornstarch
C-Sucrose
D-Dextrin
E-Glucose
F-Potato starch (cooked)
G-Potato starch (raw) with lysine
H-Cornstarch with lysine
I-Sucrose with lysine
J-Dextrin with lysine
K-Glucose with lysine
L-Cooked potato starch with lysine
[3] Crisco, Procter and Gamble Company, Cincinnati.
[4] See Experimental section in text.

tein. The minerals were furnished by USP 14 salt mixture.

Feeding experiments. Ninety-six male Sprague-Dawley weanling rats were divided into 12 groups of 8 each according to body weight and litter origin. Each of the 12 experimental diets were fed simultaneously when the rats weighed 70–80 gm at 4 weeks of age. Water and food were supplied ad libitum. Body weight and food consumption were recorded weekly during the 9-week period. The influence of various carbohydrates on the digestibility of wheat gluten and on the availability of lysine was investigated according to the method of Mitchell ('24, '54). At the end of 9 weeks, the animals were then fed a protein-free ration.

Feces excreted during the first three days of the experiment of the protein-depletion period were discarded. Thereafter, the feces from each rat were collected daily for 5 days for the determination of metabolic nitrogen and lysine. After the protein-depletion period, each group was fed its previous test ration containing wheat gluten or wheat gluten with lysine and the respective carbohydrates. The feces were again collected for a 5-day period for determination of total nitrogen and lysine. In changing from one diet to another, at least three days were allowed to elapse before the feces were again collected.

Method for collection and treatment of feces. The animals were placed in metabolic cages; the feces were collected daily, preserved with 2 N HCl, composited for the 5-day collection period, and stored in the refrigerator. The 5-day collection of feces from each rat in each period was homogenized with 2 N HCl in a glass homogenizer. The homogenates were diluted to 100 ml with 2 N HCl except for the homogenates of feces from the rats fed raw-potato starch, which were diluted to 250 ml. For the analysis of lysine, 20 ml of the homogenates were transferred to a bottle, 20 ml of concentrated HCl added and the bottle sealed and autoclaved for 10 hours at 120°C. The HCl was evaporated under reduced pressure, the residue thus obtained dissolved in distilled water, deodorized with 0.5 gm of activated charcoal, filtered by suction, and diluted to volume. The lysine content of the feces was determined microbiologically by the method of Dunn et al. ('49). For determination of total nitrogen, 2 ml of the homogenate were transferred to a micro Kjeldahl flask. The total nitrogen was determined by the micro-Kjeldahl method ('60). All data were analyzed statistically by analysis of variance and Duncan's multiple range test. (Steel, '60).

RESULTS

The average increase in weights of the rats fed the different carbohydrate diets is shown in table 2. Without lysine supplementation the growth responses of rats fed sucrose were lower ($P = 0.05$) than the responses of the rats fed glucose and dextrin. There were no significant differences in growth responses of rats fed diets containing raw-potato starch, cooked potato starch, cornstarch, or dextrin. Addition of 0.7% of L-lysine to the diet greatly increased the rate of growth of rats regardless of the source of the carbohydrates. With lysine supplementation, however, rats fed cooked-potato starch grew better than those fed raw-potato starch ($P = 0.05$); the growth rates of rats fed the other 4 sources of carbohydrates were similar.

The ingestion of raw-potato starch caused a distention of the cecum and intestine. Diarrhea was a problem in the

TABLE 2

Total weight gain and protein-efficiency ratios for rats fed various carbohydrate diets[1,2]

Carbohydrate sources	Total weight gain	Total protein eaten	Protein-efficiency ratio[3]	Food eaten daily/rat
	gm	*gm*		*gm*
	Series 1 (without lysine)			
Potato starch, raw	123.0 ± 10.4[4] bc*	196.8	0.72 ± 0.05[4] d*	14.9
Cornstarch	105.0 ± 5.6 ab	128.3	0.83 ± 0.05 cdf	11.3
Sucrose	94.4 ± 4.9 a	117.8	0.80 ± 0.05 cd	10.3
Dextrin	124.7 ± 7.2 bc	138.4	0.90 ± 0.03 bc	12.2
Glucose	131.9 ± 6.8 c	141.1	0.93 ± 0.03 bf	12.4
Potato starch, cooked	110.7 ± 6.0 abc	116.0	0.96 ± 0.04 bf	10.2
	Series 2 (with 0.7% L-lysine)			
Potato starch, raw	303.6 ± 5.5 d	282.4	1.08 ± 0.03 c	24.9
Cornstarch	320.0 ± 4.9 de	198.7	1.61 ± 0.07 a	17.5
Sucrose	322.0 ± 11.3 de	192.8	1.67 ± 0.06 a	17.0
Dextrin	322.8 ± 6.0 de	204.8	1.58 ± 0.04 a	18.0
Glucose	310.0 ± 5.1 de	201.7	1.54 ± 0.03 a	17.7
Potato starch, cooked	327.0 ± 6.8 e	197.1	1.66 ± 0.07 a	17.3

[1] Eight rats/group.
[2] Nine weeks on experiment.
[3] Weight gain in grams/gram of protein eaten.
[4] Mean ± standard error.
* Numbers that do not contain the same subscript are statistically different at the 0.05 level.

rats fed the potato starch. Also, the rats fed this ration excreted white, bulky feces in large amounts.

Table 2 also shows the protein efficiency ratio for rats fed each of the experimental diets. These were determined by dividing the grams of protein eaten by the average gain in weight during a period of 9 weeks. Lysine supplementation in the diets containing cornstarch, sucrose, dextrin, glucose, and cooked-potato starch greatly increased the protein efficiency ratio for rats. With lysine supplementation, the protein efficiency ratio for rats fed raw-potato starch was found to be the lowest (P 0.05) of all the groups, whereas the other carbohydrates tested were similar in protein-efficiency ratio. Without lysine supplementation, the protein-efficiency ratio for rats fed raw-potato starch was lower than that for the dextrin, glucose, and cooked-potato starch groups (P 0.05), and the protein-efficiency ratio for rats fed sucrose was lower than that for the rats fed glucose and cooked-potato starch.

The digestibility of protein as affected by the various carbohydrates is shown in table 3. Rats fed raw-potato starch excreted larger amounts of fecal-food nitrogen and of metabolic nitrogen. Such nitrogen excreted by the rats fed cooked-potato starch and dextrin was about one-half as much as the excreted nitrogen of those rats fed raw-potato starch and twice as much as the excreted nitrogen of the rats fed cornstarch, sucrose, and glucose. As a result of the large amount of fecal-nitrogen excretion, the digestibility of protein was lowest when the rats were fed the diet containing raw-potato starch. The protein was slightly more digestible when the rats were fed diets containing cooked-potato starch and dextrin. The highest protein digestibility ($P = 0.05$) occurred when the rats were fed sucrose, glucose, or cornstarch. Lysine supplementation greatly increased the digestibility of protein in rats fed raw-potato starch and slightly increased the digestibility of protein in rats fed cooked-potato starch and dextrin, but did not noticeably affect the digestibility of protein when the other three carbohydrates were used.

The lysine availability for rats fed various carbohydrate diets is shown in table 4. In both series, when raw-potato starch was used as the source of carbohydrate in the diets, the lysine availability was low, particularly when the diets were not supplemented with L-lysine. The lysine availability for rats fed cooked-potato starch

TABLE 3

Digestibility of protein by rats fed various carbohydrates

Carbohydrate sources	Total N intake	Total N in feces	Metabolic fecal N	Fecal food N[2]	Total N absorbed[3]	True digestibility of protein[4]
	gm	*gm*	*gm*	*gm*	*gm*	%
			Series 1 (without lysine)			
Potato starch, raw	2.494	0.518	0.197	0.321	2.173	87.13 ± 0.58[5]
Cornstarch	2.136	0.118	0.017	0.071	2.065	96.68 ± 0.10
Sucrose	2.348	0.101	0.038	0.063	2.285	97.31 ± 0.18
Dextrin	3.012	0.225	0.059	0.166	2.846	94.50 ± 0.05
Glucose	2.902	0.124	0.037	0.087	2.815	97.00 ± 0.13
Potato starch, cooked	2.254	0.258	0.105	0.153	2.101	93.21 ± 0.25
			Series 2 (with 0.7% L-lysine)			
Potato starch, raw	4.218	0.639	0.374	0.265	3.953	93.72 ± 0.70
Cornstarch	3.138	0.161	0.069	0.092	3.046	97.06 ± 0.08
Sucrose	3.476	0.173	0.081	0.092	3.384	97.35 ± 0.17
Dextrin	3.381	0.259	0.131	0.128	3.253	96.21 ± 0.05
Glucose	3.466	0.159	0.062	0.097	3.369	97.20 ± 0.08
Potato starch, cooked	3.487	0.331	0.144	0.187	3.300	94.64 ± 0.37

[1] Five-day period.
[2] Fecal food N = total N in feces − metabolic N.
[3] Total N absorbed = total N intake − fecal food N.
[4] True digestibility of protein = $\frac{\text{total N absorbed}}{\text{total N intake}}$.
[5] Mean ± standard error.
* Numbers that do not contain the same subscript are statistically different at the 0.05 level.

TABLE 4

Lysine availability for rats fed various carbohydrate diets[1]

Carbohydrate sources	Total lysine intake	Total lysine in feces	Metabolic fecal lysine	Fecal food lysine[2]	Total lysine absorbed[3]	Total lysine availability[4]
	mg	*mg*	*mg*	*mg*	*mg*	%
			Series 1 (without lysine)			
Potato starch, raw	238	119	49	70	168	70.5 ± 6.1[5]
Cornstarch	193	31	10	20	173	89.5 ± 1.0
Sucrose	200	23	7	16	184	91.9 ± 1.6
Dextrin	273	72	28	45	229	83.6 ± 3.0
Glucose	224	24	9	15	209	93.3 ± 0.8
Potato starch, cooked	208	73	37	36	172	82.5 ± 1.6
			Series 2 (with 0.7% L-lysine)			
Potato starch, raw	1185	156	81	76	1110	93.6 ± 1.0
Cornstarch	848	50	21	29	814	96.6 ± 0.6
Sucrose	980	58	19	39	941	96.0 ± 0.9
Dextrin	944	103	40	62	882	93.4 ± 1.0
Glucose	1010	47	14	33	977	96.7 ± 0.3
Potato starch, cooked	1023	116	40	77	946	92.5 ± 0.3

[1] Five-day period.
[2] Fecal food lysine = total lysine in feces − metabolic fecal lysine.
[3] Total lysine absorbed = total lysine intake − fecal food lysine.
[4] True lysine availability = $\frac{\text{total lysine absorbed}}{\text{total lysine intake}}$.
[5] Mean ± standard error.
* Numbers that do not contain the same subscript are statistically different at the 0.05 level.

True digestibility of protein[4]	Total lysine availability[4]
%	%
97.13 ± 0.58[b,c]*	70.5 ± 6.1[d]*
96.68 ± 0.10 [b]	89.5 ± 1.0 [b]
97.31 ± 0.18 [b]	91.9 ± 1.6 [b]
94.50 ± 0.05 [a]	83.6 ± 3.0 [a]
97.00 ± 0.13 [b]	93.3 ± 0.8 [bc]
93.21 ± 0.25 [a]	82.5 ± 1.6 [a]
93.72 ± 0.70 [a]	93.6 ± 1.0 [bc]
97.06 ± 0.08 [b]	96.6 ± 0.6 [c]
97.35 ± 0.17 [b]	96.0 ± 0.9 [c]
96.21 ± 0.05 [b]	93.4 ± 1.0 [bc]
97.20 ± 0.08 [b]	96.7 ± 0.3 [c]
94.64 ± 0.37 [a]	92.5 ± 0.3 [bc]

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and dextrin diets was higher than that of the raw-potato starch groups, whereas the lysine availability for rats fed cornstarch, sucrose, and glucose diets was the highest ($P < 0.05$) of all groups. Lysine supplementation increased the lysine availability in rats fed diets containing raw-potato starch, cornstarch, sucrose, dextrin, and glucose.

DISCUSSION

Since lysine supplementation did not produce much improvement in the digestibility of protein in wheat gluten for rats fed most of the carbohydrate diets but markedly increased their growth responses, it appears that the added lysine had an effect on the utilization of the other amino acids in wheat gluten. The increase in body weight of rats may have been caused by the added lysine combining with the other amino acids from wheat gluten to increase the formation of tissue protein. Previous studies in our laboratory[5] indicated that, although wheat gluten was low in methionine content, supplementation of methionine to a diet containing wheat gluten did not support better growth of the rats. This observation shows that lysine is the first limiting amino acid in wheat gluten.

The inferiority of sucrose to glucose and dextrin as shown by the slower growth rate of rats is in accord with many of the observations reported previously (Monson et al., '50; Harper et al., '53). Harper and Kitayama ('53) postulated that the slower growth of rats fed sucrose in comparison with the growth rate of rats fed cornstarch was caused by the lower availability of certain amino acids. In the present study the digestibility of protein and the availability of lysine in rats fed sucrose and cornstarch were practically equal. Possibly the lower food intake of the sucrose group might be the principal factor that caused the slower growth of the rats.

The protein-efficiency ratio, the digestibility of protein, and the availability of lysine varied to a greater extent among the rats fed the 6 different carbohydrate diets without lysine supplementation than among the rats fed the same diets with lysine supplement. Evidently the nature of the carbohydrate affects the utilization of protein more markedly when the protein in the diets is of poor quality or when the protein is inadequate. Harper et al. ('53) reported that when rats were fed various carbohydrates with 9% of casein as the source of protein, sucrose was inferior to cornstarch by the criterion of growth rate. When the casein level in the diets was increased to 18%, there was no marked difference in growth responses of rats fed these two carbohydrates. In planning diets for underfed groups it may be important to consider not only the amount and quality of protein but the nature of the carbohydrate as well.

Experimenters have reported (Langworthy and Deuel, '20, '22) that certain starches, mainly derived from tubers, were more poorly utilized than the starch derived from cereals. Harper ('52) explained that the lower growth rate of rats fed a raw-potato starch diet was caused by the poor utilization of raw-potato starch and the decrease in the digestion of the dietary protein. Booher et al. ('51) and Jelinek et al. ('52) hypothesized that a heat-labile substance present in raw-potato starch, which was digestive-resistant, was responsible for stimulating peristalsis. As a result, the swift passage of food through the digestive canal caused diarrhea and low protein utilization. The data reported in this paper confirm these interpretations. The digestibility of protein was lowest in rats fed a diet containing raw-potato starch. When the potato starch was cooked, the digestibility of the protein was improved, the amount of feces excreted by the rats was reduced, and the diarrhea disappeared.

Rats fed the diet containing raw-potato starch would be expected to grow more slowly than the rats fed cooked-potato starch. Such, however, was not the case in the unsupplemented groups. The discrepancy in the growth of rats fed raw-potato starch may be explained by the compensating effect of their high food intake. The daily food intake of rats fed the diet containing raw-potato starch was 30% greater than that of rats fed the diet containing cooked-potato starch. The inclusion of indigestible raw-potato starch lowered the energy-to-protein ratio of

[5] Unpublished data.

the diet and made it essentially a higher protein diet. Thus more protein and lysine were available for the synthesis of tissue protein, making more rapid growth possible. When 0.7% of L-lysine was added to the diets, the total lysine ingested by the rats was augmented. Under such conditions, the rats fed raw-potato starch, despite their high food intake, showed a lower weight gain than the rats fed the cooked-potato starch diet.

The higher levels of nitrogen and lysine excreted in the feces of rats fed diets containing raw-potato starch, dextrin, and cooked-potato starch were caused partly by the low digestibility of the protein and partly by the great amount of metabolic nitrogen and lysine excreted by the rats fed these carbohydrates. If the metabolic fecal nitrogen and lysine are derived mainly from the intestinal secretions and intestinal microorganisms, it appears that the nature of the carbohydrate also has an effect on the excretion of nitrogen and amino acids in the feces of the rats. Similar results have been reported by Harper et al. ('52).

In table 4, it is apparent that the metabolic fecal lysine excreted by rats fed diets with lysine supplementation was higher than that excreted by rats fed diets without lysine supplementation. This may be explained by the fact that rats in the lysine-supplemented groups are larger animals than those in the unsupplemented groups. Since larger animals contributed more intestinal secretions and intestinal microorganisms, the feces of the experimental rats contained a larger amount of lysine. When L-lysine was included in the diets, the lysine intake of rats in various groups was greatly augmented; the fecal-food lysine excreted by rats in such groups was only slightly increased. This indicated that the added lysine was almost completely absorbed by rats regardless of the source of the carbohydrate. The increase in lysine availability for rats with lysine supplementation may have been caused by the larger doses of lysine ingested by the rats rather than by any change in metabolic effect.

SUMMARY

The effect of various carbohydrates (raw-potato starch, cornstarch, sucrose, dextrin, glucose, and cooked-potato starch on utilization of dietary protein as w as the availability of lysine from whe gluten were determined in rats.

Without lysine supplementation, t digestibility of protein by rats fed ra potato starch was 87% and by those f cooked-potato starch, 93%. With the oth three sources of carbohydrates tested, t digestibility of wheat gluten-protein rang from 94 to 97%. With the addition 0.7% of L-lysine to the diets, the diges bility of the protein for the rats fed ra potato starch was 94%, for the rats f cooked-potato starch, 95%, and for tho fed dextrin, 96%. There was no signi cant change in the digestibility of the p tein with L-lysine supplementation wh the rats were fed the other three carboh drates tested.

Raw-potato starch, cooked-potato starc dextrin, sucrose, cornstarch, and gluco without the addition of lysine, had diff ent effects on the availability of lysi The availability of lysine increased in t order indicated. With the addition 0.7% of L-lysine, the lysine availability the rats fed cornstarch, sucrose, and g cose was similar and was considera higher than the lysine availability in t rats fed raw-potato starch, cooked-pot starch, and dextrin.

Lysine supplementation increased by sine availability, weight gain, food inta and protein efficiency ratio of rats fed sources of carbohydrates.

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LITERATURE CITED

Booher, L. E., I. Behan, E. McMeans and H. Boyd 1951 Biological utilization of unm fied and modified food starches. J. Nutri 45: 75.

Deshpande, P. D., A. E. Harper, M. Collins C. A. Elvehjem 1957 Biological availab of isoleucine. Arch. Biochem. Biophys. 341.

Dunn, M. S., M. P. J. Reiner amino acids i liver, muscle University of

Gupta, J. D., A. C. A. Elvehj of lysine. J.

Guthneck, B. T gert 1953 food by the

Harper, A. E., 1952 The i on levels of white rats.

Harper, A. E., influence o utilization o rat. I. Con in 9% case

Harper, A. E., Benton and of various low protei Comparison drates. Gr

Heller, B. S., A. J. Sic Utilization rat. V. E in meat.

Jehnck, B., 1952 The starch as rat. Cana

Mann, M. S., M. N. Camien, E. A. Murphy and P. J. Reiner 1949 Percentages of twelve amino acids in blood, carcass, heart, kidney, liver, muscle, and skin of eight animals. University of California Publ. Physiol., 8: 327.

Mehta, J. D., A. M. Dakroury, A. E. Harper and C. A. Elvehjem 1958 Biological availability of lysine. J. Nutrition, 64: 259.

Mitchneck, B. T., B. A. Bennett and B. S. Schweigert 1953 Utilization of amino acids from food by the rats. II. Lysine. Ibid., 49: 289.

Harper, A. E., M. C. Katayama and B. Jelinek 1952 The influence of dietary carbohydrate on levels of amino acids in the feces of the white rats. Canad. J. Med. Sci., 30: 576.

Harper, A. E., and M. C. Katayama 1953 The influence of various carbohydrates on the utilization of low protein rations by the white rat. I. Comparison of sucrose and cornstarch in 9% casein rations. J. Nutrition, 49: 261.

Harper, A. E., W. J. Monson, D. A. Arata, D. A. Benton and C. A. Elvehjem 1953 Influence of various carbohydrates on the utilization of low protein rations by the white rat. II. Comparison of several proteins and carbohydrates. Growth and liver fat. Ibid., 51: 523.

Heller, B. S., M. R. Chutkow, C. H. Lushbough, A. J. Siedler and B. S. Schweigert 1961 Utilization of amino acids from foods by the rat. V. Effects of heat treatment on lysine in meat. Ibid., 73: 113.

Jelinek, B., M. C. Katayama and A. E. Harper 1952 The inadequacy of unmodifed potato starch as dietary carbohydrate for the albino rat. Canad. J. Med. Sci., 30: 447.

Langworthy, C. F., and H. J. Deuel, Jr. 1920 Digestibility of raw corn, potato, and wheat starches. J. Biol. Chem., 42: 27.

——— 1922 Digestibility of raw rice, arrowroot, canna, cassava, taro, tree-fern, and potato starches. J. Biol. Chem., 52: 251.

Mitchell, H. H. 1924 A method of determining the biological value of protein. Ibid., 58: 873.

Mitchell, H. H., and M. H. Bert 1954 The determination of metabolic fecal nitrogen. J. Nutrition, 52: 483.

Monson, W. J., L. S. Dietrich and C. A. Elvehjem 1950 Studies on the effect of different carbohydrates on chick growth. Proc. Soc. Exp. Biol. Med., 75: 256.

Monson, W. J., E. A. Harper, D. A. Benton and C. A. Elvehjem 1954 The effect of level of dietary protein on the growth of chicks fed purified diets containing sucrose or dextrin. J. Nutrition, 53: 563.

Association of Official Agricultural Chemists 1960 Official and Tentative Method of Analysis, ed. 9. Washington, D. C.

Steel, R. G. D., and J. H. Torrie 1960 Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York.

Womack, M., and M. W. Marshall 1955 Starches, sugars, and related factors affecting liver fat and nitrogen balances in adult rats fed low levels of amino acids. J. Nutrition, 57: 193.

Yoshida, M., and H. Morimoto 1955 Utilization of sweet potato starch by rats and its effect on the digestion of dietary protein. Ibid., 57: 565.

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oxidase and less malic dehydrogenase. Differences in acid phosphatase activity in the two sample types (Table I) were not great enough to be judged significant.

If one considers that *change* in peroxidase activity may be a general indicator of differentiation in plant root material regardless of whether the direction is toward or away from further differentiation, then our results mesh with those of Avers and Grimm(5) who found that peroxidase activity of roots increased as differentiation into root hairs began. Reduced malic dehydrogenase activity in our cultured material is in keeping with the idea that the cultured material may be likened to tumors and that aerobic metabolism is reduced in both plant and animal tumor tissue(6,7). Our findings with malic dehydrogenase are the first in which reduction of activity of a Krebs cycle component has been measured directly. Whether activity of other enzymes in the cycle is similarly reduced remains to be shown.

Summary. Activity of 7 enzymes in carrot taproot secondary phloem and tissue in culture derived from secondary phloem was measured. Peroxidase, acid phosphatase, and malic dehydrogenase were present in both preparations but the cultured material contained more peroxidase and less malic dehydrogenase. Possible interpretations are discussed. Alkaline phosphatase, glyceraldehyde-3-phosphate dehydrogenase, glucose-6-phosphate dehydrogenase, and aldolase were not found in either the taproots or cultured material.

1. Steward, F. C., Mapes, M. O., Mears, K., Am. J. Botany, 1958, v45, 705.

2. Kato, H., Takeuchi, M., Plant Cell Physiol., 1963, v4, 243.

3. White, P. R., The Cultivation of Animal and Plant Cells, 2nd ed., The Ronald Press, New York, 1963.

4. Anonymous, Worthington Enzymes, Worthington Biochemical Corp., Freehold, N. J., 1963.

5. Avers, C. J., Grimm, R. B., Am. J. Botany, 1959, v46, 190; Avers, C. J., Grimm, R. B., J. Exp. Botany, 1959, v10, 341.

6. Warburg, O. H., Weiterentwicklung der zellphysiologischen Methoden, Interscience, New York, 1962.

7. Steward, F. C., Mapes, M. O., Kent, A. E., Holsten, R. D., Science, 1964, v143, 20.

Received October 25, 1965. P.S.E.B.M., 1966, v121.

Proc. Soc. Exp. Biol. Med., 121, 1966

Paper Electrophoresis of Serum in Rats Fed Various Carbohydrate Diets.*† (30820)

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The levels of free plasma amino acids, following protein meals, have been studied in swine(1) and rats(2) and found to correspond approximately to the amino acid composition of the ingested protein. The concentration of serum albumin is also related to the quality and quantity of dietary protein and has been found to decrease markedly in cases of protein malnutrition(3). Alterations in the electrophoretic pattern of specific serum protein fractions in the rat, due to type of protein in the diet, have been reported (4).

In addition to protein composition, Guggenheim *et al*(5) have shown that the nature of the carbohydrate fed exerts an influence upon the levels of free plasma amino acids. Previous studies(6) have demonstrated that the type of carbohydrate also influences protein utilization. Most methods which have been used to evaluate protein utilization have been based on standard procedures of nitrogen balance, growth, protein digestibility, and

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er the taproots or cultured

C., Mapes, M. O., Mears, K., Am.
v45, 705.
Takeuchi, M., Plant Cell Physiol,
., The Cultivation of Animal and
ed., The Ronald Press, New York.
Worthington Enzymes, Worthing
Corp., Freehold, N. J., 1963.
Grimm, R. B., Am. J. Botany,
vers, C. J., Grimm, R. B., J. Exp.
341.
H., Weiterentwicklung der zell
ethoden, Interscience, New York.
C., s, M. O., Kent, A. E.,
ence, 1964, v143, 20.
25, 1965. P.S.E.B.M., 1966, v121.

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TABLE I. Effect of Dietary Treatment on the Serum Composition of Rats.*

Diet	Total protein	Albumin	Alpha-1	Alpha-2	Beta	Gamma
	Protein fraction (g %)					
		Series I (no lysine)				
Potato starch (raw)	5.65 ± .25	2.87 ± .30	.76 ± .06	.48 ± .21	1.11 ± .26	.50 ± .07
" " (cooked)	5.42 ± .30	2.96 ± .19	.63 ± .02	.53 ± .98	.98 ± .02	.30 ± .03
Cornstarch	6.68 ± .29	3.99 ± .24	.88 ± .03	.51 ± .01	.87 ± .06	.40 ± .05
Sucrose	5.66 ± 1.15	3.16 ± .15	.77 ± .07	.49 ± .01	.90 ± .09	.31 ± .09
Glucose	6.47 ± .10	4.56 ± .07	.52 ± .03	.37 ± .04	.69 ± .03	.30 ± .02
Dextrin	6.79 ± .10	3.88 ± .15	.80 ± .04	.52 ± .05	1.22 ± .09	.30 ± .07
		Series II (0.7% L-lysine added)				
Potato starch (raw)	7.39 ± .14	4.65 ± .11	.84 ± .08	.40 ± .04	1.10 ± .08	.38 ± .03
" " (cooked)	7.49 ± .20	4.48 ± .17	.99 ± .15	.57 ± .04	1.09 ± .06	.35 ± .03
Cornstarch	8.05 ± .16	4.92 ± .38	1.33 ± .12	.53 ± .03	.87 ± .16	.37 ± .05
Sucrose	8.37 ± .14	4.94 ± .36	1.39 ± .17	.56 ± .03	1.19 ± .07	.27 ± .06
Glucose	8.35 ± .30	5.28 ± .09	.91 ± .19	.51 ± .04	.78 ± .11	.37 ± .04
Dextrin	7.88 ± .31	4.84 ± .11	1.03 ± .13	.58 ± .05	1.38 ± .08	.49 ± .05

* Average values of 8 rats per group and standard error of mean.

availability of amino acids. The present study was initiated in order to determine whether the influence of various carbohydrates on utilization of dietary protein as previously determined(6) was accompanied by an alteration in serum protein patterns.

Methods. Ninety-six weanling male rats of the Sprague-Dawley strain, weighing 70-80 g, were divided into 2 series of 6 groups with 8 rats in each group. The rats were fed the experimental diets *ad libitum* for a period of 12 weeks.

The diets were the same as those previously described(6) and were divided into 2 series of 6 groups each. Series I contained the following respective carbohydrates: raw-potato starch, cooked-potato starch, sucrose, cornstarch, dextrin, and glucose. Series II contained the same sources of carbohydrates as in Series I except that 0.7% of L-lysine HCl was added to each group. The diets were composed of 24% wheat gluten, 8% hydrogenated vegetable oil, 2% cod liver oil, 4% salt mixture (USP 14), vitamins in sucrose 1%, and carbohydrates 61%. The addition of L-lysine to the diet in Series II was compensated for by subtracting the same percentage of carbohydrate. Vitamins included were as described previously(6).

At the end of the 12-week period, the rats were anesthetized by injection of sodium amytal and blood samples were collected by heart puncture. After standing for a few hours, the blood samples were centrifuged and the resulting serum drawn off for subsequent analysis. Serum protein fractions were separated by paper electrophoresis in a Durrum cell in sodium veronal buffer (pH = 8.6, $\mu = 0.75$) for 16 hours and dyed with bromphenol blue.

The paper strips were scanned on a Spinco integrating analytrol (Beckman Instrument Co.) and the percentage distribution of each protein fraction calculated. Total serum protein was determined by the micro-Kjeldahl method(7) and the gram per cent of each protein fraction determined by multiplying total serum protein by the relative percentage of the individual fraction.

Results and discussion. Five serum protein fractions designated as albumin and alpha-1, alpha-2, beta, and gamma globulin were separated by paper electrophoresis. The total protein and the specific protein fractions, in grams per 100 ml of serum, are shown in Table I. Without L-lysine supplementation, rats fed either raw or cooked potato starch exhibited significantly lower ($P<0.05$) total serum protein levels than those which had received cornstarch, glucose, or dextrin. No differences in serum protein levels were noted in rats fed cornstarch, sucrose, glucose, or dextrin. With the addition of 0.7% L-lysine to the diets, total serum protein in rats fed raw potato starch was lower ($P<0.05$) than that of rats fed cornstarch, glucose, or sucrose. Lysine supplementation increased the total serum protein in the rats regardless of the

type of carbohydrate in the diet.

Of the 5 serum protein fractions, the type of dietary carbohydrate affected the albumin level most prominently. Without L-lysine supplementation, serum albumin was decreased ($P<0.05$) by the feeding of both raw and cooked potato starch or sucrose whereas glucose caused an increase ($P<0.05$) in the serum albumin level. Lysine supplementation increased the level of serum albumin in all of the 6 groups and appeared to negate any effect of dietary carbohydrate.

Significant differences were also noted with respect to the levels of the globular proteins in the serum from rats fed the various carbohydrates. In Series I, the levels of alpha-1, alpha-2, and beta globulin in the serum of the glucose fed rats were lowered ($P<0.05$) in comparison with rats receiving other carbohydrate sources while in Series II, supplemented with dietary L-lysine, an increase in the alpha-1 protein fraction was noted in the case of the rats receiving cornstarch, sucrose, and glucose. Alpha-1 protein was increased by addition of dietary L-lysine ($P<0.05$) in all cases except in those animals which consumed either form of potato starch or dextrin. No differences due to dietary treatment were noted in levels of serum gamma globulin.

In previous studies(6), it was noted that the nature of the carbohydrate in the diet affected protein utilization by the rat and that, in this respect, protein availability was markedly affected by either raw or cooked potato starch as compared to cornstarch, sucrose, glucose, and dextrin. The lowering of total protein and albumin levels in the serum of rats fed raw or cooked potato starch in the present study appears also to be correlated with lowered protein utilization by the rats in these groups. It has been suggested(8,9) that the levels of free plasma amino acids may be useful as an index for evaluation of the nutritive adequacy of protein. Longnecker and Hause(10) reported that the free amino acid levels in the plasma of dogs reflected the protein quality of the diet. This study indicates that serum protein and albumin levels may also be additional factors which can be used in conjunction with free plasma amino acid levels for evaluation of protein utilization.

Summary. The effect of various carbohydrates on the serum protein and serum protein fractions in rats have been investigated. Without L-lysine supplementation, rats fed raw or cooked potato starch showed the lowest serum protein and serum albumin concentration; whereas rats fed glucose showed the highest serum albumin concentration. Lysine supplementation increased serum protein and serum albumin concentration in rats regardless of the sources of carbohydrates. The type of carbohydrate in the diet influences the serum protein pattern in rats.

1. Puchal, F., Hays, V. W., Spear, V. C., Jones, J. D., Catron, D. V., J. Nutrition, 1962, v76, 11.

2. Wheeler, P., Morgan, A. F., ibid., 1958, v64, 137.

3. Flodin, N. W., J. Agr. & Feed Chem., 1953, v1, 222.

4. Erwin, E. S., Proc. Soc. Exp. Biol. and Med., 1960, v103, 396.

5. Guggenheim, K., Halevy, S., Friedmann, N., Arch. Biochem. and Biophys., 1960, v91, 6.

6. Chang, Yet-Oy, J. Nutrition, 1962, v78, 21.

7. Association of Official Agricultural Chemists. Official Methods of Analysis, 1960, 9th ed., Washington, D. C.

8. Smith, R. E., Scott, H. M., J. Nutrition, 1965, v86, 37.

9. ———, ibid., 1965, v86, 45.

10. Longnecker, J. B., Hause, N. L., Arch. Biochem. and Biophys., 1959, v84, 46.

Received October 25, 1965. P.S.E.B.M., 1966, v121.

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Progr. biochem. Pharmacol., vol. 2, pp. 182–202 (Karger, Basel/New York 1967)

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The Manifold Effects of Different Dietary Carbohydrates[1]

L. Cohen, E. G. Perkins and Ljubica Dobrilovic

The bioconversion of dietary carbohydrate to fatty acids has been known for decades [1]. It has also become well known that the replacement of dietary fat with carbohydrate can influence the variety of fatty acids which accumulate although the mechanism by which this occurs is unclear [2]. Most such studies have been confined to the measurement of tissue fatty acids, although recently attention also has been given to the effect of such substitutions on the fatty acids in the serum lipids [3]. Generally these studies have been short term and have devoted little attention to the effects of feeding different carbohydrates. The primary purpose of the present study was to define the tissue fatty acid and serum lipid changes associated with the feeding of moderate amounts of eight different dietary carbohydrates to rats for most of their lives. In the course of these studies other unexpected and interesting observations were made, and will also be described.

Methods

Seventy-two 2-month-old male, Sprague-Dawley rats were divided into 8 equal groups of comparable average weight. Each group was fed a diet of ground rat chow mixed with 20% by weight of one of the carbohydrates listed in Table I. In addition, one group of 5 rats was fed only a diet of rat chow. These diets were provided for 18

[1] Supported by grants from the Chicago, Illinois, and American Heart Associations and United States Public Health Service (H-01119).

months. All animals were offered and consumed the same amount of food daily; most of their lives the animals consumed 20 g of diet per day per rat. The animals were housed in an air-conditioned room and their cage positions were changed weekly. They were weighed weekly.

Table I. Varieties of carbohydrate added to rat chow[1] (rat chow, 80%; carbohydrate, 20%)

Name	Source and description
Monosaccharides	
Cerelose	α-glucose hydrate. It is derived from corn sugar, but has not been crystallized. It is said to be 99.8% pure, containing 1 part per million of maltose, and of a trisaccharide. Its ash content is 0.02%. It is said to be fat free. Corn Products Sales Co., 10 E. 56th St., New York 22, N.Y.
Fructose	d( ) levulose, C. P. Nutritional Biochemicals Co., 21010 Miles Avenue, Cleveland, Ohio.
Galactose d(+)	Anhydrous. Nutritional Biochemicals Co.
Disaccharides	
Sucrose d(+)	Nutritional Biochemicals Co.
Granulated sugar	It is derived from sugar cane and contains no additives. It is reportedly 99.5% pure sucrose. Invert sugar present is 0.010% ± 0.005%; the ash content is 0.007% ± 0.002% and the moisture content is 0.020% ± 0.005%. Colonial Sugar Co.
Beta-lactose	Milk sugar; U.S.P.; Hydrate; Nutritional Biochemicals Co.
Oligosaccharide	
Dextrin	It is derived from corn starch, roasted in the presence of HCl and then allowed to repolymerize. The amount of maltose and trisaccharides is said to be small. If 2 g are added to 100 ml water, 65% is soluble and therefore of 'short' chain length. Corn Products Co.
Polysaccharide	
Pectin	Citrus, N.F., M.W. 150,000–300,000; moisture < 10%. Nutritional Biochemicals Co. Two hundred ml of water were added to the diet containing pectin which made it palatable.

[1] Rat chow, Ralston Purina Company. Analysis: protein 23.0%; fiber, 6.0%; ash, 9.0%; carbohydrate, 57.1%; and fat, 4.9%. The diet contained 0.04% Lieberman-Burchard positive sterols, each rat consumed 16 mg of such sterols per day.

Adding 20 parts of carbohydrate to 80 parts of rat chow reduced the protein and fat in the diet, respectively, to 18.4 and 3.6%.

Progr. biochem. Pharmacol., Vol. 2, pp. 182-202 (Karger, Basel/New York 1967

49

Animals were bled by tail vein, on three occasions, using light ether anesthesia, after 9, 14, and 18 months of feeding. Serum was collected in the usual way and analyzed for total cholesterol [4] and total fatty acids [5].

Two-thirds of the animals survived the duration of the study. They were sacrificed by exanguination. The livers, epididymal fat pads and testes were collected, trimmed and weighed. The livers were homogenized and extracted with $CHCl_3:CH_3OH$ (1:1). The extract was washed twice with water, evaporated to dryness, *in vacuo*, and stored at —20°C under nitrogen. The major lipid classes were separated by silicic acid chromatography [6]. The cholesterol, cholesterol ester, phospholipid and triglyceride fractions were weighed and the latter three were analyzed for their fatty acid composition, after hydrolysis and esterification [7], by gas-liquid chromatography [8]. Each fraction resulting from silicic acid chromatography was monitored by thin layer chromatography[9]. Samples were rechromatographed when necessary. The epididymal fat pad was similarly treated but the column chromatographic step was omitted. Rat chow was also analyzed for its total fat, sterol and fatty acid composition (Table II).

Table II. The fatty acid composition of rat chow[1]

No. of Carbons	Percent	No. of Carbons	Percent
9:0	0.10	17:0	0.42
10:0	0.39	18	0.27
12:0	0.22	18:0	7.77
14:0	1.85	18:1	33.68
14:1	0.31	18:2	24.78
15:0	0.19	18:3	1.83
16:0	23.18	20:0	0.81
16:1	3.10	20:1	1.10

[1] The rat chow used contained 4.85% total lipids; of this esterified sterol was 1.5%, total sterol 1.77%, triglycerides 25.3%, and the remainder calculated as phospholipids. Each rat consuming 16 g of rat chow consumed 16 mg of Lieberman-Burchard positive sterols per day.

The water consumption and output of stool (dried residue) for each group of rats were also determined. Each was measured daily for four to six days during each of three different seasons. The mean

water consumption and stool output per day per 100 g of rat were then calculated.

The protein efficiency ratio was determined by calculating the ratio of the grams of body weight gain to grams of protein consumed in a six month period.

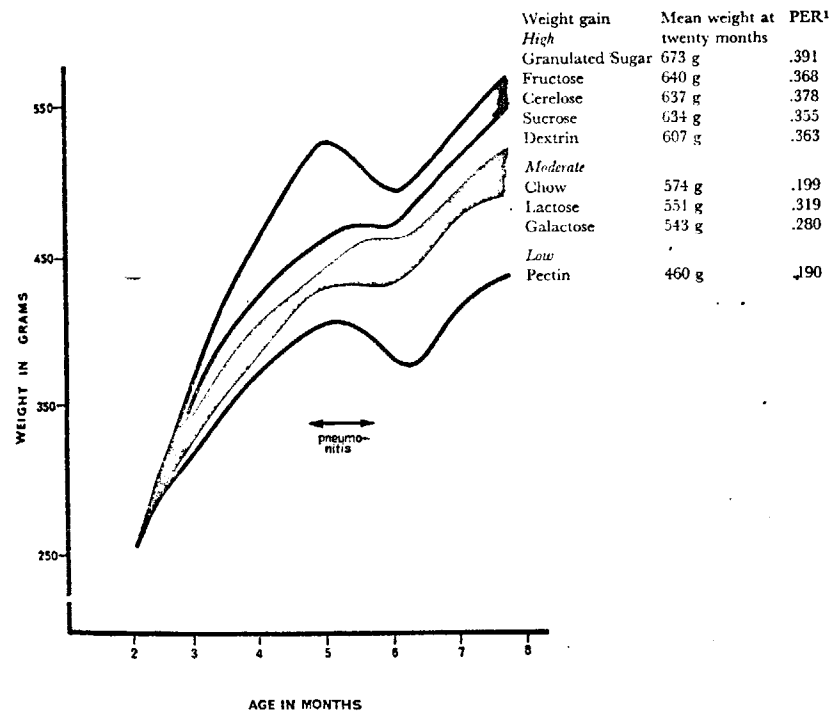

Fig. 1. The effect of dietary carbohydrate variety on the weight gain of rats. The weight gain was divided into three groupings, labelled high, moderate and low. Only the upper and lower curves for each weight gain group are shown for simplicity. The maximum weights obtained the week before sacrifice are also shown. [1]PER, refers to the protein efficiency ratio, which is the ratio of the body weight gain in grams to grams of protein consumed, during the six month period, between the second and eighth months of the study. The arrows demarcate a period during which the rat colony was affected by pneumonitis.

Results

Growth and protein efficiency ratio (PER). Despite comparable food intake, growth and PER differences were evident and are shown in Fig. 1. Three levels of weight gain, *high, moderate* and *low*, and protein efficiency ratios, could be distinguished. Those consuming glucose- or fructose-containing *mono-, di-* or *oligo*saccharides enjoyed the greatest weight gain, and had the highest PER. Rats consuming galactose and betalactose had a moderate weight gain and PER; their weight gain was comparable to that of the rat consuming unsupplemented chow. The rats consuming the pectin supplement grew the least, presumably because of the low digestibility and absorption of this carbohydrate [10]. The PER ratios were comparable in the pectin and chow groups, indicating that unsupplemented chow was, in this regard, the least efficient of the diets provided.

None of the rats appeared to have evidence of malnutrition, and all groups of animals but one appeared to age in an expected manner. The one remarkable exception was the group fed pectin. While these animals were smaller by some 200 g, all were obviously healthy and each in fact had the appearance of animals about six months of age; their coats of hair were sleek-white, unlike the bristly, red-pocked coats of the other groups.

Water consumption and stool residue. Ad libitum water consumption was found to be inversely proportional to the weight gain; for example, the pectin low-weight gain group drank almost twice the volume of water consumed by the high-weight-gain group, and the moderate weight gain groups were intermediate in this regard. These measurements are summarized at the top of Table III. This relationship held no matter the season.

The output of stool residue was also inversely proportional to the weight gain, in all three seasons. The stool residue in the pectin group was more than twice that of the high weight gain group and half again that produced by the rats of moderate weight gain. These stool weights are summarized at the bottom of Table III.

The relationship between stool residue and water consumption was approximately linear as shown in Fig. 2 which suggests the two are related; this will be discussed later. The volume of water consumed daily was equal to approximately 15 times the dry weight of the stool.

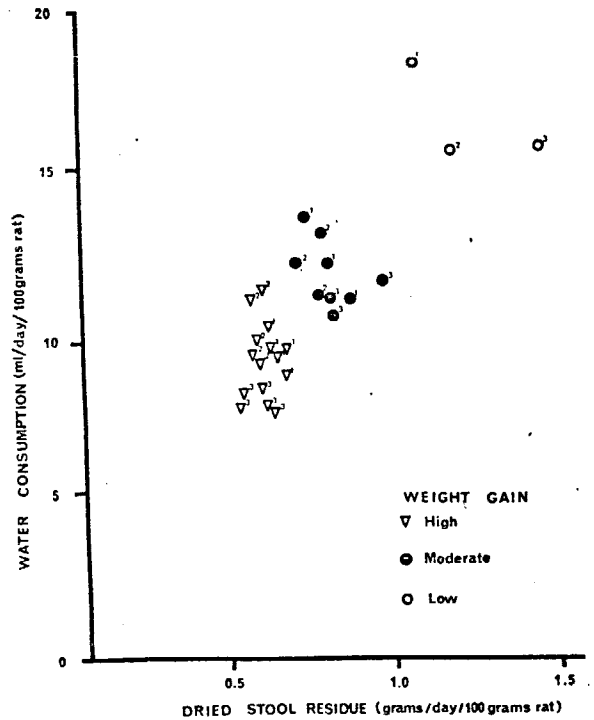

Fig. 2. Interrelationships of water consumption, dried stool residue and weight gain in the rat. The superscripts 1, 2 and 3 refer to the three different periods during which the water consumption and the dried stool residue were measured for each group of rats consuming a different carbohydrate. Each symbol represents one of the dietary groups comprising each of the weight gain groups shown in the legend. These data correspond to those listed in Table III.

Since both the water consumption and dried stool weight are related to the amount of weight gain, and the weight gain differences were determined by the carbohydrates added to the diet, it follows that differences in both the water consumption and stool residue were also determined by the carbohydrates added to the diet.

Table III. The influence of carbohydrate variety on water consumption and stool dry weight

Weight gain group	High					Moderate		Low	
Carbohydrate supplement	Granulated sugar	Sucrose	Fructose	Cerelose	Dextrin	Chow	Galactose	Beta Lactose	Pectin
	Water consumption per day per 100 g of rat[1]								
Winter	9.48	9.33	10.3	9.85	9.38	12.2	13.6	11.1	18.2
Spring	11.1	9.42	11.4	9.87	9.30	11.3	12.2	13.1	15.5
Fall	8.3	7.89	7.66	8.37	7.86	11.2	10.7	11.7	15.6
	Dry stool weight per day per 100 g of rat[2]								
Winter	0.62	0.65	0.63	0.68	0.61	0.81	0.74	0.87	1.08
Spring	0.58	0.58	0.61	0.59	0.59	0.78	0.71	0.79	1.19
Fall	0.55	0.54	0.62	0.61	0.62	0.81	0.82	0.97	1.45

[1] milliliters [2] grams

Organ weights. The variety of dietary carbohydrate was also an important determinant of the relative weights reached by the liver and testes. The mean relative liver weight in the galactose-fed rats was significantly greater than in fructose- ($p < 0.05$) and cerelose-fed ($p < 0.02$) animals. Similarly, the dextrin- and pectin-fed animals had relative testicular weights which were significantly greater (all p-values < 0.002) than in the fructose- and sucrose-fed rats. The relative organ weight data are shown in Table IV.

The ratios of the relative liver and testicular weights, shown also in Table IV, and the low correlation coefficient of these relative weights ($r = 0.247$), suggest that in the same animal, the final relative weights of these organs need not necessarily be comparably influenced by the same carbohydrate variety.

Serum lipids. Serum cholesterol determinations were made after 9, 14 and 18 months of feeding; serum total fatty acids were determined at 9 and 18 months; these data are summarized in Table V. The major finding was a steady increase in these lipid levels with increasing age in every group; a virtual doubling of the serum lipids occurred in the last 9 months of their lives. This increase was independent of the carbohydrate provided.

The variety of dietary carbohydrate however did appear to be of some importance in determining the level of the lipid increases. For example, the final serum cholesterol levels in the cerelose and pectin groups were significantly lower than the levels found at the end of the study in the groups fed granulated sugar ($p < 0.02$) and beta-lactose ($p < 0.02$). The serum total fatty acids in the groups fed glucose or pectin were also significantly lower than found in the groups fed dextrin ($p < 0.02$) or beta-lactose ($p < 0.02$).

The serum lipid levels seemed not to be importantly influenced by the level of weight gain. For example, despite the marked difference in weight gain throughout the study, the serum lipid levels in the groups fed cerelose and pectin were comparable.

Composition of liver lipids. The dietary carbohydrate was of some importance also in determining particularly the proportion of triglycerides and free cholesterol found in the liver lipids. The distributions of the major classes of liver lipids for each diet group are shown in Table VI. The triglyceride portion was significantly lower in the cerelose and galactose-fed rats, than in the fructose-, sucrose- and lactose-fed rats ($p < 0.01$ to < 0.03). The cholesterol portion

Table 3

Table IV. The influence of dietary carbohydrate variety on relative organ weights

Weight gain group	Carbohydrate supplement	Liver weight / Body weight × 100 (Mean % ± SD[2])		Testicle weight / Body weight × 100 (Mean % ± SD)		Liver weight (g)[3] / Testicle weight (g) (Mean ± SD)	
		No.[1]		No.		No.	
High	Granulated sugar	4	3.33 ± 0.35	4	0.93 ± 0.05	5	3.55 ± 0.17
	sucrose	6	3.35 ± 0.37	6	0.75 ± 0.02	6	4.57 ± 0.79
	cerelose	5	3.04 ± 0.28	5	0.84 ± 0.12	5	3.60 ± 0.26
	fructose	7	2.99 ± 0.10	7	0.69 ± 0.15	7	4.63 ± 1.04
	dextrin	4	3.38 ± 0.36	5	1.08 ± 0.10	4	3.13 ± 0.17
Moderate	chow	5	3.60 ± 0.14	5	0.92 ± 0.16	5	4.16 ± 0.83
	galactose	6	3.83 ± 0.32	6	0.95 ± 0.28	6	4.20 ± 0.89
	lactose	6	3.40 ± 0.32	6	0.83 ± 0.03	6	4.01 ± 0.54
Low	pectin	7	3.13 ± 0.27	6	1.12 ± 0.10	6	2.85 ± 0.33

[1] number of animals studied [2] standard deviation [3] g, grams

(2)

Table V. Effect of age and dietary carbohydrate variety on the serum cholesterol and total fatty acids

Weight gain group	High						Moderate		Low
Carbohydrate supplement	Granulated sugar	Cerelose	Dextrin	Sucrose	Fructose	Chow	Beta Lactose	Galactose	Pectin
				Cholesterol mg/100 ml (Mean ± SD[1])					
Months of feeding									
9	73.1 (21.1)[1]	68.6 (13.3)	72.0 (15.5)	111 (33.2)	95.8 (20.7)	77.4 (13.8)	84.3 (10.8)	98.0 (18.2)	88.3 (16.8)
No.[2]	7	8	6	8	8	5	7	7	9
14	153 (36.5)	97.8 (27.9)	147 (32.4)	168 (51.5)	127 (18.9)	150 (28.0)	138 (36.7)	110 (38.8)	126 (20.5)
No.	5	6	4	6	7	5	7	6	7
18	170 (16.3)	134 (16.4)	187 (60.7)	239 (93.3)	176 (59.2)	202 (47.5)	190 (28.1)	149 (28.7)	127 (26.4)
No.	4	4	4	6	7	5	6	6	7
				Total fatty acids meq/liter (Mean ± SD)					
9	6.13 (1.30)	4.31 (2.50)	6.80 (2.10)	8.86 (3.80)	6.27 (1.10)	6.09 (1.67)	6.60 (1.60)	6.04 (1.10)	4.32 (1.20)
No.[2]	7	8	6	8	8	5	7	7	9
18	14.7 (1.41)	12.6 (1.00)	18.1 (3.00)	19.4 (8.06)	14.6 (3.00)	14.6 (3.32)	18.3 (3.00)	15.2 (2.80)	12.2 (1.73)
No.	4	4	4	6	6	5	6	6	7

[1] Standard deviation [2] The number of animals assayed

beta-lactose. Furthermore, despite the great variation in the proportion of arachidonic acid found esterified with cholesterol among the rats within each group (Fig. 4B) the proportion of this fatty acid which became esterified was also very likely influenced by the carbohydrate variety present in the diet.

The liver *phospholipid fatty acid* distribution was quite different from that in the diet. It was comparable to that recorded in the literature for chow fed animals [12]. Despite these shifts from the diet composition, to a fatty acid composition characteristic for phospholipids, one could not help but be struck by the relative lack of influence of carbohydrate variety in influencing the magnitude of the differences between the phospholipid and diet fatty acid composition. Though there were some differences it was not possible to be certain any were significant.

Sucrose and granulated sugar. The attention in the literature [13] recently given sucrose as responsible for higher blood lipid levels as well as perhaps being implicated in coronary disease, should not obscure the fact that sucrose and common table sugar though very similar are not identical. It was therefore particularly interesting to contrast the effects of these two almost identical carbohydrates. The adipose tissue fatty acid composition in these two groups was essentially comparable; there was a slightly higher mean level in the C 16:0 fraction, and a lower mean level of the C 16:1 fatty acids in the sucrose-fed rats but these were of doubtful significance. However, a small amount (2.2 ± 0.64%) of C 18:3 was found only in the liver triglycerides of the group fed granulated sugar. Despite considerable variation among rats in each group, the C 18:0 fraction in the liver cholesterol esters was less than half that in the granulated sugar group, but about 50% greater in the C 18:2 fractions; and the mean C 20:4 fraction in the sucrose-fed rats was six times greater than in the rats consuming granulated sugar. The

Fig. 3. Comparisons of the fatty acid composition of the diet and the epididymal fat pads. The four fatty acids in the epididymal fat (open bars) whose proportions differed from that in the diet (hatched bars) are shown. Each open bar represents the mean value for each diet group. The line above or within the bar represents one standard deviation. High, moderate, and low refer to weight gain groups. Non, refers to the number of fat pads analyzed. The groups receiving the different carbohydrates are denoted as follows: *GS* granulated sugar; *C* cerelose (glucose); *D* dextrin; *S* sucrose; F fructose; *L* beta-lactose; *G* galactose; P pectin. *Ch* indicates the group fed only rat chow. See the text for additional explanation.

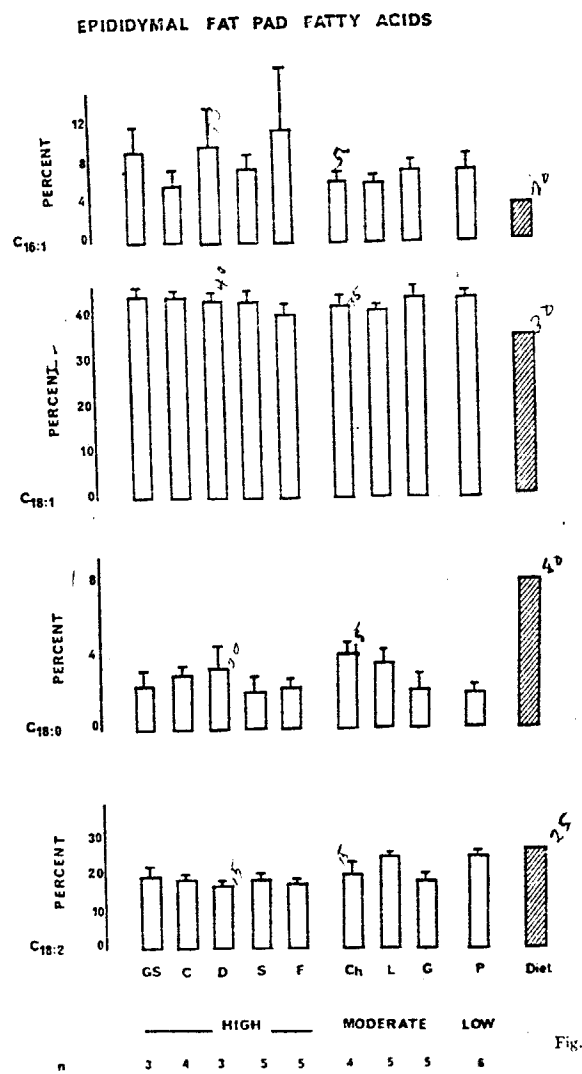

Fig. 3

Table VI. The influence or carbohydrate variety on the distribution of liver lipids

Weight gain group	Carbohydrate supplement	No.[2]	Distribution of liver lipids[1] Cholesterol esters	Triglycerides	Cholesterol	Phospholipids
High	granulated sugar	3	6.30 ± 4.76	25.2 ± 10.80	5.91 ± 1.52	62.5 ± 13.3
	sucrose	5	5.71 ± 4.37	29.1 ± 8.13	10.3 ± 7.68	54.9 ± 15.6
	cerelose	4	3.60 ± 3.79	13.2 ± 3.79	24.3 ± 8.12	58.9 ± 7.50
	fructose	4	4.10 ± 1.34	30.0 ± 3.60	11.5 ± 4.24	54.4 ± 6.24
	dextrin	3	4.90 ± 3.61	21.4 ± 8.05	12.0 ± 9.05	61.7 ± 9.63
Moderate	chow	4	6.60 ± 6.15	23.2 ± 8.03	15.8 ± 11.70	54.4 ± 12.9
	galactose	5	5.70 ± 4.00	14.8 ± 1.73	10.1 ± 4.69	69.4 ± 3.61
	lactose	5	9.78 ± 4.50	31.8 ± 8.94	17.7 ± 15.90	40.8 ± 17.0
Low	pectin	5	2.70 ± 1.59	24.6 ± 8.75	7.7 ± 2.32	65.0 ± 11.0

[1] mean percent ± standard deviation [2] number of animals studied

of the liver lipids was significantly greater in the cerelose-fed rats than in those consuming galactose or pectin ($p < 0.02$).

Adipose tissue fatty acids. Two important observations were made. Despite the low fat content of the diet five of nine fatty acids in the adipose tissue of all groups were present in proportions comparable to those found in the diet. The four major adipose tissue fatty acids, whose proportions differed from those in the diet, are shown in Fig. 3; two were higher (C 16:1 and C 18:1) and two were lower (C 18:0 and C 18:2). The second observation was that the quality of the dietary carbohydrate appeared to be a determinant of the degree to which the fatty acid distribution in the diet and adipose tissue differed; for example, the fructose-fed rats had a significantly greater proportion of C 16:1 than found in 5 of the 7 other groups fed different carbohydrates ($p < 0.02$); and the C 18:2 proportions found in the groups fed lactose and pectin, though comparable to one another, were significantly greater (all p values < 0.02) than found in all the other groups fed different carbohydrates.

Liver lipid fatty acids. Eight of the ten fatty acids in the *liver triglyceride* fatty acids were comparable to those in the diet. Two differed; the proportions of C 18:2 and C20:4. These are shown in Fig. 4A. The C 18:2 proportion was lower and the C 20:4 was higher than found in the diet. Three points are worth making: one is that since C 20:4 (arachidonic acid) was absent from the diet it must have been synthesized; presumably the precursor in the diet was C 18:2 (linoleic acid) [11]. It is of interest in this regard that the sum of the mean porportions of these two liver triglyceride fatty acids, in virtually every group, was about equal to the proportion of C 18:2 present in the diet (about 25%). Finally, the C 20:4 proportion that was present in the liver triglycerides appeared to be influenced by the carbohydrate in the diet; for example, the proportion in the pectin-fed rats was twice that present in the rats fed cerelose ($p < 0.05$).

While the *liver cholesterol esters* also had a fatty acid composition not unlike that of the diet there were two exceptions: the proportions of C 18:0 (Stearic) and C 20:4 (arachidonic acids). These are shown in Fig. 4B. The C 18:0 proportion was comparable to that in the diet except in the groups fed sucrose, fructose, and pectin. The proportion of this fatty acid was significantly higher ($p < 0.05$) in these three groups than found in the group fed dextrin; the same was true when pectin and sucrose fed groups were compared with

Fig. 4 A

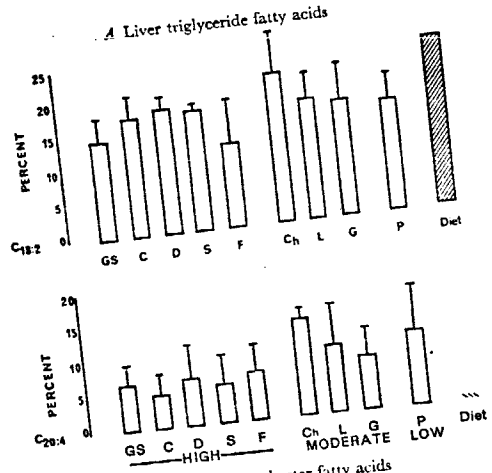

Fig. 4 B

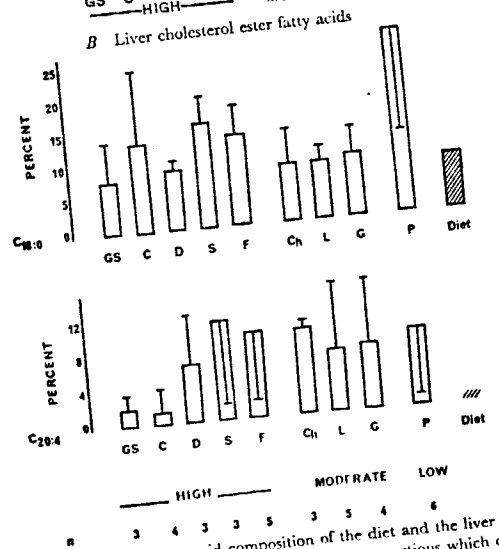

Fig. 4. Comparison of the fatty acid composition of the diet and the liver triglycerides and cholesterol esters. As in Fig. 3 only the fatty acid proportions which differed from those in the diet are shown. See the legend of Fig. 3 for the explanation of abbreviations, etc.

appearance of small amounts of behenic acid (1.54 ± 1.10%) in the liver phospholipids was evident only in the granulated sugar group. Finally, although the saturated: unsaturated fatty acid ratios for these two sugars were comparable in the liver triglyceride and cholesterol esters, and differed only slightly in the adipose tissue triglycerides (granulated sugar, 0.322; sucrose, 0.414), this ratio was strikingly different in the liver phospholipids (granulated sugar, 1.22; sucrose, 0.66).

Discussion

Non-lipid studies. This study demonstrates several influences in the rat of chronically consumed dietary carbohydrates including some, to the authors' knowledge, not previously described. It has been demonstrated that the dietary carbohydrate variety plays an important role in determining many characteristics of the rat: the rate at which they grow; their utilization of protein for growth, the final relative size of their livers and testes; their water consumption; and, the non-aqueous residue in their stool. The interrelationships of these gross observations are speculative. It is possible, however, that differences in carbohydrate digestibility and/or an alteration in the intestinal bacterial mass promoted by the carbohydrates may have altered the bulk of the stool. A bulkier stool residue could increase the colonic osmotic pressure, reduce fecal water resorption, and result in increased water consumption. The crude wet stool weights support this interpretation. This would also explain the linear relationship between the stool residue and water consumption noted in Fig. 2.

If the well known protein-sparing effect of dietary carbohydrates is different for different carbohydrates, the differences in the PER could be explained. Such a possibility is also suggested by other studies [14–16]. The nutritional implication of this observation is not minor. Furthermore, different protein-sparing effects of the various carbohydrates used in this study could also result in a significant reduction in the production and possible output of metabolic water, and explain in part the differences in water consumption. GAMBLE was the first to demonstrate the reduced water requirement associated with feeding carbohydrate [17]. Such differences in protein utilization could also contribute to differences in weight gain and organ size.

Lipid studies. The important role of dietary carbohydrate in influencing serum lipoproteins and lipoprotein lipid concentrations is now well known. AHRENS [18] and GOFMAN [19] have led in clarifying the important influence of the amount of dietary carbohydrate on the serum lipids and lipoproteins. Others have suggested that the role of not only the amount but also the variety of dietary carbohydrate may be important [20, 21]. The present study indicates that certain dietary carbohydrate varieties may be of influence on the serum lipid levels. The mechanisms of action by which these effects are produced by the different carbohydrates are not known. The studies of KRITCHEVSKY *et al.* [22] suggest that the intestinal flora may play an important role, as nonabsorbable antibiotics were able to abolish serum cholesterol differences associated with feeding different carbohydrates for a short period; such a mechanism may be operative when cholesterol is added to the diet which was not the case in the present study.

The present study also underscores the importance of carbohydrate variety in controlling the fatty acid composition of complex lipids in the liver and adipose tissue. The contribution of exogenous fatty acids to the fatty acids found in glycerides and other complex tissue lipids is well known [23]; as shown here the *variety* of carbohydrate is also important. Furthermore, if a single carbohydrate is chronically consumed it need not represent $^3/_4$ of the total calories to exert an influence on the distribution of the saturated and unsaturated fatty acids present in the liver and adipose tissue.

A comparable study of the influence of dietary carbohydrate on the composition of tissue fatty acids was reported by MACDONALD in 1962 [24]. He reported a greater amount of liver lipid accumulated when sucrose rather than maize starch or a starch hydrolysate was fed. He reported further that both the adipose tissue and liver fatty acids varied with the variety of carbohydrate. There were several differences between his and our study; he provided three different carbohydrates to groups of protein-starved rabbits all of whom lost weight and suffered high mortality, and no separation was made of the major lipid classes from the liver prior to fatty acid analysis. Nevertheless, both MACDONALD's and the present studies establish that the lipid response in two different species is related to the type of carbohydrate consumed.

There are many possible mechanisms by which carbohydrates might influence lipid metabolism. Three of these might be mentioned here. One possibility, already noted, is an alteration in the intestinal flora. The phenomenon of diet-induced changes in the species comprising the fecal bacterial mass has been known for at least forty years [25, 26], and redemonstrations of this phenomenon with novel experimental diets continue to appear [27]. Alteration of the flora might influence the absorption of the fecal sterols, by virtue of the bacterial conversion of the sterols to non-absorbable varieties [28].

Another possibility is suggested by the studies of VAGELOS and others [29]. Fatty acid (palmitate) biosynthesis has been found to be stimulated by several tricarboxylic acid cycle intermediates, but particularly citrate. Citrate appears to produce this effect by altering certain physical characteristics of an enzyme, acetyl-CoA carboxylase, which is responsible for the initial carboxylation of acetyl-CoA to malonyl-CoA; this facilitates the subsequent enzymatic reactions of fatty acid synthesis. It is possible that various dietary carbohydrates might increase the availability of Krebs-cycle or other intermediates potentiating fatty acid synthesis, or that the individual carbohydrates might also influence the enzymes responsible for fatty acid synthesis directly and thereby determine the fatty acid which will be formed. The peculiar localization of carbohydrates such as fructose in the semen and allantoic fluid, glycogen in muscle and liver, and lactose in the lactating breast, may well serve a role in directing the variety of fatty acids which are synthesized in these organs. These data certainly suggest that the study of *in vitro* fatty acid synthesis in the presence of a variety of carbohydrates might be of interest.

A third possibility to explain the appearance of unique fatty acids on carbohydrate feeding is that there are impurities in the refined and chemically pure carbohydrates which are fed. For example, there is evidence from the present study that despite the obvious similarities, recrystallized purified large crystal sucrose produces different effects on lipid metabolism from those produced by the feeding of ordinary commercial granulated sugar available to the general population. This suggests that impurities are present in one or both which account for these differences.

There is one additional point. Lipids comprise a major part of the membranes of the cells, including the endoplasmic reticulum and the many intra-cytoplasmic organelles. It is likely that the metabolic activity of each membrane is influenced by its structure.

While the knowledge of the structure-function relationships of membranes is still in a primitive state, it is known that the fatty acid composition of the mitochondrion influences its gross appearance and its ability to synthesize ATP [30]. Thus, if the dietary carbohydrate influences the complex lipids comprising the membranes of such organelles, it is likely that the functioning of these organelles will also be changed. This may be a means by which cellular adaptation to nutrients in the diet may occur.

Summary

The influence of the consumption over a period of eighteen months of eight different dietary carbohydrates on the rats' growth rate, water consumption, stool output, organ size, serum lipids, liver lipid distribution, and liver lipid and adipose tissue fatty acid composition was studied. Generally speaking, glucose- and fructose-containing carbohydrates supported weight gain more adequately than galactose-containing carbohydrates which in turn were more efficient than pectin. Weight gain was found to be inversely related to water consumption and the dried stool output. Relative liver and testes weights were not comparably influenced by the different carbohydrates. There was no correlation of serum lipid levels and weight gain, but of those carbohydrates associated with the greatest weight gain, cerelose (glucose) consumption was associated with the lowest serum lipids. The distribution of some of the major lipid classes in the liver was markedly effected by dietary carbohydrate variety, as was the fatty acid composition of adipose tissue and liver.

Acknowledgements

The authors are indebted to Mrs. J. DJORDJEVICH for aid in preparing this manuscript, to Mr. C. HILL for his technical assistance, to Dr. RICHARD J. JONES and Mr. LAWRENCE FRAZIER for many valuable contributions.

References

1. DUELL, H. J. JR.: The Lipids, vol. II, p. 538 (Interscience Press, New York 1955).
2. LONGENECKER, H. E. Desposition and utilization of fatty acids. I. Fat synthesis from high carbohydrate and protein diets in fasted rats. J. biol. Chem. *128:* 645 (1939).
3. MACDONALD, I.: The lipid response of young women to dietary carbohydrates. Amer. J. clin. Nutr. *16:* 458 (1965).
4. COHEN, L.; BATRA, K. V. and JONES, R. J.: Determination of total, ester and free cholesterol in serum and serum lipoproteins. Clin. chim. Acta *6:* 613 (1961).
5. ALBRINK, M. J.: The microtitration of total fatty acids of serum with notes on the estimation of triglycerides. J. lipid. Res. *1:* 53 (1959).
6. FILLERUP, D. L. and MEAD, J. F.: Chromatographic separation of the plasma lipids. Proc. Soc. exp. Biol., N.Y. *83:* 574 (1957).
7. AHMED, M. E.; BLACK, L. M.; PERKINS, E. G.; WALKER, B. L. and KUMMEROW, F. A.: Lipid in potato yellow dwarf virus. Biochem. biophys. Res. Comm. *17:* 103 (1964).
8. WITTING, L. A.; HARVEY, C. C.; CENTURY, B. and HORWITT, M. K.: Dietary alteration of fatty acids of erythrocytes and mitochondria of brain and liver. J. lipid. Res. *2:* 412 (1961).
9. STAHL, E.: Thin layer chromatography, p. 147 (Academic Press, New York 1965).
10. FISHER, H.; GRIMINGER, P. and WEISS, H. S.: Avian atherosclerosis: Retardation by pectin. Science *146:* 1063 (1964).
11. NUGTEREN, D. H.: Conversion *in vitro* of linoleic acid into γ-linolenic acid by rat liver enzymes; in Biochemical Problems of Lipids, vol. I, p. 460 (Elsevier, New York 1963).
12. GETZ, G. S.; BARTLEY, W.; STIRPE, F.; NOTTON, B. M. and RENSHAW, A.: The lipid composition of rat liver. Biochem. J. *80:* 176 (1961).
13. YUDKIN, J.: Dietary fat and dietary sugar in relation to ischaemic heart disease and diabetes. Lancet *II:* 4 (1964).
14. REUSSNER, G. JR.; ANDROS, J. and THUISSEN, R. JR.: Studies on the utilization of various starches and sugars in the rat. J. Nutr. *80:* 291 (1963).
15. HOWE, E. E. and GILFILLAN, E. W.: Protein, carbohydrate and fat content of the diet of the rat as related to growth. J. Nutr. *79:* 395 (1963).
16. CHANG, VET-OY: Effect of carbohydrates on utilization of protein and lysine by rats. J. Nutr. *78:* 21 (1962).
17a. GAMBLE, J. L.: The water requirements of castaways. Proc. Amer. Phil. Soc. *88:* 151 (1944).
17b. Nutrition Reviews. *20:* 171 (1962); *22:* 102 (1964).
18. AHRENS, E. H. JR.: Nutritional factors and serum lipid levels. Amer. J. Med. *23:* 928 (1957).
19. GOFMAN, J. W.: Diet in the prevention and treatment of myocardial infarction. Amer. J. Cardiol. *1:* 271 (1958).
20. COHEN, L.; DOBRILOVIC, L. and JONES, R. J.: Disparate changes in lipoprotein lipid with the substitution of dietary carbohydrate for animal fat. J. lab. clin. Med. *58:* 893 (1961).
21. JONES, R. J. and COHEN, L.: Chemistry and therapy of chronic cardiovascular diseases (C. C. Thomas, Springfield 1961).
22. KRITCHEVSKY, D.; KOLMAN, R. R.; GUTTMACHER, R. M. and FORBES, M.: Influence of dietary carbohydrate and protein on serum and liver cholesterol in germ-free chickens. Arch. Biochem. *85:* 444 (1959).
23. HIRSCH, J. and GOLDRICK, R.: Studies on composition and metabolism of adipose tissue in man; in ROBERTS' Fat as a Tissue, p. 314 (McGraw-Hill, New York, 1962).

24. MACDONALD, I.: Some influences of dietary carbohydrate on liver and depot lipids. J. Physiol. *162:* 334 (1962).
25. BERTHER, L. F.: Bacteria of the intestinal tract; in JORDAN's The Newer Knowledge of Bacteriology and Immunology, p. 639 (University of Chicago Press, Chicago 1928).
26. GYORGI, P.: Bacterial symbiosis in the gastrointestinal tract; in BROCK's Recent Advances in Human Nutrition, p. 252 (Little, Brown and Co., Boston 1961).
27. GRABER, C. D.; O'NEAL, R. M. and RABIN, E. R.: Effect of high fat diets on intestinal microflora and serum cholesterol in rats. J. Bact. *89:* 47 (1965).
28. JONES, R. J.; REISS, O. K. and GOLDEN, M. F.: Influence of a brain extract upon cholesterol metabolism. International Symposium on Drugs Affecting Lipid Metabolism (Elsevier, Amsterdam 1964).
29. VAGELOS, P. R.; MARTIN, D. B.; GOLDMAN, P. and ALBERTS, A. W.: Fatty acid biosynthesis and its control; in RODAHL's Fat as a Tissue, p. 149 (McGraw-Hill, New York 1962).
30. ITO, T. and JOHNSON, R. M.: Effects of a nutritional deficiency of unsaturated fats on rat liver mitochondria. J. biol. chem. *239:* 3201 (1964).

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Discussion

D. KRITCHEVSKY (Philadelphia, Pa.): It is well documented that sucrose is more cholesteremic than glucose in cholesterol-fed rats, rabbits and chickens. Your data suggest that, in long-term feeding, sucrose retains its hypercholesteremic effect even in the absence of dietary cholesterol. We have fed chickens cholesterol plus various combinations of carbohydrates (glucose, fructose, sucrose, lactose, glucose plus fructose and sorbitol). Over a 30 day period of study, sucrose is consistently the most hypercholesteremic carbohydrate. This result has been observed in several different experiments.

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Effects of N-cyclohexyl linoleamide (Linolexamide) on Experimental Atherosclerosis in Rabbits

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During the course of an investigation on cholesterol-lowering agents, N-cyclohexyl linoleamide (Linolexamide[1]) was found to have a remarkable blood cholesterol-lowering effect on experimental hypercholesterolemia in laboratory animals. The present study deals with the effect of Linolexamide on experimental hypercholesterolemia and vascular lesions in cholesterol-fed rabbits, and an attempt is also made to compare its effects with those of linoleic acid, ethyl linoleate and safflower oil under similar experimental conditions.

Linolexamide was prepared from purified linoleic acid and cyclohexylamine. The physical constants and analytical data are shown in Table I. Linoleic acid used in this study was purified from natural sources, and ethyl linoleate was prepared from this linoleic acid in our laboratory.

Male white rabbits, weighing 2 to 3 kg, were housed in screen-bottomed individual cages and given 50 g of commercial rabbit pellet chow and 20 g of a cholesterol-containing diet, which consisted of 8% of cholesterol, 32% of hydrogenated coconut oil and 60% of commercial diet, daily for a week. The animals were then divided into 8 groups of 6 or 8 animals each, so that the distribution of plasma cholesterol levels was comparable among the groups. During the following 9 weeks, the animals of each group received 50 g of commercial diet and 20 g of cholesterol diet supplemented with the test materials shown in Table II. Plasma total cholesterol

[1] Suggested international non-proprietary name.

50

EXPERIMENTAL AND MOLECULAR PATHOLOGY 2, 74-82 (1963)

Experimental Eosinophilia IV. Eosinotactic Influences of Polysaccharides[1]

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INTRODUCTION

Although the association of eosinophilic granular cell responses with hypersensitivity reactions and antigen-antibody union (Samter *et al.*, 1953; Litt, 1960; Cohen *et al.*, 1961) is a well documented phenomenon, eosinophilia may arise from chemotactic stimuli not dependent upon immune mechanisms (Godlowski, 1951; Archer, 1960). It was therefore of interest to observe selective eosinophil cell influences effected by polysaccharides administration in the rabbit. In only some of these instances could the existence of natural conditions that might allow for the possible participation of responsible polysaccharides in immune pathways be demonstrated.

MATERIALS AND METHODS

The basic experimental procedure was based upon the demonstration of cellular responses within regional lymph nodes draining subcutaneous sites of induced inflammation.

One hundred and twenty-four male albino rabbits weighing 2 to 2.5 kg were divided into experimental groups of five or six with the members of each receiving one of sixteen substances described below. All injections of these materials in either aqueous or saline solution or suspension were given in total volumes of 0.5 ml directly into the foot pad of one hind extremity. Four hours later the rabbit was sacrificed by air embolism and the popliteal lymph nodes from both the treated and untreated hind extremities were immediately exposed, removed and prepared for histologic study. Tissue sections were routinely stained with hematoxylin and eosin. Giemsa stain and Undritz's modification II of the peroxidase reaction (Undritz, 1952) were additionally employed for selective granule staining and verification of eosinophil cell identification.

The absence of protein contaminants in polysaccharides was ascertained by negative test results with biuret reagent and with potassium ferrocyanide. The presence or absence of polysaccharide components in experimental materials was determined by test procedures involving acid hydrolysis and reduction of copper sulfate.

SUBSTANCES ADMINISTERED

I. Glycogen

A. Forty-eight rabbits each received an aqueous solution of 10 mg of oyster glycogen (Nutritional Biochemicals—N.B.). Of these, six received only the glycogen.

[1] This study was supported (in part) by research grants from the Northeastern Pennsylvania Heart Association and the National Heart Institute (Grant No. H-3890), United States Public Health Service.

The remaining forty-two rabbits were divided into groups of six and each group additionally received one of the following chemical agents intravenously (iv) immediately prior to the glycogen and at 1 hour intervals thereafter and/or incorporated into the glycogen solution given directly into the foot pad (fp).

1. Antihistaminic agent: Tripelennamine (Pyribenzamine, Ciba), 1 mg/kg iv and 1 mg fp.
2. Inhibitors of histamine liberation:
   (a) Disodium ethylenediamine tetraacetic acid (EDTA—Endrate Disodium, Abbott), 15 mg fp.
   (b) Iodoacetic acid (N.B.), 0.5 mg fp.
3. Antihistamine-antiserotonin agent: Promethazine (Phenergan, Wyeth), 1 mg/kg iv and 1 mg fp.
4. Antiserotonin agent: methyl lysergic acid butanol amide tartrate (UML-491, Sandoz), 1.5 mg/kg iv and 1 mg fp.
5. Antiheparin agents: 3 mg/kg iv and 3 mg fp.
   (a) Protamine (Lilly).
   (b) Hexadimetrine bromide (Polybrene, Abbott).

B. One group of six rabbits each received 10 mg of glycogen of rabbit liver origin (N.B.).

II. Other Polysaccharides

Five rabbits each received 10 mg of one of the following polysaccharides:

1. Amylodextrin, soluble wheat starch (N.B.).
2. Amylopectin, potato origin (N.B.).
3. Inulin, dahlia tuber origin (N.B.).
4. Laminarin, prepared from algae.[2]
5. Dextran, mol. wt. 60,000–90,000 (N.B.).
6. Dextran, mol. wt. 5–40 million (N.B.).

III. Monosaccharides

Five rabbits each received 10 mg of dextrose and 5 received 10 mg of levulose.

IV. Other Histamine-Releasing Agents (Van Arsdel and Beall, 1960):

Each of five rabbits received 10 mg of one of the following:

1. Acacia, Gum Arabic.
2. Polyvinylpyrrolidone (Mann Biochemicals).
3. Polymyxin B (Pfizer).
4. Stilbamidine Isothionate (Delta Chemical).
5. Peptone (Bacto Peptone, Difco).
6. Trypsin (National Drug).

Serologic Studies

Sera from normal animals were studied for possible content of both naturally occurring or preformed antibody to the injected polysaccharides and cross-reacting blood group isoagglutinins.

[2] Prepared and kindly supplied by Dr. N. R. DiLuzio, Department of Physiology, University of Tennessee Medical Units, Memphis, Tennessee.

I. *Precipitation Reaction Tests*

Tests for precipitation reactions between serum and polysaccharides by agar diffusion were carried out in 3 mm glass tubes according to the method of Preer (1956). Where precipitin band formation was demonstrated tests for reactions of identity between the polysaccharide and blood group substances, A (hog stomach)[3] and B (horse stomach)[3], by immunodiffusion according to the method of Ouchterlony (1953) were carried out.

II. *Absorption and Elution Techniques*

Sera demonstrating (a) precipitation reactions with a polysaccharide and (b) anti-A erythrocyte agglutinating activity were subjected to absorption and elution techniques with human blood group A and group B erythrocytes. The modification of the Landsteiner-Miller procedure described by Springer *et al.* (1959) was employed. Three-tenths milliliter of fresh, three times washed, packed A or B cells were added to 1 ml of serum subjected to previous absorption with group O cells. The serum and erythrocytes were kept at room temperature for 2 hours with frequent shaking. The erythrocytes were then centrifuged and washed four times. Five-tenths milliliter saline was added to the cells and the mixture shaken at 56° C for 15 minutes. Eluate-containing supernatants were removed after centrifugation and immediately employed in diffusion tests with the polysaccharide.

III. *Hemagglutination Inhibition Tests*

Normal rabbit sera found to have agglutinins against human blood group A erythrocytes were employed in standard hemagglutination inhibition tests according to the method described by Kabat (1961). Serum samples each containing 10 hemagglutinating units were added to varying dilutions of a polysaccharide test solution, mixed, and held at 37° C for 30 minutes prior to the addition of group A cells.

IV. *Polysaccharide Granule Aggregation*

Normal sera were studied for their ability to induce aggregate formation of polysaccharide granules according to the method described by Nelson (1958). Ten milligrams of the test polysaccharide was added to 1 ml saline and 0.5 ml serum and kept at 37° C for 1 hour with frequent shaking. Agglutination or granule clumping was determined by microscopic examination.

V. *Hemagglutinating Antibody Tests*

Small amounts of serum antibody not capable of detection by precipitin test procedures were sought by hemagglutination techniques. The polysaccharides were adsorbed to group O treated human erythrocytes at 37° C according to the method described by Kabat (1961). To 0.5 ml quantities of varying dilutions of the sera were added 0.2 ml of washed polysaccharide-treated cells. These suspensions were kept at 37° C for 2 hours and hemagglutination determined both macro- and microscopically.

[3] Kindly supplied by Dr. Kenneth C. Robbins of the Michael Reese Research Institute, Chicago, Illinois.

RESULTS

Eosinophilia

Eosinophilic granular cell infiltrations found in serial sections of popliteal lymph nodes occupied both peripheral, cortical and medullary sinuses. These consisted of diffuse uniform cell accumulations occupying portions of and often entire sinuses (Fig. 1). Similar histologic involvement was never observed within the popliteal lymph nodes draining contralateral untreated foot pad sites. Deposits of glycogen or starch could not be demonstrated within involved areas by means of Best's carmine or iodine histochemical staining techniques.

Results of histologic examinations of the popliteal nodes of members of those groups receiving a polysaccharide, a monosaccharide, or histamine-liberating agent are summarized in Table I. The lymph nodes of all animals receiving glycogen exhibited similar degrees of eosinophil granular cell involvement regardless of the simultaneous administration of any chemical agent.

Serologic Studies

I. *Precipitin Tests*

Results of agar gel double-diffusion tests are given in Table II.

TABLE I

The Number of Rabbits Demonstrating Eosinophilic Granular Cell Infiltrations into the Cortical and Medullary Sinuses of Popliteal Lymph Nodes

Substance administered[a]	No. rabbits[b] exhibiting eosinophilia		
	Negative	Equivocal	Diffuse infiltrations
Glycogen			
(a) oyster			6
(b) rabbit liver			6
Starch			
Amylodextrin			5
Amylopectin			5
Inulin			5
Laminarin			5
Dextran			
(a) Low mol. wt.	4	1	
(b) High mol. wt.			5
Dextrose	5		
Levulose	3	2	
Acacia		5	
Polymyxin B		5	
Polyvinylpyrolidone	5		
Stilbamadine	5		
Peptone	5		
Trypsin	5		

[a] Members of each group received 10 mg of one of the polysaccharides, monosaccharides, or histamine-liberating agents by foot pad injection.

[b] Each of the glycogen-treated groups was comprised of 6 rabbits. Groups of 5 rabbits each received one of the other listed agents.

FIG. 1. Popliteal lymph node sections taken from a rabbit that had received an ipsilateral foot pad injection of 10 mg of amylodextrin. Hematoxylin and eosin. (A) Accumulations of eosinophilic granular leucocytes (darker shaded cells) completely occupy the peripheral cortical lymph sinus, × 250. (B) Eosinophilic granular cells are seen interspaced among lymphoid cellular elements within portions of a medullary sinus, × 970.

II. Absorption and Elution Tests

Absorption of normal rabbit sera with group O and A erythrocytes did not effect any change in their originally-demonstrated capacity to precipitate with amylodextrin, amylopectin, and laminarin. Saline eluates of serum-treated group A erythrocytes: (1) did not precipitate with amylodextrin and laminarin, but (2) did develop precipitate bands with amylopectin in two instances. Negative results were obtained with group O and B cell eluates.

III. Hemagglutination Inhibition Tests

The polysaccharides were ineffective in reducing anti-A hemagglutinating capacity of the normal rabbit serum samples. In only one instance this was accomplished with amylodextrin and in a minimum concentration of 1 mg per ml.

IV. Polysaccharide Granule Agglutination

Granule aggregate formation was noted only with starch amylodextrin and starch amylopectin when treated with normal rabbit sera.

V. Hemagglutinating Antibody Tests

Normal rabbit sera that failed to demonstrate precipitation with glycogens, inulin, laminarin, and dextrans also failed to agglutinate group O erythrocytes coated with these respective polysaccharides.

TABLE II

NUMBER OF NORMAL SERA WITHIN EACH GROUP OF 5 RABBITS THAT DEMONSTRATED PRECIPITATE BAND FORMATION IN AGAR WITH THE CORRESPONDING SUBSTANCE PRIOR TO ITS FOOT PAD INJECTION

Substance	Precipitate band formation	
	No. rabbit sera	No. bands
Glycogen		
(a) oyster	0	0
(b) rabbit liver	0	0
Starch		
Amylopectin	3	2
Amylodextrin	5	4
Inulin	0	0
Laminarin	3	2
Dextran		
(a) low mol. wt.	0	0
(b) high mol. wt.	0	0
Acacia	2	2
Polymyxin B	3	3

DISCUSSION

The source of eosinophilic granular cells occupying regional lymph node sinuses was not apparent from histologic study. Possibilities include transport by hematogenous routes, by lymphatic drainage from primary sites of inflammatory stimulation, or both. Eosinophils were observed in both subcutaneous sites of treated foot pads and the lumen of small blood vessels within the popliteal nodes.

The ability of crude liver extract to influence eosinophil cell responses apart from a demonstrable role of immune reactant has been reported (Dzury and Cohen, 1958). That this may be related to its glycogen content is of interest since this polysaccharide has resembled antigen-antibody precipitate by other parameters of physiologic activity (Rocha e Silva, 1950; Waalkes and Coburn, 1959, 1960) These have included leukocyte and platelet-effects and histamine-, and serotonin-release. However, like antigen-antibody union (Cohen *et al.*, 1961) the eosinotactic inflammatory influences of glycogen were not blocked by inhibitors of histamine-release or histamine-, serotonin-, or heparin-chemical antagonists. It is also noteworthy that similar histopathologic alterations could not be duplicated by non-polysaccharide macromolecular or colloidal histamine-liberating agents.

Eosinotactic effects also resulted from the administration of amylodextrin, amylopectin, inulin, laminarin, and high molecular weight dextran. This could not be related to any uniformity of chemical structure. Though the molecular weight range of these substances varies from only 2600 (laminarin) and 5000 (inulin) to 5–40 million (dextran), other dextrans of 60,000–90,000 mol. wt. were ineffective. The polyglucoses of glycogen, amylodextran, amylopectin, and dextrans are linked at 1, 4, and 6 positions and laminarin at 1 and 3, while inulin is a polyfructose. Comparable cellular inflammatory responses were not effected by monosaccharide components in equivalent total dosages.

Since antigen-antibody union can play an etiological role in eosinophilia (Samter *et al.*, 1953; Litt, 1960; Cohen *et al.*, 1961) it was of considerable interest to note that amylodextrin and in some instances amylopectin and laminarin reacted with normal serum in agar diffusion. However, this was not found to be the case for glycogen, inulin, and dextran. Cross-reactions of glycogen (Heidelberger *et al.*, 1954) and amylopectins (Kabat and Mayer, 1961) with antisera to bacterial fractions have been demonstrated, yet there is no evidence for their ability to stimulate antibody formation. In a study of reactions of laminarin with normal serum differences in the nature of resultant precipitates from those of true polysaccharide antigen-antibody systems were noted (Allen and Kabat, 1957). Therefore, it is not clear whether all of these precipitation and granule agglutination reactions indicate valid instances of preformed serum antibodies to polysaccharide.

That normal sera could contain precipitating antibody to polysaccharides might be explained on the basis of prior exposure either through diet or to antigenically related infecting parasitic helminths or bacteria (Kabat, 1961; Heidelberger *et al.*, 1954; Springer *et al.*, 1961). The presence of blood group antigen in rabbits related to human group A (Wheeler *et al.*, 1939) suggested the possibility of polysaccharide-participation in cross-reactions with isoagglutinins. However, evidence for this based on data obtained from serologic tests has been inconclusive.

In vivo conditions favorable for possible participating antigenic roles in reactions with some normal sera have been demonstrated for amylodextrin and amylopectin. However, there was no apparent relationship noted between other eosinotactic polysaccharides and their ability to react with serum of individual animals. Furthermore, preformed precipitins for polymyxin B were noted in sera taken from some animals fed antibiotic-containing pellets. Yet this polypeptide-containing, carbohydrate-free material evoked only equivocal degrees of eosinophilia.

Whether chemotactic or immunologic mechanisms may explain the etiology of polysaccharide-influenced eosinophilic cellular inflammatory responses can be a matter only for speculation in the light of this experimental data.

SUMMARY

The administration of heterologous and homologous species glycogen, soluble starch amylodextrin, starch amylopectin, laminarin, inulin, or high molecular weight dextran into the rabbit foot pad resulted in 4-hour eosinophilic granular cell infiltrations into the sinuses of regional popliteal lymph nodes. Similar histologic alterations could not be demonstrated with component monosaccharides or with other non-carbohydrate macromolecular histamine-releasing agents including acacia, stilbamadine, peptone, trypsin, polyvinylpyrrolidone, or polymyxin B.

The eosinotactic effects of glycogen were studied through the use of chemical inhibitors and antagonists. These effects could not be related to release of histamine, serotonin, or heparin.

Precipitation of amylodextrin and amylopectin by normal sera suggests the possibility of participation of some polysaccharides in immune reactions with preformed antibody. However, the eosinotactic effects of polysaccharides could not be related to any delineated immunologic phenomena or chemotactic action.

ACKNOWLEDGMENT

Arlene R. Gallia and Louise T. Gatto, student laboratory assistants, participated in the conduct of experimental procedures. Students were aided (in part) through National Science Foundation Undergraduate Research Participation Grant (No. NSF-G 16064).

REFERENCES

Allen, P. Z., and Kabat, E. A. (1957). Studies on the capacity of some polysaccharides to elicit antibody formation in man. *J. Exptl. Med.* **99**, 383-394.

Archer, R. K. (1960). Eosinophil leucocyte-attracting effect of histamine in skin. *Nature* **187**, 155-156.

Cohen, S. G., Kantor, M., and Gatto, L. (1961). Experimental eosinophilia III. Regional lymph node responses to reactions of tissue sensitization. *J. Allergy* **32**, 214-222.

Dzury, D. S., and Cohen, S. G. (1958). Experimental eosinophilia. studies on the origin and relationship of tissue eosinophil cells to peripheral blood eosinophils. *J. Allergy* **29**, 340-357.

Godlowski, Z. Z. (1951). Prevention of hormonal eosinopenia and lymphopenia by inhibition of clotting in blood. *Brit. Med. J.* **1**, 854-855.

Heidelberger, M., Aisenberg, A. C., and Hassid, W. Z. (1954). Glycogen, an immunologically specific polysaccharide. *J. Exptl. Med.* **99**, 343-353.

Kabat, E. A., and Mayer, M. M. (1961). "Experimental Immunochemistry." 2nd ed., pp. 127, 119, 415-416. Charles C Thomas, Springfield, Illinois.

Litt, M. (1960). Studies in experimental eosinophilia II. Induction of peritoneal eosinophilia by the transfer of tissues and tissue extracts. *Blood* **16**, 1330-1337.

Nelson, A. R., Jr. (1958). An alternative method for the properidin system. *J. Exptl. Med.* **108**, 515-535.

Ouchterlony, Ö. (1953). Antigen-antibody reaction in gels IV. Types of reactions in coordinated systems of diffusion. *Acta Pathol. Microbiol. Scand.* **32**, 231-240.

Preer, J. R., Jr. (1956). A quantitative study of a technique of double diffusion in agar. *J. Immunol.* **77**, 52-60.

Rocha e Silva, M. (1950). The role played by leukocytes and platelets in anaphylactic and peptone shock. *Ann. N.Y. Acad. Sci.* **50**, 1045-1067.

Samter, M., Kofold, M. A., and Pepper, W. (1953). A factor in anaphylactically shocked guinea pigs which can induce eosinophilia in normal animals. *Blood* **8**, 1078-1090.

Springer, G. F., Horton, R. E., and Forbes, M. (1959). Origin of antihuman group B agglutinins in white leghorn chickens. *J. Exptl. Med.* **110**, 221-244.

Springer, G. F., Williamson, P., and Brandes, W. C. (1961). Blood group activity of gram-negative bacteria. *J. Exptl. Med.* **113**, 1077-1093.

UNDRITZ, E. (1952). "Sandoz Atlas of Haemotology." P. 28. Sandoz, Basle, Switzerland.

VAN ARSDEL, P. P., JR., and BEALL, G. N. (1960). The metabolism and functions of histamine. *Arch. Intern. Med.* **106**, 714-723.

WAALKES, T. P., and COBURN, H. (1959). Comparative effects of glycogen and antigen-antibody reactions on serotonin and histamine in the rabbit. *Proc. Soc. Exptl. Biol. Med.* **101**, 122-125.

WAALKES, T. P., and COBURN, H. (1960). Studies on the relationship of the release of serotonin and histamine by chemical means to anaphylaxis in the rabbit. *J. Allergy* **31**, 395-407.

WHEELER, K. M., SAWIN, P. B., and STUART, C. A. (1939). Group-specific agglutinins in rabbit serums for human serums V. Inheritance of the A character. *J. Immunol.* **36**, 349-359.

54

THE OCCURRENCE OF A CALCINOSIS SYNDROME IN THE COTTON RAT[1]

I. THE EFFECT OF DIET ON THE ASH CONTENT OF THE HEART

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ONE FIGURE

(Received for publication January 24, 1952)

INTRODUCTION

A colony of cotton rats *(Sigmondon hispidus hispidus)* is maintained in this laboratory for use in a study of the effect of nutrition on the production of dental caries. During the past three years autopsy of the experimental animals has shown that this species, as raised in our colony, is affected by a calcinosis syndrome which is primarily associated with the consumption of partially purified diets. The lesions observed in our cotton rats are similar to those recently described by Harris and Wulzen ('50) in guinea pigs fed diets deficient in the anti-stiffness factor. Since many of the dietary factors which modify the course of the syndrome in guinea pigs behave similarly with cotton rats, it is felt that a report of our studies may help elucidate the cause of the calcinosis syndrome found in the two species.

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We are indebted to Merck and Co., Rahway, N. J., for the crystalline vitamins; to Abbott Laboratories, North Chicago, Ill., for the halibut liver oil; and to the Wilson Laboratories, Chicago, Ill., for the 1: 20 liver extract used in these experiments.

Source of the experimental animals

There are only a few laboratories in this country which use cotton rats, and they have not reported the occurrence of similar pathology in their animals. Therefore, a brief consideration of the pertinent facts concerning the management of the stock colony is included in this report. The original diet fed to the stock colony from 1944 to 1949 consisted primarily of dehydrated commercial dog food[2] (table 1). After the supply of this diet was exhausted, several different

TABLE 1

Composition of stock colony diets

DIET 1 — 1944 TO 1949	%	DIET 2 — 1949 TO 1951[2]	%
"Ideal" dog food	71.5	Whole yellow corn	30
Yellow corn	11.0	Wheat germ flakes	10
Casein	7.0	Linseed oil meal	18
Alfalfa leaf meal	7.0	Alfalfa leaf meal	15
Corn oil[1]	3.5	Skim milk powder	15
		Sodium chloride	1
DIET 3 — 1951		Meat scraps	7
Rockland guinea pig pellets		Corn oil	4

[1] Occasionally included 0.6% wheat germ oil.

[2] Diet 2 contains approximately 50% carbohydrate, 24% protein, 7% fat, 1.1% calcium, 0.9% phosphorus.

diets were used, with particular attention being given to the mineral and vitamin content. The composition of the second diet, which was used from approximately 1949 to 1950, is given in table 1. During these trial periods it was noted that guinea pig pellets were quite acceptable to cotton rats, and these have been used since the latter part of 1950. These pellets have maintained an excellent reproduction performance in the colony.

Stock colony animals which ranged in age from 6 months to one and one-half years have been routinely autopsied. Although these animals occasionally showed the presence of

[2] Ideal.

pathological changes in the kidney, liver, and uterine horns, the cardiac and skeletal muscles were normal. These tissues, especially the cardiac muscle, became progressively calcified in our weanling cotton rats which consumed diets of partially purified foods (Constant et al., '52).

EXPERIMENTAL AND RESULTS

Weanling cotton rats were housed in individual wire cages and received food and water ad libitum. They were given two drops of haliver oil weekly.

The basal diet consisted of 24% vitaminized casein,[3] 67% sucrose, 4% salts IV,[4] and 5% corn oil. Modifications of this diet were made by altering the sucrose level. This diet (for purposes of brevity referred to as "high Mg basal") contained 0.56% calcium, 0.48% phosphorus and 0.040% magnesium. Data from representative experiments, which show the varied effect of diet on the development of the calcinosis syndrome, as represented by the ash content of the heart, and on growth, are summarized in table 2 and figure 1.

The basal diet supplemented with 4% 1:20 liver extract provided adequate levels of the nutrients required by cotton rats for growth, as recently reviewed by Schweigert ('48), and resulted in weight gain equivalent to that obtained when the stock diet was fed (McIntire et al., '44; Schweigert, '48). Our animals grew well when fed the basal diet with liver extract (fig. 1F) but in 7 to 10 weeks their hearts invariably showed grossly observable pathological changes and a high ash content of 9 to 19% on a fat-moisture-free basis (table 2). When the calcium level was reduced to 0.3% or 0%, the ash

[3] Provides the following levels of B vitamins/kg of diet: 3.8 mg thiamine hydrochloride, 3.8 mg pyridoxine hydrochloride, 4.5 mg riboflavin, 38 mg nicotinamide, 30 mg calcium pantothenate, 1.5 gm inositol, 1.5 gm choline, 450 mg para-aminobenzoic acid.

[4] Hegsted et al., J. Biol. Chem., *138*: 459, 1941. Salts IV at 4% provides 40 mg % of magnesium, 0.56% calcium, and 0.2% phosphorus. By combining the regular salts IV with Mg-free salts IV, it was possible to feed any level of magnesium desired and maintain the same amounts of the other ions.

Casein, at the 24% level, supplies approximately 0.03% Ca and 0.2% P.

TABLE 2

Effect of diet on the ash content of the heart

RATION FED	WEEKS ON EXPER.	NO. OF ANIMALS	% ASH [1] (range)	Ca, P, and Mg levels in diets		
				Ca	P	Mg
				%	%	%
High mg basal with L.E.[2]	7–10	8	9–19	0.58	0.48	0.04
—— (0.3% Ca)	7–10	4	5–6	0.30	0.48	0.04
—— (0 % Ca)	7–10	9	4–6	0.02	0.48	0.04
—— minus L.E.	3–5	6	6–16	0.58	0.48	0.04
Intermediate mineral basal	5	3	6–7	0.32	0.34	0.02
Low mg basal — sucrose	3–5	8	23–36	0.58	0.48	0.01
—— + 10% casein	3–5	3	8–19	0.58	0.56	0.01
—— + 6% gelatin	3–5	3	20–25	0.58	0.48	0.01
—— (0.3% Ca)	4	3	16–25	0.30	0.41	0.01
Low mg basal — dextrin	5–6	11	8–21	0.58	0.48	0.01
—— + vit. E	5–6	4	11–14	0.58	0.48	0.01
—— + B vitamins	5–6	4	5–14	0.58	0.48	0.01
Low mineral basal	11	5	3–5	0.03	0.27	0.01
—— + $CaCl_2$	11	5	6–13	0.32	0.27	0.01
—— + $Ca(NO_3)_2$, 1% NaCl	11	4	7–9	0.32	0.27	0.01
—— + $CaCO_3$	11	5	6–24	0.32	0.27	0.01
Oatmeal diets 1 and 2[3]	5–6	15	3–6	0.03	0.28	0.07
Oatmeal — milk	11	7	5–6	0.50	0.64	0.07

[1] Moisture and fat-free basis.
[2] Four per cent 1:20 liver extract.
[3] Includes ash data from animals fed oatmeal diet 2 plus 0.4 or 0.8% calcium as $CaCO_3$ or 0.44% P (3 litter mates/group).

content of the hearts was low, 4 to 6%. The femur ash content of animals fed 0.56%, 0.3% or 0% calcium ranged from 66 to 69%, 64 to 65%, and 57 to 61%, respectively. The minerals mobilized from the bones of the low calcium group were not

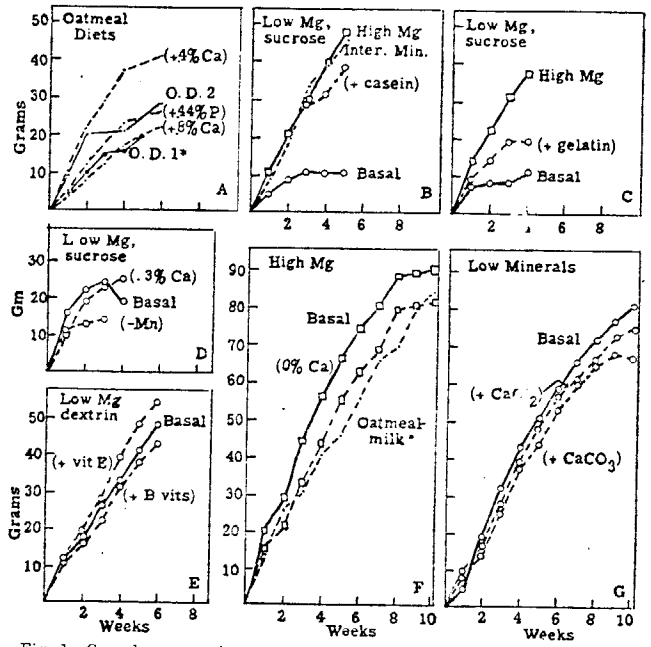

Fig. 1 Growth curves of cotton rats fed oatmeal or partially synthetic diets. (Figs. A–G contain data from representative experiments and, with the exception of the * curves, comparison is made only between litter mates.)

redeposited in the soft tissues. Animals fed the basal diet without liver extract grew well (figs. 1B and 1C) and had 6 to 16% heart ash content in three to 5 weeks. However, reducing the salts IV level from 4% to 2% (intermediate mineral basal — 0.3% Ca, 0.02% Mg) resulted in a low heart ash content of 6 to 7% despite the fact that the magnesium

content of the diet was similarly lowered. These experiments showed that although the basal diet was adequate for the growth of cotton rats, it did not prevent the development of calcinosis. Liver extract at the 4% level did not prevent the lesion. A calcium level of 0.3% and a Ca : Mg ratio of 7.5 : 1 prevented the syndrome for at least 10 weeks.

During the course of these studies we found that partially purified diets with lower (10 mg %) magnesium content (referred to as "low Mg basal — sucrose") consistently produced severely calcified hearts. The heart ash content of animals in several experiments ranged from 23 to 36% on a fat-moisture-free basis within three to 5 weeks. These animals might show good growth gains for the first two weeks, after which their growth was retarded or they lost weight (figs. 1B, 1C and 1D). The mortality rate was high after approximately 4 to 5 weeks. The addition of 10% crude casein resulted in improved growth (fig. 1B) and decreased heart ash (8 to 19% ash). Six per cent gelatin reduced the ash content (20 to 25% ash), but at this level it was not as effective as the slightly higher level of casein. Dietary modifications which imposed additional metabolic demands likewise increased the degree to which the animals were affected. Thus, a concurrent manganese deficiency increased the mortality rate and the calcification of the skeletal muscle, while the addition of 1% sodium acetate to the low magnesium diet greatly increased the pathological involvement of the liver. When the calcium content was reduced to 0.3%, the loss of weight after the third week was prevented (fig. 1D) and the heart ash content was somewhat reduced (16 to 25% ash). However, the animals were in poor health.

Substituting dextrin for the sucrose of this low magnesium basal resulted in nearly normal growth (fig. 1E) and a decreased ash content of the heart (8 to 21% ash). These data also include those obtained from animals fed the dextrin diet supplemented with 0.016 mg % B_{12} or 0.3% cystine. Supplementation of the dextrin diet with 10 mg % alpha-tocopherol did not further decrease the calcinosis (table 2). Supplementation with twice the level of B vitamins (excluding para-aminobenzoic acid) and including 2 mg folic acid and 0.3 mg biotin per kilogram ration tended to reduce the ash content of the heart (range of 4.8 to 14.4% ash), but the effect was erratic and not completely preventive.

The foregoing experimental diets contained moderate levels of all minerals except magnesium, which was present at a level of 10 mg %. When the level of other minerals was reduced (low mineral basal) by reducing salts IV from 4% to 1% and using Ca-free salts IV, and various calcium salts were added to give 0.3% calcium, striking results were obtained. The animals grew well for 11 weeks (fig. 1G). With the exception of the animals fed the basic calcium salt, the heart ash content was less than 13% at the end of the experimental period.

We found that cotton rats which consume diets with a high content of natural foods have markedly decreased incidence of the calcinosis syndrome. This is shown in part by the stock colony animals, which consumed natural diets with calcium and phosphorus levels of 1.0 to 1.4% and 0.8 to 1.0%, respectively, without manifesting any gross evidence of muscle pathology. The diets containing oatmeal used in these studies showed a marked tendency to protect against calcinosis. Each of the oatmeal diets contained 50% oatmeal. In addition the diets contained: group 1, 8.5% casein and 41.5% dextrin; group 2, 8.5% casein, 14.5% dextrin, 18% sucrose and 9% corn oil; group 2a, the same as group 2 plus 0.44% P as Na_2HPO_4; group 2b, the same as group 2 plus 0.8% calcium; group 2c, the same as group 2 plus 0.4% calcium as calcium carbonate. Animals fed the low calcium diets, groups 1, 2, and 2a, and the high calcium diet, group 2b, grew poorly (fig. 1A). The femur ash contents of these animals were lower than those of group 2c (65%) and were as follows: group 1, 52%; 2, 52%; 2a, 57%; and 2b, 57%. The mobilized minerals were not redeposited in the cardiac muscle, for the highest ash content found was 6% (group 2a). The protective effect of oatmeal was further shown when animals were fed

the oatmeal-milk diet, which consisted of 50% oatmeal and 50% dried whole milk. This diet contained 0.5% calcium and 0.64% phosphorus. At the end of 11 weeks the ash content of the hearts of these animals was still low (5 to 6% ash).

Since decreasing the magnesium content of the diet increased the severity of the calcinosis, and magnesium deficiency in white rats has been shown to produce a several-fold increase in the calcium content of the kidney (Tufts and Greenberg, '37), the kidneys of cotton rats were ashed. The average ash content of the kidneys was similar in all groups, ranging from 5.5 to 7.0%, and was not correlated with the severity of the syndrome.

That nutrition may alter the course of experimentally produced infections has been shown by many studies (Clark et al., '49), although the dietary influence in many cases has not been well defined. In the present experiments cultures of cecal contents for *Salmonella* were negative, as were tests for *Bartonella*. No correlation could be found between the severity of the syndrome and the blood cell picture, including hemoglobin, total red blood and white blood cells, and differential counts. No abscesses have been found in our cotton rats, in contrast to the high incidence found in guinea pigs (Harris and Wulzen, '50). Only rarely did even the cotton rats most severely affected show evidence of pneumonia. Subcutaneous injections of magnesium- or manganese and magnesium-deficient mice with 1:20 homogenates of affected liver or muscle were without effect. Mice fed a magnesium-deficient diet containing 1 to 2% dried feces from affected cotton rats did not develop the syndrome.

Attempts to produce calcinosis in other species have been essentially negative. Diets which produce the severest lesions and maximum stress in cotton rats were without effect when fed to white rats or hamsters for 10 to 12 weeks, and resulted in the sporadic and unreproducible occurrence of cardiac lesions in mice.

DISCUSSION

Petering et al. ('48) noted that oatmeal delayed or alleviated the wrist stiffness syndrome in guinea pigs; Hogan et al. ('50) found that an increased dietary level of phosphate resulted in a more extensive calcification of the soft tissues of their deficient guinea pigs; Van Wagtendonk and Wulzen ('46) were not able to cure or prevent the wrist stiffness syndrome in guinea pigs with vitamin E. Similar observations have been made with regard to the effect of diet on the calcinosis syndrome in cotton rats. Cotton rats, however, do not show evidence of any wrist stiffness. It has been shown further, by the use of cotton rats from our colony, that the ratio and level of calcium, phosphorus and magnesium are important factors in determining the acuteness of the disease. The high level of calcium and phosphorus consumed by stock colony animals and the failure of high levels of magnesium in partially purified diets to prevent the syndrome indicate that natural rations contain a factor or produce digestive conditions which are lacking in the experimental diets. The factor or conditions provided by dextrin or additional casein in alleviating the syndrome cannot be explained at the present time.

SUMMARY

Survey data have been presented on the effect of diet on the development of a calcinosis syndrome which produced a markedly increased ash content of the hearts of cotton rats. The alleviating effect of oatmeal, the detrimental influence of high phosphorus, and the failure of vitamin E to cure the syndrome in guinea pigs apparently apply also to cotton rats. The syndrome in cotton rats was primarily associated with the feeding of partially purified diets. Preliminary studies have failed to establish an infective agent as the cause of this syndrome.

Dietary conditions which increased the severity of the syndrome were (a) increased calcium and phosphorus; or (b) decreased magnesium levels; or (c) basic mineral mixture; or

(d) increased metabolic demands imposed by manganese deficiency or the feeding of sodium acetate. Dietary modifications found to alleviate the syndrome were dextrin or additional protein. Supplementation of partially purified diets with B vitamins, cystine, B_{12} or 1:20 liver extract had little effect on the syndrome. A high level of magnesium did not prevent it.

LITERATURE CITED

CLARK, P. F., L. S. MCCLUNG, H. PINKERTON, W. H. PRICE, H. A. SCHNEIDER AND W. TRAGER 1949 Influence of nutrition in experimental infection. Bact. Rev., *13:* 99–134.

CONSTANT, M. A., P. H. PHILLIPS AND D. M. ANGEVINE 1952 The occurrence of a calcinosis syndrome in the cotton rat. II. Pathology. J. Nutrition, *47:* 327.

HARRIS, P. N., AND R. M. WULZEN 1950 Pathologic changes induced in guinea pigs by administration of diets deficient in the anti-stiffness factor. Am. J. Path., *26:* 595–615.

HOGAN, A. G., W. O. REGAN AND W. B. HOUSE 1950 Calcium phosphate deposits in guinea pigs and the phosphorus content of the diet. J. Nutrition, *41:* 203–214.

MCINTIRE, J. M., B. S. SCHWEIGERT AND C. A. ELVEHJEM 1944 The nutrition of the cotton rat *(Sigmondon hispidus hispidus)*. Ibid., *27:* 1–9.

PERERING, H. G., L. STUBBERFIELD AND R. A. DELOR 1948 Studies on the guinea pig factor of Wulzen and van Wagtendonk. Arch. Biochem., *18:* 487–494.

SCHWEIGERT, B. S. 1948 Nutritional requirements of the cotton rat and hamster. Vitamins and Hormones, *VI:* 55–67.

TUFTS, E. V., AND D. M. GREENBERG 1937 The biochemistry of magnesium deprivation. I. Chemical changes resulting from magnesium deprivation. J. Biol. Chem., *122:* 693–714.

VAN WAGTENDONK, W. J., AND R. WULZEN 1946 A dietary factor essential for guinea pigs. VIII. The isolation of the anti-stiffness factor from cane juice. Ibid., *164:* 597–602.

THE OCCURRENCE OF A CALCINOSIS SYNDROME IN THE COTTON RAT[1]

II. PATHOLOGY

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ELEVEN FIGURES

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The occurrence of diffuse calcification in cotton rats which consumed partially purified diets has been reported (Constant and Phillips, '52). Although the acuteness and extent of the disease could be modified by altering several dietary factors, the lesions remained essentially the same. Certain similarities were observed between the effect of diet upon the development of the calcinosis in guinea pigs deficient in the anti-stiffness factor and its effect upon the syndrome in our cotton rats. On the basis of the description by Harris and Wulzen ('50), the microscopic appearance of the lesions was similar. The purpose of the present report is to describe the pathologic alterations that have been observed in the tissues of cotton rats affected by the calcinosis syndrome.

EXPERIMENTAL

Material

The experimental procedure and the dietary modifications that alter the course of this syndrome in cotton rats have

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55

THE OCCURRENCE OF A CALCINOSIS SYNDROME IN COTTON RATS

IV. THE EFFECT OF DIET AND THE AGE OF THE ANIMALS ON THE DEVELOPMENT OF THE DISEASE AND ON THE URINARY EXCRETION OF VARIOUS METABOLITES [1]

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SEVEN FIGURES

(Received for publication July 16, 1953)

INTRODUCTION

During the course of nutritional studies in which weanling cotton rats from our colony were used, we observed a diffuse calcification confined primarily to the cardiac and skeletal muscles but frequently affecting the livers of experimental animals if they were fed semi-purified diets. A survey of the effects of many types of diets on the development of this disease and the growth of the animals (Constant and Phillips, '52) and a description of the pathology of the lesions (Constant, Phillips and Angevine, '52) have been reported. Several similarities were noted between the lesions and the effect of diet on the disease in cotton rats, and in guinea pigs fed diets deficient in the anti-stiffness factor.

Since growth rate changes only partially reflected the development of the disease, and the involvement of the heart

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made routine blood samples from this source impractical, urinary analyses were made in order to find other criteria which would permit a clinical pursuit of the development of the disease. The urinary excretion levels of creatine and phosphorus were followed in each case, and such other urinary analyses and enzyme studies were made as were suggested by the data obtained, in order to gain further information on this disease. Young and adult animals were fed synthetic or stock diets to determine whether the cotton rats were more susceptible to the development of the disease during their rapid growth period. White rats were also used to compare the effects of similar diets on their urinary excretion levels of the various substances studied with the results obtained when cotton rats were used.

EXPERIMENTAL

In the experiments in which young (three- to 4-week-old) cotton rats were used, litter mates were distributed one each per lot. The white rats were weanling male rats of the Sprague-Dawley strain. The animals were kept in individual metabolism cages 6 inches in diameter and given food and water ad libitum. Haliver oil supplements were given every 8th day. The basal diet contained crude casein (24%), sucrose (67%), modified salts IV (4%) and corn oil (5%). The mineral mix provided 20 mg of Mg, 560 mg of Ca and 280 mg of P per 100 gm of ration in all experiments except the first, in which the magnesium level was 10 mg %. The following levels of B vitamins per kilogram of ration were used and are slightly higher than the amounts shown to be required by this species (Schweigert, '48): thiamine HCl, 3.8 mg; pyridoxine HCl, 3.8 mg; riboflavin, 4.5 mg; nicotinamide, 38.0 mg; calcium pantothenate, 30.0 mg; choline, 1.5 gm; inositol, 1.5 gm; *p*-aminobenzoic acid, 0.45 gm. Finely ground guinea pig pellets [2] were used as the stock diet and contained approximately 1.4% Ca, 0.5% P and 0.3% Mg.

[2] Rockland.

Urine was collected under toluene, acidified, taken to 100 ml in volume and filtered through no. 1 filter paper. Creatine and creatinine were determined by the alkaline-picrate method. In the first two experiments the conversion of creatine to creatinine was accomplished by autoclaving the samples with 1N HCl. Since it was found that autoclaving for 45 minutes with picric acid gave more uniform and reproducible results, this procedure was used in the remainder of the experiments. Phosphorus was determined by the Fiske-Subbarow method cited by Hawk, Oser and Sommerson, '48). Calcium determinations were made by precipitating as the oxalate at pH 4.0 and titrating with 0.01N $KMNO_4$. Bisulfite-binding substances were determined by the method of Shils et al. ('41) and the Benedict-Francke method cited by Hawk et al. ('48) was used for uric acid determinations. Heart and abdominal muscle samples were dried at 100°C., extracted in a Goldfish extractor with ether and dry ashed at 550°C.

RESULTS AND DISCUSSION

In a preliminary experiment determinations were made of the creatine excretion levels of cotton rats fed the basal ration (0.01% Mg) with vitamin E (0.05%), manganese (0.03%) or methionine (0.2%) supplements, and a weekly supplement of β-carotene in olive oil in place of haliver oil, or the basal ration containing 1% salts IV (0.01% Mg) with 1.4% $CaCO_3$ added (low minerals). Two litters of 6 animals each were used. The creatine excretion data in figure 1 showed that all animals, with the exception of one in the low minerals group and another in the manganese-supplemented group, excreted increasing amounts of creatine starting about the 14th day. The fact that the excretion level of these two animals continued to be 1.0 mg/24 hours or less for the entire period suggested that the creatine excretion level of cotton rats should normally be low. The weight gains and heart ash data which are given in table 1 showed that all animals of litter 347 were more severely affected than those of litter 324 and demon-

strated the necessity of having litter-mate controls in these studies.

In the previous survey report (Constant and Phillips, '52) it was found that vitamin E had no beneficial effect on growth or the ash content of the heart, a concurrent manganese deficiency increased the severity of the disease, and a reduction of minerals other than calcium resulted in better growth

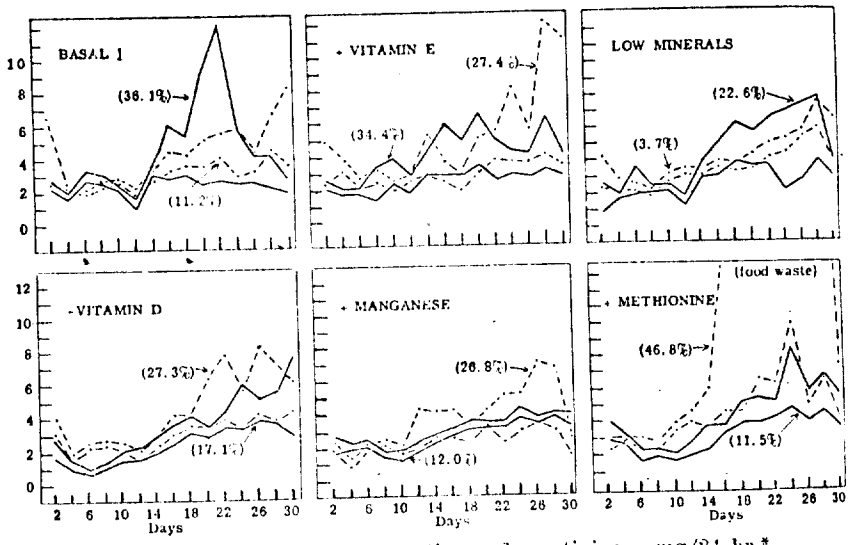


Fig. 1 Daily excretion of creatine and creatinine — mg/24 hr.*

* Data from two 24-hour collections are averaged for each point. Each pair of broken or solid lines designates excretion data on individual male and female rats which are further identified in table 1. Total creatinine values are graphed on upper curve, "free" creatinine values on lower curve; thus, distance between points on a given pair of solid or broken curves equals creatine, expressed as creatinine. Data in parentheses equal % ash content of heart.

and a reduced ash content of the heart. Although vitamin E reduced the creatinuria of guinea pigs deficient in the anti-stiffness factor (van Wagtendonk et al., '44), it apparently had no effect on the creatine excretion levels of cotton rats. The present experiment indicated that the calcinosis was not due to excessive intakes of vitamin D, or to an increased requirement of the cotton rat for manganese or methionine.

In the second experiment the effect of increasing levels of magnesium on the severity of the disease and the excretion of chloride, phosphorus and creatine was studied. The basal ration which contained 0.02% Mg was supplemented with $MgSO_4 \cdot H_2O$ to provide an additional 0.04% and 0.08% Mg.

TABLE 1

The variation in the susceptibility of litters to the disease

RATION	WEIGHT GAIN Litter no. 324	WEIGHT GAIN Litter no. 347	HEART ASH Litter no. 324	HEART ASH Litter no. 347
	gm	*gm*	%	%
Basal	34 ♂	27 ♀	11.2	36.1
+ vitamin E	27 ♂	27 ♀	27.4	34.4
avitaminosis D	21 ♀	43 ♂	17.1	27.3
+ manganese	35 ♀	29 ♂	12.0	26.8
+ methionine	29 ♀	23 ♂	11.5	46.8
range	(23–43) 5 ♂	(21–35) 5 ♀	(11–27)	(27–47)
Low minerals	59 ♂	47 ♀	3.7	22.6

TABLE 2

Composition of diets which were fed to weanling white rats

LOT	1	2	3	4	5	6	7	8
Mg (mg %)	40	10	10	10	10	10	10	10
Ca pantothenate (mg/kg)	30	30	10	10	30	10	30	10
Weekly haliver oil supplement[1]	*	*	*	**	**	**	**	**
Other changes					(1)	(2)	(3)	(4)

(1) Biotin, 10 mg/kg; (2) cortisone, 2 mg/10 gm; (3) No KI; (4) iodinated casein, 0.05%.

[1] All animals were given two drops of haliver oil every 8th day starting * immediately or ** on the 24th day.

The 4th rat of each of the 4 litters used in this experiment was fed cortisone in the ration at an initial level of 3.0 mg/10 gm of ration. The level was reduced to 1.5 mg on the 14th day because of the very poor growth of the animals, but they still failed to grow. The data in figure 2 show that food consumption and growth increased with increasing levels of

Mg in the ration and decreased in the cortisone-supplemented group.

Although there was a marked difference in the chloride excretion per 48 hours, the data in figure 2 show that, on the basis of food consumed, there was no difference between the

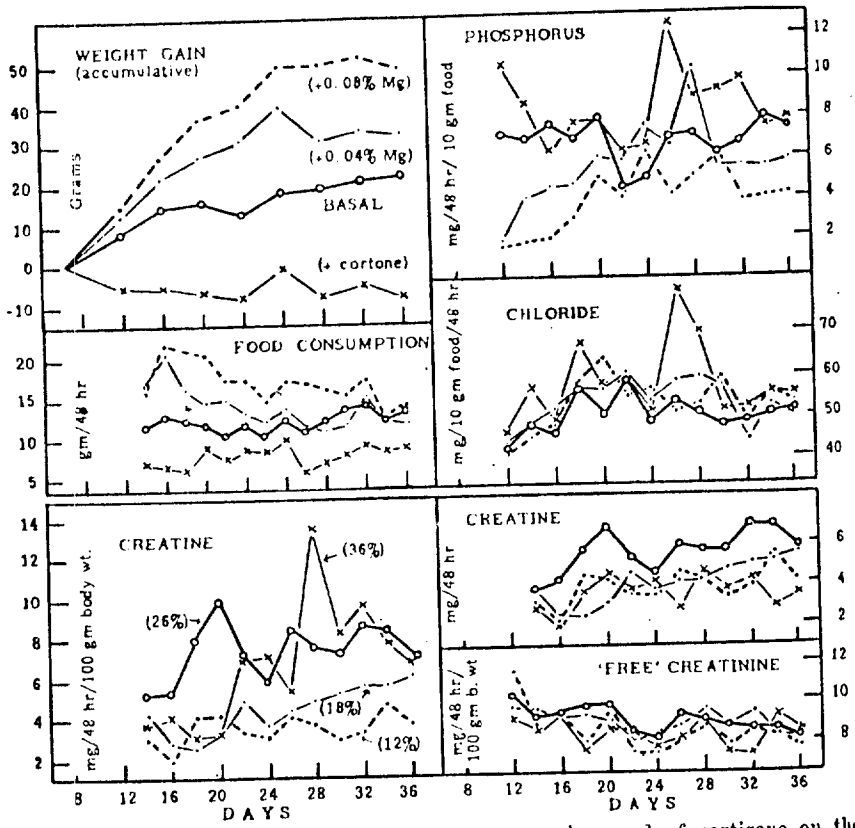


Fig. 2 The effect of increasing levels of magnesium and of cortisone on the disease.

various groups. On the other hand, the phosphorus excretion data computed on the basis of food consumed showed that the animals were divided into two groups, low Mg and high Mg. This finding has been repeated in each of the experiments where a high Mg ration was used as a positive control. The basal group excreted the greater amount of creatine

throughout the experiment. Although cortisone increased catabolism (Ingle, '50), and these animals showed considerably poorer growth and more extensive calcification than the basal group, their creatine excretion was not greater than that of the basal group. Beard ('46) reported that creatine excretion increased with increased protein intake. In an attempt to assess this influence on creatine excretion, the data were computed on the basis of 100 gm of body weight. These data (fig. 2) showed a direct correlation with the terminal severity of the disease, as indicated by the ash content of the hearts of these animals. In addition to lesions of the heart and skeletal muscle, the animals fed cortisone showed large (1-cm) areas of calcification of the subcutaneous fat in the thoracic region.

The previously reported studies had shown that when dextrin replaced the dietary sucrose, the animals showed normal growth despite the low level of magnesium in the diet. In the third experiment, 4 litters of 4 animals each were fed the basal ration with sucrose, or with dextrin, or with dextrin and 0.08% Mg, or with sucrose and 0.05% $ZnCl_2$. The weight gains, the ash, and the calcium content of cardiac and skeletal muscle are given in figure 3. These data confirm the previous findings; namely, that the substitution of dextrin for sucrose resulted in normal growth despite the low magnesium content of the diet (Constant and Phillips, '52). The heart ash content was reduced from an average of 27% to 13%; the calcium content of heart and skeletal muscle was reduced from 4.2% to 2.1% and 3.8% to 1.7%, respectively. The ash content of the abdominal muscle was not reduced. The presence of additional Mg (0.08%) in the diet resulted in a further decrease in the ash content of the heart to 7.2% and the calcium content to 1.0%. The ash content of the abdominal muscle was reduced from 10% to 4% and the calcium content to 0.1%.

The food consumption data (fig. 3) show that the presence of dextrin or dextrin and Mg prevented the decline in appetite which occurred when the low magnesium-sucrose diet was fed. The presence of Mg in the diet resulted in a consistently

greater excretion of calcium, whereas there was no difference in the calcium excretion of animals fed the low magnesium-sucrose or dextrin diets. The phosphorus excretion of animals fed the basal diet with dextrin and Mg was consistently lower than that of the animals fed the low Mg diets. The presence of dextrin tended to decrease the phosphorus excretion. From the 12th to the 20th day the amounts excreted

RATION	Graph Symbol	No. of rats	Weight Init	Weight Final	HEART % Ash[1]	HEART Calcium[2]	ABDOMINAL MUSCLE % Ash[1]	ABDOMINAL MUSCLE Calcium[2]
			gm	gm	Ave. ± S.D.	Ave. ± S.D.	Ave. ± S.D.	Ave. ± S.D.
BASAL		4	48	53	26.7 ± 8.4[3]	4.2 ± 1.0	9.9 ± 1.3	3.8 ± 2.1
" with dextrin		4	49	77	13.2 ± 8.7	2.1 ± 1.4	10.1 ± 3.2	1.7 ± 0.7
" " " + Mg		4	44	73	7.2 ± 3.1	1.0 ± 0.8	4.3 ± 1.4	0.1 ± <0.1
" + Zn		4	45	57	23.4 ± 1.1	5.8 ± 0.8	13.2 ± 4.4	2.1 ± 0.4

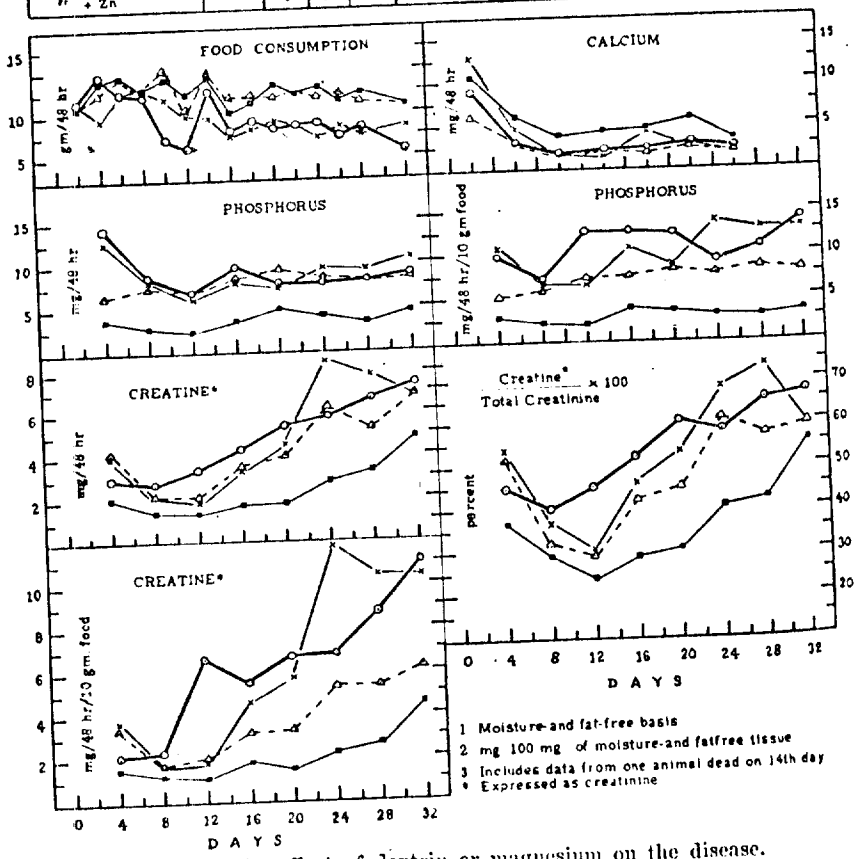


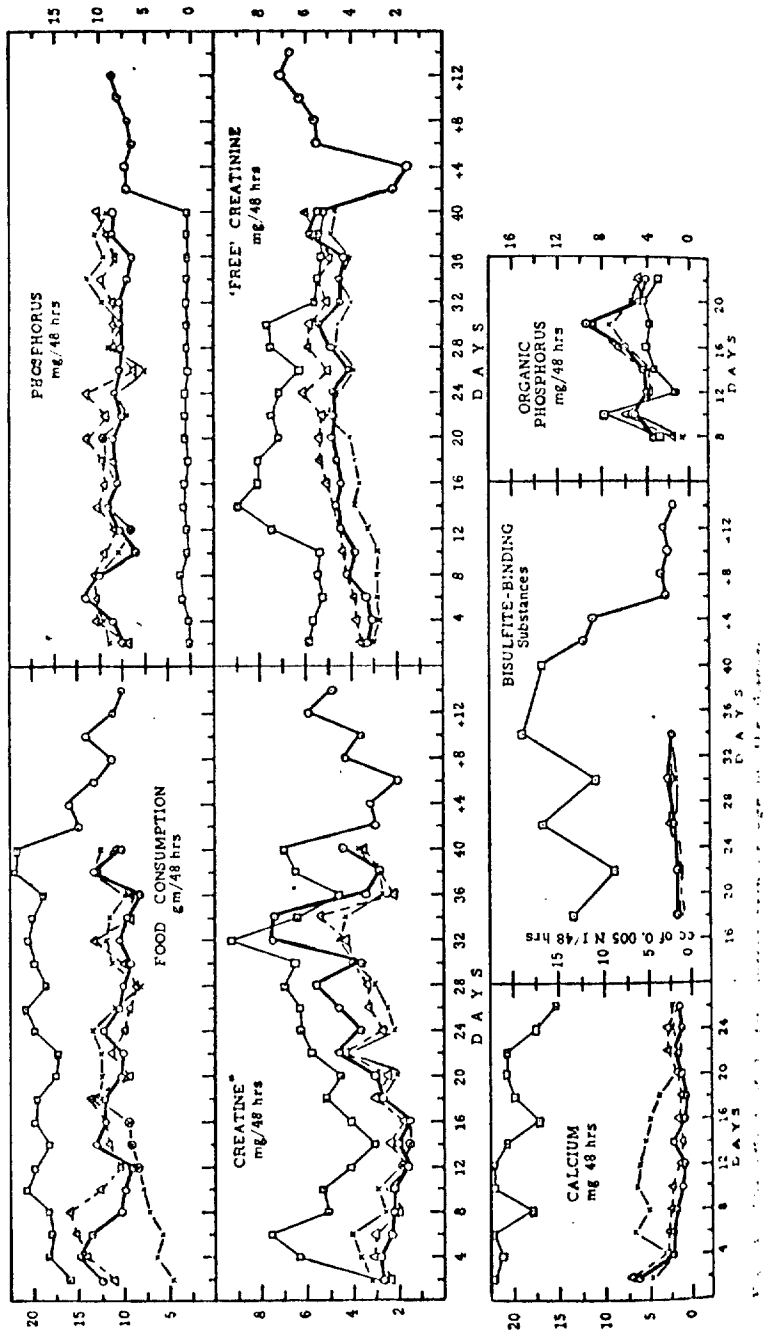
Fig. 3 The effect of dextrin or magnesium on the disease.

by the low Mg-dextrin group were less than the range of amounts excreted by the animals fed the low Mg-sucrose ration. The creatine excretion data in figure 3 show that there was an increase in creatine excretion with time in all groups. However, the excretion level of the basal sucrose group was at all times greater than that of the group fed the basal ration with dextrin and Mg. The excretion values of the low Mg-dextrin group tended to be intermediate. The differences between the groups were increased when the effect of protein was taken into account by computing the data on the basis of the amount of food consumed. Calculations on the percentage of creatine excreted in relation to total creatinine showed high values for the basal ration, low values for the basal with dextrin and Mg, and intermediate values for the basal with dextrin.

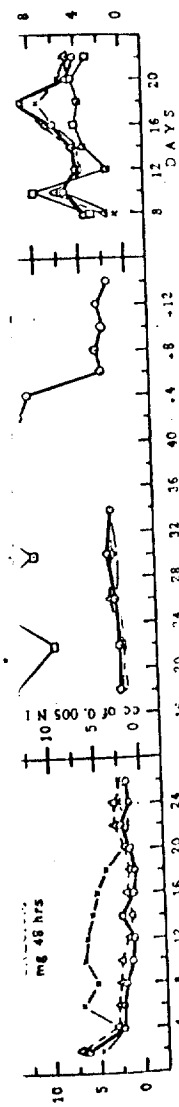
In the 4th experiment animals were fed the basal diet supplemented with biotin (5 mg/kg) or aureomycin. The initial level of aureomycin was 250 mg/kg, which was reduced to 50 mg/kg on the 4th day and to 0 mg/kg on the 12th day because of severe constipation apparently caused by the aureomycin. These animals did not defecate until after they had been on the basal ration for approximately 4 days. The feces were slate grey and were decreased in quantity for almost the remainder of the experiment. Aureomycin (50 mg/kg) was added to the diet again on the 18th day. The basal diet was fed to a negative control group and the stock diet to the positive control group. At the end of the experiment, animals which had been fed the stock diet were switched to the basal diet and kept on experiment an additional two weeks to compare the susceptibility of animals two months old with that of weanlings.

Analyses of cardiac and abdominal muscle showed (fig. 4) that supplements of biotin or aureomycin did not prevent or reduce the calcinosis. Although one group had been fed the stock diet during their period of rapid growth before the basal diet was fed, these animals showed an average of 13% ash in the heart, which is approximately three times that of

RATION	Graph Symbol	No. of rats	WEIGHT Initial	WEIGHT Final	HEART % Ash[1] Ave. ± S.D.	HEART Calcium[2] Ave. ± S.D.	ABDOMINAL MUSCLE % Ash[1] Ave. ± S.D.	ABDOMINAL MUSCLE Calcium[2] Ave. ± S.D.
BASAL	○—○	4	gm 45	gm 78	26.6 ± 4.5	4.3 ± 0.2	12.1 ± 1.9	1.9 ± 0.5
" + Biotin	△—△	4	45	81	31.5 ± 8.5	4.3 ± 0.9	11.3 ± 2.6	1.7 ± 0.9
" + Aureomycin	×—×	4	42	70	27.4 ± 6.2	4.2 ± 1.4	9.3 ± 1.0	1.0 ± 0.7
STOCK DIET, then BASAL	□—□	4	44	89 (85)	13.2 ± 2.8	1.5 ± 0.5	6.6 ± 1.1	0.5 ± 0.1



animals maintained continuously on the stock diet (fig. 6). Despite the considerably greater food consumption of animals fed the stock diet and the higher level of phosphorus in that diet, the phosphorus excretion (fig. 4) was very low, averaging approximately 0.5 mg/48 hours for the entire period, compared to 5 to 10 mg for the groups of animals fed



RATION	Graph Symbol	No of rats	WEIGHT Initial	WEIGHT Final	HEART % Ash[1]	HEART Calcium[2]	ABDOMINAL MUSCLE % Ash[1]	ABDOMINAL MUSCLE Calcium[2]
			gm	gm	Ave ± S.D.	Ave ± S.D.	Ave ± S.D.	Ave ± S.D.
BASAL[3]	-o-	3	201	186	6.6 ± 0.8	0.4 ± <0.1	5.0 ± 0.2	0.1 ± <0.1
+ with dextrin + Mg	-o-	3	175	185	6.6 ± 2.4	0.2 ± <0.1	4.6 ± <0.1	0.1 ± <0.1
STOCK DIET (S.D.)	-x-	3	178	—	4.8 ± 0.1	0.2 ± <0.1	4.8 ± 0.2	0.1 ± <0.1

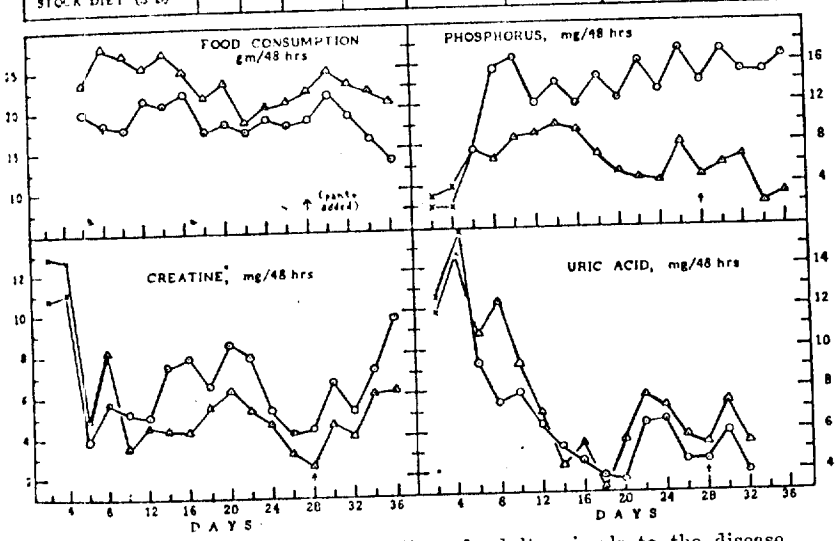


Fig. 5 The decreased susceptibility of adult animals to the disease. Footnotes 1, 2 and * are the same as for figure 3.

[3] Adult animals (6 months old) were fed pantothenate-deficient diets until the 28th day.

the low Mg basal. Immediately upon being switched to the basal diet, the phosphorus excretion rose to approximately 7 mg/48 hours. The creatine and "free" creatinine excretion of animals fed the stock diet was quite variable but in almost all instances was greater than that of animals fed the synthetic diets. The free creatinine excretion dropped sharply to approximately 2 mg/48 hours for 4 days after feeding of

the basal diet and then rose sharply to 6 or 7 mg. The creatine level also dropped to 2 to 3 mg for 6 days and then rose sharply but irregularly to 5 or 6 mg/48 hours. This rise was earlier and sharper than that shown by animals fed the basal diet at weaning age. The calcium excretion of animals fed the stock diet was several times that of animals fed the synthetic diets.

The decrease in appetite shown by animals fed the low magnesium diets and the interrelationship of magnesium to thiamine-containing enzymes suggested the possibility of a thiamine deficiency. Shils et al. ('41) showed that the excretion of bisulfite-binding substances was greatly increased in thiamine-deficient white rats. However, it was found that the excretion of these substances by animals fed the stock diet was considerably greater than that of animals fed synthetic diets (fig. 4) and dropped gradually to a low level after the animals were switched from the stock diet to the basal. Preliminary determinations of organic phosphorus excretion indicated that the excretion level of this substance may also be less when diets are fed which do not precipitate the calcinosis. These findings were checked further in the 6th experiment.

Since animals which were two months old were as susceptible to the development of the disease as weanlings, adults 6 months old were used in the 5th experiment. These animals were fed the basal diet (0.02% Mg) or the basal diet with dextrin and Mg (total of 0.1%). Both diets were deficient in calcium pantothenate (see discussion on enzyme studies). They were fed the stock diet for 4 days prior to being fed the synthetic diet. All animals fed the basal diet had a few white linear streaks observable grossly in the heart muscle. One of the animals fed the basal with dextrin and Mg had an atypical lesion which resembled a scar, and the ash content of the heart of this animal was 10% compared to 4.8 and 5.1% for the other two animals of this group and 5.7 to 7.6% for the basal group. The calcium content was 0.5% compared to 0.1% for the other two animals and 0.4 to 0.5% for the basal

group. In comparison to animals three weeks or two months old, adults were much less susceptible to the development of the calcinosis. There was no increase in the ash or calcium content of the abdominal muscle (fig. 5).

The food consumption of adults fed the low magnesium basal was less than that of adults fed the basal with magnesium. The excretion data given in figure 5 show that phosphorus excretion rose abruptly when the synthetic diet was fed and the same increased excretion by the low Mg group was observed as in the case of weanlings. There was a definite tendency for the low Mg group to excrete greater amounts of creatine than the high Mg group, although this difference was not as pronounced as in weanlings. The uric acid excretion of the animals was considerably greater when they were fed the stock diet than when they were fed the synthetic diets.

In the last experiment in which cotton rats were used, the animals were fed the basal diet supplemented with vitamin A[4] (10,000 U.S.P. units/kilogram) or calcium pantothenate (total of 70 mg/kg). The basal diet plus Mg (total of 0.1%) or with dextrin and Mg (total of 0.1%) was used as the positive control. In a preliminary experiment, 8 pairs of weanling male white rats were fed the basal diet containing one-half as much magnesium (0.01%) or otherwise modified as shown in table 2. The cotton rats were fed the stock diet for 8 days and the white rats for 4 days prior to feeding the experimental rations.

The data on the ash and calcium content of cardiac and skeletal muscle which are given in figure 6 for each group of cotton rats showed that the vitamin A or calcium pantothenate supplements had no beneficial effect on growth or deposition of minerals in muscle tissue. Supplementation of the diet with Mg improved growth and reduced the ash and calcium content of the tissues to the range of values obtained for animals maintained on the stock diet. Although the pairs of white rats fed cortisone or iodinated casein grew very poorly,

[4] Nopcay "10" Type IV, 10,000 U. S. P. units/gram; Nopco Chemical Co., Harrison, N. J.

the ash and calcium contents of cardiac and skeletal muscle were not increased thereby. All of the cotton rats fed the high magnesium diet with sucrose had a few short white linear streaks observable grossly on the heart, whereas none was visible on the hearts of animals fed the high Mg diet

RATION	No of rats	Graph Symbol	WEIGHT Initial	WEIGHT Final	HEART % Ash[1]	HEART Calcium[2]	ABDOMINAL MUSCLE % Ash[1]	ABDOMINAL MUSCLE Calcium[2]
			gm	gm	Ave. ± S.D.	Ave. ± S.D.	Ave. ± S.D.	Ave. ± S.D.
BASAL	3		55	75	31.3 ± 3.6	4.2 ± 0.6	12.7 ± 5.8	[illegible]
" + Vitamin A	3		50	68	31.3 ± 5.3	7.4 ± 0.6	11.7 ± 5.4	[illegible]
" + Calcium pantothenate	3		48	74	31.8 ± 3.7	7.2 ± 1.1	9.9 ± 2.8	[illegible]
" + Magnesium	3		48	106	5.8 ± 0.5	0.2 ±<0.1	4.8 ± 0.3	[illegible]
" with dextrin + Magnesium	3		63	103	5.1 ± 0.6	0.2 ±<0.1	4.6 ± 0.2	[illegible]
STOCK DIET (S.D.)	7		(3 mos old)		3.5 ± 1.4	0.1 ±<0.1	5.8 ± 1.5	[illegible]
WHITE RATS	16		37	127	4.1 ± 0.8	0.1 ±<0.1	6.6 ± 3.3	[illegible]
(cortone)	–		–	64				
(iodinated casein)	–		–	75				

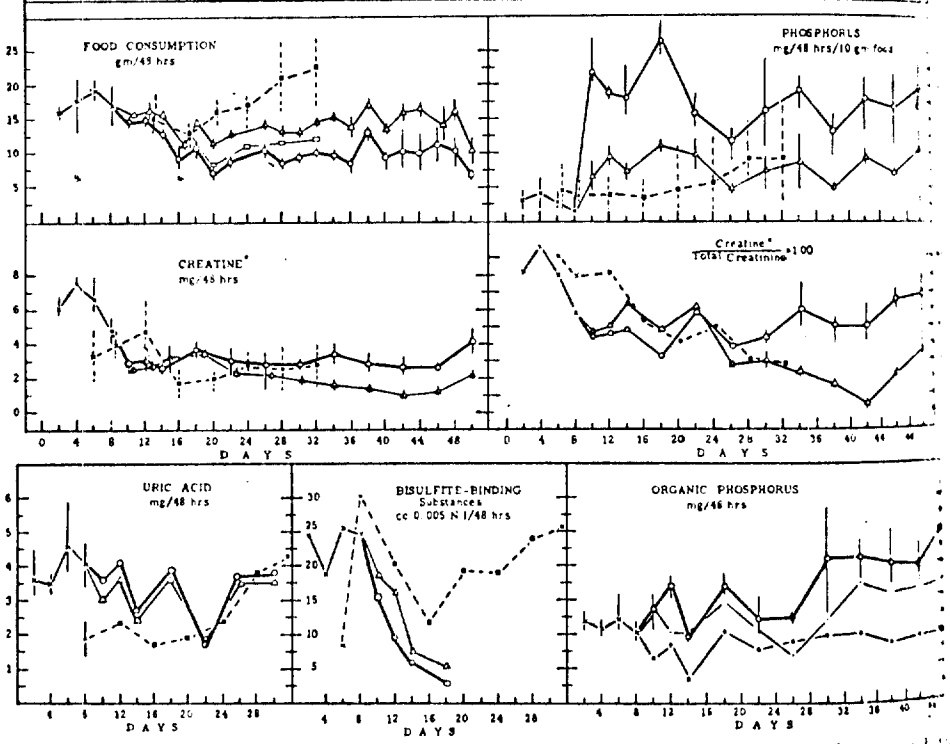


Fig. 6 Comparison of the effect of low Mg diets on the ash content of tissues and the urinary excretions of white rats and cotton rats.

Footnotes 1, 2 and * are as for figure 3.

The values for the three groups of cotton rats fed low Mg diets, two groups fed high Mg diets and 8 pairs of white rats are averaged and plotted. The maximum and minimum average of the subgroups are given on the vertical lines.

with dextrin. The presence of these lesions was not reflected by an increase in the ash or calcium content of the heart and, as noted previously (Constant et al., '52), the early lesions may not be accompanied by calcium deposition.

The food consumption data in figure 5 again showed the effect of magnesium in maintaining the appetite. The amount of food consumed during the first week of feeding the synthetic diets to white rats and cotton rats was similar. Thereafter, food consumption by the white rats increased until it was approximately one and one-half to two times that of the cotton rats. Although 14 of the 16 white rats were fed diets containing one-half as much magnesium as was contained in the low Mg diets fed to the cotton rats, there was no immediate rise in phosphorus excretion upon switching from the stock diet to the synthetic diet. The white rats fed iodinated casein excreted the greater amounts of phosphorus in the last 4 collection periods. Otherwise, all pairs of animals excreted less than 4 mg/10 gm food. The vitamin supplements to the diets of the cotton rats modified the excretion levels of phosphorus by the low Mg groups, but the levels of excretion were not reduced to the level shown by the high Mg groups.

As in previous experiments, cotton rats fed the low Mg basal ration excreted greater amounts of creatine than those fed the Mg-supplemented ration. The excretion levels of the white rats were low for two of the early collections but rose when the food consumption increased. If the data were calculated to take into account the difference in protein consumption, the amounts of creatine excreted by white rats would be less than the creatine excretion by cotton rats fed the Mg-supplemented ration. The low Mg cotton rat groups excreted the highest percentage of creatine. The white rats showed a gradual decline in the percentage of creatine excreted, and when the experiment was terminated the values were similar to those of the cotton rats fed the Mg-supplemented diets.

The excretion of uric acid by white rats was less than that of cotton rats when the stock diet was fed and in the early

collection periods when synthetic diets were fed, but it increased when food consumption increased. There was no difference in the uric acid excretion of cotton rats fed the low or high Mg diets. The drop in the excretion level of bisulfite-binding substances which was noted previously (fig. 4) was again observed. The data obtained with white rats indicated that the excretion level was influenced by the food consumption level. However, the low level excreted by cotton rats indicated that according to this criterion the cotton rats were not suffering from thiamine deficiency. The previous indication that the organic phosphorus excretion may also be affected by diet or the disease was checked in this experiment. The data (fig. 6) showed that the organic phosphorus excretion of animals fed the basal diet with dextrin and Mg was less than that of animals fed the basal diet with sucrose and Mg. Although there were no overlapping values for the last 4 collections of samples, a definite conclusion as to the effect of dextrin on the excretion of this substance cannot be made until the experiment is repeated.

In view of the fact that an increase in the protein content of the diet showed some beneficial effect on this disease (Constant and Phillips, '52) and that Williams and Elvehjem ('49) have shown that xanthine oxidase activities may be used as an assay for protein quality, studies were made of the xanthine oxidase activities of liver homogenates by the method of Axelrod and Elvehjem ('41). Control flasks containing homogenates of the livers of cotton rats fed the stock diet, or white rats two to three months old fed the stock diet, were used in each series of determinations. Duplicate flasks were used for endogenous respiration and xanthine oxidase activity determinations. Only the values for endogenous respiration are shown in figure 7, for in approximately 80 to 90% of the determinations there was a marked decrease in oxygen uptake when the xanthine substrate was added and the inhibition remained for the duration of the test (240 minutes).

The high endogenous respiration of homogenates of the livers of cotton rats compared to that of the livers of white rats of similar age is shown in figure 7. The maximum oxygen uptake by liver homogenates in the case of white rats was shown in the first 10 minutes, gradually declined and

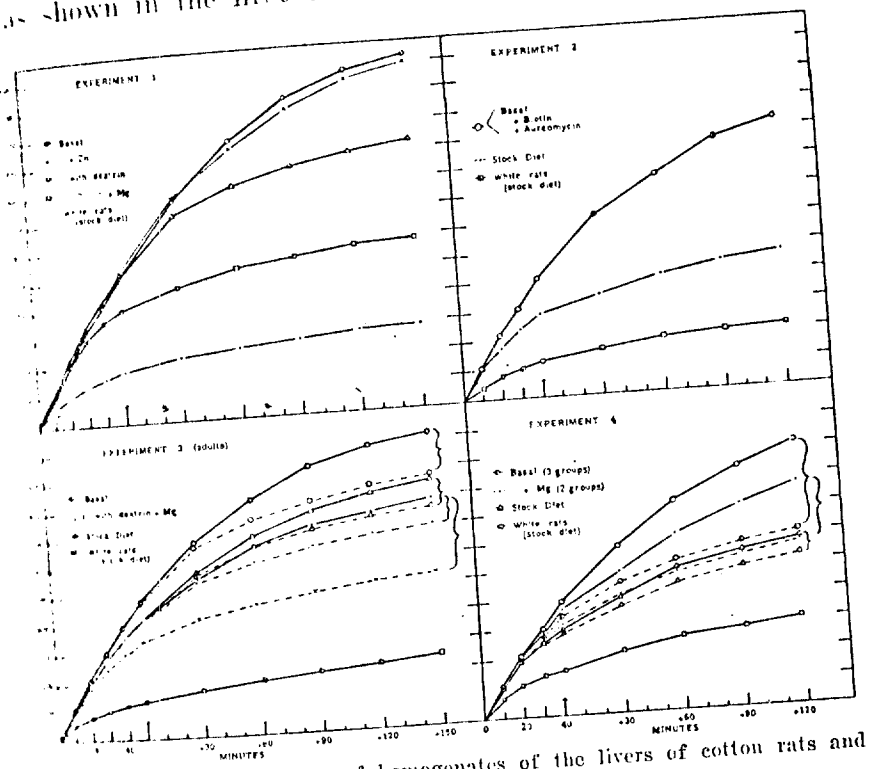


Fig. 7 The oxygen uptake of homogenates of the livers of cotton rats and white rats—ml O_2 per flask (284 mg of tissue).
Dotted lines represent values obtained when 100 μg of calcium pantothenate was added in vitro. Animals used in experiments 1, 2, 3 and 4 of these studies were those used in experiments 3, 4, 5 and 6 of the urinary excretion studies.

leveled off at approximately 40 to 50 minutes. The oxygen uptake of homogenates of livers from weanling and adult cotton rats was linear in all cases for the first 30 minutes and in the case of animals fed low Mg diets it continued at the same rate for 50 minutes. The data in figure 7 show that the oxygen uptake of homogenates of the livers of cotton

rats fed the stock diet or the basal diet supplemented with magnesium tapered off more rapidly than that of the livers of animals fed the low Mg diets.

In an attempt to modify the oxygen uptake, several substances were tested. Of these substances — vitamin C, glutathione, pyruvic acid, NaF, iodoacetic acid, magnesium, methionine, sodium citrate, folic acid or calcium pantothenate — only calcium pantothenate produced a significant and consistent change in the rate of oxygen uptake. When 100 μg of calcium pantothenate were added per flask with the xanthine, the oxygen uptake gradually decreased and was significantly less at the end of 60 minutes. If calcium pantothenate was added at zero time, the accumulative decrease was considerably greater but it still took approximately 40 to 50 mintues before the rate of uptake was significantly less than that of flasks containing a similar volume (0.1 ml) of water. This procedure, however, did not reduce the oxygen uptake to the level shown by homogenates of the livers of white rats. The addition of calcium pantothenate to the homogenates of livers of white rats fed the stock diets had no effect on the oxygen uptake. Dietary supplements of biotin, aureomycin, vitamin A or calcium pantothenate had no effect on the endogenous respiration of the liver homogenates.

In addition to the similarities between the calcification disease in guinea pigs and cotton rats (Constant et al., '52; Constant and Phillips, '52), several other similarities have been observed in the present studies. Guinea pigs fed diets deficient in the anti-stiffness factor showed high respiration of liver homogenates (van Wagtendonk and Wulzen, '50), similar to that of the deficient cotton rats. It is of interest to note the low xanthine oxidase activity of guinea pig livers (Richert and Westerfeld, '51) and the difficulty in demonstrating xanthine oxidase activity in homogenates of livers of cotton rats. Roine and Ettala ('52) have recently reported the toxicity of aureomycin (100 mg/kg) for guinea pigs, and in the present study a low tolerance for aureomycin was found with cotton rats. Generalized hair loss is a non-specific

symptom of ill cotton rats. Cotton rats fed the low Mg diets began losing excessive quantities of hair after the first week and those fed Mg-supplemented diets did not start shedding excessively until about the 4th week. Hair loss never reached the stage of alopecia. A more marked loss of hair by severely deficient guinea pigs was observed by van Wagtendonk and Wulzen ('50). An observation made with cotton rats which has not been reported for guinea pigs was a decrease in the color of the feces, which were brown at the beginning of the experiment and progressively faded to colorless in almost all animals fed the low Mg diets for three weeks or longer. The loss in color of the feces was considerably slower when high Mg diets were fed and often there was no appreciable change.

The actual amounts of creatine excretion varied somewhat from experiment to experiment but the increased excretion of creatine by animals fed low Mg diets (0.02% Mg) compared to animals fed diets containing 0.1% Mg was similar. Substituting β-carotene in olive oil for the haliver oil supplement had only a very temporary reducing effect on creatine excretion, and supplementing the diet with stabilized vitamin A had no effect on the calcinosis. The early decrease in appetite and poor growth suggested a possible increase in the requirement for B vitamins, but the addition of vitamin supplements to a low Mg-dextrin diet was previously found to have no alleviating effect on the disease (Constant and Phillips, '52) and in the present studies supplements of biotin or calcium pantothenate had no effect. Although aureomycin has been shown to have a sparing action on some B vitamins in sucrose-containing diets (Lih and Baumann, '51), supplementing the low magnesium diet with aureomycin was not beneficial. Many of the supplements modified the creatine excretion levels of cotton rats but none was as effective as Mg in reducing the creatine excretion.

White rats which consumed the same basal diet containing 0.01% Mg instead of 0.02% Mg did not show the immediate rise in phosphorus excretion which was found in each

experiment with cotton rats. Dogs (Kruse et al., '34) which were fed diets containing 1 to 2 mg % of Mg did not show this immediate phosphaturia. White rats required approximately 5 mg % of Mg when the diet contained 0.8% calcium for normal growth (Tufts and Greenberg, '38), whereas the low Mg basal ration which resulted in extensive calcification in cotton rats contained 20 mg % of Mg and 0.56% Ca. The generalized calcification which occurred when cotton rats were fed diets containing 20 mg % was not found when white rats were fed diets containing 2 mg % Mg (Lowenhaupt et al., '50). However, it has been found that calves on Mg-low diets showed calcification of arteries and frequently of skeletal muscle (Moore et al., '38) and that guinea pigs which developed lesions similar to those in cotton rats (Harris and Wulzen, '50) showed a marked improvement in growth when the dietary levels of K and Mg were raised to 1% and 0.3%, respectively (Roine et al., '49).

The increased creatine excretion and the immediate phosphaturia which occurred when cotton rats were fed diets containing 20 mg% of Mg, when compared to the effects of diets containing 0.1% Mg, suggest that the calcinosis syndrome was due to a magnesium deficiency. However, the alleviating effect of dextrin, protein or decreased dietary minerals other than calcium indicates that the intake of other nutrients may have been imbalanced or suboptimum. This suggestion is further borne out by the fact that lesions were observed grossly when a partially purified diet containing 0.1% Mg and sucrose was fed. To date animals fed a synthetic diet with dextrin and 0.1% Mg have not been kept on experiment longer than 50 days and it is not known whether lesions would eventually develop.

Although these studies have been of considerable value in obtaining information concerning the disease, histopathologic examination of the tissues must be used as the final criterion for determining its presence.

SUMMARY

Several experiments have been conducted to study the effect of diet and age on the susceptibility of cotton rats to development of the calcinosis disease.

Partially purified diets which were low in Mg (0.02%) precipitated and increased the severity of the disease compared to diets containing 0.1% Mg. An immediate phosphaturia occurred when cotton rats were fed the low Mg diets which was not shown by white rats fed diets containing 0.01% Mg or by cotton rats fed diets containing 0.1% Mg. The creatine excretion of cotton rats fed the low Mg diets also was increased, starting at approximately the second week.

Supplementation of the low Mg diets with biotin, aureomycin, vitamin A, calcium pantothenate, vitamin E, manganese, Zn or methionine or omission of the vitamin D supplement did not improve the growth of the cotton rats or reduce the deposition of minerals in cardiac and abdominal muscles. Some of these supplements reduced the phosphaturia and creatinuria, but the reduction was not as great as that due to a dietary supplement of Mg. The substitution of dextrin for sucrose retarded the development of the disease.

Young animals were more susceptible to the disease than adults.

Several similarities have been noted between the disease in guinea pigs and in cotton rats.

LITERATURE CITED

Axelrod, A. E., and C. A. Elvehjem 1941 The xanthine oxidase content of rat liver in riboflavin deficiency. J. Biol. Chem., *140*: 725–738.

Beard, H. H. 1946 Recent developments in creatin-creatinine metabolism with possible clinical application. Quarterly, Chicago Med. School, 7 (3).

Constant, M. A., and P. H. Phillips 1952 The occurrence of a calcinosis syndrome in the cotton rat. I. The effect of diet on the ash content of the heart. J. Nutrition, *47*: 317–326.

Constant, M. A., P. H. Phillips and D. M. Angevine 1952 The occurrence of a calcinosis syndrome in the cotton rat. II. Pathology. Ibid., *47*: 327–339.

Harris, P. N., and R. M. Wylzen 1950 Pathologic changes induced in guinea pigs by administration of diets deficient in the anti-stiffness factor. Am. J. Path., *26*: 595–615.

HAWK, P. B., B. L. OSER AND W. H. SUMMERSON 1948 Practical Physiological Chemistry. The Blakiston Co., Philadelphia, Pa., pp. 844 and 889.

INGLE, D. J. 1950 The biologic properties of cortisone: a review. J. Clin. Endocrinology, *10:* 1312–1354.

KRUSE, H. D., M. M. SCHMIDT AND E. V. MCCOLLUM 1934 Studies on magnesium deficiency in animals. V. Changes in the mineral metabolism of animals following magnesium deprivation. J. Biol. Chem., *106:* 553–571.

LIH, H., AND C. A. BAUMANN 1951 Effects of certain antibiotics on the growth of rats fed diets limiting in thiamine, riboflavin, or pantothenic acid. J. Nutrition, *45:* 143–152.

LOWENHAUPT, E., M. P. SCHULMAN AND D. M. GREENBERG 1950 Basic histologic lesions of magnesium deficiency in the rat. Arch. Path., *49:* 427–433.

MOORE, L. A., E. T. HALLMAN AND L. B. SHOLL 1938 Cardiovascular and other lesions in calves fed diets low in magnesium. Ibid., *26:* 820–838.

RICHERT, D. A., AND W. W. WESTERFELD 1951 Xanthine oxidases in different species. Proc. Soc. Exp. Biol. Med., *76:* 252–254.

ROINE, P., A. N. BOOTH AND C. A. ELVEHJEM 1949 Importance of potassium and magnesium in nutrition of the guinea pig. Ibid., *71:* 90–91.

ROINE, P., AND T. ETTALA 1952 Toxicity of aureomycin to guinea pigs. Nature, *169:* 1014.

SCHWEIGERT, B. S. 1948 Nutritional requirements of the cotton rat and hamster. Vitamins and Hormones, *VI:* 55–67.

SHILS, M. E., H. G. DAY AND E. V. MCCOLLUM 1941 The effect of thiamine deficiency in rats on the excretion of pyruvic acid and bisulfite-binding substances in the urine. J. Biol. Chem., *139:* 145–161.

TUFTS, E. V., AND D. M. GREENBERG 1938 The biochemistry of magnesium deficiency. II. The minimum magnesium requirement for growth, gestation and lactation and the effect of the dietary calcium thereon. Ibid., *122:* 715–726.

VAN WAGTENDONK, W. J., V. SCHOCKEN AND R. WULZEN 1944 A dietary factor essential for guinea pigs. II. A comparative study of the creatine excretion of animals on a diet deficient in this factor and in vitamin E. Arch. Biochem., *1:* 373–377.

VAN WAGTENDONK, W. J., AND R. WULZEN 1950 Physiological and chemical aspects of the anti-stiffness factor essential for guinea pigs. Vitamins and Hormones, *VIII:* 70–124.

WILLIAMS, J. N., JR., AND C. A. ELVEHJEM 1949 The relation of amino acid availability in dietary protein to liver enzyme activity. J. Biol. Chem., *181:* 559–564.

RELATION OF CARBOHYDRATE TO INTESTINAL SYNTHESIS OF BIOTIN AND HATCHABILITY IN MATURE FOWL[1,2]

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FIVE FIGURES

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It is well-recognized that changes in the kind of dietary carbohydrate may be reflected in the amount of vitamins synthesized by intestinal microorganisms (Guerrant, Dutcher and Tomey, '35; Morgan, Cook and Davison, '38; Mannering, Orsini and Elvehjem, '44; Najjar and Barret, '45; and Teply, Krehl and Elvehjem, '47). Evidence has been presented which shows that biotin is synthesized in animals (McElroy and Jukes, '40; Wegner et al., '41; Nielsen et al., '42; Mitchell and Isbell, '42) and in humans (Gardner et al., '43, '45, '46; Oppel, '42); it is synthesized by the bacteria of the intestinal tract (Landy et al., '42; Thompson, '42; Burkholder and McVeigh, '42).

The laying hen appeared to be a desirable experimental animal for studying intestinal synthesis since the vitamin content of the egg could be determined and would be a measure of the biotin available for metabolism. The use of the egg

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[3] General Education Board Fellow.

as a measure of intestinal synthesis eliminates certain difficulties associated with collecting and assaying urine and feces in the chicken. Cravens, Sebesta, Halpin and Hart ('42) reported that biotin was necessary for normal embryonic development in the domestic fowl.

The present investigation was initiated to determine the effect of different carbohydrates on intestinal synthesis of biotin in the mature fowl. Sucrose, dextrin, and combinations of sucrose with lactose or dried whey were tested. Egg production, hatchability of fertile eggs, and biotin content of the eggs were the criteria used in evaluating the extent of intestinal synthesis and absorption of biotin.

MATERIALS AND METHODS

Twenty-four Single Comb White Leghorn pullets were placed in individual laying cages with raised screen bottoms, after being reared on the Experiment Station range. All birds were artificially inseminated weekly with mixed semen from New Hampshire cockerels. All eggs laid were marked with the hen's number and the date and settings were made weekly. The eggs were candled on the sixth and seventeenth days of incubation, at which times the infertile eggs and dead embryos were removed. All eggs which were removed on candling, and also those which failed to hatch, were broken and the age of the embryo at the time of death was estimated. Any gross abnormalities were recorded.

The pullets were fed an all-mash laying ration for 4 weeks prior to the start of the experiment, during which period data were collected on egg production and hatchability. At the end of this pre-experimental period the 24 birds were divided into 6 groups of 4 pullets each. At the end of the ninth week of the experimental period, the pullets which had been receiving diets B33 and B35 were placed on the practical all-mash diet and received the latter during the final week of the experiment. This change was necessitated by the poor condition of the birds at the end of the ninth week.

The materials used in this study were sucrose, dextrin (prepared by autoclaving moist cornstarch 2 hours with subsequent drying and grinding), lactose and dried whey. The composition[4] of the diets is shown in table 1. Crystalline B-vitamins were added in amounts which were thought to meet

TABLE 1

Composition of diets.

	B31	B32	B33	B34	B35
Sucrose — %	63		48	63	38
Dextrin — %		63			
Purified casein — %	18	18	18	18	18
Gelatin — %	5	5	5	5	5
Salts IV — %	5	5	5	5	5
Liver fraction "L" — %	4	4	4	4	4
Fish oil (3000 A) (400 D) — %	2	2	2	2	2
Soybean oil — %	3	3	3	3	3
Lactose — %			15		
Dried whey — %					25
Choline — %	0.2	0.2	0.2	0.2	0.2
Oyster shell			Ad libitum		
All vitamins listed below added as indicated in mg per kg of ration					
Biotin				0.2	
Thiamine HCl	4	4	4	4	4
Riboflavin	6	6	6	6	6
Ca pantothenate	15	15	15	15	15
Niacin	100	100	100	100	100
2-methyl-1,4-naphthoquinone	0.5	0.5	0.5	0.5	0.5
Pyridoxine HCl	4	4	4	4	4
Alpha tocopherol	3	3	3	3	3

the requirements of the pullets with the exception of biotin and pteroylglutamic acid. Liver fraction "L" was used as a source of the latter vitamin. Biotin was omitted from all diets except B34 since this vitamin was the one under study. A practical all-mash diet was used as a positive control in addition to diet B34. Oyster shell and tap water were supplied ad libitum.

[4] We are indebted to the Western Condensing Co., for the lactose and dried whey; to Wilson Laboratories, Chicago, Illinois, for the liver fraction "L"; to Merck and Co., Rahway, N. J., for the biotin.

Eggs which were to be assayed for biotin were set aside on the last 2 days of each week. The microbiological method of Luckey, Moore and Elvehjem ('46), with slight modification, was used. The egg whites and egg yolks were separated and those from each group of birds were pooled for assay. After thorough mixing, approximately 10 gm were weighed out. An equal number of milliliters of 4N sulfuric acid was added and the sample autoclaved for 2 hours at 15 pounds pressure. In preliminary assays on eggs it was determined that hydrolysis with 4N, 8N or 12N sulfuric acid, as outlined above, gave approximately the same results. The 4N acid was selected since the sample would contain sodium sulfate on neutralization. After autoclaving, the pH was adjusted to 4–5 with 4N sodium hydroxide and the samples were filtered through Whatman no. 1 filter paper. The filtrate was neutralized to pH 6.8–7.0 and preserved under toluene in the refrigerator. Dilutions were made as needed. The organism used for the assay was Streptococcus faecalis R. The extent of growth of the organism was measured by turbidity using the Evelyn colorimeter. Satisfactory recoveries of biotin were obtained when known amounts of the vitamin were added to the samples. The biotin content of the egg whites and yolks is expressed in millimicrograms per gram on a fresh weight basis.

EXPERIMENTAL RESULTS

Egg production. The production of pullets fed the various diets is shown in figure 1. The egg production of pullets fed diets B31 and B32 and those fed the practical all-mash diet followed the same general trend throughout the 10-week period although a slight drop in the egg production of pullets fed sucrose, diet B31, may be noted during the last week of the experiment. The egg production of pullets fed sucrose plus biotin, diet B34, was somewhat erratic. The fluctuations in this case were traceable to 1 pullet and are more apparent due to the small number of pullets in the group; this pullet would lay a clutch of eggs and go out of production, then come back in production and repeat the same procedure.

The rather high levels of lactose and dried whey appeared to cause a decrease in egg production. The production of pullets fed diets B33, lactose, and B35, dried whey, decreased during the third and fourth weeks of the experimental period and remained at a fairly low level for the rest of the 10-week period. This low production was unfortunate because an insufficient number of eggs were laid to obtain a measure of

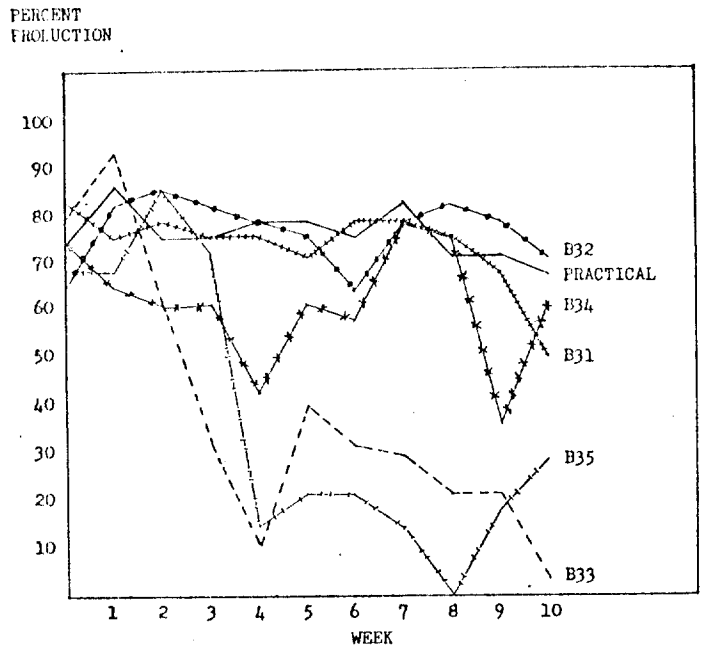

Fig. 1 Effect of diet on egg production: diet B31, sucrose; B32, dextrin; B33, lactose; B34, biotin; B35, dried whey.

hatchability and for biotin assay in the case of pullets fed dried whey (diet B35).

Hatchability. The per cent hatchability of fertile eggs is shown in figure 2. Hatchability of eggs from pullets fed diet B31, sucrose, decreased to zero by the third week and remained at this level. Hatchability of eggs from pullets fed diet B32, dextrin, ranged from 60 to 85% during the 10-week period. This was somewhat lower than that of the positive control

groups which were fed diet B34 and the practical all-mash diet. Hatchability of eggs from pullets fed diet B34, sucrose plus biotin, was approximately the same as that of those fed the practical all-mash diet. The results with respect to hatchability obtained with diets B33, lactose, and B35, dried whey, were somewhat inconclusive after the third and fourth weeks due to the poor egg production which resulted from the

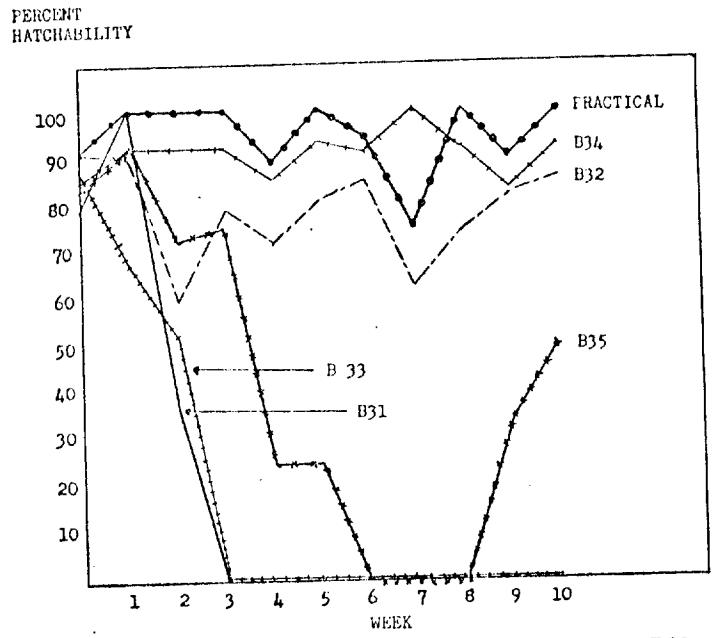

Fig. 2 Effect of diets on hatchability: diet B31, sucrose; B32, dextrin; B33, lactose; B34, biotin; B35, dried whey.

amounts of these materials used in this study. This was discussed above.

Biotin content of egg white and yolks. At the beginning of the experiment the biotin content of the egg whites (fig. 3) varied from 65 to 105 mμg per gram expressed on a fresh weight basis. The biotin content of egg whites from hens fed diet B34 decreased from 65 to 17 mμg per gram during the first week and the presence of the vitamin was not detected in egg whites from these birds after this time. The level of biotin in

the whites of eggs from pullets fed diet B32 decreased from 88 to 22 mμg per gram during the first week and remained at the latter figure until the end of the third week; during the fourth week there was a further decrease from 22 to about 4–5 mμg of biotin per gram and it remained at this level through the eighth week.

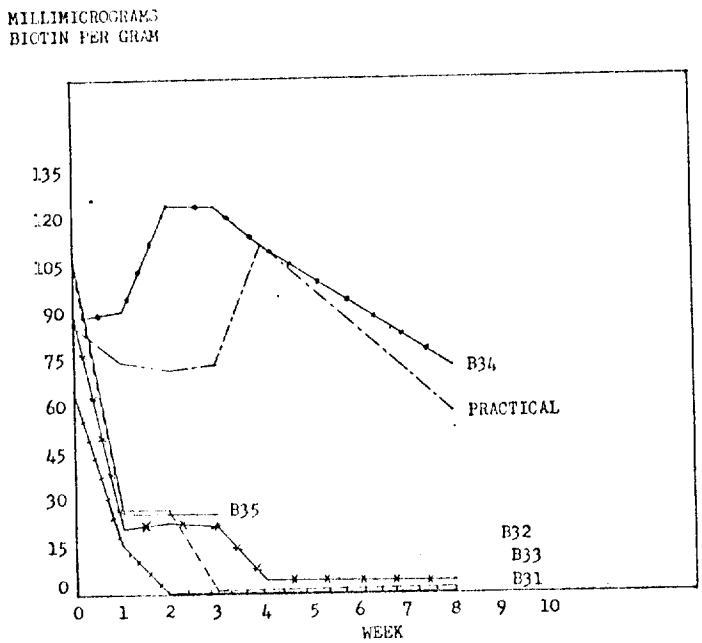

Fig. 3 Effect of diet on biotin content of egg white: diet B31, sucrose; B32, dextrin; B33, lactose; B34, biotin; B35, dried whey.

The biotin content of egg whites from pullets fed diet B33 decreased from 105 to 27 mμg per gram during the first week and remained at the latter figure throughout the second week. At the end of the third week and thereafter, biotin could not be detected in egg whites from pullets fed diet B33. There was a decrease in the biotin content of egg whites from pullets fed diet B35 from 105 to 26 mμg of biotin per gram during the first week and it remained at the latter figure until the end of the third week. No eggs were analyzed from

pullets fed diet B35 after the third week due to the poor egg production of the birds. The feeding of 200 μg of biotin with sucrose, diet B34, was sufficient to maintain a slightly higher level of the vitamin in egg whites than was maintained in egg whites of pullets fed the practical all-mash diet (fig. 3).

The egg yolks from the pullets used in this study contained approximately 500 mμg of biotin per gram at the start of the

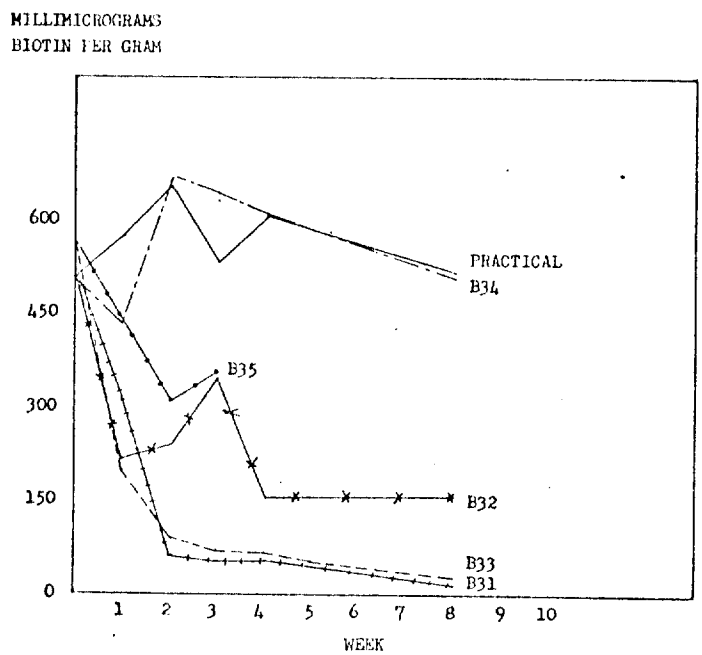

Fig. 4 Effect of diet on biotin content of egg yolk: diet B31, sucrose; B32, dextrin; B33, lactose; B34, biotin; B35, dried whey.

experiment (fig. 4). The biotin content of the yolks from pullets fed diets B31, sucrose, and B33, lactose, decreased from about 500 to 50–70 mμg of biotin per gram during the first 2 weeks of the experiment. The egg yolks from pullets fed diet B32, dextrin, showed a decrease of from 500 to approximately 150 mμg of the vitamin per gram and remained at the lower level. The biotin content of egg yolks from pullets fed diet B35 followed the same general trend as those

TABLE 2

Effect of diet on incidence of syndactyly and other skeletal deformities.

HEN	DIET	NO. OF FERTILE EGGS	EMBRYOS DYING		NUMBER OF EMBRYOS SHOWING			
			Before 8 days	After 8 days	Beak parrot	Crooked tibia	Short twisted tarso metatarsus	SYNDACTYLY
1	B-31	49	19	18	8	7	7	7
2		13	3	7	3	6	6	6
3		16	14	0	0	0	0	0
4		35	18	14	8	2	2	5
	Total	113	54	39	19	15	15	18
5	B-32	40	2	2	0	0	0	0
6		48	2	6	0	0	0	0
7		49	2	4	0	0	0	0
8		43	7	9	0	0	0	0
	Total	180	13	21	0	0	0	0
9	B-33	28	9	11	3	5	5	4
10		13	7	1	0	0	0	0
11		11	1	4	4	4	4	3
12		9	1	7	1	2	2	4
	Total	61	18	23	8	11	11	11
13	B-34	37	2	3	0	0	0	0
14		42	1	0	0	0	0	0
15		33	0	4	0	0	0	0
16		6	0	0	0	0	0	0
	Total	118	3	7	0	0	0	0
17	B-35	26	10	4	0	2	2	1
18		15	1	0	0	0	0	0
19		6	0	0	0	0	0	0
20		8	1	1	0	0	0	0
	Total	55	12	5	0	2	2	1
21	Practical	42	1	2	0	0	0	0
22	Control	39	0	1	0	0	0	0
23	Diet	49.	0	2	0	0	0	0
24		37	0	3	0	0	0	0
	Total	177	1	8	0	0	0	0

from pullets fed diet B32. Diet B34 and the practical all-mash diet supported about the same concentration of the vitamin in the egg yolks.

Embryonic mortality. The classification of the embryos that died during the incubation period is shown in table 2. There was a high incidence of micromelia and syndactyly in dead embryos from pullets fed diet B31. The micromelic embryos

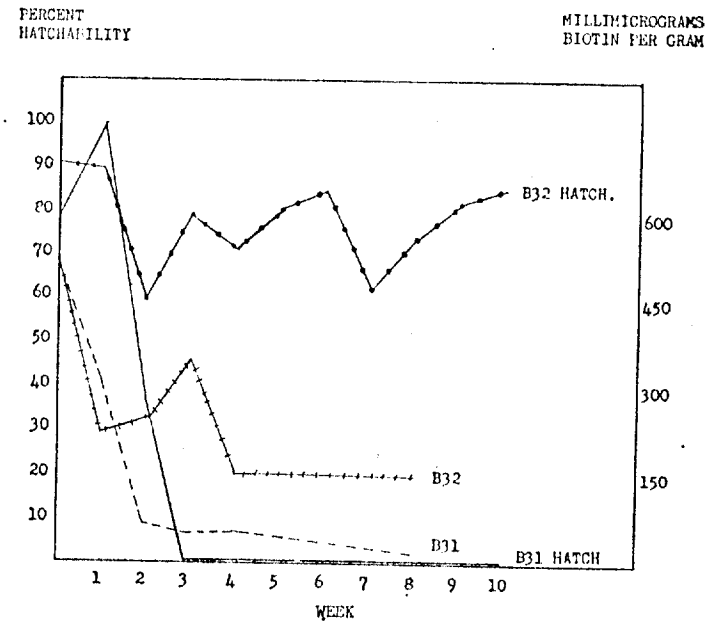

Fig. 5 Relation of carbohydrate to biotin content of egg yolk and hatchability: diet B31, sucrose; B32, dextrin.

were characterized by a parrot beak which was generally associated with a crooked tibia and a short twisted tarsometatarsus. Syndactyly and micromelia were not observed in dead embryos from pullets fed diet B32, diet B34 or from those fed the practical all-mash diet. The incidence of micromelia and syndactyly was high in the dead embryos from pullets fed diet B33 and was low in those from pullets fed diet B35, although these results are inconclusive due to the poor egg production of the birds.

DISCUSSION OF RESULTS

It may be noted from figures 2, 3, 4 and 5 that dextrin (diet B32) maintains a higher level of biotin in the egg yolk than does sucrose (diet B31) and that this is associated with a much higher per cent hatchability. The biotin content of egg yolks from pullets fed diet B31 decreased from about 500 to 50 mμg of biotin per gram during the first 3 weeks and had decreased to about 16 mμg of the vitamin per gram by the end of the eighth week. The decrease in the per cent hatchability of eggs from pullets fed diet B31 paralleled the decrease in biotin content of the yolk (fig. 5). The per cent hatchability was 79 at the start of the experiment. This increased to 100% for some unknown reason during the first week and decreased to zero by the end of the third week. It is apparent therefore that there was an insufficient amount of biotin to support embryonic development in the eggs from pullets fed diet B31. Thus it is indicated that eggs containing 50 mμg of biotin per gram of yolk and an undetectable amount in the white will not support normal embryonic development. In the case of pullets fed diet B32 there was a decrease in the biotin content of the yolk to about 150 mμg by the end of the fourth week and this amount of biotin was found in the yolks at the end of the eighth week. Hatchability of eggs from pullets fed diet B32 was 91% at the start of the experiment and varied from 60 to 85% in the course of the 10-week period. It is realized that the per cent hatchability of eggs from pullets fed diet B32 is lower than that of pullets fed diet B34 and also lower than that of pullets fed the practical all-mash diet. Embryonic abnormalities which appeared to be identical with those previously described by Cravens et al. (44), as occurring in eggs from hens fed a low-biotin diet, were noted in eggs from pullets fed diet B31 but did not appear in eggs of pullets fed diet B32 (table 2). From these data it is indicated that eggs which contain 150 mμg of biotin per gram of yolk on a fresh weight basis will support embryonic development even though hatchability may be somewhat reduced.

Supplementation of the sucrose diet with biotin (diet B34) resulted in a per cent hatchability and biotin content of the eggs comparable to those obtained by feeding a practical all-mash diet (figs. 2, 3 and 4). These data indicate that the deficiency which existed in the pullets fed diet B31 was a biotin deficiency.

The results obtained with dextrin (diet B32) as compared to those obtained with sucrose (diet B31) suggest that there is a marked synthesis of biotin in the intestinal tract. Further, there is good evidence that the biotin synthesized in the intestinal tract of the pullets fed dextrin was absorbed and deposited in the egg. This latter fact was established through biotin analyses of the eggs and by the fact that the biotin in the eggs was available to the embryo as evidenced by the per cent hatchability.

This is in agreement with the work of Mannering et al. ('44) in which it was reported that dextrin favored the synthesis of riboflavin in the rat, and also with the work of Teply et al. ('47) in which it was reported that dextrin diets produced the greatest synthesis of niacin and folic acid in the rat.

The data presented in this paper indicate that the laying hen is a desirable animal for studying intestinal synthesis since a determination of vitamin content of the egg offers a method of evaluating the extent of synthesis and subsequent absorption of vitamins from the intestinal tract which is difficult with other animals.

The studies reported herein shed no light on the important problem of where synthesis and absorption take place in the intestinal tract. Studies by Johansson et al. ('47) which were conducted on the birds used in the present investigation indicate that there was a marked difference in the intestinal flora of birds fed the various diets. Dextrin appeared to favor a biotin synthesizing organism while sucrose favored a biotin-utilizing organism.

The biotin content of the eggs did not appear to be related to the egg production of the hens in the cases of pullets fed

diets B31 (sucrose) or B32 (dextrin). This is in agreement with the work of Cravens et al. ('42).

Results obtained by replacing a part of the sucrose with lactose (diet B33) or with dried whey (diet B35) are somewhat inconclusive due to the poor egg production of the pullets fed these diets. It is probably true that the level of lactose and dried whey used in these diets was too high for best results and suggests that the laying pullet may not tolerate lactose in quantities equal to that of the rat (Geyer et al., '46). Lactose, in the quantity used in diet B33, did not appear to favor the intestinal synthesis of biotin (figs. 2, 3 and 4). The biotin content and per cent hatchability of eggs from pullets fed diet B33 (lactose) followed the same general trend as those of pullets fed diet B31 (sucrose). Embryonic abnormalities, which were associated with a low biotin diet, were also observed in embryos from pullets fed diet B33 (table 2).

The results obtained with diet B35 (dried whey) were quite inconclusive after the third or fourth week due to the poor egg production of the pullets fed this diet (fig. 1). There was a higher level of biotin in the egg yolks and whites and a higher per cent hatchability of eggs from pullets fed diet B35 during the first 3 weeks than was noted in eggs from pullets fed diets B31 and B33. This may have been traceable to the fact that 25% dried whey contributed approximately 40 μg of biotin per kilogram of diet. A small number of embryos exhibiting symptoms of a biotin deficiency were noted in the eggs of 1 pullet fed this diet (table 2).

The term "micromelia" is used in contrast to the term "chondrodystrophy" in referring to the characteristic embryonic abnormality associated with a deficiency of biotin in the diet of the mother hen. It is recognized that the term "chondrodystrophy" designates a certain histopathologic picture. It should be pointed out, however, that from a gross macroscopic study the micromelia embryos recorded in table 2 of the present report and described by Cravens et al. ('44) are indistinguishable from the chondrodystrophy which has been shown to have a hereditary basis (Lamoreaux, '42).

The occurrence of micromelia and syndactyly in embryos from pullets fed diet B31 extends and confirms the previous work of Cravens et al. ('44). It may be noted from table 2 that the characteristics which describe micromelia: parrot beak, crooked tibia and short-twisted tarsometatarsus appeared to occur in about the same frequency as syndactyly. This indicates that syndactyly is a definite symptom of biotin deficiency in the developing chick embryo as was also observed by Cravens et al. ('44). Micromelia and syndactyly were observed among embryos which lived beyond the eighth day of incubation from pullets fed diets B31 and B33. The fact that eggs from these birds were low in biotin has been discussed earlier. The incidence of micromelia and syndactyly appeared to be fairly well distributed among the individual pullets fed diets B31 and B33 (table 2).

SUMMARY

Evidence is presented to show that dextrin favors the intestinal synthesis of biotin in the mature fowl and sucrose does not promote the synthesis of this vitamin. The results obtained with lactose and dried whey indicate that lactose does not stimulate the synthesis of biotin in the intestinal tract of the laying pullet.

The laying hen is a desirable animal for studying intestinal synthesis. A determination of the vitamin content of the egg offers a method of evaluating the extent of synthesis and subsequent absorption of vitamins from the intestinal tract which is difficult in other animals.

There is a failure of embryonic development when the egg yolk contains as little as 50 mμg of biotin per gram on a fresh weight basis. Eggs which contain 150 mμg of biotin per gram will support normal embryonic development.

LITERATURE CITED

Burkholder, P. R., and I. McVeigh 1942 The synthesis of vitamins by intestinal bacteria. Nat. Acad. Sci., *28*: 285.

CRAVENS, W. W., E. E. SEBESTA, J. G. HALPIN AND E. B. HART 1942 Effect of biotin on reproduction in the domestic fowl. Proc. Soc. Exp. Biol. Med., *50:* 101.

CRAVENS, W. W., W. H. MCGIBBON AND E. E. SEBESTA 1944 Effect of biotin deficiency on embryonic development in the domestic fowl. Anat. Rec., *90:* 55.

GARDNER, J., A. L. NEAL, W. H. PETERSON AND H. T. PARSONS 1943 Biotin, pantothenic acid, and riboflavin balances of young women on a milk diet. J. Am. Diet. Assoc., *19:* 683.

GARDNER, J., H. T. PARSONS AND W. H. PETERSON 1945 Human biotin metabolism on various levels of biotin intake. Arch. Biochem., *8:* 339.

——————— 1946 Human utilization of biotin from various diets. Am. J. Med. Sci., *211:* 198.

GEYER, R. P., R. K. BOUTWELL, C. A. ELVEHJEM AND E. B. HART 1946 The effect of fat on the utilization of galactose by the albino rat. J. Biol. Chem., *162:* 251.

GUERRANT, N. B., R. A. DUTCHER AND L. F. TOMEY 1935 The effect of the type of carbohydrate on the synthesis of B vitamins in the digestive tract of the rat. J. Biol. Chem., *110:* 233.

JOHANSSON, K. R., S. K. SHAPIRO AND W. B. SARLES 1947 The flora of hens as influenced by various carbohydrates in a biotin-deficient ration. Soc. Am. Bact., Abst. of Proc., 47th General Meeting, p. 35.

LAMOREAUX, W. F. 1942 Hereditary chondrodystrophy in the fowl. J. Heredity, *33:* 275.

LANDY, M. L., D. M. DICKINS, M. M. BICKING AND W. R. MITCHELL 1942 Use of avidin in studies on the biotin requirement of microorganisms. Proc. Soc. Exp. Biol. Med., *49:* 441.

LUCKEY, T. D., P. R. MOORE AND C. A. ELVEHJEM 1946 A differential, microbiological assay for O heterobiotin. Proc. Soc. Exp. Biol. Med., *61:* 97.

MANNERING, G. J., D. ORSINI AND C. A. ELVEHJEM 1944 Effect of the composition of the diet on the riboflavin requirement of the rat. J. Nutrition, *28:* 141.

MCELROY, L. W., AND T. H. JUKES 1940 Formation of the anti egg-white-injury factor (biotin) in the rumen of the cow. Proc. Soc. Exp. Biol. Med., *45:* 296.

MITCHELL, H. K., AND E. R. ISBELL 1942 Intestinal bacterial synthesis as a source of B vitamins for the rat. Univ. of Texas Pub., no. 4237, p. 125.

MORGAN, A. F., B. B. COOK AND H. G. DAVISON 1938 Vitamin B_2 deficiencies as affected by dietary carbohydrate. J. Nutrition, *15:* 27.

NAJJAR, V. A., AND R. BARRETT 1945 The synthesis of B vitamins by intestinal bacteria. Vitamins and Hormones, *3:* 23; Academic Press, New York.

NIELSEN, E., G. M. SHELL AND W. H. PETERSON 1942 Response of bacteria, yeast and rats to peroxide-treated biotin. Intestinal synthesis of biotin in the rat. J. Nutrition, *24:* 523.

OPPEL, T. W. 1942 Studies of biotin metabolism in man. Am. J. Med. Sci., *204:* 806.

TEPLY, L. J., W. A. KEEHL AND C. A. ELVEHJEM 1947 The intestinal synthesis of niacin and folic acid in the rat. Am. J. Physiol., *148:* 91.

THOMPSON, R. C. 1942 Synthesis of B vitamins by bacteria in pure culture. Univ. of Texas Pub no. 4237, p. 87.

WEGNER, M. I., A. N. BOOTH, C. A. ELVEHJEM AND E. B. HART 1941 Rumen synthesis of the vitamin B complex on natural rations. Proc. Soc. Exp. Biol. Med., *47:* 517.

THE EFFECT OF PTEROYLGLUTAMIC ACID AND AN UNIDENTIFIED FACTOR ON EGG PRODUCTION AND HATCHABILITY [1]

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Bauernfeind and Norris ('39) and Schumacher, Heuser and Norris ('40) reported a factor necessary for breeding hens which they designated Factor R and which subsequently has been suggested to be a mixture of folic acid conjugates (Charkey, Daniel, Farmer, Norris and Heuser, '47). The essential nature of a factor present in the *Lactobacillus casei* ε eluate fraction of solubilized liver extract for breeding hens was established by Cravens, Sebesta, Halpin and Hart ('42). The identification of the *L. casei* factor as pteroylglutamic acid was established by Angier et al. ('46). Since synthetic pteroylglutamic acid (PGA) has become available, Taylor ('47) and Schweigert, German, Pearson and Sherwood ('48) have published reports on the quantitative needs of the breeding hen for this vitamin. Taylor ('47) reported that 0.12 mg per kilogram of ration was essential for egg production, while an amount somewhat in excess of this was required for satisfactory hatchability. Schweigert et al. ('48), on the other hand, reported that the requirement is between 0.12 and 0.42 mg per kilogram of ration.

During the course of studies designed to determine the effect of pteroylglutamic acid on egg production and hatch-

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ability it became obvious that the results were complicated by a deficiency of an unidentified factor or factors. Data will be presented in this report indicating the importance of such a factor or factors for hatchability. Further, some of the data may shed some light on the quantitative needs of the breeding hen for pteroylglutamic acid.

EXPERIMENTAL

Single Comb White Leghorn pullets were used in all experiments. The birds were housed in individual-cage laying-batteries with raised screened floors. They were artificially

TABLE 1

Composition of experimental diets

INGREDIENTS	BASAL DIETS				VITAMINS, CHOLINE AND INOSITOL ADDED TO ALL BASAL DIETS	
	B20	B22	B23	B24		
	%	%	%	%		*mg/kg of diet*
Dextrin	64	67	..		Riboflavin	6.0
Sucrose	..	..	67	67	Thiamine-HCl	4.0
Casein [1]	18	18	18	18	Ca pantothenate	15.0
Gelatin	5	5	5	5	Niacin	100
Salts IV	5	5	5	5	Pyridoxine-HCl	4.0
Soybean oil	3	3	3	3	2-Methyl 1,4-naphthoquinone	0.5
Vitamins A and D (400D-3000A)	2	2	2	2	Biotin	0.2
Liver fraction L	3.0	..	..	..	Alpha-tocopherol	3.0
Fish solubles (50% solids)		..	..	3.0	Choline	2,000
					Inositol	1,000

[1] Vitamin test. General Biochemicals, Inc.

inseminated weekly with pooled semen from New Hampshire males. All birds were weighed weekly. Eggs were collected daily, marked with the hen's number and recorded. Settings of eggs were made weekly. All eggs failing to hatch were broken to determine fertility and to make gross examinations of the embryos for developmental abnormalities.

The composition of the basal diets used is given in table 1. Feed, water and oyster shell were supplied ad libitum.

Experiment 1 was designed to obtain information on the pteroylglutamic acid requirements of the breeding chicken. Five groups of 4 birds each were fed the following rations for 11 weeks: Group 1, diet B20 (which served as a control since this diet had proved satisfactory in previous experiments); group 2, diet B22; groups 3–5, ration B22 with pteroylglutamic acid additions at levels of 0.1, 0.25 and 1.0 mg per kilogram of diet, respectively. The hatchability and egg production data are presented in table 2.

Experiment 2 was similar in design to experiment 1. The following diets were fed to 6 groups of three birds each for 14 weeks: Group 1, diet B20; group 2, B22; and groups 3–6,

TABLE 2

Effect of pteroylglutamic acid on egg production and hatchability

PGA SUPPLEMENT TO DIET B22:	EXPERIMENT 1		EXPERIMENT 2	
	Egg production	Hatchability	Egg production	Hatchability
mg/kg	%	%	%	%
0	51	40	36	32
0.10	59	56	40	35
0.20			39	28
0.25	61	51		
0.50			31	60
1.0	51	66	51	20
3% Liver L	57	83	55	71

diet B22 supplemented with 0.1, 0.2, 0.5 and 1.0 mg of pteroylglutamic acid per kilogram of diet, respectively. The hatchability and egg production data are given in table 2.

It is obvious from the results shown in table 2 that none of the diets fed in experiments 1 and 2, except perhaps the control, was adequate for good hatchability. It is also obvious that the response of the birds to the same diets in the two experiments was radically different. The possible significance of this observation will be discussed later, but suffice it to point out here that the results of experiments 1 and 2 show definitely that some unidentified factor, or factors, needed by breeding hens is missing from diet B22 supplemented with

PGA. It was also suspected that intestinal synthesis on the dextrin rations used in these experiments might be one factor responsible for certain of the erratic results observed, since Couch et al. ('48a) have shown that dextrin promotes intestinal synthesis of biotin in the mature fowl. Thus an experiment was designed to take these factors into consideration.

In the third experiment sucrose was compared with dextrin as a carbohydrate source and condensed fish solubles was tested as a source of unidentified factors. Additional purified casein was also added to eliminate the possibility that insufficient protein or amino acids might be affecting the results. The diets fed and the resulting egg production and hatchability for experiment 3 may be seen in table 3.

TABLE 3

Rations fed and per cent of egg production and hatchability (Experiment 3)

GROUP [1]	BASAL DIET	SUPPLEMENT TO BASAL DIET	EGG PRODUCTION	HATCHABILITY		
				Start of exp.	End of exp.	Ave.
			%	%	%	%
1	B22	None	59	75	29	62
2	B23	None	36	90	0.0	37
3	B22	4.0 mg per kg PGA	62	100	40	72
4	B23	4.0 mg per kg PGA	[2]	74	8.0	65
5	B22	6% casein [3]	51	87	17	45
6	B22	6% casein plus 4.0 mg per kg PGA	79	100	29	73
7	B23	6% casein plus 4.0 mg per kg PGA	61	96	20	76
8	B22	3% liver fraction L	82	75	91	83
9	B23	3% liver fraction L	75	89	88	84
10	B23	3% liver fraction L plus 6% casein	76	74	87	94
11	B22	4.0 mg per kg PGA plus 3% fish solubles	67	75	90	92
12	B23	same as group 11	76	90	71	86
13	Practical ration		68	95	95	93

[1] Four birds in each group.

[2] Not presented because of loss of two birds in this group.

[3] Vitamin test casein. General Biochemicals, Inc.

From the data shown in this table it appeared that satisfactory egg production and hatchability could be obtained using either sucrose or dextrin as a source of carbohydrate when liver fraction L or fish solubles and 4.0 mg of pteroylglutamic acid per kilogram of ration were included in the diets. With the results of experiment 3 at hand it appeared that a diet was available (group 12 of experiment 3) which

TABLE 4

Rations fed and per cent of egg production and hatchability (Experiment 4)

GROUP [1]	BASAL DIET	SUPPLEMENT TO BASAL DIET	EGG PRODUCTION	HATCHABILITY		
				Start of exp.	End of exp.	Ave.
			%	%	%	%
2	I23	4.0 mg per kg PGA	48.5	75	0	27
3	I22	Same as group 2	70.8	86	36	54
4	I24	None	50.9	89	0	26
5	I24	0.25 mg per kg PGA	74.7	90	0	46
6	I24	0.50 mg per kg PGA	70.5	95	0	65
7	I24	0.75 mg per kg PGA	73.8	100	0	53
8	I24	1.0 mg per kg PGA	69.9	89	0	75
9	I24	4.0 mg per kg PGA	62.8	100	40	76
10	I22	3% fish solubles	55.7	87	31	53
11	I22	Same as group 10 plus 4.0 mg per kg PGA	59.5	95	36	80
12	I23 less gelatin	Same as group 2	43.9	100	0	41
13	Practical control		55.1	100	67	96

[1] Four birds in each group.

would make possible studies on the quantitative needs of breeding chickens for pteroylglutamic acid. Experiment 4 was then set up to gain evidence on this point and to obtain further evidence on the unidentified factor as well as the possible effect of the source of carbohydrate on the reaction of the birds to the diets used. The diets fed and the egg production and hatchability data for experiment 4 are given in table 4.

RESULTS AND DISCUSSION

Unidentified factors

The results of experiments 1 and 2, given in table 2, are of interest because they emphasize the need of breeding hens for some unidentified factor which is essential for embryonic development. Further, the data indicate that the bird may be depleted of this factor in a relatively short period of time. It was mentioned previously that a marked difference in response of the birds to identical rations in experiments 1 and 2 was observed. In experiment 2 the birds depleted much more quickly and uniformly than in experiment 1. In experiment 2 hatchability of all groups dropped to zero by the 7th week, while in experiment 1 only two groups dropped to zero by the 6th week and even in these hatchability again improved as the experiment progressed.

Data which suggest that diets containing dextrin may promote the synthesis of an unidentified factor or factors in the intestinal tract are given in tables 3 and 4. A comparison of the hatchability of the eggs from groups 3 and 4 of experiment 3 (table 3) shows that those of group 3 which were produced by hens receiving dextrin as a carbohydrate hatched better than those from hens receiving sucrose as a carbohydrate. The results of experiment 4, given in table 4, further indicate that dextrin stimulates the intestinal synthesis of an unidentified factor or factors essential for the breeding hen. The hens of group 3, which received dextrin as a carbohydrate, produced eggs which hatched significantly better than those from hens of group 2, which were receiving sucrose as a carbohydrate.

Further evidence showing the importance in the diet of breeding hens of an unidentified factor in fish solubles and liver fraction L is given in the results of experiments 3 and 4, presented in tables 3 and 4. In every case a rapid decline (to less than 20% in 4 weeks) in hatchability occurred when a sucrose diet containing no fish solubles or liver fraction L, but containing 4.0 mg per kilogram of pteroylglutamic acid

was fed to the birds. The drop in hatchability was prevented by the addition of either fish solubles or liver fraction L. Two birds of group 4 were discarded at 4 weeks as they had lost considerable weight and had developed anorexia. The injection of 0.1 ml of reticulogen[2] brought about a dramatic recovery, but obviously the two birds had to be eliminated from the group.

The addition of casein did not improve hatchability. It is interesting to note, however, that the addition of 6% purified casein (group 5) appeared to result in a more rapid depletion of the birds (to 13% in 4 weeks) and that this effect was apparently overcome by the addition of pteroylglutamic acid (group 6). The birds of group 12 which received the same diet as group 2, except that gelatin was omitted, did not deplete any more rapidly than those of group 2.

There is some evidence from these experiments that a deficiency of the factor found in fish solubles and liver fraction L resulted in a decline in egg production. For example, group 4 of experiment 3 produced eggs at a very low rate and, as mentioned previously, two of the birds in this group showed a pronounced decline in weight. By comparing the rate of production of the groups of birds receiving dextrin and no fish solubles with that of the groups receiving a similar diet containing sucrose (tables 3 and 4), it may be seen that the dextrin-fed birds produced at a higher rate, as would be expected if intestinal synthesis made available to the bird a limited quantity of the factor yet one sufficient to support egg production. It is believed, however, that additional data are necessary to demonstrate the possible effect of this factor on egg production, since with such great individual variations, the numbers involved in the present experiments are inadequate to determine significance.

The studies reported herein dealing with an unidentified factor or factors essential for the breeding bird are of interest chiefly because they demonstrate that the laying bird can be depleted very quickly of an unidentified factor es-

[2] Lilly.

sential for hatchability. Numerous reports dealing with unidentified factors essential for breeding hens have appeared, including those of McGinnis et al. ('44), Bird et al. ('46), Bethke et al. ('46), and Cravens et al. ('46). Rubin et al. ('47) presented data which indicated that 15–16 weeks of feeding all-vegetable diets deficient in an unidentified factor were required to deplete the birds. Rubin, Bird and Rothchild ('46) suggested that intestinal synthesis of an unidentified factor might occur in the mature bird since hen feces were found to be a good source of unidentified chick growth factors essential in all-vegetable chick rations. McGinnis, Stevens and Groves ('47) presented evidence which they interpreted as indicating that the growth factors were synthesized, for the most part, by microorganisms after the feces were voided and not in the intestinal tract of the hen. Similar results have been reported by Kennard et al. ('48). The data presented herein are in agreement with the work of Rubin, Bird and Rothchild ('46), and these data further suggest that such synthesis may be, in part, responsible for the prolonged depletion period required when diets are employed which promote intestinal synthesis. It is probable, however, that other unrecognized conditions affect the rate of depletion since, even with the purified diets employed in the present study, considerable variation in degree and time of depletion was observed. It seems logical to conclude, nevertheless, that rapid depletion may be obtained if conditions are right, since there is abundant evidence that the hen may be quickly depleted of water soluble vitamins, and the unidentified factor, or factors, from cow manure, fish solubles, fish meal and certain liver fractions is water soluble.

Pteroylglutamic acid

The results of experiment 4, given in table 4, fail to provide clear-cut data on the pteroylglutamic acid requirements of the breeding hen. It would appear from these data that between 0.5–1.0 mg per kilogram of diet is essential for good hatchability, but even at a level of 4.0 mg per kilogram hatchability was not maintained at a high level for the 12-week

experiment. A rapid falling off in hatchability had occurred on the 0.25, 0.50 and 0.75 mg levels by the 9th week of the experiment, while a similar fall in hatchability occurred at the 10th and 11th weeks for the 1.0 and 4.0 mg levels, respectively. The significance of these results is not apparent at this time and the possibility exists that the fall in hatchability at 11 and 12 weeks is related to some environmental conditions, since the control group (13) dropped to 67% during the last week of the experiment. Of course, the possibility also exists that the birds had become depleted of a second factor by this time, or that 3% fish solubles supplied inadequate quantities of the factor to support hatchability. It is obvious, however, that further experiments are necessary to determine the reason for the pronounced drop in hatchability observed during the latter phases of experiment 4.

There is no question but that the birds in group 4, experiment 4, produced eggs at a significantly lower rate than did the birds receiving an adequate amount of pteroylglutamic acid. The average production of the birds of group 4, given in table 4, does not give a true picture of the egg production data of this group, since there was a gradual decline in egg production to 11% by the end of the experiment. The drop in production was most rapid after the 8th week. No such decline was observed in group 5, which received 0.25 mg of pteroylglutamic acid per kilogram of diet. Neither was there a pronounced decline in the production of group 10, which was fed a diet deficient in pteroylglutamic acid with dextrin as the carbohydrate. These results suggest that 0.25 mg of this vitamin per kilogram of ration is adequate for egg production and, further, that with the rations containing carbohydrates that promote intestinal synthesis pteroylglutamic acid may be synthesized in sufficient quantities to meet the needs of the laying bird.

The results of this study are not in agreement with the results reported by Schweigert et al. ('48) regarding the pteroylglutamic acid requirements of the breeding bird. It appears likely that the higher requirement indicated in the

present study (0.5–1.0 mg per kilogram) results from the use of a type of basal diet which does not favor intestinal synthesis of the vitamin. That intestinal synthesis was a factor affecting hatchability may be seen by comparing the results of groups 4 (sucrose) and 10 (dextrin), table 4. Hatchability of eggs from the birds of group 10 declined rapidly, but after an apparent adjustment period it again increased, only to decline again. Such variations have been noted in studies on the effects of various carbohydrate sources on the intestinal synthesis of biotin (Couch et al., '48b). The diets employed by Schweigert et al. would appear to favor such synthesis and thus might lead to the suggestion of requirements far below the true optimum.

SUMMARY

1. Four experiments with breeding chickens have been described which demonstrate the importance for hatchability of an unidentified factor or factors.

2. Fish solubles and liver fraction L are good sources of the factor or factors.

3. The breeding hen may be depleted of the factor in from 4–6 weeks when purified diets are used.

4. Data are presented which indicate that dextrin favors the intestinal synthesis of the factor in the mature fowl.

5. The pteroylglutamic acid requirement of the laying bird is probably not over 0.25 mg per kilogram of diet.

6. The pteroylglutamic acid requirement of the breeding bird appears to be approximately 0.5–1.0 mg per kilogram of diet when a diet is employed which does not favor intestinal synthesis of the vitamin.

7. Evidence is presented which indicates that pteroylglutamic acid synthesis in the intestinal tract of the breeding hen is favored by diets containing dextrin as a carbohydrate.

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LITERATURE CITED

ANGIER, R. B., J. H. BOOTHE, B. L. HUTCHINGS, J. H. MOWAT, J. SEMB, E. L. R. STOKSTAD, Y. SUBBAROW, C. W. WALLER, D. B. COSULICH, M. J. FAHRENBACH, M. E. HULTQUIST, E. KUH, E. H. NORTHEY, D. R. SEEGER, J. P. SICKLES AND J. M. SMITH, JR. 1946 The structure and synthesis of the liver L. casei factor. Science, *103:* 667.

BAUERNFEIND, J. C., AND L. C. NORRIS 1939 The role of the antidermatosis vitamin and a new water soluble growth factor in the nutrition of the mature fowl. J. Nutrition, *18:* 579.

BETHKE, R. M., D. C. KENNARD AND V. D. CHAMBERLIN 1946 The relation of nutrition to production and hatchability of chicken eggs. II. Effects of choline chloride, methionine, and certain animal and marine products. Poultry Sci., *25:* 579.

BIRD, H. R., M. RUBIN, D. WHITSON AND S. K. HAYNES 1946 Effectiveness of dietary supplements in increasing hatchability of eggs and viability of progeny of hens fed a diet containing a high level of soybean oil meal. Ibid., *25:* 285.

CHARKEY, L. W., LOUISE J. DANIEL, FLORENCE A. FARMER, L. C. NORRIS AND G. F. HEUSER 1947 Further studies on factor R. Proc. Soc. Exp. Biol. Med., *64:* 102.

COUCH, J. R., W. W. CRAVENS, C. A. ELVEHJEM AND J. G. HALPIN 1948a The relation of carbohydrate to intestinal synthesis of biotin and hatchability in mature fowl. J. Nutrition, *35:* 57.

COUCH, J. R., M. L. SUNDE, W. W. CRAVENS, C. A. ELVEHJEM AND J. G. HALPIN 1948b Effect of oats, oat products and fat on the intestinal synthesis of biotin in mature fowl. (In press.)

CRAVENS, W. W., J. G. HALPIN AND W. H. MCGIBBON 1946 The use of various vitamin supplements in rations for laying and breeding hens. Poultry Sci., *25:* 99.

CRAVENS, W. W., E. E. SLBESTA, J. G. HALPIN AND E. B. HART 1942 Effect of Lactobacillus casei eluate fraction on reproduction in the domestic fowl. Proc. Soc. Exp. Biol. Med., *51:* 106.

KENNARD, D. C., R. M. BETHKE AND V. D. CHAMBERLIN 1948 Built-up litter as a source of dietary factors essential for hatchability of chicken eggs. Poultry Sci., *27:* 177.

MCGINNIS, J., G. F. HEUSER AND L. C. NORRIS 1944 The need for a new unidentified factor for reproduction in chickens. Ibid., *23:* 553.

MCGINNIS, J., JOAN M. STEVENS AND K. GROVES 1947 The in vitro synthesis of a chick growth promoting factor in hen feces. Ibid., *26:* 432.

RUBIN, M., H. R. BIRD AND I. ROTHCHILD 1946 A growth promoting factor for chicks in the feces of hens. Ibid., *25:* 526.

RUBIN, M., A. C. GROSCHEL AND H. R. BIRD 1947 Chick growth factor in cow manure. VI. Effect on hatchability and storage in hens. Proc. Soc. Exp. Biol. Med., *66:* 36.

SCHUMACHER, A. E., G. F. HEUSER AND L. C. NORRIS 1940 The complex nature of the alcohol precipitate factor required by the chick. J. Biol. Chem., *135*: 213.

SCHWEIGERT, B. S., H. L. GERMAN, P. B. PEARSON AND R. M. SHERWOOD 1948 Effect of pteroylglutamic acid intake on the performance of turkeys and chickens. J. Nutrition, *35*: 89.

TAYLOR, L. W. 1947 The effect of folic acid on egg production and hatchability. Poultry Sci., *26*: 372.

63

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STUDIES ON THE BIOSYNTHESIS OF B-VITAMINS.

Part IV.

EFFECT OF DIFFERENT CARBOHYDRATES AND SOME INORGANIC AND ORGANIC NITROGENOUS COMPOUNDS ON THE BIOSYNTHESIS OF NICOTINIC ACID IN ALBINO RATS.

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INTRODUCTION.

IN a previous communication by De and Datta (1951) has been reported the influence of different nitrogenous precursors on the microbial synthesis of nicotinic acid in the rat intestine. The present investigation aims to study the effect of the dietary carbohydrate component on the biosynthetic process.

Very little work is reported in this line. Krehl *et al.* (1946, 1946*a*, 1946*b*) by growth studies on rats have observed marked superiority of dextrin, glucose and starch over sucrose in protecting against the deleterious effect by corn and they had attributed this effect to their influence in decreasing the tryptophane requirement. Schweigert and Pearson (1948) found that the diet with cerelose as the sole carbohydrate and with casein from 12 to 18 per cent level produced more growth rate, more nicotinic acid and N^1-methyl-nicotinamide excretions in rats than the other diet prepared with sucrose. This beneficial effect of cerelose was suggested to be due to its capacity for producing favourable medium for the microbial synthesis

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of nicotinic acid or its precursor in the intestine. Since in almost all the above-mentioned experiments only the growth rates as influenced by the carbohydrates have been studied and, moreover, as some protein components which possibly supplied some nicotinic-acid precursors were also included in the diets of the rats, the actual effect of the carbohydrate on the intestinal synthesis of this growth factor by microflora cannot, therefore, be known from their results.

In the present investigation, diets free from nitrogen and nicotinic acid have been prepared with different carbohydrate sources, such as glucose, sucrose, starch, agar-agar and dextrin, and their effects on the biosynthesis of nicotinic acid in rats studied.

The present work deals with the study of the effect of some inorganic nitrogenous compounds on the biosynthesis of nicotinic acid. Huff and Perlzweig (1942) in their attempts in this line have shown that ammonium lactate increased the nicotinic-acid output only when given intraperitoneally but not when given orally, thus indicating the incapability of the intestinal microflora to synthetize nicotinic acid from this compound. On the contrary, Ellinger and Abdul Kader (1919) have shown that *B. coli* isolated from rat fæces can easily produce nicotinamide when incubated with the above ammonium salt. The present investigation is an attempt to get additional information in this line and, therefore, two inorganic nitrogenous compounds, i.e. potassium nitrate and ammonium sulphate, have been chosen for this part of the work.

The present communication also reports as to whether the laboratory method of synthetizing nicotinamide, as devised by Bovarnick (1943, 1944) by heating aspargine with the amino acids, such as glutamic acid, methionine, ornithine, proline, etc., can be duplicated in the animal body when the above compounds are fed along with the basal diet.

Experimental.

Experiment with different carbohydrate diets.—The experiments of this series were carried out with 15 adult rats weighing between 180 g. and 230 g. After a fasting period of two days, they were first given the starch diet D-I and after a preliminary period of four days on this diet their urine and fæces were collected for three consecutive days. The rats were then allowed a rest on the laboratory stock diet for seven days and after this, experiment was started with the dextrose diet D-II as before. The procedure was repeated with the sucrose, dextrin and agar-agar.

Experiment with nitrate and ammonium ions, asparagine and methionine.—Three groups of adult rats, each consisting of six, were selected. One group (group A) was first given the basal diet of the composition shown in Table III and the basal excretion was collected as in the previous experiment. After the basal period, the rats were given a daily supplement of 500 mg. potassium nitrate and excretions on this supplement were measured for three days after preliminary period of four days. The rats of group B were similarly treated and after giving them a daily supplement of 500 mg. ammonium sulphate the excretions were measured for three days. The rats of group C were given supplements of 10 mg. of asparagine and 10 mg. asparagine *plus* 10 mg. methionine, the excretions were collected as before.

Nicotinic acid of urine and fæces were estimated by methods of De and Banerjee (1949) and De, Malaker and Paul (1949). The values represent nicotinic acid, nicotinuric acid, nicotinamide and trigonelline. These comprise of N^1-methylnicotinamide, and all these have been expressed as total nicotinic acid.

TABLE I.

Showing the composition of the nitrogen-free and nicotinic-acid-free diets based on different carbohydrates.

(The figures represent the weights in g.)

Nature of the dietary components.	Diet D-I.	Diet D-II.	Diet D-III.	Diet D-IV.	Diet D-V.
Starch	82	...	...	...	...
Dextrose	...	82	...	...	...
Sucrose	...	...	82	...	...
Dextrin	...	...	...	82	...
Agar-agar	...	...	...	...	82
Ground-nut oil	12	12	12	12	12
Salt mixture (Osborne and Mendel) ...	4	4	4	4	4
Cod-liver oil	2	2	2	2	2

In addition to the above ingredients, the rats were also given the following vitamin supplements per rat daily :—

	Per day.
Thiamine	15 μg.
Riboflavin	10 μg.
Pyridoxin	15 μg.
Choline	1 mg.
Ca-pantothenate	0·5 mg.

RESULTS AND DISCUSSION.

The data presented in Table II show that the total nicotinic-acid excretion on nitrogen-free and nicotinic-acid-free diets based on agar-agar, glucose, dextrin, starch and sucrose was 78·0 μg., 75·3 μg., 73·4 μg., 72·9 μg. and 62·7 μg., respectively. Of the five carbohydrate sources, agar-agar produced comparatively the maximum elimination and sucrose the minimum, while the values of others stand very much close to that of agar-agar. The differences between the excretions on sucrose diet on the one hand and the other four carbohydrate diets on the other were found to be statistically significant and thus indicate the superiority of agar-agar, glucose, dextrin and starch over sucrose in promoting microbial synthesis of growth factor. Since the diets employed were free from nitrogen and nicotinic acid, excretions of nicotinic acid on such diets denote the endogenous fractions as previously reported by De and Banerjee (*loc. cit.*) and De and Datta (*loc. cit.*) arising out of the biosynthesis in the intestine as well as in the tissues. These seem to vary only to a limited extent when the dietary carbohydrate source is either starch, dextrin, dextrose or agar-agar.

TABLE II.

Showing the effect of different carbohydrate sources on the biosynthesis of nicotinic acid.

(The figures indicate the average values of the total urinary and fæcal excretions of nicotinic acid in μg. per rat daily.)

Nature of the diet.	Total nicotinic-acid output in μg.
Starch D–I	72·9 ± 1·892
Dextrose D–II	75·3 ± 2·021
Sucrose D–III	62·7 ± 1·763
Dextrin D–IV	73·4 ± 1·938
Agar-agar D–V	78·0 ± 1·658

TABLE III.

Showing the effect of potassium nitrate, ammonium sulphate, asparagine and asparagine plus *methionine on the biosynthesis of nicotinic acid.*

(The figures represent the average values of the total urinary and fæcal nicotinic acid in μg. per rat daily.)

Group of the rats.	Diet.	Supplement.	Period. *	Total nicotinic acid in μg.
Group A ...	Basal †	*Nil*	P–II	58·8 ± 1·236
	Basal †	500 mg. potassium nitrate.	P–IV	63·2 ± 1·452
Group B ...	Basal †	*Nil*	P–II	61·6 ± 1·098
	Basal †	500 mg. ammonium sulphate.	P–IV	79·2 ± 1·684
Group C ...	Basal †	*Nil*	P–II	64·5 ± 1·376
	Basal †	10 mg. asparagine.	P–IV	60·8 ± 1·421
	Basal †	10 mg. asparagine *plus* 10 mg. methionine.	P–VI	67·2 ± 1·624

* P–II, P–IV and P–VI indicate the experimental periods. Those which are not indicated should be regarded as the preliminary periods for the succeeding ones.

† The basal diet was composed of 73 g. corn starch, 9 g. sugar, 12 g. ground-nut oil, 4 g. Osborne and Mendel salt mixture and 2 g. cod-liver oil. The vitamin supplements were the same as shown in Table I.

On the basis of these results the observations of Krehl *et al.* (*loc. cit.*) that the corn diet could produce even better growth when prepared with dextrin, dextrose or starch than when prepared with sucrose may be explained as due to the capacity of the former three carbohydrates to produce more favourable medium than the latter for the growth of intestinal microflora.

Effect of potassium nitrate and ammonium sulphate.—The results presented in Table III show that the daily feeding of 500 mg. of potassium nitrate along with the basal diet did not affect nicotinic-acid excretion to any appreciable extent, the values were found to be 63·2 μg. and 58·8 μg., respectively, for diets with and without nitrate supplement. When, however, nitrogen was administered in the form of ammonium ion, such as ammonium sulphate, some stimulation of nicotinic-acid synthesis was noted, the excretion values obtained being in the region of 79·2 μg. and 61·6 μg., respectively, for diets with and without ammonium-sulphate supplement. This result supports the observations of Ellinger and Abdul Kader (*loc. cit.*) on nicotinamide synthesis in ammonium-lactate medium by *B. coli* isolated from rat fæces. This falls almost under the same category as plant nutrition in which case also it has been noted by Chibnall (1939) and Vickery *et al.* (1938, 1939, 1940) that in the synthesis of amide and amino acids, as required for the synthesis of proteins and other complex nitrogenous compounds, the absorption of nitrogen in the form of ammonia is primarily necessary and even when nitrate is added it must pass by reduction through nitrite and ammonia before absorption and for these processes the soil micro-organisms take an active part. The present findings of the ineffectiveness by the nitrate ion as opposed to stimulation by the ammonium ions in promoting the biosynthesis of nicotinic acid suggests that intestinal microflora, unlike the soil microflora, cannot reduce nitrate to ammonium ions.

Effect of asparagine and asparagine plus *methionine.*—The results presented in Table III further show that asparagine when given alone or in combination with methionine does not affect the nicotinic-acid excretion to any appreciable extent and this indicates that the animals cannot perform the drastic chemical reactions as are necessary for preparing nicotinamide from the above two compounds in the laboratory as processed by Bovarnick (*loc. cit.*). Similar results have also previously been reported by De and Datta (1950) showing that the feeding of nicotine, 3-3' dipyridyl and quinoline do not enhance the nicotinic-acid synthesis in the body of rats although in the laboratory this growth factor can be easily prepared by oxidizing these compounds with permanganate, chromic acid and other oxidizing agents.

Summary.

1. The effect of the carbohydrate sources, such as starch, glucose, sucrose, dextrin and agar-agar, on the biosynthesis of nicotinic acid has been studied by feeding the rats nitrogen-free and nicotinic-acid-free diets based on these carbohydrate sources.

2. The nicotinic-acid excretion on starch, dextrin, agar-agar and glucose was found to be much higher than that on sucrose and the significance of these findings has been discussed.

3. The intestinal microflora can easily utilize the ammonium salt for the biosynthesis of nicotinic acid but fails to do so with nitrate.

4. Asparagine and asparagine *plus* methionine failed to stimulate the biosynthesis of nicotinic acid in the animals although in laboratory synthetic process these could be successfully utilized.

REFERENCES.

BOVARNICK, M. R. (1943) ... *Jour. Biol. Chem.*, **151**, p. 467.
Idem (1944) ... *Ibid.*, **153**, p. 1.
CHIBNALL, A. C. (1939) ... *Ibid.*, **128**, p. 703.
DE, H. N., and BANERJEE, S. N. (1949). *Ind. Jour. Med. Res.*, **37**, p. 293.
DE, H. N., and DATTA, P., Jr. (1950). *Current Science*, **19**, p. 279.
Idem (1951) ... *Ind. Jour. Med. Res.*, **39**, p. 63.
DE, H. N., MALAKER, M. C., and PAUL, A. K. (1949). *Ind. Med. Gaz.*, **84**, p. 542.
ELLINGER, P., and ABDIL KADER, M. M. (1949). *Biochem. Jour.*, **45**, p. 276.
HUFF, W. J., and PERLZWEIG, A. W. (1942). *Jour. Biol. Chem.*, **142**, p. 101.
KREHL, W. A., HENDERSON, L. M., HUERGA, J. DE LA, and ELVEHJEM, C. A. (1946). *Ibid.*, **166**, p. 531.
KREHL, W. A., SARMA, P. S., and ELVEHJEM, C. A. (1946*a*). *Ibid.*, **162**, p. 403.
KREHL, W. A., SARMA, P. S., TEPLEY, L. J., and ELVEHJEM, C. A. (1946*b*). *Jour. Nutr.*, **31**, p. 85.
SCHWEIGERT, B. S., and PEARSON, P. B. (1948). *Jour. Biol. Chem.*, **172**, p. 485.
VICKERY, H. B., and PUCHER, G. W. (1939). *Ibid.*, **128**, p. 703.
VICKERY, H. B., PUCHER, G. W., LEVENWORK, C. S., and WAKEMAN, A. J. (1938). *Ibid.*, **125**, p. 527.
Idem (1940) ... *Connecticut Agric. Exptl. Statn. Bull.*, **422**, p. 163.

70

Lee DeCarli, Miss Elsie Puro, and M.

GRAPHY

., *Biochem. J.*, **28**, 916 (1934).
D. N., and Stare, F. J., *J. Biol. Chem.*, 173.

B., *J. Biol. Chem.*, **129**, 303 (1939).
r, I. G., *J. Am. Chem. Soc.*, **75**, 1010 (195.
iol. Chem., **230**, 307 (1958).
Jr., *J. Biol. Chem.*, **79**, 65 (1928).
J., *J. Biol. Chem.*, **210**, 455 (1954).
Med., **88**, 317 (1955).
asson, J., and Finch, C. A., *J. Biol. Chem.*

H., and Lowry, O. H., *J. Biol. Chem.*, 198.

Nutr., **37**, 411 (1949).
, American Society Clinical Investigation.

J Biol Chem, 230(1), 1958

AMINO ACID IMBALANCE ON LOW FIBRIN DIETS*

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Young growing rats fed on a 9 per cent casein diet containing choline and supplemented with methionine and tryptophan develop a partial deficiency of threonine which is characterized by the accumulation of fat in the liver (1, 2). The condition was shown to be due to an amino acid imbalance (3). Later, other proteins such as fibrin, egg albumin, and those of beef and pork were studied (4). With all proteins except fibrin, the addition of methionine and tryptophan to the low protein diets precipitated a threonine deficiency and fat accumulated in the liver to the extent of 25 to 30 per cent expressed on a dry weight basis. No accumulation of fat was observed in the livers of rats receiving fibrin even when the protein level was reduced from 9 to 6 per cent in the diet. A very poor rate of gain was observed, however, when the diet contained only 6 per cent of fibrin. Since fibrin contains nearly twice as much threonine as is present in most of the other proteins studied, and since with the low level of fibrin the need for threonine may be less because of the low rate of gain, an investigation of amino acid imbalances and liver fat deposition has been undertaken with rats fed on diets containing low levels of fibrin.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain, weighing 40 to 50 gm., were used. The rats were housed in individual suspended cages and fed *ad libitum* during the experimental period of 2 weeks. At the end of the experiments in which liver fat deposition was studied, the animals were stunned by a blow on the head and decapitated. Livers were removed immediately and stored at −4° until the estimations of fat were carried out. Fat was determined by ether extraction of the dried and ground liver.

The basal diet used in the various experiments consisted of the following:

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5

Fibrin,[1] 6; corn oil, 5; Salts 4 (5), 4; vitamin mixture, 0.25; choline chloride, 0.15 per cent; and the various amino acid supplements as indicated. Sucrose or dextrin, as indicated in the text, was added to make the ration 100 per cent. The vitamin mixture provided, in mg. per 100 gm. of diet, the following: thiamine, 0.5; riboflavin, 0.5; niacin, 2.5; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12}, 0.002; and inositol, 10.0. 50 mg. of ascorbic acid were added to each kilo of the diet to prevent possible losses of thiamine (6). 2 drops of halibut liver oil fortified with vitamins E and K were administered weekly to each rat (7).

Results

The effect on growth of supplementing the basal diet with the limiting amino acids is shown in Table I. These supplements were such that, together with the amino acids from fibrin, they should have met the requirements of the rat.

When methionine, which was calculated to be the most limiting amino acid, was added, growth was depressed. Phenylalanine had a similar but less pronounced growth-retarding effect. When histidine, which was considered to be the next limiting amino acid, was added, growth was not depressed; instead a slight growth stimulation resulted. When combinations of these amino acids were used, they proved more deleterious than the individual amino acids (Groups 5 and 6). A mixture of leucine, isoleucine, and valine, together with methionine, phenylalanine, and histidine, had a distinct growth-promoting effect (Group 10). Again incomplete combinations (Groups 7 to 9) were ineffective in overcoming the growth depression.

Lysine and threonine, deficiencies of which have been implicated in the accumulation of fat in the liver, were ineffective in overcoming the imbalance (compare Groups 5, 14, and 15).

Further information on the role of the individual amino acids in either creating or correcting the imbalance was obtained from the results given in Table II. Methionine and phenylalanine which depressed growth when added to the 6 per cent fibrin diet were found to be necessary for maximal growth when the other four amino acids were present (Groups 1 to 5). Growth was severely retarded if histidine, leucine, isoleucine, or valine was omitted from the mixture.

Results showing the effect on the accumulation of fat in the liver when the various imbalanced diets were fed are given in Table III. It may be seen that in groups fed on the basal diet there was a slight tendency towards

[1] Ground beef blood fibrin, Armour and Company, Chicago, Illinois.

TABL

Effect of Limiting Amino Acid
Per Cent Fibri

Group No.	Diet
1	6% fibrin-sucrose (basal)
2	Basal + 0.4% DL-methionine
3	" + 0.6% DL-phenylalanine
4	" + 0.2% L-histidine·HCl
5	" + 0.4% DL-methionine + (
6	" + 0.4% " + (2% L-histidine·HCl
7	No. 6 + 0.4% L-leucine
8	" 6 + 0.4% DL-isoleucine
9	" 6 + 0.6% DL-valine
10	" 6 + 0.4% L-leucine + 0.4% valine
11	Basal + 0.4% L-leucine + 0.4% valine
12	Basal + 0.4% L-leucine + 0.4% valine + 0.2% L-histidine·HC
13	No. 5 + 0.1% L-arginine
14	" 5 + 0.5% L-lysine·HCl
15	" 5 + 0.4% DL-threonine
16	" 5 + 0.1% DL-tryptophan

TABL

Effect of Omitting Single Amino Acid
Fibrin Diet Supplemente

Group No.	Diet
1	6% fibrin-sucrose (basal)
2	Basal + 0.4% DL-methionine + (
3	" + 6 amino acids*
4	No. 3 minus methionine
5	" 3 " phenylalanine
6	" 3 " histidine
7	" 3 " leucine
8	" 3 " isoleucine
9	" 3 " valine

* The following amino acids were added
ine, 0.6 per cent; L-histidine·HCl, 0.2
ine, 0.4 per cent; and DL-valine, 0.6 p

, 4; vitamin mixture, 0.25; choline chlori
is amino acid supplements as indicat
in the text, was added to make the ra
xture provided, in mg. per 100 gm. of d
oflavin, 0.5; niacin, 2.5; calcium pantoth
iotin, 0.01; folic acid, 0.02; vitamin B
g. of ascorbic acid were added to each k
losses of thiamine (6). 2 drops of halib
E and K were administered weekly to each

Results

plementing the basal diet with the limiting
le I. These supplements were such that
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balance was obtained from the results given
phenylalanine which depressed growth when
diet were found to be necessary for maximal
amino acids were present (Groups 1 to 5).
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on the accumulation of fat in the liver when
were fed are given in Table III. It may be
basal diet there was a slight tendency towards

nour and Company, Chicago, Illinois.

TABLE I

Effect of Limiting Amino Acids on Growth of Rats Fed on 6 Per Cent Fibrin-Sucrose Diet

Group No.	Diet	Gain in weight
		gm. per 2 wks.
1	6% fibrin-sucrose (basal)	14.2 ± 1.0
2	Basal + 0.4% DL-methionine	9.6 ± 1.2
3	" + 0.6% DL-phenylalanine	12.1 ± 0.9
4	" + 0.2% L-histidine·HCl	16.3 ± 1.3
5	" + 0.4% DL-methionine + 0.6% DL-phenylalanine	5.6 ± 0.6
6	" + 0.4% " + 0.6% " + 2% L-histidine·HCl	5.6 ± 0.9
7	No. 6 + 0.4% L-leucine	8.6 ± 0.6
8	" 6 + 0.4% DL-isoleucine	7.3 ± 1.1
9	" 6 + 0.6% DL-valine	5.0 ± 0.7
10	" 6 + 0.4% L-leucine + 0.4% DL-isoleucine + 0.6% DL-valine	30.6 ± 1.8
11	Basal + 0.4% L-leucine + 0.4% DL-isoleucine + 0.6% DL-valine	10.6 ± 1.2
12	Basal + 0.4% L-leucine + 0.4% DL-isoleucine + 0.6% DL-valine + 0.2% L-histidine·HCl	16.3 ± 1.3
13	No. 5 + 0.1% L-arginine	6.1 ± 0.6
14	" 5 + 0.5% L-lysine·HCl	6.0 ± 0.4
15	" 5 + 0.4% DL-threonine	5.5 ± 1.0
16	" 5 + 0.1% DL-tryptophan	5.5 ± 0.7

TABLE II

*Effect of Omitting Single Amino Acid on Growth of Rats Fed on 6 Per Cent Fibrin Diet Supplemented with Six Amino Acids**

Group No.	Diet	Growth
		gm. per 2 wks.
1	6% fibrin-sucrose (basal)	13.5 ± 1.2
2	Basal + 0.4% DL-methionine + 0.6% DL-phenylalanine	5.0 ± 0.8
3	" + 6 amino acids*	29.0 ± 2.2
4	No. 3 minus methionine	20.1 ± 1.8
5	" 3 " phenylalanine	12.0 ± 0.4
6	" 3 " histidine	8.0 ± 1.2
7	" 3 " leucine	3.0 ± 0.9
8	" 3 " isoleucine	4.5 ± 1.0
9	" 3 " valine	5.0 ± 0.9

* The following amino acids were added: DL-methionine, 0.4 per cent; DL-phenylalanine, 0.6 per cent; L-histidine·HCl, 0.2 per cent; L-leucine, 0.4 per cent; DL-isoleucine, 0.4 per cent; and DL-valine, 0.6 per cent.

fatty infiltration. However, the liver fat values were normal when growth was retarded as a result of imbalances. The growth stimulation obtained when six amino acids were included (Group 7) was accompanied by an

TABLE III

*Growth and Liver Fat in Rats Fed on 6 Per Cent Fibrin Diet Supplemented with Amino Acids**

Group No.	Diet	Growth	Liver fat
		gm. per 2 wks.	*per cent dry weight*
1	6% fibrin-sucrose (basal)	14.2 ± 1.0	18.6 ± 2.3
2	Basal + 0.4% DL-methionine	9.6 ± 1.2	11.4 ± 2.0
3	" + 0.1% DL-tryptophan	12.6 ± 1.2	14.5 ± 1.1
4	" + 0.4% DL-methionine + 0.1% DL-tryptophan	9.0 ± 1.1	12.5 ± 2.1
5	Basal + 0.4% DL-methionine + 0.1% DL-phenylalanine	6.6 ± 0.8	13.5 ± 1.6
6	No. 5 + 0.2% L-histidine·HCl	6.3 ± 1.0	7.8 ± 1.1
7	Basal + 6 amino acids*	30.6 ± 1.8	37.6 ± 1.8
8	No. 7 + 0.6% L-lysine·HCl	34.3 ± 0.4	25.9 ± 1.6
9	" 7 + 0.5% DL-threonine	29.0 ± 1.0	29.5 ± 2.5
10	" 7 + 0.6% L-lysine·HCl + 0.5% DL-threonine	33.5 ± 1.5	19.9 ± 1.2

* See the footnote to Table II.

TABLE IV

*Growth and Accumulation of Liver Fat in Rats Fed 6 Per Cent Fibrin-Dextrin Diet Supplemented with Amino Acids**

Group No.	Diet	Growth	Liver fat
		gm. per 2 wks.	*per cent dry weight*
1	6% fibrin-dextrin (basal)	29.3 ± 2.6	17.6 ± 1.6
2	Basal + 0.4% DL-methionine	28.8 ± 3.6	18.1 ± 2.9
3	" + 0.4% " + 0.6% DL-phenylalanine	16.5 ± 1.0	12.1 ± 1.7
4	Basal + 6 amino acids*	39.2 ± 1.0	24.5 ± 2.2
5	No. 4 + 0.6% L-lysine·HCl	43.8 ± 3.1	16.1 ± 0.8
6	" 4 + 0.5% DL-threonine	40.5 ± 1.7	19.9 ± 1.2
7	" 4 + 0.6% L-lysine·HCl + 0.5% DL-threonine	42.5 ± 2.7	16.1 ± 1.1

* The amino acid supplements were the same as those in Table II.

accumulation of fat in the liver. These fatty livers responded more to lysine than to threonine. The effect of lysine and threonine together was more pronounced than that of either of the individual amino acids. In no case, however, were the liver fat values reduced quite to normal (Groups 8, 9, and 10).

In other experiments dextrin was used as the dietary carbohydrate

place of sucrose and the various growth rate was twice that ob suboptimal. The results given i by methionine was not as seve the results for growth, as well a lowed a pattern similar to that

The results presented in this that is more complex than any data, methionine and phenylala limiting for growth in the diet considered to be followed by h this order. However, from Ta methionine and phenylalanine stimulation and that histidine w I and II) indicated that, of the least deficient (Groups 1, 3, an which they became limiting for histidine, all approximately equa methionine.

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ed (Group 7) was accompanied by an

BLE III
ts Fed on 6 Per Cent Fibrin Diet
with Amino Acids*

	Growth	Liver fat
	gm. per 2 wks.	per cent dry weight
	14.2 ± 1.0	18.6 ± 2.3
	9.6 ± 1.2	11.4 ± 2.0
	12.6 ± 1.2	14.5 ± 1.1
+ 0.1% DL-tryp-	9.0 ± 1.1	12.5 ± 2.1
0.1% DL-phenyl-	6.6 ± 0.8	13.5 ± 1.6
	6.3 ± 1.0	7.8 ± 1.1
1	30.6 ± 1.8	37.6 ± 1.8
	34.3 ± 0.4	25.9 ± 1.6
	29.0 ± 1.0	29.5 ± 2.5
0.5% DL-threonine	33.5 ± 1.5	19.9 ± 1.2

TABLE IV
Fat in Rats Fed 6 Per Cent Fibrin-Dextrin
nted with Amino Acids*

	Growth	Liver fat
	gm. per 2 wks.	per cent dry weight
	29.3 ± 2.6	17.6 ± 1.6
	28.8 ± 3.6	18.1 ± 2.9
+ 0.6% DL-phen-	16.5 ± 1.0	12.1 ± 1.7
	39.2 ± 1.0	24.5 ± 2.2
	43.8 ± 3.1	16.1 ± 0.8
	40.5 ± 1.7	19.9 ± 1.2
- 0.5% DL-threonine	42.5 ± 2.7	16.1 ± 1.1

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place of sucrose and the various groups were repeated. In this case the growth rate was twice that obtained with sucrose although it was still suboptimal. The results given in Table IV show that the imbalance caused by methionine was not as severe as when sucrose was used. Otherwise the results for growth, as well as for liver fat, for the various groups followed a pattern similar to that described for the sucrose series.

DISCUSSION

The results presented in this paper illustrate an amino acid imbalance that is more complex than any reported previously. From the analytical data, methionine and phenylalanine appeared to be the amino acids most limiting for growth in the diet containing 6 per cent of fibrin; these were considered to be followed by histidine, leucine, isoleucine, and valine in this order. However, from Table I it can be seen that the addition of methionine and phenylalanine caused a growth depression rather than a stimulation and that histidine was without effect. Further studies (Tables I and II) indicated that, of the six amino acids tested, methionine was the least deficient (Groups 1, 3, and 4 in Table II) and that the sequence in which they became limiting for growth was leucine, isoleucine, valine, and histidine, all approximately equally so, followed by phenylalanine and then methionine.

The difference between the experimental results and the theoretical sequence suggests either that the accepted amino acid requirements are too high or that the availability of some of these amino acids from fibrin is not complete; perhaps it is a combination of both.

Several instances have been reported in which the addition of a small amount of the second most limiting amino acid to a low protein diet has been found to increase the severity of the deficiency of the amino acid that was most limiting for growth (8, 9). The situation in the present instance, although more complex, follows this general pattern. In the earlier example, addition of a single amino acid that was not limiting created a more severe deficiency of the single amino acid that was most limiting. In this case, there are four almost equally limiting amino acids. The addition of the next most limiting amino acids, methionine and phenylalanine, either alone or in combination apparently increased the severity of the deficiencies of the four most limiting amino acids, leucine, isoleucine, valine, and histidine.

The exact quantitative relationships necessary for the creation of an amino acid imbalance have not been established. However, from the present studies (Table II), as well as from previous observations on amino acid imbalances (10–13), it is evident that supplementation with a mixture of all the essential amino acids except the most limiting is a reliable and reproducible method of creating a severe imbalance.

The data further provide an explanation for the previous observations of Winje and coworkers (4) that no fatty livers were produced when rats were fed a low protein diet containing fibrin. Dietary deficiencies of one or two specific amino acids may cause the development of fatty livers in rats fed low protein diets containing choline (1, 2). Therefore, when the need for such amino acids is increased, as is the case when growth is improved, partial deficiencies would occur resulting in the development of fatty livers. As long as growth remained poor and certain minimal amounts of the "lipotropic" amino acids were provided, normal liver metabolism might be expected. Reference to Table III shows that when the imbalance was corrected by the addition of six amino acids and growth was improved, fat accumulated in the liver. When growth was poor, the quantity of liver fat was not abnormal. Since the quantities of lysine and threonine in the diets of Groups 1 to 7 (Table III) were the same, it follows that, when growth was improved, more of each of these amino acids were used for body protein synthesis and less were available for their "lipotropic" function. When additional quantities of lysine and threonine were provided, liver fat approached a normal value. In this case, in contrast to the results with a 9 per cent casein diet, lysine was required as well as threonine.

It may be pointed out that growth in all these experiments was suboptimal. When sucrose was replaced by dextrin, much better growth was obtained (compare Tables I and IV). This would be in agreement with the observation that the protein requirement, expressed as a percentage of the diet, is greater when sucrose is the dietary carbohydrate (14).

SUMMARY

Amino acid imbalances have been created by supplementing a low protein diet containing 6 per cent of fibrin with various combinations of amino acids.

When methionine and phenylalanine, the amino acids calculated to be most limiting for growth, were added, either alone or together, a complex imbalance involving leucine, isoleucine, valine, and histidine was created.

Supplementation of the 6 per cent fibrin diet with the six amino acids stimulated growth but at the same time caused fat to accumulate in the liver. These fatty livers responded to lysine and to threonine. A combination of lysine and threonine, however, was more effective than either of the individual amino acids.

When sucrose was replaced by dextrin, although growth was substantially improved, the same effects of the various amino acid supplements on growth and liver fat deposition were observed.

1. Singal, S. A., Hazan, S. J
Chem., **200**, 867 (1953).
2. Harper, A. E., Monson, W
383 (1953).
3. Harper, A. E., Benton, D.
209, 159 (1954).
4. Winje, M. E., Harper, A.
J. Nutr., **54**, 155 (1954).
5. Hegsted, D. M., Mills, R.
138, 459 (1941).
6. Kandutsch, A. A., and Ba
7. Harper, A. E., Benton, D.
209, 171 (1954).
8. Elvehjem, C. A., and Krel
9. Harper, A. E., *Nutr. Rev.*,
10. Pecora, L. J., and Hundle
11. Deshpande, P. D., Harpe
Nutr., **57**, 415 (1955).
12. Deshpande, P. D., Harper
13. Sauberlich, H. E., *J. Nutr*
14. Harper, A. E., Monson, W
A., *J. Nutr.*, **51**, 523 (195

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BIBLIOGRAPHY

1. Singal, S. A., Hazan, S. J., Sydenstricker, V. P., and Littlejohn, J. M., *J. Biol. Chem.*, **200**, 867 (1953).
2. Harper, A. E., Monson, W. J., Benton, D. A., and Elvehjem, C. A., *J. Nutr.*, **50**, 383 (1953).
3. Harper, A. E., Benton, D. A., Winje, M. E., and Elvehjem, C. A., *J. Biol. Chem.*, **209**, 159 (1954).
4. Winje, M. E., Harper, A. E., Benton, D. A., Boldt, R. E., and Elvehjem, C. A., *J. Nutr.*, **54**, 155 (1954).
5. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, **138**, 459 (1941).
6. Kandutsch, A. A., and Baumann, C. A., *J. Nutr.*, **49**, 209 (1953).
7. Harper, A. E., Benton, D. A., Winje, M. E., and Elvehjem, C. A., *J. Biol. Chem.*, **209**, 171 (1954).
8. Elvehjem, C. A., and Krehl, W. A., *Borden's Rev. Nutr. Res.*, **16**, 69 (1955).
9. Harper, A. E., *Nutr. Rev.*, **14**, 225 (1956).
10. Pecora, L. J., and Hundley, J. M., *J. Nutr.*, **44**, 101 (1951).
11. Deshpande, P. D., Harper, A. E., Quiros-Perez, F., and Elvehjem, C. A., *J. Nutr.*, **57**, 415 (1955).
12. Deshpande, P. D., Harper, A. E., and Elvehjem, C. A., *J. Nutr.*, **62**, 503 (1957).
13. Sauberlich, H. E., *J. Nutr.*, **59**, 353 (1956).
14. Harper, A. E., Monson, W. J., Arata, D. A., Benton, D. A., and Elvehjem, C. A., *J. Nutr.*, **51**, 523 (1953).

70

CYTES AND GLUCOSE

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GRAPHY

., *Biochem. J.*, **28**, 916 (1934).
D. N., and Stare, F. J., *J. Biol. Chem.*, 173.

B., *J. Biol. Chem.*, **129**, 303 (1939).
, I. G., *J. Am. Chem. Soc.*, **75**, 1010 (195
iol. Chem., **230**, 307 (1958).
Jr., *J. Biol. Chem.*, **79**, 65 (1928).
J., *J. Biol. Chem.*, **210**, 455 (1954).
Med., **88**, 317 (1955).
sson, J., and Finch, C. A., *J. Biol. Chem.*

H., and Lowry, O. H., *J. Biol. Chem.*, 198.

Nutr., **37**, 411 (1949).
, American Society Clinical Investigation,

J Biol Chem, 230(1), 1958

AMINO ACID IMBALANCE ON LOW FIBRIN DIETS*

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Young growing rats fed on a 9 per cent casein diet containing choline and supplemented with methionine and tryptophan develop a partial deficiency of threonine which is characterized by the accumulation of fat in the liver (1, 2). The condition was shown to be due to an amino acid imbalance (3). Later, other proteins such as fibrin, egg albumin, and those of beef and pork were studied (4). With all proteins except fibrin, the addition of methionine and tryptophan to the low protein diets precipitated a threonine deficiency and fat accumulated in the liver to the extent of 25 to 30 per cent expressed on a dry weight basis. No accumulation of fat was observed in the livers of rats receiving fibrin even when the protein level was reduced from 9 to 6 per cent in the diet. A very poor rate of gain was observed, however, when the diet contained only 6 per cent of fibrin. Since fibrin contains nearly twice as much threonine as is present in most of the other proteins studied, and since with the low level of fibrin the need for threonine may be less because of the low rate of gain, an investigation of amino acid imbalances and liver fat deposition has been undertaken with rats fed on diets containing low levels of fibrin.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain, weighing 40 to 50 gm., were used. The rats were housed in individual suspended cages and fed *ad libitum* during the experimental period of 2 weeks. At the end of the experiments in which liver fat deposition was studied, the animals were stunned by a blow on the head and decapitated. Livers were removed immediately and stored at −4° until the estimations of fat were carried out. Fat was determined by ether extraction of the dried and ground liver.

The basal diet used in the various experiments consisted of the following:

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5

Fibrin,[1] 6; corn oil, 5; Salts 4 (5), 4; vitamin mixture, 0.25; choline chloride, 0.15 per cent; and the various amino acid supplements as indicated. Sucrose or dextrin, as indicated in the text, was added to make the ration 100 per cent. The vitamin mixture provided, in mg. per 100 gm. of diet, the following: thiamine, 0.5; riboflavin, 0.5; niacin, 2.5; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12}, 0.002; and inositol, 10.0. 50 mg. of ascorbic acid were added to each kilo of the diet to prevent possible losses of thiamine (6). 2 drops of halibut liver oil fortified with vitamins E and K were administered weekly to each rat (7).

Results

The effect on growth of supplementing the basal diet with the limiting amino acids is shown in Table I. These supplements were such that, together with the amino acids from fibrin, they should have met the requirements of the rat.

When methionine, which was calculated to be the most limiting amino acid, was added, growth was depressed. Phenylalanine had a similar but less pronounced growth-retarding effect. When histidine, which was considered to be the next limiting amino acid, was added, growth was not depressed; instead a slight growth stimulation resulted. When combinations of these amino acids were used, they proved more deleterious than the individual amino acids (Groups 5 and 6). A mixture of leucine, isoleucine, and valine, together with methionine, phenylalanine, and histidine, had a distinct growth-promoting effect (Group 10). Again incomplete combinations (Groups 7 to 9) were ineffective in overcoming the growth depression.

Lysine and threonine, deficiencies of which have been implicated in the accumulation of fat in the liver, were ineffective in overcoming the imbalance (compare Groups 5, 14, and 15).

Further information on the role of the individual amino acids in either creating or correcting the imbalance was obtained from the results given in Table II. Methionine and phenylalanine which depressed growth when added to the 6 per cent fibrin diet were found to be necessary for maximal growth when the other four amino acids were present (Groups 1 to 5). Growth was severely retarded if histidine, leucine, isoleucine, or valine was omitted from the mixture.

Results showing the effect on the accumulation of fat in the liver when the various imbalanced diets were fed are given in Table III. It may be seen that in groups fed on the basal diet there was a slight tendency towards

[1] Ground beef blood fibrin, Armour and Company, Chicago, Illinois.

TABL

Effect of Limiting Amino Acid
Per Cent Fibri

Group No.	Diet
1	6% fibrin-sucrose (basal)
2	Basal + 0.4% DL-methionine
3	" + 0.6% DL-phenylalanine
4	" + 0.2% L-histidine·HCl
5	" + 0.4% DL-methionine + (
6	" + 0.4% " + (2% L-histidine·HCl
7	No. 6 + 0.4% L-leucine
8	" 6 + 0.4% DL-isoleucine
9	" 6 + 0.6% DL-valine
10	" 6 + 0.4% L-leucine + 0.4% valine
11	Basal + 0.4% L-leucine + 0.4% valine
12	Basal + 0.4% L-leucine + 0.4% valine + 0.2% L-histidine·HC
13	No. 5 + 0.1% L-arginine
14	" 5 + 0.5% L-lysine·HCl
15	" 5 + 0.4% DL-threonine
16	" 5 + 0.1% DL-tryptophan

TABL

Effect of Omitting Single Amino Acid
Fibrin Diet Supplemente

Group No.	Diet
1	6% fibrin-sucrose (basal)
2	Basal + 0.4% DL-methionine + (
3	" + 6 amino acids*
4	No. 3 minus methionine
5	" 3 " phenylalanine
6	" 3 " histidine
7	" 3 " leucine
8	" 3 " isoleucine
9	" 3 " valine

* The following amino acids were added
ine, 0.6 per cent; L-histidine·HCl, 0.2
ine, 0.4 per cent; and DL-valine, 0.6 p

, 4; vitamin mixture, 0.25; choline chlor
as amino acid supplements as indicat
in the text, was added to make the ra
xture provided, in mg. per 100 gm. of d
oflavin, 0.5; niacin, 2.5; calcium pantoth
iotin, 0.01; folic acid, 0.02; vitamin B
g. of ascorbic acid were added to each k
losses of thiamine (6). 2 drops of halibu
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Results

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diet were found to be necessary for maximal
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were fed are given in Table III. It may be
basal diet there was a slight tendency towards

mour and Company, Chicago, Illinois.

TABLE I

Effect of Limiting Amino Acids on Growth of Rats Fed on 6 Per Cent Fibrin-Sucrose Diet

Group No.	Diet	Gain in weight
		gm. per 2 wks.
1	6% fibrin-sucrose (basal)	14.2 ± 1.0
2	Basal + 0.4% DL-methionine	9.6 ± 1.2
3	" + 0.6% DL-phenylalanine	12.1 ± 0.9
4	" + 0.2% L-histidine·HCl	16.3 ± 1.3
5	" + 0.4% DL-methionine + 0.6% DL-phenylalanine	5.6 ± 0.6
6	" + 0.4% " + 0.6% " + 2% L-histidine·HCl	5.6 ± 0.9
7	No. 6 + 0.4% L-leucine	8.6 ± 0.6
8	" 6 + 0.4% DL-isoleucine	7.3 ± 1.1
9	" 6 + 0.6% DL-valine	5.0 ± 0.7
10	" 6 + 0.4% L-leucine + 0.4% DL-isoleucine + 0.6% DL-valine	30.6 ± 1.8
11	Basal + 0.4% L-leucine + 0.4% DL-isoleucine + 0.6% DL-valine	10.6 ± 1.2
12	Basal + 0.4% L-leucine + 0.4% DL-isoleucine + 0.6% DL-valine + 0.2% L-histidine·HCl	16.3 ± 1.3
13	No. 5 + 0.1% L-arginine	6.1 ± 0.6
14	" 5 + 0.5% L-lysine·HCl	6.0 ± 0.4
15	" 5 + 0.4% DL-threonine	5.5 ± 1.0
16	" 5 + 0.1% DL-tryptophan	5.5 ± 0.7

TABLE II

*Effect of Omitting Single Amino Acid on Growth of Rats Fed on 6 Per Cent Fibrin Diet Supplemented with Six Amino Acids**

Group No.	Diet	Growth
		gm. per 2 wks.
1	6% fibrin-sucrose (basal)	13.5 ± 1.2
2	Basal + 0.4% DL-methionine + 0.6% DL-phenylalanine	5.0 ± 0.8
3	" + 6 amino acids*	29.0 ± 2.2
4	No. 3 minus methionine	20.1 ± 1.8
5	" 3 " phenylalanine	12.0 ± 0.4
6	" 3 " histidine	8.0 ± 1.2
7	" 3 " leucine	3.0 ± 0.9
8	" 3 " isoleucine	4.5 ± 1.0
9	" 3 " valine	5.0 ± 0.9

* The following amino acids were added: DL-methionine, 0.4 per cent; DL-phenylalanine, 0.6 per cent; L-histidine·HCl, 0.2 per cent; L-leucine, 0.4 per cent; DL-isoleucine, 0.4 per cent; and DL-valine, 0.6 per cent.

fatty infiltration. However, the liver fat values were normal when growth was retarded as a result of imbalances. The growth stimulation obtained when six amino acids were included (Group 7) was accompanied by an

TABLE III

*Growth and Liver Fat in Rats Fed on 6 Per Cent Fibrin Diet Supplemented with Amino Acids**

Group No.	Diet	Growth	Liver fat
		gm. per 2 wks.	*per cent dry weight*
1	6% fibrin-sucrose (basal)	14.2 ± 1.0	18.6 ± 2.3
2	Basal + 0.4% DL-methionine	9.6 ± 1.2	11.4 ± 2.0
3	" + 0.1% DL-tryptophan	12.6 ± 1.2	14.5 ± 1.1
4	" + 0.4% DL-methionine + 0.1% DL-tryptophan	9.0 ± 1.1	12.5 ± 2.1
5	Basal + 0.4% DL-methionine + 0.1% DL-phenylalanine	6.6 ± 0.8	13.5 ± 1.6
6	No. 5 + 0.2% L-histidine·HCl	6.3 ± 1.0	7.8 ± 1.1
7	Basal + 6 amino acids*	30.6 ± 1.8	37.6 ± 1.8
8	No. 7 + 0.6% L-lysine·HCl	34.3 ± 0.4	25.9 ± 1.6
9	" 7 + 0.5% DL-threonine	29.0 ± 1.0	29.5 ± 2.5
10	" 7 + 0.6% L-lysine·HCl + 0.5% DL-threonine	33.5 ± 1.5	19.9 ± 1.2

* See the footnote to Table II.

TABLE IV

*Growth and Accumulation of Liver Fat in Rats Fed 6 Per Cent Fibrin-Dextrin Diet Supplemented with Amino Acids**

Group No.	Diet	Growth	Liver fat
		gm. per 2 wks.	*per cent dry weight*
1	6% fibrin-dextrin (basal)	29.3 ± 2.6	17.6 ± 1.6
2	Basal + 0.4% DL-methionine	28.8 ± 3.6	18.1 ± 2.9
3	" + 0.4% " + 0.6% DL-phenylalanine	16.5 ± 1.0	12.1 ± 1.7
4	Basal + 6 amino acids*	39.2 ± 1.0	24.5 ± 2.2
5	No. 4 + 0.6% L-lysine·HCl	43.8 ± 3.1	16.1 ± 0.8
6	" 4 + 0.5% DL-threonine	40.5 ± 1.7	19.9 ± 1.2
7	" 4 + 0.6% L-lysine·HCl + 0.5% DL-threonine	42.5 ± 2.7	16.1 ± 1.1

* The amino acid supplements were the same as those in Table II.

accumulation of fat in the liver. These fatty livers responded more to lysine than to threonine. The effect of lysine and threonine together was more pronounced than that of either of the individual amino acids. In no case, however, were the liver fat values reduced quite to normal (Groups 8, 9, and 10).

In other experiments dextrin was used as the dietary carbohydrate

place of sucrose and the variou growth rate was twice that ob suboptimal. The results given i by methionine was not as seve the results for growth, as well a lowed a pattern similar to that

The results presented in this that is more complex than any data, methionine and phenylala limiting for growth in the diet considered to be followed by h this order. However, from Ta methionine and phenylalanine stimulation and that histidine w I and II) indicated that, of the least deficient (Groups 1, 3, an which they became limiting for histidine, all approximately equa methionine.

The difference between the quence suggests either that t high or that the availability not complete; perhaps it is a Several instances have been r amount of the second most limi found to increase the severi is most limiting for growth (8, though more complex, follows ple, addition of a single amino ere deficiency of the single an there are four almost equal next most limiting amino ac or in combination apparen of the four most limiting an dine.

The exact quantitative relati amino acid imbalance have no at studies (Table II), as we imbalances (10–13), it is evi the essential amino acids lucible method of creating

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ces. The growth stimulation obtained
d (Group 7) was accompanied by an

BLE III
s Fed on 6 Per Cent Fibrin Diet
*with Amino Acids**

	Growth	Liver fat
	gm. per 2 wks.	*per cent dry weight*
	14.2 ± 1.0	18.6 ± 2.3
	9.6 ± 1.2	11.4 ± 2.0
	12.6 ± 1.2	14.5 ± 1.1
0.1% DL-tryp-	9.0 ± 1.1	12.5 ± 2.1
0.1% DL-phenyl-	6.6 ± 0.8	13.5 ± 1.6
	6.3 ± 1.0	7.8 ± 1.1
	30.6 ± 1.8	37.6 ± 1.8
	34.3 ± 0.4	25.9 ± 1.6
	29.0 ± 1.0	29.5 ± 2.5
.5% DL-threonine	33.5 ± 1.5	19.9 ± 1.2

ABLE IV
Fat in Rats Fed 6 Per Cent Fibrin-Dextrin
*ted with Amino Acids**

	Growth	Liver fat
	gm. per 2 wks.	*per cent dry weight*
	29.3 ± 2.6	17.6 ± 1.6
	28.8 ± 3.6	18.1 ± 2.9
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place of sucrose and the various groups were repeated. In this case the growth rate was twice that obtained with sucrose although it was still suboptimal. The results given in Table IV show that the imbalance caused by methionine was not as severe as when sucrose was used. Otherwise the results for growth, as well as for liver fat, for the various groups followed a pattern similar to that described for the sucrose series.

DISCUSSION

The results presented in this paper illustrate an amino acid imbalance that is more complex than any reported previously. From the analytical data, methionine and phenylalanine appeared to be the amino acids most limiting for growth in the diet containing 6 per cent of fibrin; these were considered to be followed by histidine, leucine, isoleucine, and valine in this order. However, from Table I it can be seen that the addition of methionine and phenylalanine caused a growth depression rather than a stimulation and that histidine was without effect. Further studies (Tables I and II) indicated that, of the six amino acids tested, methionine was the least deficient (Groups 1, 3, and 4 in Table II) and that the sequence in which they became limiting for growth was leucine, isoleucine, valine, and histidine, all approximately equally so, followed by phenylalanine and then methionine.

The difference between the experimental results and the theoretical sequence suggests either that the accepted amino acid requirements are too high or that the availability of some of these amino acids from fibrin is not complete; perhaps it is a combination of both.

Several instances have been reported in which the addition of a small amount of the second most limiting amino acid to a low protein diet has been found to increase the severity of the deficiency of the amino acid that was most limiting for growth (8, 9). The situation in the present instance, although more complex, follows this general pattern. In the earlier example, addition of a single amino acid that was not limiting created a more severe deficiency of the single amino acid that was most limiting. In this case, there are four almost equally limiting amino acids. The addition of the next most limiting amino acids, methionine and phenylalanine, either alone or in combination apparently increased the severity of the deficiencies of the four most limiting amino acids, leucine, isoleucine, valine, and histidine.

The exact quantitative relationships necessary for the creation of an amino acid imbalance have not been established. However, from the present studies (Table II), as well as from previous observations on amino acid imbalances (10–13), it is evident that supplementation with a mixture of all the essential amino acids except the most limiting is a reliable and reproducible method of creating a severe imbalance.

The data further provide an explanation for the previous observations of Winje and coworkers (4) that no fatty livers were produced when rats were fed a low protein diet containing fibrin. Dietary deficiencies of one or two specific amino acids may cause the development of fatty livers in rats fed low protein diets containing choline (1, 2). Therefore, when the need for such amino acids is increased, as is the case when growth is improved, partial deficiencies would occur resulting in the development of fatty livers. As long as growth remained poor and certain minimal amounts of the "lipotropic" amino acids were provided, normal liver metabolism might be expected. Reference to Table III shows that when the imbalance was corrected by the addition of six amino acids and growth was improved, fat accumulated in the liver. When growth was poor, the quantity of liver fat was not abnormal. Since the quantities of lysine and threonine in the diets of Groups 1 to 7 (Table III) were the same, it follows that, when growth was improved, more of each of these amino acids were used for body protein synthesis and less were available for their "lipotropic" function. When additional quantities of lysine and threonine were provided, liver fat approached a normal value. In this case, in contrast to the results with a 9 per cent casein diet, lysine was required as well as threonine.

It may be pointed out that growth in all these experiments was suboptimal. When sucrose was replaced by dextrin, much better growth was obtained (compare Tables I and IV). This would be in agreement with the observation that the protein requirement, expressed as a percentage of the diet, is greater when sucrose is the dietary carbohydrate (14).

SUMMARY

Amino acid imbalances have been created by supplementing a low protein diet containing 6 per cent of fibrin with various combinations of amino acids.

When methionine and phenylalanine, the amino acids calculated to be most limiting for growth, were added, either alone or together, a complex imbalance involving leucine, isoleucine, valine, and histidine was created.

Supplementation of the 6 per cent fibrin diet with the six amino acids stimulated growth but at the same time caused fat to accumulate in the liver. These fatty livers responded to lysine and to threonine. A combination of lysine and threonine, however, was more effective than either of the individual amino acids.

When sucrose was replaced by dextrin, although growth was substantially improved, the same effects of the various amino acid supplements on growth and liver fat deposition were observed.

1. Singal, S. A., Hazan, S. J
Chem., **200**, 867 (1953).
2. Harper, A. E., Monson, W
383 (1953).
3. Harper, A. E., Benton, D.
209, 159 (1954).
4. Winje, M. E., Harper, A.
J. Nutr., **54**, 155 (1954).
5. Hegsted, D. M., Mills, R.
138, 459 (1941).
6. Kandutsch, A. A., and Ba
7. Harper, A. E., Benton, D.
209, 171 (1954).
8. Elvehjem, C. A., and Krel
9. Harper, A. E., *Nutr. Rev.*,
10. Pecora, L. J., and Hundle
11. Deshpande, P. D., Harpe
Nutr., **57**, 415 (1955).
12. Deshpande, P. D., Harper
13. Sauberlich, H. E., *J. Nutr*
14. Harper, A. E., Monson, W
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BIBLIOGRAPHY

1. Singal, S. A., Hazan, S. J., Sydenstricker, V. P., and Littlejohn, J. M., *J. Biol. Chem.*, **200**, 867 (1953).
2. Harper, A. E., Monson, W. J., Benton, D. A., and Elvehjem, C. A., *J. Nutr.*, **50**, 383 (1953).
3. Harper, A. E., Benton, D. A., Winje, M. E., and Elvehjem, C. A., *J. Biol. Chem.*, **209**, 159 (1954).
4. Winje, M. E., Harper, A. E., Benton, D. A., Boldt, R. E., and Elvehjem, C. A., *J. Nutr.*, **54**, 155 (1954).
5. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, **138**, 459 (1941).
6. Kandutsch, A. A., and Baumann, C. A., *J. Nutr.*, **49**, 209 (1953).
7. Harper, A. E., Benton, D. A., Winje, M. E., and Elvehjem, C. A., *J. Biol. Chem.*, **209**, 171 (1954).
8. Elvehjem, C. A., and Krehl, W. A., *Borden's Rev. Nutr. Res.*, **16**, 69 (1955).
9. Harper, A. E., *Nutr. Rev.*, **14**, 225 (1956).
10. Pecora, L. J., and Hundley, J. M., *J. Nutr.*, **44**, 101 (1951).
11. Deshpande, P. D., Harper, A. E., Quiros-Perez, F., and Elvehjem, C. A., *J. Nutr.*, **57**, 415 (1955).
12. Deshpande, P. D., Harper, A. E., and Elvehjem, C. A., *J. Nutr.*, **62**, 503 (1957).
13. Sauberlich, H. E., *J. Nutr.*, **59**, 353 (1956).
14. Harper, A. E., Monson, W. J., Arata, D. A., Benton, D. A., and Elvehjem, C. A., *J. Nutr.*, **51**, 523 (1953).

Tolerance, Toxicity, and Caloric Availability of Intravenously Injected Tapioca Dextrin Solution in Rabbits*,†

By LAWRENCE E. DETRICK, ALVIN E. LEWIS, DOROTHY N. HIGHBY, BONNIE RHODES, and THOMAS J. HALEY‡

Dutch strain rabbits were given eleven consecutive daily intravenous injections (via an indwelling plastic catheter) of tapioca dextrin solution containing amino acid hydrolysate, B-complex vitamins, and adequate potentially available calories for complete parenteral nutrition. Intravenous tapioca dextrin was shown not to be a source of high caloric intake. The intravenous regime was well tolerated by the majority of animals. Recovery occurred in those animals developing symptoms of toxicity if the infusions were not continued too long after their onset. Hydropic degeneration found in the epithelium of the proximal convoluted tubules of the kidney at the end of the injections was reversible during an oral feeding recovery period. Hyaline degeneration was not observed in the glomeruli of the kidney or the blood vessel in the lungs, and foam cells were absent in the reticulo-endothleial system.

COMPLETE parenteral nutrition is limited by the lack of suitable means for providing an adequate caloric intake (1). Isotonic glucose intravenously administered for complete caloric requirement is accompanied by a fluid intake which is prohibitive, and hypertonic glucose scleroses vessels. Many carbohydrates have been tried as an intravenous source of calories but are not utilized in quantity unless previously reduced to their basic glucose units (2). Rice, *et al.* (3) have devised a parenteral solution containing invert sugar, amino acids, ethanol, and water which has 1000 calories per liter.

Dextran, a polysaccharide, labeled with C^{14} and injected intravenously, has been reported to be subject to complete oxidation in the mouse (4). Engstrand and Åberg (5) have found 28 per cent of the intravenously infused dextran to be excreted in the alimentary canal of animals in nine hours, but the ultimate fate of dextran and other plasma substitutes remains to be determined (6).

Intravenous fat emulsions have been administered for their high caloric value, but the problems associated with the production of a suitable stable emulsion which will withstand the rigors of rough handling, temperature changes, and storage remain to be solved (7).

Preliminary studies made elsewhere indicated that macromolecular tapioca dextrin, given intravenously to rabbits or intraperitoneally to rats, provided an adequate source of carbohydrate (8). That investigation compared the utilization of intravenous 36 per cent tapioca dextrin with that of 38.5 per cent glucose in rabbits; when calculated as glucose, from 13 to 17 per cent of the former and 46 per cent of the latter solution appeared in the urine in twenty-four hours. Rats on an inadequate basal diet received intraperitoneal injections of a 35 per cent tapioca dextrin solution and derived a body weight benefit from the injections. However, when used clinically, tapioca dextrin appeared in the urine faster than it could be utilized by the body.

Inasmuch as there were no data available on the effect of continuous intravenous administration of tapioca dextrin on the body weight curve or on its acute and chronic toxic effects, these factors have been investigated, since such information is necessary before more extensive clinical trials with tapioca dextrin can be undertaken.

EXPERIMENTAL

Adult male and female Dutch rabbits (average weight, 2,100 Gm.) were given 11 daily intravenous infusions (Table I) via an indwelling polyethylene catheter (9). The daily injection of R4 solution (see Table I) supplied an adequate potentially-available caloric intake, but no change was made in the daily dosage to compensate for subsequent body weight losses. Half of the animals were sacrificed at the end of the injection period. Their tissues were examined grossly, and samples of the kidney, spleen, liver, lung, pancreas, adrenal gland, and muscle were taken for histological study. Sections were stained routinely with hematoxylin and eosin, but liver and kidney slices were also fixed in absolute alcohol and studied for glycogen with Best's carmine stain. The remainder of the animals

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TABLE

Infusion so
Tapioca de
Casein hyd
Thiamin,
Riboflavin,
Nicotinam
Glucose (a
Tonicity[a]
Calories pe

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TABLE II.—CHE
TAPIO

Solids
Ash
Chloride
Sulfate, 0.0

Potassium
Calcium
Phosphorus
Magnesium
Sodium
Silicon
Iron
Boron
Aluminum
Copper
Strontium
Lead
Manganese
Nickel
Chromium

TABLE I.—COMPONENTS AND CHARACTERISTICS OF PARENTERAL SOLUTIONS R1 TO R6

	Animal Group		
	A	B	C
Infusion solution	R1–R4	R5	R6
Tapioca dextrin, % (w/v)	30	0	0
Casein hydrolysate, % (w/v)	4.3	4.3	4.3
Thiamin, % (w/v)	0.005	0.005	0.005
Riboflavin, % (w/v)	0.01	0.01	0.01
Nicotinamide, % (w/v)	0.05	0.05	0.05
Glucose (anhyd.), % (w/v)	0	0	10
Tonicity[a]	11.5	5.0	14.0
Calories per 150 cc.	216	26	82

[a] Tonicity is expressed in terms of anhydrous glucose solution. A tonicity of 11.5 is equivalent in tonicity to an 11.5% anhydrous glucose solution. Since a 5.3% solution of anhydrous glucose is isotonic with blood, solution R4 is hypertonic and has an osmotic pressure of approximately 2.1 times that of blood.

were allowed a recovery period, given the regular colony diet of Albers Green rabbit pellets, greens, and high protein pellets (Albers Calf Manna), then were sacrificed and similarly studied for tissue changes. The body weight was observed daily as a manifestation of the caloric availability of the intravenously administered nutrient solution. The animals' physical condition, alertness, general activity, and body weight curves were correlated daily as an expression of the over-all nutritional and chronic toxic effects of the experimental procedure.

Source and Chemical and Physical Properties of 36% Tapioca Dextrin Solution (8).—Tapioca dextrin is obtained by acid hydrolysis of a starch derived from the root of the cassava plant. The starch has been reported to contain approximately 80% amylopectin (highly branched polysaccharide of glucose units) and 20% amylose (linear polysaccharide of glucose units). The chemical constituents and the physical properties of 36% tapioca dextrin solution are tabulated in Tables II and III, respectively.

A 36% parenteral tapioca dextrin solution is a clear, amber, sterile, nonpyrogenic, slightly viscous solution suitable for intravenous injection. Physical characteristics resulting from a reduction of tapioca dextrin from 36-30% and the inclusion of amino acids and vitamins were not determined. Specifications for the components, viscosity characteristics, and caloric value of parenteral solutions R1 to R6 are given in Table I. All solutions were infused at a *p*H of 6. Animal groups A, B, and C refer to groups of animals which received intravenous feeding and whose average body weight curves are illustrated as curves *A*, *B*, and *C*, respectively, in Fig. 1.

TABLE II.—CHEMICAL CONSTITUENTS OF 36% TAPIOCA DEXTRIN SOLUTION

	Gm./100
Solids	36.00
Ash	0.06
Chloride	0.02
Sulfate, 0.01 *M*	0.004
	P. P. M.
Potassium	220
Calcium	100
Phosphorus	45
Magnesium	39
Sodium	5–10
Silicon	5–10
Iron	5–10
Boron	0–5
Aluminum	0–5
Copper	0–5
Strontium	0–5
Lead	0–1
Manganese	0–1
Nickel	0–1
Chromium	0–1

RESULTS

Acute Toxicity Due to High Dosage Rate.—The potential toxic effect of a high intravenous dosage rate became apparent almost immediately. Daily infusions of 150 cc. of R1 solution at a rate of 50 cc./hr. resulted in death on the ninth day. The body weight curve fell steadily for five days (similar to curve *A*, Fig. 1); the general appearance, alertness, and activity of the animal were normal. On the sixth and seventh days the weight curve deviated to the right (flattened out and slightly elevated), then rose precipitously on the eighth and ninth days almost reaching its initial level, and was accompanied by progressive physical deterioration. By the ninth day the animal was almost too weak to stand. It had an unkempt appearance and the abdomen was distended, tense, and tender. The rabbit appeared in a stupor unless moved or aroused. Moving the animal initiated intermittent tonic and clonic convulsions that progressed until death.

Post mortem examination showed an edematous abdominal skin, muscles, and viscera with an abnormal amount of straw-colored, protein-containing fluid in the pleural and peritonea cavities.

TABLE III.—PHYSICAL PROPERTIES OF 36% TAPIOCA DEXTRIN SOLUTION

*p*H	4.7
Freezing point, ° C.	0.7
Specific gravity	1.12
Rotation spec. $[\alpha]_D^{25}$	180
Spec. cond., micromhos	400
Viscosity, centipoises at 25° C.	14
Titratable acidity, ml. of 1 *N* NaOH/L.	22
Reducing sugars, Gm. glucose/100 ml.	1.5
Nitrogen, Gm./100 ml.	0.004
Apparent mol. wt. of dextrin (freezing point)	2200

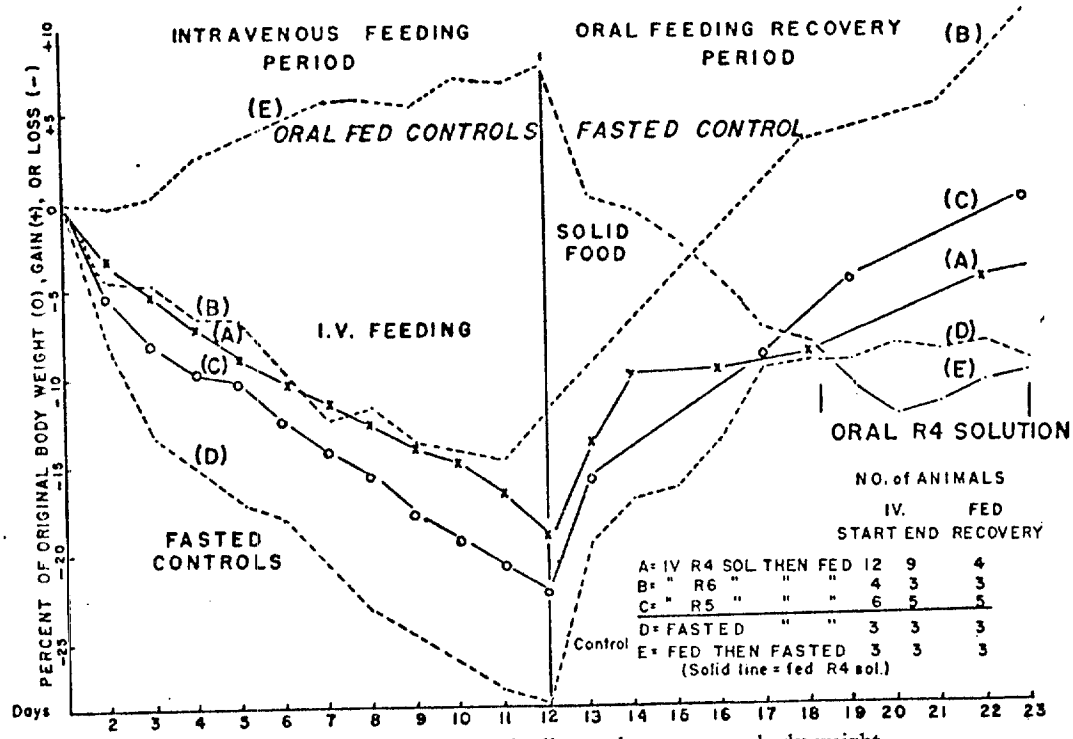

Fig. 1.—Effect of intravenous feeding and recovery on body weight.

Histological sections of the lung and spleen were essentially normal, but the individual liver cells were generally pale and contained small vacuoles. There was a marked generalized swelling and pallor of the tubular epithelium of the kidney. The cytoplasm of these cells was either clear or composed of large confluent empty vacuoles, but the nuclei were intact and neither the collecting ducts nor the glomeruli were involved. The vacuoles in the kidney and liver sections did not contain glycogen.

Tolerance to Lower Dosage Rate.—The majority of normal animals tolerated repeated daily infusions of 150 cc. of R5 solution administered in two 3-hour injection periods with one hour intervening. Tolerance was accompanied by a steady fall in body weight (without edema) as illustrated by curve *A*, Fig. 1. However, 2 of 12 animals developed the previously described chronic toxic symptoms even when the R5 solution was given at this reduced rate. Recovery occurred if only 3 injections were given after these toxic symptoms appeared, and the animal was placed on the regular colony diet. Under similar conditions, 6 infusions after the onset of toxicity resulted in death of the animal five days after the injections were stopped.

Nutritional Value of Intravenously Injected Tapioca Dextrin.—The group average percentage of the initial body weight loss, as illustrated in Fig. 1, served as the criterion of caloric availability for prolonged and repeated intravenous infusions of tapioca dextrin or control solutions (Table I).

Of the twelve animals which started (group A, Fig. 1), nine completed the 11 consecutive daily injections of R4 solutions (Table I). Five animals were sacrificed; four were placed on an oral diet during the recovery period and then sacrificed for recovery studies. The body weight of the group A animals steadily decreased throughout the infusion period. A daily injection of 216 potential calories as R4 solution resulted in a group average loss of 19% of the original body weight on the twelfth day of the experiment.

Curve *B*, Fig. 1, shows the effect on body weight of a group of animals similarly injected with a solution (R6) containing 10% anhydrous glucose in place of tapioca dextrin and all other constituents remaining the same (Table I). A daily injection of 82 available calories as R6 solution resulted in body weight curve *B* which does not differ significantly from that of group A. This group of animals was inadvertently fed on the eleventh day of the experiment; therefore, they received only 10 instead of the usual 11 intravenous injections.

Animals represented by curve *C* (Fig. 1) derived their daily exogenous caloric intake (26 calories, Table I) from the amino acids present in parenteral solution R5, since both glucose and dextrin were omitted. The body weight curve for animal group C (−22% on twelfth day) lies significantly below that of group A and above the curve for group D (−30% on the twelfth day), the fasted controls.

An orally-fed group E and a fasted control group D (Fig. 1) which were reversed during the recovery period, were included for comparative purposes.

Histological Change and Reversibility of Tissue Damage.—Kidney changes after 11 tapioca dextrin infusions were similar to, but less intense than, those reported following the onset of the symptoms of toxicity. The remainder of the organs were essentially normal. Foam cells were not observed in the reticulo-endothelial system but liver glycogen

was depleted after 8 injections. Complete reversibility of tissue damage occurred fourteen to twenty-six days after injections were discontinued.

DISCUSSION

Although tapioca dextrin injections were not a high source of calories, the body weight curve was maintained well above the starvation level. Acid hydrolysis used in the preparation of tapioca dextrin liberated reducing sugars that accounted for from 1.8 to 2.25 Gm. of nutritionally available glucose administered daily. The dextrin can be metabolized if taken orally (see curve *E*, Fig. 1). An average of 15 cc. of R4 solution was consumed daily. The favorable weight gain of animal group A over that of fasted group D, Fig. 1, cannot be interpreted as tissue utilization, pending further investigation repeating the technique of Engstrand and Åberg (5), who demonstrated that intravenously injected dextran was excreted into the gastrointestinal tract. Since tapioca dextrin is utilized when given orally, any of the infusion material excreted into the gastrointestinal tract could be digested, reabsorbed, and utilized as glucose. However, from liver glycogen sections, it is known that neither the glucose molecules in the infusion solution nor that postulated as of possible intestinal origin were sufficient to prevent depletion of liver glycogen during the injection period.

Since curve *C* (amino acid control group) is significantly above curve *D* (fasted control group), Fig. 1, it would appear that the amino acid content of solutions R4 and R6 furnished a significant fraction of the body weight gain of curves *A*, *B*, and *C*. This would confirm the observation of Elman (10) that intravenous amino acids are burned for calories when the amount of available carbohydrate is inadequate. The fluid intake was the same for all 3 daily infusion solution dosages. The absence of this hydrophilic property in the latter two solutions (R5 and R6) would increase the tendency for fluid escape into the tissues, thus falsely elevating body weight curves *B* and *C*, Fig. 1.

The small molecular size of the tapioca dextrin particles used (2,200 mol. wt. by freezing point) may be of significance in the tolerance, relative lack of toxicity, and reversibility of the histological changes observed. Spinco analytical ultracentrifuge examination showed tapioca dextrin to be highly polydisperse, in the range of very low molecular weight, probably well below the permeability factor for the glomeruli of the kidney (11). The employment of such low-range molecular weight material precludes the danger of increased toxicity from and reactions to intravenously administered molecular particle sizes above 100,000 mol. wt. (12). On the other hand, in the preparation of Dextran, particles below 40,000 mol. wt. are removed, not because they were observed to enhance the toxic effect, but rather because they were excreted so rapidly in the urine (13). Lippman, Marti, and Oliver (14) suggest a saturation of the tubular absorption capacity by small molecular weight oxypolygelatin to be the principal mechanism responsible for proteinuria, glomerular and tubular damage and azotemia; also they suggest that it might be possible to eliminate the proteinuria and principal toxicity by fractionation to remove the molecules of small molecular size. In the present experiment only small particle-sized tapioca dextrin was used. Eleven consecutive daily intravenous injections of 45 Gm. of this material were tolerated by the majority of animals. During the injection period no untoward effects were observed; the toxic syndrome, previously discussed in connection with injection rates of 50 cc./hr., was lacking; and the hydropic degeneration observed in the epithelium of the proximal tubules of the kidney was reversible. On the basis of reported physico-chemical relationship of macromolecular size to toxicity, reactions, and excretions, it is suggested that the small size of the tapioca dextrin molecule *per se* is of significance in the results reported.

Histological changes observed at the end of the period of injections, but which were reversible during the recovery period, appear comparatively mild to those reported by Hartman (15) for other intravenously injected macromolecular substances given in smaller doses but as larger particle sizes. In the present study, massive foam cell formation in the reticulo-endothelial system or a hyaline degeneration involving the glomeruli of the kidney or the blood vessel walls in the lungs could not be demonstrated.

CONCLUSIONS

1. An intravenously injected tapioca dextrin solution is not a source of high caloric intake.

2. A parenteral solution of tapioca dextrin, amino acid hydrolysate, and vitamins, when intravenously injected at a rate of 25 cc. per hour, in two 3-hour injection periods with a one-hour rest period intervening, and in daily doses of 150 cc., has been shown to be well tolerated by the majority of normal rabbits for eleven consecutive days.

3. This histological kidney damage observed after 11 daily injections of parenteral tapioca dextrin has been found to be reversible during the oral feeding recovery period.

REFERENCES

(1) Madden, S. C., Basset, S. H., Remington, J. H., Martin, F. J. C., Woods, R. R., and Shull, F. W., *Surg. Gynecol. Obst.*, **82**, 131(1946).

(2) Elman, R., "Parenteral Alimentation in Surgery," Paul B. Hoeber, Inc., New York, 1947, p. 15.

(3) Rice, C. O., Strickler, J. H., and Erwin, P. D., *Arch. Surg.*, **64**, 20(1952).

(4) Cargill, W. H., and Bruner, H. D., *J. Pharmacol. Exptl. Therap.*, **103**, 339(1951).

(5) Engstrand, L., and Åberg, B., *Lancet*, **258**, 1071 (1950).

(6) Stormont, R. T., *J. Am. Med. Assoc.*, **147**, 658 (1951).

(7) Van Itallie, T. B., Waddel, W. R., Geyer, R. P., and Stare, F. J., *Arch. Internal. Med.*, **89**, 353(1952).

(8) Cherkin, A., "Report on the Use of Tapioca Dextrin," Don Baxter, Inc., Glendale, Calif., 1950.

(9) Detrick, L. E., and Rhodes, B., THIS JOURNAL, **41**, 283(1952).

(10) Elman, R., *Proc. Soc. Exptl. Biol. Med.*, **36**, 867 (1937).

(11) Ingelman, B., and Halling, M. S., *Arkiv Kemi, Mineral. Geol.*, **1**, 61(1949).

(12) Ingelman, B., *Upsala Läkarefören. Forh.*, **54**, 107 (1949).

(13) Bull, J. P., Ricketts, C. R., Squire, J. R., Maycock, W. d'A., Spooner, S. J. L., Mollison, P. L., and Paterson, J. C. S., *Lancet*, **256**, 134(1949).

(14) Lippman, R. W., Marti, H. U., and Oliver, J., *Federation Proc.*, **11**, 96(1952).

(15) Hartman, F. W., *Arch. Surg.*, **63**, 728(1951).

carbohydrates, the relative liver weight of sucrose-fed group was higher than that of the groups fed other carbohydrates. After protein repletion, liver weight remained high in sucrose group and heart weight also increased. The relative weight of pancreas also increased in all the groups during protein repletion. No significant difference in the relative weights of adrenal, kidney and spleen was observed during protein regeneration also.

Influence of quality of carbohydrates on body weight changes and nitrogen metabolism during protein depletion and regeneration — The results given in Table 2 show that during protein inanition fall in body weight was greater in sucrose-fed group ($P<0{\cdot}05$) than in corn starch-fed group. Higher rate of endogenous nitrogen excretion and greater loss of carcass nitrogen were observed in the sucrose group. Among complex carbohydrates, tapioca starch-fed animals showed greater loss in body weight but not statistically significant ($P>0{\cdot}05$) and lower nitrogen retention than corn starch and dextrin. On the

TABLE 1 — INFLUENCE OF FEEDING DIFFERENT TYPES OF CARBOHYDRATES ON ORGAN WEIGHTS IN PROTEIN DEPLETED AND REPLETED RATS, EXPRESSED AS PERCENTAGE OF BODY WEIGHT

(Values are averages of 4 rats with SE)

Carbohydrate source	Liver	Heart	Pancreas
	DURING PROTEIN DEPLETION		
Corn starch	3·5 ± 0·12	0·413 ± 0·001	0·279 ± 0·021
Dextrin	3·6 ± 0·14	0·423 ± 0·011	0·282 ± 0·022
Tapioca	3·1 ± 0·11	0·442 ± 0·009	0·289 ± 0·023
Sucrose	5·1 ± 0·15	0·593 ± 0·031	0·252 ± 0·031
	DURING PROTEIN REPLETION		
Corn starch	3·3 ± 0·21	0·431 ± 0·002	0·319 ± 0·121
Dextrin	3·6 ± 0·10	0·485 ± 0·001	0·421 ± 0·022
Tapioca	4·0 ± 0·23	0·466 ± 0·002	0·349 ± 0·013
Sucrose	4·9 ± 0·15	0·572 ± 0·013	0·312 ± 0·112

CARBOHYDRATES ON BODY WEIGHT GAINS, N EFFICIENCY, CARCASS ... EXCRETION IN PROTEIN DEPLETED AND REPLETED RATS

(Averages of 4 rats with SE)

	Retention of carcass nitrogen/ 100 g. of body wt g.	Carcass moisture g./100 g.	Carcass fat g./100 g.	Endogenous nitrogen (fecal + urinary) g.	Total N excretion through urine + feces mg./100 g. of body wt	Nitrogen retention mg./100 g. of body wt per day
PROTEIN DEPLETION						
07	—	64·7	5·2	0·882	—	—
09	—	64·8	4·9	0·913	—	—
07	—	63·9	4·1	1·123	—	—
5	—	67·8	3·1	1·369	—	—
PROTEIN REPLETION						
99	1·12	60·1	18·81	—	87 ± 4	50 ± 3
90	1·09	63·5	17·19	—	114 ± 8	48 ± 2
98	0·92	63·4	16·96	—	146 ± 17	48 ± 3
3	0·71	65·9	11·40	—	159 ± 16	38 ± 2

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81

Influence of Different Types of Carbohydrates on the Progress of Protein Depletion & Repletion in Rats

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Groups of young adult albino rats were maintained on protein-free diet containing different types of carbohydrates such as corn starch, dextrin, tapioca starch and sucrose for 21 days. From the fall in body weight the protective capacity of different carbohydrates in decreasing order was corn starch > dextrin > tapioca starch > sucrose. Proportionate loss of body nitrogen and fat and decrease in the level of liver xanthine oxidase and succinic dehydrogenase activity were in the above order. Fasting blood glucose level was lower and blood pyruvic acid level higher in all the groups compared to the control group of animals, sucrose having the highest. Liver glycogen level was increased in all groups, the accumulation being significantly higher in sucrose-fed group. Glucose-6-phosphatase activity in the liver, however, was found to be lower in all cases; in sucrose-fed group it was relatively higher than in others. Pancreatic amylase activity decreased in protein depleted condition, the least enzyme activity being found in sucrose-fed group. On protein repletion all the above parameters normalized except in sucrose-fed group in which the weight gain and nitrogen retention were the lowest, and the rise in liver fat, blood pyruvic acid level and liver glycogen associated with protein depletion was not significantly reduced. Glucose-6-phosphatase activity rose to a very high level and amylase activity remained very low.

THE interaction of carbohydrates and protein in metabolism is well documented. It has been reported from this laboratory[1] and by others[2-4] that the nature of carbohydrates may alter the metabolism of both carbohydrate and protein during the growth of rats. It is not clear in what way the type of carbohydrate influences the growth stimulating effect of protein diets. It has, however, been repeatedly shown[5] that complex carbohydrates such as starches and dextrin have greater capacity to utilize dietary protein than simpler carbohydrates like sucrose, lactose, etc. However, such protein sparing action of complex carbohydrates is greater in animals fed on low protein diet[6]. That the nature of carbohydrates influences the hepatic carbohydrate metabolism has been indicated by significant difference in glucose tolerance[7], liver glycogen[8], activity of certain glycolytic enzymes[9,10], e.g. glucose-6-phosphatase and fructose-1-6-diphosphatase, and blood level of pyruvate and lactate[11].

In protein deficiency states as well as in kwashiorkor conditions carbohydrate metabolism is significantly deranged[12,13]. It was, therefore, of considerable interest to find out the relative influence of different types of carbohydrates on the metabolism of protein and carbohydrates during protein depletion and protein repletion. The present paper reports the findings of investigations in which protein-free diets containing corn starch, tapioca starch, dextrin and sucrose were fed to young adult rats and the same diets supplemented with 18 per cent protein were fed during protein repletion.

*Findings reported in this paper were presented at the Physiology Section of the Indian Science Congress held in January 1968.

Materials and Methods

Equal number of young adult male and female albino rats (120-150 g.) bred and reared in our colony were grouped and housed in individual metabolic cages. The composition (g./100 g.) of the protein-free diet was as follows: mineral mixture (USP XIV), 4; refined arachis oil, 9; vitamin mixture[14], 2; choline chloride, 0·2; and carbohydrate to make up 100.

The animals were maintained on protein-free diets for 21 days. Test diets and water were supplied *ad libitum*. Daily feed consumption and biweekly body weights were recorded. Towards the end of the experimental period, collection of urine and stool from representative groups of animals was made daily for the determination of nitrogen. A few animals from each group were sacrificed for the determination of tissue weights, carcass nitrogen, glucose and pyruvic acid in the blood and glycogen and activity of certain enzymes in the liver. For the determination of initial value of carcass nitrogen, a group of animals of the same body weight were sacrificed at the start of the experiment.

The remaining animals were then maintained on the above diet but supplemented with casein protein at 18 per cent level for another 21 days for protein regeneration. Daily feed consumption and biweekly body weights were recorded. Urine and feces were collected as indicated earlier[1]. At the end of the experimental period, the animals were bled by cardiac puncture, and blood and the different tissues were removed for analysis; half the number of animals were sacrificed for blood glucose and pyruvic acid and liver glycogen after 12 hr fasting period.

Analysis — Nitrogen in the feed, feces and urine was determined by the macro-Kjeldahl procedure.

other hand, the protective influence of corn starch and dextrin was indicated by reduced loss in body weight and lower nitrogen excretion.

During protein repletion gain in body weight was faster and greater in corn starch and dextrin-fed animals than sucrose ($P<0.05$) and tapioca ($P>0.05$)-fed animals. Nitrogen efficiency ratio and nitrogen retention were also greater in corn starch and dextrin-fed animals than in sucrose or tapioca-fed animals.

The total nitrogen and non-protein nitrogen in plasma and liver of different groups of animals are recorded in Table 3. In protein depleted condition, sucrose-fed animals showed highest fall in both liver and plasma nitrogen but maintained relatively greater NPN level. This trend was observed during protein repletion also.

Influence of quality of carbohydrates on certain enzyme activities — The activities of some enzymes in the liver and pancreas during both protein depletion and repletion are recorded in Table 4. In protein depleted animals the activity of xanthine oxidase and succinate dehydrogenase in the liver was considerably reduced. In tapioca starch-fed animals, the activity of these enzymes showed values similar to those of corn starch or dextrin-fed animals, but lower values were observed if the values are expressed as units per 100 g. of body weight. Glucose 6-phosphatase activity was also greatly reduced in all the groups, but relatively higher level was maintained in sucrose-fed group ($P<0.02$).

After protein repletion, both corn starch and dextrin groups regained the activity of all enzymes but lower values of xanthine oxidase were indicated in sucrose-fed animals. Tapioca-fed animals showed lower value when the enzyme activity was expressed per 100 g. of body weight. Succinate dehydrogenase activity was also low in sucrose-fed group but glucose-6-phosphatase activity rose to a very high level ($P<0.01$).

Pancreatic amylase activity was found to be low in all the groups, the sucrose-fed animal having the lowest value. Protein feeding significantly raised the enzyme activity in all other groups excepting in sucrose-fed animals in which normal amylase activity was not regained.

Influence of nature of carbohydrates on the liver fat and on the blood pyruvic acid and glucose and liver glycogen in fasting condition — Data presented in Table 5 indicate a significant fall in fasting blood glucose level in all the groups during protein depletion and a little rise in blood pyruvic acid level. Both liver glycogen and liver fat tended to increase. This increase was highest in sucrose-fed group. After protein feeding, however, all these parameters became normal in all the groups excepting sucrose-fed group where higher levels of pyruvic acid, glycogen and fat were maintained. Similar trend of results was observed when liver glycogen was determined under fed condition.

TABLE 3 — INFLUENCE OF DIFFERENT TYPES OF CARBOHYDRATES ON TOTAL NITROGEN AND NPN OF PLASMA AND LIVER IN PROTEIN DEPLETED AND REPLETED RATS

(Values are averages of 4 rats with SE)

Carbohydrate source	Plasma		Liver	
	N mg./100 ml.	NPN mg./100 ml.	N mg./g. tissue	NPN mg./g. tissue
DURING PROTEIN DEPLETION				
Corn starch	726·42 ± 4·20	35·6 ± 6·2	28·06 ± 1·6	3·4 ± 0·3
Dextrin	735·49 ± 3·20	39·4 ± 2·1	27·09 ± 1·25	4·2 ± 0·5
Tapioca	702·90 ± 5·00	35·6 ± 5·1	24·90 ± 1·50	3·3 ± 1·1
Sucrose	625·51 ± 7·00	41·9 ± 3·9	20·90 ± 0·39	4·9 ± 0·3
DURING PROTEIN REPLETION				
Corn starch	1027·92 ± 20	30·1 ± 0·2	38·69 ± 0·19	1·9 ± 0·1
Dextrin	992·01 ± 15	32·2 ± 0·9	36·91 ± 0·12	2·1 ± 0·3
Tapioca	925·52 ± 12	36·4 ± 1·2	28·80 ± 0·16	2·9 ± 0·2
Sucrose	866·09 ± 9	38·9 ± 0·4	25·60 ± 3·11	3·2 ± 0·9

Discussion

From the present studies it is observed that loss of body weight, rate of nitrogen excretion (via urine and feces), loss of carcass nitrogen and accumulation of fat are more pronounced in sucrose-fed animals during protein deficiency than when other complex carbohydrates are fed. This supports our previous observation[1] that sucrose, one of the simpler carbohydrates, tends to intensify the catabolism of the body protein. The greater endogenous nitrogen

TABLE 4 — INFLUENCE OF DIFFERENT TYPES OF CARBOHYDRATES ON SOME HEPATIC AND PANCREATIC ENZYMES IN PROTEIN DEPLETED AND REPLETED RATS

(Values are averages of 4 rats with SE)

Carbohydrate	G-6-pase		Xanthine oxidase		Succinic dehydrogenase		Pancreatic amylase unit
	Unit/100 g. body wt	Unit/g. liver protein	Unit/100 g. body wt	Unit/g. liver protein	Unit/100 g. body wt	Unit/g. liver protein	
DURING PROTEIN DEPLETION							
Corn starch	24 ± 3	39 ± 2	18 ± 2	30 ± 1	11 ± 1	19 ± 1	18 ± 2
Dextrin	23 ± 2	39 ± 1	21 ± 1	35 ± 2	14 ± 1	22 ± 2	24 ± 2
Tapioca starch	28 ± 2	57 ± 1	13 ± 1	27 ± 1	9 ± 1	19 ± 1	16 ± 3
Sucrose	61 ± 4	92 ± 3	6 ± 1	10 ± 1	8 ± 1	13 ± 1	6 ± 2
DURING PROTEIN REPLETION							
Corn starch	39 ± 2	49 ± 1	36 ± 1	43	19 ± 1	24 ± 3	62 ± 4
Dextrin	44 ± 2	52 ± 1	40 ± 2	48	20 ± 2	24 ± 2	72 ± 5
Tapioca starch	56 ± 1	77 ± 2	27 ± 3	40	18 ± 1	25 ± 1	52 ± 4
Sucrose	129 ± 4	162 ± 5	24 ± 1	31	15 ± 1	24 ± 2	36 ± 3

TABLE 5 INFLUENCE OF DIFFERENT TYPES OF CARBOHYDRATES ON LIVER FAT AND GLUCOSE AND PYRUVIC ACID LEVELS OF BLOOD AND HEPATIC GLYCOGEN IN PROTEIN DEPLETED AND REPLETED RATS*

(Values are averages of 4 rats with SE)

Carbohydrate	Protein depleted group				Protein repleted group			
	Glucose mg./100 ml. blood	Pyruvic acid mg./100 ml. blood	Liver glycogen mg./100 g. liver tissue	Liver fat g. %	Glucose mg./100 ml. blood	Pyruvic acid mg./100 ml. blood	Liver glycogen mg./100 g. liver tissue	Liver fat g./100 g. tissue
Corn starch	67 ± 3	1·06	397 ± 22	5·4	99 ± 5	0·82	247 ± 21	3·3
Dextrin	72 ± 4	0·94	525 ± 45	5·0	109 ± 3	0·72	217 ± 17	3·2
Tapioca	66 ± 2	1·03	580 ± 20	5·9	105 ± 4	0·74	255 ± 15	4·9
Sucrose	59 ± 3	1·56	1027 ± 42	7·1	96 ± 5	1·32	450 ± 30	6·0

*All data collected under fasting condition.

excretion in sucrose provides additional evidence for the above view.

Womack and Marshall[24] have reported that although negative nitrogen balance decreased in protein depleted rats fed extra starch or dextrin compared to the level found after feeding sucrose and low levels of amino acids, the results are not influenced by the type of carbohydrate fed and substitution of potato starch for sucrose did not improve nitrogen balance. On the other hand, the observation of Macdonald and Gharavi[25] that sucrose aggravates the protein deficiency condition in rabbits is similar to our findings. It is known that xanthine oxidase[26] and succinate dehydrogenase[27] of liver are reduced in protein inanition. Such reduction is greater in sucrose-fed animals. In tapioca starch-fed animals, although the specific activity of these enzymes is normal, lower values are observed when the enzyme activity is expressed per 100 g. of body weight, indicating lower/slower metabolic adaptation in the system.

Excessive accumulation of hepatic fat[28,29] and glycogen[30] in liver and higher fasting blood pyruvic acid and lower glucose level[13], which are signs of protein deficiency, are intensified in sucrose-fed animals. However, Stewart and Heard[31] have observed that decreasing the protein content and increasing carbohydrate content of the diet of pigs cause high blood glucose levels in the animals. Fasting liver glycogen level is slightly above normal in all the groups and highest accumulation of liver glycogen[1] is in sucrose-fed group. The cause of lower fasting blood glucose may thus be less mobilization of liver glycogen due to lowered activities of glycolytic enzymes. Further, the activity of hepatic glucose-6-phosphatase, which helps in the liberation of glucose, is reduced in protein deficiency due probably to lack of specific protein[32]. This observation is also supported by the findings of others[33]. However, the activity of this enzyme is significantly higher in sucrose-fed group and such increase in activity may be due to the fructose molecule in sucrose. It has been reported by various workers[16,32] that fructose stimulates glucose-6-phosphatase activity by stimulating specific protein synthesis. After repletion with 18 per cent casein protein this enzyme activity increases in all the groups, but such an increase is significantly greater in sucrose-fed animals as a result of the double effect of the availability of specific protein and stimulation of the formation of glucose from fructose.

Higher pyruvic acid level of blood in protein deficiency may be due to metabolic 'block' in the pathway responsible for the oxidation of pyruvate via citric acid cycle as suggested by Whitehead and Harland[13]. An accumulation of pyruvic acid may be also due to the delayed progress of metabolite round the TCA cycle.

After repletion of protein, pyruvic acid, glucose and glycogen levels become normal in all the groups, but in sucrose-fed group both pyruvic acid and liver glycogen levels are still higher as explained by Dodds *et al.*[11] and Smith *et al.*[34]. After protein repletion, when the fat content of all the groups becomes normal, it is still higher in sucrose-fed group. Such increase in liver fat in sucrose-fed group has been reported[35], and may be explained on the basis of increased lipogenesis by the presence of fructose molecule in sucrose. Increase in relative liver size in sucrose-fed group may also be due to deposition of fat as reported by earlier investigators[36].

Reduced activity of pancreatic amylase is found in all protein-deficient groups; after protein repletion, amylase activity rises in all the groups, excepting sucrose-fed group. This observation also confirms the findings of Howard and Yudkin[20], indicating lowered adopted response of the enzyme to sucrose feeding.

The above findings suggest that because of the interdependence among many of the reactions associated with the metabolism of protein and that of carbohydrates, it is possible that dietary protein modifies responses of enzyme synthesis to changes in dietary carbohydrates.

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References

1. ESH, G. C. & BANDYOPADHYAYA, PRABHATI, *Indian J. Biochem.*, **5** (1968), 173.
2. HARPER, A. E. & KATAYAMA, M. C., *J. Nutr.*, **49** (1953), 261.
3. NAGDY, AL. S., MILLER, D. S., QUREESHI, R. U. & YUDKIN, J., *Nature, Lond.*, **209** (1966), 81.

4. Cohen, A. M. & Teitelbaum, A., *Metabolism*, **15** (1966), 81.
5. Reussner, G. (Jr), Andros, J. & Theissen (Jr), *J. Nutr.*, **80** (1963), 391.
6. Spivey, H. E., Katayama, M. C., Yoshida, H. & Harper, A. E., *Am. J. Physiol.*, **193** (1958), 479.
7. Cohen, A. M. & Teitelbaum, A., *Am. J. Physiol.*, **206** (1964), 105.
8. Landau, B. R., Hastings, A. B. & Zettu, S., *J. biol. Chem.*, **233** (1958), 1957.
9. Freedland, R. A. & Harper, A. E., *J. biol. Chem.*, **234** (1959), 1350.
10. Fitch, W. M. & Chaikoff, L., *J. biol. Chem.*, **235** (1960), 554.
11. Dodds, C., Miller, L. & Rose, E. M., *Lancet*, **II** (1960), 178.
12. Waterlow, J. C. & Weisz, T., *J. clin. Invest.*, **35** (1956), 346.
13. Whitehead, R. G. & Harland, P. S. E., *Br. J. Nutr.*, **20** (1966), 825.
14. Esh, G. C. & Som, J. M., *Ann. Biochem. exp. Med.*, **23** (1963), 9.
15. Hawk, E. A. & Elvehjem, C. A., *J. Nutr.*, **49** (1953), 495.
16. Freedland, R. A. & Harper, A. E., *J. biol. Chem.*, **233** (1958), 1.
17. Litwack, G., Bothwell, J. W., Williams, J. N. & Elvehjem, C. A., *J. biol. Chem.*, **200** (1953), 303.
18. Slater, E. S. & Bonner, W. D., *Biochem. J.*, **52** (1952), 185.
19. Smith, B. W. & Roe, J. H., *J. biol. Chem.*, **179** (1949), 53.
20. Howard, F. & Yudkin, J., *Brit. J. Nutr.*, **17** (1964), 281.
21. Nelson, N., *J. biol. Chem.*, **153** (1944), 375.
22. Friedeman, T. E. & Haugen, G. E., *J. biol. Chem.*, **147** (1943), 415.
23. Carrol, N. V., Langley, R. W. & Roe, J. H., *J. biol. Chem.*, **220** (1956), 583.
24. Womack, M. & Marshall, M. W., *J. Nutr.*, **57** (1965), 193.
25. Macdonald, I. & Gharavi, E. M., *Proc. Nutr. Soc.*, **19** (1960), xxix.
26. Esh, G. C. & Som, J. M., *Ann. Biochem. exp. Med.*, **17** (1957), 117.
27. Williams, J. N. (Jr), *J. Nutr.*, **73** (1961), 210.
28. Litwack, G., Hankes, L. V. & Elvehjem, C. A., *Proc. Soc. exp. Biol. Med.*, **81** (1952), 44.
29. Wikramanayake, T. W., *Br. J. Nutr.*, **20** (1966), 641.
30. Durbin, P. A. J., Heard, C. R. C. & Platt, B. S., *Proc. Nutr. Soc.*, **19** (1960), 35.
31. Stewart, R. J. C. & Heard, C. R. C., *Proc. Nutr. Soc.*, 18 (1959), x.
32. Freedland, R. A. & Harper, A. E., *J. biol. Chem.*, **233** (1958), 1041.
33. Mukherjee, K. L. & Nath, R. L., *Bull. Calcutta Sch. trop. Med. Hyg.*, **5** (1957), 170.
34. Smith, L. H., Ettinger, R. H. & Seligson, D., *J. clin. Invest.*, **32** (1953), 273.
35. Mohamed, A. Antar & Margaret, A. Ohlson, *J. Nutr.*, **85** (1965), 329.
36. Allen, R. J. L. & Leahy, J. S., *Br. J. Nutr.*, **20** (1966), 339.
37. Ju, S. J. & Nasset, E. S., *Proc. Soc. exp. Biol. Med.*, **100** (1959), 834.

Short Communications

Isolation & Purification of a New Toxic Factor from *Lathyrus sativus*

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A water soluble aliphatic amino acid glycoside with a nitrile group has been isolated from aqueous extracts of *Lathyrus sativus*. It is toxic to day-old chicks at a dose of 50 mg./100 g. body weight and produces paralysis of the limbs within 5-10 min. of intraperitoneal injection.

EXCESSIVE consumption of *Lathyrus sativus* seeds is known[1] to produce neurolathyrism, a crippling disease in man. One of the toxins responsible for neurolathyrism has been identified[2], isolated[3,4] and characterized[1,5] as N-oxalyl α,β-diaminopropionic acid (BOAA), which exists in two isomeric forms (α and β)[6,7]. Oral feeding as well as parenteral administration of the seed extracts to experimental animals for several years did not produce any permanent disability. Even with BOAA, no residual or cumulative effect could be induced in chicks by continuous administration[8]. Thus, the production of symptoms similar to human lathyrism in experimental animals with BOAA was not successful. However, Rao *et al.*[9] could produce complete flaccid paralysis of the hind limbs in monkeys by injecting BOAA intrathecally. Chemical and biological investigations[10] have indicated the presence of more than one toxic factor in the seeds of *L. sativus*. With a view to studying the nature of other toxic factors, a systematic chemical investigation of the seeds of *L. sativus* has been undertaken. The isolation of a new toxin is reported in this communication.

Whole *L. sativus* seed powder (3 kg.) was stirred with 4 litres of water twice for 24 hr at room temperature and filtered. Alcohol was added to the filtrate to 30 per cent concentration by volume and the precipitated proteins were removed by centrifugation. The supernatant was concentrated on a water-bath. Vacuum concentration was not possible even with defoaming agents due to the heavy frothing nature of the solution. To the concentrate, absolute methanol (10 vol.) was added, when a slimy precipitate was obtained. This precipitate was triturated repeatedly with fresh quantities of absolute methanol (6×50 ml), when a brown hygroscopic powder was obtained. TLC examination revealed the hygroscopic compound to be a mixture of several amino acids and inorganic salts. Hence, the powder was dissolved in minimum amount of water, and passed over a column of alumina (48×2 cm.) and eluted with 30 per cent alcohol. The effluent

91

THE NUTRITION OF THE MOUSE

II. EFFECT OF DIET ON THE BACTERIAL FLORA OF THE INTESTINE AND THE CECUM [1]

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It is well-known that diet influences the intestinal flora of several species of animals, as is shown by the work of Tissier ('08) and later Sanborn ('31) with humans, Belonovsky ('07) with mice, Herter and Kendall ('09–'10) with cats and monkeys, Hull and Rettger ('15, '17) and Porter and Rettger ('40) with rats, and Crecelius and Rettger ('43) with guinea pigs. In general, all of the species responded similarly whenever either of 2 types of diets was fed. Meat or a high protein diet produced proteolytic flora consisting largely of coliform organisms; a variation in the carbohydrate induced a change toward a fermentative flora. For example, milk or lactose, and sometimes dextrin, encouraged an aciduric bacterial population; when dextrin was fed, there was produced a type of flora which synthesized some of the B vitamins (Guerrant et al., '35; Schweigert et al., '45).

Belonovsky ('07) studied only the effect of lactose and milk on the bacteria of the feces and upon the ease of implantation of certain strains of bacteria in the intestine. Little attention was paid to the effect of varying the diet on the predominating

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Dextrin

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flora or the effect that such a change would have in the nutrition of the mouse.

It has been shown in this laboratory that considerable difference exists in the nutritional requirements of several highly inbred strains of mice. In general, the requirements of the C_{57} strain (black, with low incidence of spontaneous mammary tumors) are lower than those for the A strain (albino, highly susceptible to spontaneous mammary tumors). Both of these strains have been used in these studies of the intestinal flora, but this paper deals principally with the A strain.

The studies to be reported here were undertaken to determine the effect of 2 synthetic rations and a natural diet upon the predominating bacteria in the intestinal tract of A strain mice, and whether any such change could affect the nutrition of the host. The lower parts of the small intestine and the cecum were chosen for investigation since it was felt that these were probably the most likely sites of absorption by the mouse of any synthetic products liberated by the bacteria.

The predominating bacteria of these segments of the intestine were studied by Gram stains and by culturing serial dilutions of the contents. Direct bacterial counts were made on the samples and the entire cecal contents were weighed.

EXPERIMENTAL

Weanling mice of the A strain, 21 days old, were placed in individual screen-bottom cages, and food and water supplied *ad libitum*. Growth rates were measured by weighing the animals periodically. The stock ration [2] and 2 synthetic diets (nos. 101 and 133) were used. The composition of the synthetic rations has been described in detail elsewhere (Fenton and Cowgill, '47). Diet 101 contained 23% casein and 60% dextrose, while diet 133 contained 30% casein and 45% dextrin. In other respects the 2 diets were similar except that no. 133 also had cystine, vitamin K, biotin and folic acid added.

Representative animals from these 3 groups were killed by etherization and were opened immediately using sterile tech-

[2] Purina Laboratory Chow.

nique. The cecum and the lower half of the small intestine were removed and the contents from these segments squeezed directly into sterile, tared vials which were then plugged with cotton. The upper part of the small intestine was not studied because of the small numbers of organisms present. The large intestine was not considered to be a likely area of absorption of bacterial excretory products because on the synthetic diets this material formed into hard pellets immediately upon leaving the cecum. It seemed likely that any substances that might be produced by bacteria in this region would be lost to the host. The vials containing the samples were weighed and the samples made up to a known dilution with sterile distilled water. The vials containing the diluted samples (usually a 1 : 25 to 1 : 100 dilution, depending on the size of the sample) were stoppered and shaken thoroughly. Any clumps of contents were broken up with a sterile pipette. The samples were then further diluted to 10^{-4}, and then serially by steps of 100 until a dilution of 10^{-10} was reached.

The cultural work was done first, and the whole procedure after the removal of the contents from the body was done as quickly as possible in order to keep the sample from being exposed unduly to air and low temperatures. The cecal contents were cultured first, as the bacteria isolated from this segment seemed to be more anaerobic than those from the small intestine. Attempts to reduce dissolved air to a minimum consisted of plugging the water blanks with rubber stoppers immediately upon removal from the autoclave, and of boiling the broth for 10 minutes in a water bath, followed by immediate cooling in water just before inoculation. The inoculum was placed carefully under the surface of the broth with a pipette, and the tube was sealed with vaspar.[3] The broth was composed of 1% of each of the following: Bacto-peptone, tryptone, peptonized milk, yeast extract, beef extract, and glucose, and 0.5% of K_2HPO_4 all dissolved in liver extract made by extracting 75 gm of Bacto liver powder in a

[3] Composed of equal weights of petrolatum and paraffin.

distilled water. The pH was adjusted to 6.8 before ving.

he cecal cultures, inocula representing 10^{-8}, 10^{-9}, and ere put into the broth in duplicate while for the small ne, only dilutions 10^{-8} and 10^{-9} were cultured. The s were incubated for 1 week at 37°C. Only these higher ns were cultured, as this study was primarily concerned he predominating organisms. However, in order to nore about the distribution of coliform organisms, in ses the E C medium of Hajna and Perry ('43) was ed with dilutions of the samples from 10^{-2} to 10^{-7}. on as growth appeared in any tube, the culture was tained, the motility was determined, the pH of the as measured electrometrically, and agar shake cultures ade to indicate the degree of anaerobiosis and to permit tion if necessary. The agar shakes were saved so rtain synthetic activities of these cultures could be . The results of this phase of the work will be pre- in the following paper (Gall et al., '48).

ediately after the completion of the cultural procedure, n stain was made of the diluted material from the l vial. From this the predominating morphological nd their approximate relation to each other were noted. counts were also made on the sample after suitable di- In general the cecal content was diluted 1 : 1000, while ll intestinal content was diluted 1 : 25 to 1 : 100, de- on the expected bacterial count. The slide counts ade on 2 × 2 cm areas by mixing 0.01 ml of the diluted with 1 drop of saturated nigrosine solution in methyl spread evenly over the area. The slides were dried ickly on a hot plate, and the counts were made with a ope for which the field had been calibrated. It was see the unstained bacteria against the black back- and from the average of 10 fields on each of 2 slides 00–400 organisms), it was possible to calculate the l count per gram of original sample.

The weight of the cecal and small intestinal contents was determined to the nearest milligram on an analytical balance. The material came out of the cecum very easily, and it is felt that the weights obtained represented with fair accuracy the entire cecal contents. The weights of the contents of the small intestines were extremely variable, since the position of the lumps of food in the intestine was so irregular that the inclusion or exclusion of 1 mass of food would alter the result severalfold. Therefore not much significance is attached to these figures, and they are not reported in the table.

In addition to these main experiments on A strain mice a few preliminary studies were carried out on small groups of C_{57} and A strain mice on various other diets under investigation in the laboratory.

RESULTS

Growth rates of male mice fed the stock ration or diet 101 have been reported by Fenton et al. ('47). All 3 diets studied here supported growth about equally well. Fenton and Cowgill ('47) have reported complete failure in securing reproduction and lactation in mice raised and maintained on ration 101. The more complete diet 133 did support reproduction and lactation, but the results were still not as satisfactory as with the stock ration.

Gram stains of the cecal contents of the animals fed these 3 diets were examined microscopically, and the differences in the flora on these diets were sufficiently marked to permit identification of the diet fed, simply by studying the slide. The cecal contents of the animals on stock ration were characterized by the presence of a slender, gram-negative, curved rod and a cigar-shaped organism. Except for some gram-positive rods, probably lactobacilli, there were few other bacteria present. In contrast to this simple flora, the animals on diet 101 harbored a varied bacterial population, but there was always present in these animals a considerable number of large round cocci, occurring in pairs and short chains. This coccus was characteristic of this diet. Diet 133 also supported

a variety of organisms, but the flora differed from that on ration 101 in several ways. The large coccus found on diet 101 was absent, but in its place was found in smaller numbers an elongated coccus, occurring in pairs, and a tiny coccus forming a characteristic, closely united chain of 10–20 organisms. On diet 133 there also appeared in large numbers, a highly curved rod that formed a perfect C.

The Gram stains of the small intestinal contents from animals fed all 3 diets showed the gram-positive rod as the predominating bacterium, but on the 2 synthetic diets the coccus characteristic of that ration usually appeared.

A more complete description of the types of bacteria found on these various diets, as well as some notes on their synthetic activities, especially in regard to B vitamins, will be presented in the following publication.

As shown in table 1 the cultural differences among the 3 groups were also marked and largely confirmed the conclusions drawn from the examination of the slides. The cecal samples cultured from stock diet animals gave scanty growth, due to the predominance of the slender curved rod, an anaerobe which proved very difficult to culture. In no case did the cultures from the animals on this diet grow in as high a dilution as was expected from the slide count. Virtually the only organism isolated from these animals was the easily cultivatable gram-positive rod.

In contrast, the bacteria from the ceca of animals fed diet 101 grew very readily. In 9 out of 12 cases the cultures grew in the dilutions indicated by the slide counts. The characteristic coccus was easily isolated, as were the gram-positive rods, and the coliform organisms when the latter were present. Gas, usually found in 1 or more tubes in the series, could in some instances be attributed to coliforms. However, in some tubes only a slight amount of gas appeared. On staining these cultures only cocci were observed, and attempts to isolate coliforms from these tubes repeatedly failed. This cultural picture identified the animals which had been fed diet 101.

TABLE 7

Summary of the bacterial counts, weights of cecal contents and cultural results obtained from A strain mice

DIET NUMBER	101		133		STOOK	
Part of intestinal tract	Small	Cecum	Small	Cecum	Small	Cecum
No. of animals	12	12	12	12	9	9
No. of animals showing agreement of slide and cultural "counts"	12	9	10	3	9	0
Wt. of segment contents, *gm*	.. [1]	0.065 ± 0.021 [2]	.. [1]	0.094 ± 0.029 [2]	.. [1]	0.407 ± 0.033 [2]
Bacterial count — billions						
per gm of sample	0.710 ± 0.81 [2]	22.2 ± 11.5 [2]	0.321 ± 0.26 [2]	23.0 ± 11.8 [2]	0.875 ± 0.99 [2]	42.8 ± 11.5 [2]
per intestinal segment	.. [1]	1.5 ± 0.84 [2]	.. [1]	2.09 ± 1.15 [2]	.. [1]	17.9 ± 5.4 [2]
No. of cultures isolated; according to type						
Gram + rods						
10^{-8}	14	7	9	10	13	13
10^{-9}	7	6	3	5	8	8
10^{-10}	.. [1]	3	.. [1]	0	.. [1]	0
Round coccus						
10^{-8}	7	15	0	0	0	1
10^{-9}	3	9	0	0	0	0
10^{-10}	.. [1]	5	.. [1]	0	.. [1]	0
Elongated coccus						
10^{-8}	0	0	1	6	0	0
10^{-9}	0	0	0	3	0	0
10^{-10}	.. [1]	0	.. [1]	0	.. [1]	0
Tiny coccus						
10^{-8}	0	0	1	3	0	0
10^{-9}	0	1	0	4	0	0
10^{-10}	.. [1]	1	.. [1]	1	.. [1]	0
Curved rod						
10^{-8}	1	0	1	1	0	1
10^{-9}	0	2	0	6	0	1
10^{-10}	.. [1]	3	.. [1]	1	.. [1]	0
Coliforms						
10^{-8}	0	4	0	3	0	0
10^{-9}	0	3	0	0	0	1
10^{-10}	.. [1]	3	.. [1]	0	.. [1]	0
No. of animals showing coliform organisms 100 M or above	.. [1]	5	.. [1]	2	.. [1]	1

[1] Not computed. [2] Standard deviation.

The cecal contents from animals fed ration 133 gave cultural results somewhat different from the other 2 diets. In only 3 cases out of 12 did the cultures grow in the dilution expected from the slide counts, but the discrepancy was not as great as with the stock ration. The elongated coccus and the tiny coccus, which proved to be an anaerobe, occurred in a fair number of cultures. The gram-positive rods were also isolated regularly. The curved, C-shaped rod seen on the smears was never isolated in that morphological form.

It has commonly been thought that coliform bacteria are always among the predominating organisms in the intestinal tract, but this study indicates that with A strain mice its occurrence in large numbers varies with the diet. With the average bacterial count in the neighborhood of 22 billion per gram of cecal contents, coliforms were isolated in dilutions of 1 billion or more only 7 times. Three of these came from the 10 billion dilution and 4 from the 1 billion. These cultures represented 5 animals, 4 of which were fed diet 101. Therefore, in only 10% of the animals tested were coliforms present in the top 2 dilutions, and in only 20% of the cases in the top 3 dilutions. It is pertinent to point out that 41% of the animals on diet 101 had coliforms present in 100 million or more, as compared to much fewer in animals on the other 2 diets. Two-thirds of the animals tested in low dilutions showed less than 1 million coliform organisms per gram of cecal contents and at least 25% were below 10 thousand per gram. It is of interest to note that with the stock ration animals where the opportunity for coprophagy was the greatest, there was the mildest coliform flora.

The cultural results of the small intestinal contents on the 3 diets reflected the findings with the Gram stain. All 3 diets gave a large number of cultures of gram-positive rods, while the 2 synthetic diets showed in addition cocci of the type characteristic of the ration.

The 3 diets were compared as to bacterial count per gram of cecal material and total bacterial count per cecum. For reasons stated above, a similar study for the small intestinal

contents is not reported in table 1. The animals eating the stock ration showed a higher bacterial content when measured per gram or per cecum, although the difference is greater by far when measured by the latter criterion. This is a function of the greater content of the cecum of the animals eating the natural diet. By statistical analysis these differences were found to be highly significant ($p < 0.01$). There was little difference between the animals eating the 2 synthetic diets, however. Statistically there was no difference between the cecal count per gram on diets 101 and 133. A comparison of the total cecal counts of these diets also showed no significant difference ($p = 0.2$).

The cecal contents obtained from animals fed the stock ration were statistically highly significantly heavier than the contents from the animals on the 2 synthetic diets ($p < 0.01$). The animals on diet 133 showed greater cecal contents than those on 101 (p just equals 0.01). This should be interpreted cautiously, however, for reasons to be discussed below.

By way of preliminary investigation a few animals of both C_{57} and A strains were studied after having been maintained on the following diets: (a) 3 diets similar to no. 133, but with dextrose as the carbohydrate, and a diet identical with no. 101 (containing dextrose), but omitting roughage; (b) a diet low in riboflavin and one low in pantothenate, but in other respects similar to no. 101; (c) a diet similar to no. 101 with 1% sulfathalidine added; (d) a ration identical with no. 101, but replacing the dextrose with dextrin.

The diets listed under (a) showed a cecal flora essentially the same as that found on ration 101. The animals deficient in riboflavin and pantothenate showed the same cecal flora as those on diet 101 except that a C-shaped rod was also present. The mice fed sulfathalidine showed a flora similar to those on ration 101, but the weights of their cecal contents were somewhat greater. When the dextrose of diet 101 was replaced with dextrin, the resulting flora resembled that which was found on the dextrin containing diet no. 133. In all of these preliminary studies it must be emphasized that only a micro-

opic examination was made, thus affording a comparison on e basis of morphology alone.

A group of mice was fasted 24 hours and then fed the stock ration. Small numbers of these animals were sacrificed and their cecal contents studied at various intervals following feeding. The animals sacrificed 3.5 to 6 hours after feeding showed a C-shaped rod, which was not present in animals permitted to feed until sacrificed or in mice sacrificed up to 5 hours after feeding, following a period of fasting.

DISCUSSION

Ample evidence has been presented in the main portion of this study and also in the preliminary work reported here showing that the composition of the diet can have a marked effect upon the intestinal flora of the experimental animals. The bacteria seen on each of the 3 rations investigated have been so distinctive that it was possible to judge by microscopic examination alone which diet had been fed to any particular animal. In fact, following the initial experiments, made it a practice to give the investigator (L.S.G.) an animal for the day's work without her having any knowledge of the nature of the ration. On the basis of the Gram stain, the examiner would attempt to identify the diet fed, and in only 1 case out of 33 was an error made.

Many investigators have implied that a great deal of the intestinal synthesis should be ascribed to coliform bacteria. Our data do not warrant such conclusions, at least for this highly inbred strain of mouse. We have found appreciable numbers of coliforms only on diet 101, and here only 5 out of 12 animals showed this organism in the top dilutions.

It is important to stress that with the animals fed the stock on and diet 133, we frequently failed to obtain cultural lts commensurate with the slide count. More work is necessary to determine the cultural requirements of the bacteria which predominate on this diet.

In view of the greater roughage content of the stock ration, was to be expected that animals on this diet would have

greater cecal contents than the animals on synthetic diets. In our studies comparing 2 synthetic diets, 1 containing dextrin (no. 133) and the other dextrose (no. 101), there was a statistically significant difference between the weights of the cecal contents of the 2 groups (p just equals 0.01). However it must be stated that the animals fed diet 133 were slightly older than those eating no. 101, and for this reason the results should be interpreted with caution. Mannering et al. ('44) found that rats fed a synthetic diet containing dextrin had a larger cecal content per unit of body weight than animals fed a similar diet containing sucrose.

It must also be stated in this connection that 6 of the 9 mice fed the stock ration were older than the animals on synthetic ration, while 3 were the same age or younger. On comparison the average weights of the cecal contents of the young and old animals on stock ration were found to be so nearly alike ($0.386 \pm .038$ and $0.417 \pm .045$, respectively), that it was felt that these age differences did not seriously affect the interpretation.

The study reported here on both C_{57} and A strains of mice on different diets gives evidence that there is a flora, characteristic of mice fed a dextrose diet, which is different from that of animals maintained on a ration containing dextrin. This is in agreement with the findings of other workers on several species cited in the introduction, that certain carbohydrates induce differences in intestinal flora.

SUMMARY AND CONCLUSIONS

1. Microscopic and cultural examinations of the small intestine and the cecum of A strain mice on stock ration and 2 synthetic diets were carried out.

2. Mice fed each of these diets had a characteristic cecal flora; on the stock ration it consisted of a slender, gram-negative, curved rod; on the 2 synthetic diets cocci characterized each of them.

3. Coliforms were not found to be present in significant numbers on 2 of the diets and were found in large numbers in less than half of the mice on diet 101.

4. The mice on the stock diet had a larger bacterial population per gram of cecal contents and per cecum than the mice on synthetic diets. There was no significant difference between the 2 groups of mice on synthetic diets.

5. The weights of the cecal contents of the animals on stock ration were greater than those of the animals fed synthetic diets. There was little difference between the animals on synthetic diets with respect to weights of cecal contents.

ACKNOWLEDGMENTS

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LITERATURE CITED

BELONOVSKY, J. 1907 Influence du ferment lactique sur la flore des excréments des souris. Ann. de l'Inst. Pasteur, *21:* 991.

CRECELIUS, H. G., AND L. F. RETTGER 1943 The intestinal flora of the guinea pig. J. Bact., *46:* 1.

FENTON, P. F., AND G. R. COWGILL 1947 Reproduction and lactation in highly inbred strains of mice on synthetic diets. J. Nutrition, *33:* 703.

FENTON, P. F., G. R. COWGILL AND M. A. STONE 1947 Growth rates of mice on stock rations and on purified diets. Yale J. Biol. Med. (in press).

GALL, L. S., B. A. ILLINGWORTH, G. R. COWGILL AND P. F. FENTON 1948 The nutrition of the mouse. III. Relation of diet to the synthetic activity of the predominating flora isolated from the small intestine and cecum. J. Nutrition, *35:* 27.

GUERRANT, N. B., R. A. DUTCHER AND L. F. TOMEY 1935 Effect of the type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. J. Biol. Chem., *110:* 233.

HAJNA, A. A., AND C. A. PERRY 1943 Comparative study of the presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am. J. Pub. Health, *33:* 550.

HERTER, C. A., AND A. I. KENDALL 1909–1910 Influence of dietary alternations on the types of intestinal flora. J. Biol. Chem., *7:* 203.

HULL, T. G., AND L. F. RETTGER 1915 Influence of milk and carbohydrate feeding on the intestinal flora of white rats. Zentralbl. f. Bakt. I. Abt. Originale, *75:* 219.

——— 1917 Influence of milk and carbohydrate feeding on the character of intestinal flora. IV. Diet vs. bacterial implantation. J. Bact., *2:* 47.

MANNERING, G. J., D. ORSINI AND C. A. ELVEHJEM 1944 Effect of the composition of the diet on the riboflavin requirement of the rat. J. Nutrition, *28:* 141.

PORTER, J. R., AND L. F. RETTGER 1940 Influence of diet on the distribution of bacteria in the stomach, small intestine and cecum of the white rat. J. Inf. Dis., *66:* 104.

SANBORN, A. G. 1931 The fecal flora of adults, with particular attention to individual differences and their relationship to the effects of various diets. II. Individual differences in response to special diets. J. Inf. Dis., *49:* 37.

SCHWEIGERT, B. S., J. M. MCINTIRE, L. M. HENDERSON AND C. A. ELVEHJEM 1945 Intestinal synthesis of B vitamins by the rat. Arch. Biochem., *6:* 403.

TISSIER, H. 1908 Recherches sur la flore intestinale normale des enfants âgés d'un an à cinq ans. Ann. de l'Inst. Pasteur, *22:* 189.

Am J. Dis. Children, 39, 1930

101

THE CARBOHYDRATE METABOLISM OF THE NORMAL NEW-BORN INFANT

II. THE EFFECT ON THE CONCENTRATION OF THE BLOOD SUGAR OF FEEDING VARIOUS SUGARS TO NEW-BORN INFANTS *

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In a previous paper we reported observations on the blood sugar values during fasting in normal new-born infants.[1] In this paper we report the results of studies on the dextrose, saccharose, lactose and dextrimaltose tolerance in new-born infants. In addition, the blood sugar curve after a breast feeding was determined in eighteen infants. We found a number of reports[2] in the literature on carbohydrate tolerance in infants ranging in age from 1 month to 2 years, but a careful search failed to reveal any studies on new-born infants.

TECHNIC

In all the work done, the values of the blood sugar were estimated on blood from the great toe, according to the technic described in a previous paper.[1]

Three hours after a feeding, the blood was taken and the sugar content was determined. Immediately after the blood was taken, 2 Gm. of dextrose, saccharose, lactose or dextrimaltose per kilogram of body weight was given in a 20 per cent solution. No difficulty was encountered in getting the infants to take the solution. If vomitting occurred, the infant was excluded from the study. Two grams of carbohydrate per kilogram of body weight was decided on: first, because Rumpf[3] found that the smallest amount of dextrose that produced an alimentary glycemia in older infants was 1.3 Gm. per kilogram of body weight, and second

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1. Greenwald, H. M., and Pennell, S.: The Carbohydrate Metabolism of the Normal New-Born Infant: I. Average Concentration of the Blood Sugar in Normal New-Born Infants During Fasting, Am. J. Dis. Child. **39**:281 (Feb.) 1930.

2. Mogwitz: Monatschr. f. Kinderh. **12**:569, 1914. Bergmark: Jahrb. f. Kinderh. **80**:373, 1914. Nieman: Jahrb. f. Kinderh. **83**:1, 1916. Frank, A., and Melhorn, L.: Jahrb. f. Kinderh. **91**:313, 1920. Götzky: Ztschr. f. Kinderh. **9**:44, 1913. Bing and Winderlow: Ztschr. f. Kinderh. **9**:64, 1913. Spence, J. C.: Quart. J. Med. **14**:314, 1921. Mertz, A., and Romminger, E.: Arch. f. Kinderh. **69**:81, 1921. Rumpf, F.: Jahrb. f. Kinderh. **105**:321, 1924. Brown, M. J.: Quart. J. Med. **18**:175, 1925. Tisdall, F. F.; Drake, T. Y. H., and Brown, A.: Carbohydrate Metabolism of Normal Infant, Am. J. Dis. Child. **30**:675 (Nov.) 1925. MacLean, A. B., and Sullivan, R. C.: Dextrose Tolerance in Infants and in Young Children, Am. J. Dis. Child. **37**:1146 (June) 1929.

3. Rumpf (footnote 2, ninth reference).

because this quantity closely approximates the amount of carbohydrate usually taken by a new-born infant under normal conditions after the secretion of milk in the mother has become established. For example, it has been fairly well established that an infant weighing from 6 to 7 pounds (2.7 to 3.2 Kg.) at the fifth or sixth day of life will take from 70 to 80 Gm. of breast milk per feeding, which contains approximately from 5 to 6 Gm. of lactose; this is about 2 Gm. of carbohydrate per kilogram of body weight.

Estimations were made of the blood sugar concentration during fasting and at one-half hour, one hour and two hours after the meal. Each sugar was administered to fifteen infants so that in all, sixty infants were studied. These infants ranged in age from 2 to 10 days.

RESULTS

Blood Sugar Values after Breast Feeding.—The blood sugar during fasting was determined and estimations were made one-half hour, one

TABLE 1.—*Increase in Blood Sugar Concentration After a Breast Feeding, During First Forty-Eight Hours of Life*

Infant No.	Age in Hrs.	Blood Sugar, Per Cent				
		Fasting	½ Hour	1 Hour	1½ Hours	2 Hours
1	2½	0.060	0.066	0.080	0.090	.....
2	9	0.074	0.083	0.074	0.074	0.069
3	14	0.074	0.073	0.074	0.069	0.071
4	16	0.062	0.071	0.071	0.064	0.066
5	16	0.069	0.066	0.067	.....	0.064
6	18	0.060	0.064	0.066	0.080	0.080
7	22	0.087	0.087	0.077	0.080	0.079
8	22	0.074	0.074	0.071	0.071	0.070
9	27	0.077	0.080	0.077	0.080	.....
10	30	0.087	0.080	0.083	0.077	.....
11	33	0.071	0.074	0.087	0.111	0.100
12	33	0.077	0.080	0.080	0.074	0.080
13	36	0.077	0.087	0.077	.....	0.077
14	40	0.069	0.083	0.091	0.087	0.083
15	40	0.062	0.062	0.062	.....	0.064
16	48	0.077	0.077	0.080	.....	0.105
17	50	0.087	0.082	0.076	0.077	0.087
18	52	0.082	0.064	0.063	0.066	0.069

hour, one and one-half hours and two hours after the breast feeding. In six infants the tolerance curve was followed from the first to the sixth day of life, estimations being made at the same time each morning.

In the eighteen infants studied after a breast feeding, no rise in the blood sugar concentration occurred after a breast feeding, during the first forty-eight hours of life, except in infants Nos. 11, 13 and 15 (table 1). This is undoubtedly due to the fact that the breasts secrete little or no milk, as a rule, during the first forty-eight hours after delivery. Colostrum, while it contains as much lactose as breast milk (6.8 to 7.59 per cent [4]), is secreted in but comparatively small amounts, and it is our opinion that not enough lactose is absorbed to produce a rise in the blood sugar curve during this period. Infants Nos. 11, 13 and 15 were born of multiparous mothers whose breasts secreted a fairly large

4. Abt, I. A.: Pediatrics, Philadelphia, W. B. Saunders Company, 1923, vol. 1, p. 616.

amount of milk as early as the second day post partum. In the eighteen infants studied, a rise occurred in one and no rise in eight infants on the first day of life; on the second day there was an equal number in which no rise and a rise occurred. On the third day, six did and two did not show a rise in the blood sugar curve; on the fourth day, the breast feeding was followed by a rise in three infants and in two it was not (chart 1).

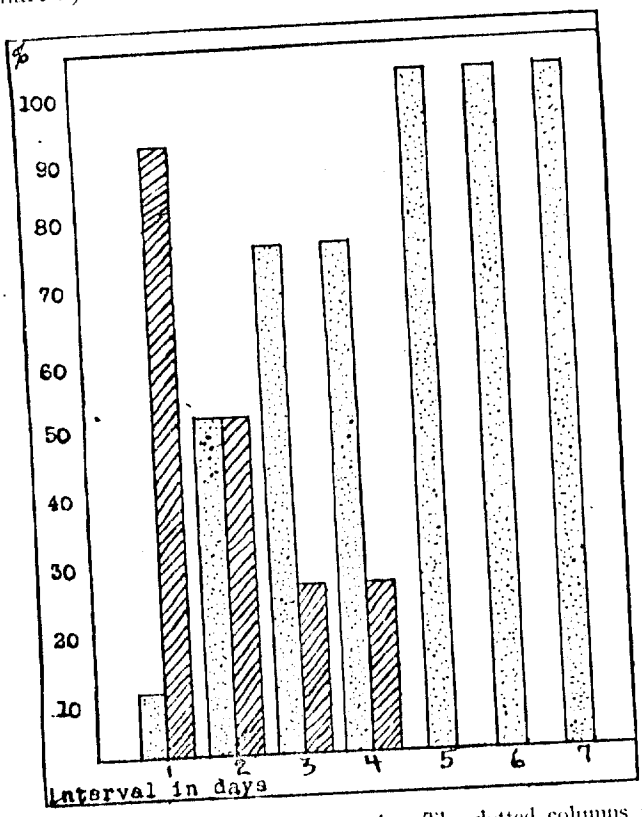

Chart 1.—Rise of blood sugar at intervals. The dotted columns indicate percentage of cases showing rise of blood sugar at various intervals; the lined columns, the percentage of cases showing no rise in blood sugar.

Table 2 is a summary of the blood sugar curves in the six breast-fed infants from the first to the sixth day of life. It will be seen that in only one infant a significant rise in the blood sugar concentration did not occur after a feeding until the fourth day of life (infant No. 4). In all the others, significant elevations occurred after the third day. In most instances the greatest value was usually reached one-half hour after the feeding. Exceptions were infants Nos. 12 and 16, in whom

the peak was not reached until two hours after a feeding. Whether this delayed rise was due to imperfect or tardy absorption in the gastro-intestinal tract or to changes in the carbohydrate metabolism is impossible to say at this time. The maximum value obtained was 0.182 per cent, one hour after a breast feeding, in infant No. 17.

The average values obtained during the first forty-eight hours of life are shown in chart 2. The average values obtained in the six infants studied from the third to the sixth day of life are shown in chart 3.

TABLE 2.—*Blood Sugar Concentration After a Breast Feeding in Infants Aged from 1 to 6 Days*

Infant	Age	Weight, Lbs. Oz.	Blood Sugar Per Cent				
			Starving	½ Hour	1 Hour	1½ Hours	2 Hours
1	16 hours....	7 10	0.069	0.066	0.077	.....	0.064
	40 hours....	7 3	0.062	0.062	0.062	.....	0.064
	3 days.....	7 5	0.069	0.111	0.095	.....	0.095
	4 days.....	7 7	0.080	0.111	0.095	.....	0.087
	5 days.....	7 10	0.066	0.118	0.111	.....	0.111
2	3 days.....	7 15	0.074	0.111	0.100	.....	0.100
	5 days.....	.....	0.083	0.154	0.182	.....	0.125
	6 days.....	.....	0.087	0.144	0.133	.....	0.083
3	3 days.....	7 6	0.077	0.077	0.095	.....	0.133
	5 days.....	.....	0.087	0.133	0.118	.....	0.095
	6 days.....	.....	0.074	0.118	0.105	.....	0.095
4	9 hours....	7	0.074	0.083	0.074	0.074	0.069
	33 hours....	.....	0.077	0.080	0.080	0.074	0.080
	57 hours....	.....	0.080	0.060	0.057	0.060	0.069
	81 hours....	.....	0.066	0.069	0.087	0.087	0.083
	105 hours....	.....	0.083	0.095	0.087	0.087	0.083
5	16 hours....	.....	0.062	0.071	0.071	0.064	0.066
	40 hours....	.....	0.069	0.083	0.091	0.087	0.083
	64 hours....	.....	0.069	0.071	0.074	0.087	0.091
	88 hours....	.....	0.069	0.080	0.087	0.111	0.091
	112 hours....	.....	0.066	0.074	0:083	0.105	0.100
6	2 days.....	.....	0.077	0.077	0.080	.....	0.105
	3 days.....	.....	0.091	0.111	0.118	0.100	0.100
	4 days.....	.....	0.091	0.125	0.118	0.095	0.091
	5 days.....	.....	0.071	0.133	0.100	0.071	0.077
	6 days.....	.....	0.095	0.166	0.154	0.105	0.077

Dextrose Tolerance.—Fifteen normal new-born infants, ranging in age from 2 to 10 days, were fed 2 Gm. of dextrose per kilogram of body weight, and the blood sugar curve was determined. In all but one a prompt and definite rise occurred in from one-half to one hour after the feeding. The highest point was 0.150 per cent in three cases. In twelve infants the blood sugar level during fasting was reached at the end of two hours. In the remaining three infants there was a decided lag. In one, the blood sugar reached a higher level two hours after the ingestion of dextrose than it did one hour after ingestion. In two infants the blood sugar was exactly the same at the end of two hours as after one hour. Maximum absorption occurred at the end of one-half hour in seven infants and at the end of one hour in an

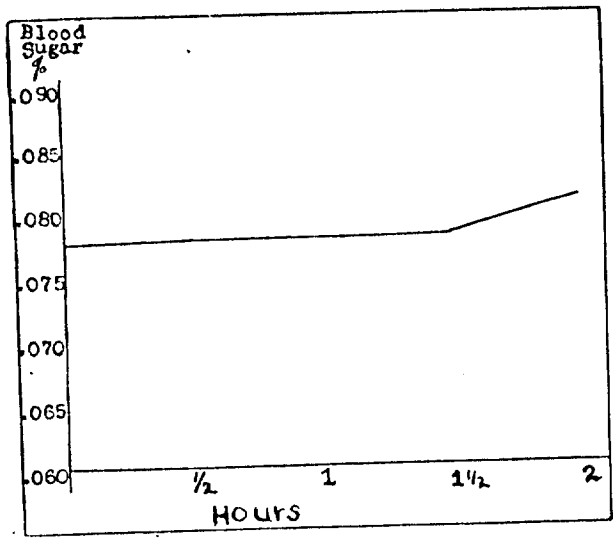

Chart 2.—Curve following breast feeding during first forty-eight hours of life.

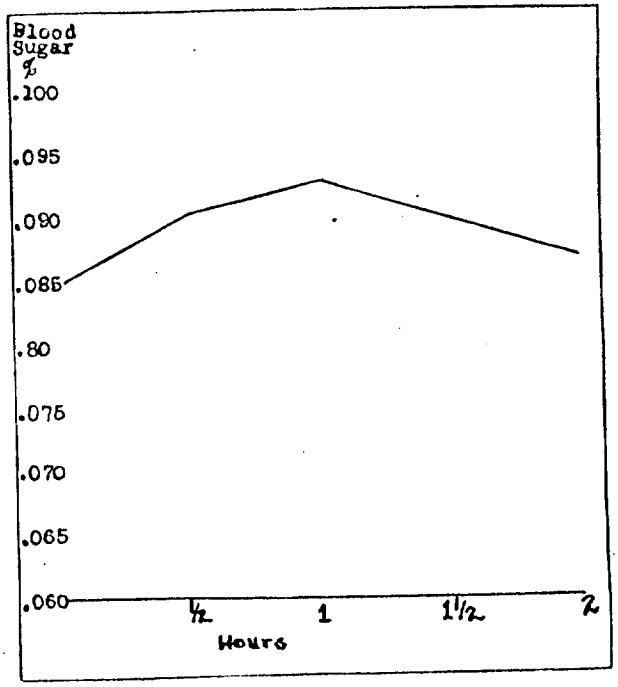

Chart 3.—Curve following breast feeding from the third to the sixth day of life.

equal number. The average value at the end of one-half hour was 112 mg.; at the end of one hour, 115 mg., and at the end of two hours, 90 mg. (table 3).

These blood sugar curves correspond closely to those obtained by MacLean and Sullivan[5] in their studies on the dextrose tolerance in infants varying in age from 5 weeks to 3 years. A comparison of the alimentary dextrose tolerance of children from 5 weeks to 3 years of age and new-born infants with that of adults is given in chart 4. The sugar tolerance curve in the new-born infant is essentially the same as in older infants and adults, except that the curve is at a lower level.

Cane Sugar Tolerance.—In only two infants were maximum values of 140 mg. or above obtained after the ingestion of cane sugar, as compared with the values obtained in three infants after the ingestion

TABLE 3.—*Dextrose Tolerance*

Infant	Age in Days	Weight	Blood Sugar Per Cent			
			Starving	½ Hour	1 Hour	2 Hours
1	7	7:4	0.069	0.111	0.133	0.157
2	7	7:5	0.077	0.080	0.091	0.091
3	6	6:15	0.080	0.111	0.125	0.125
4	7	8:0	0.062	0.091	0.133	0.154
5	6	8:8	0.069	0.144	0.144	0.083
6	6	7:10	0.083	0.125	0.144	0.105
7	5	8:6	0.062	0.118	0.154	0.069
8	5	7:13	0.062	0.111	0.095	0.095
9	4	7:13	0.069	0.100	0.125	0.105
10	9	6:2	0.077	0.154	0.118	0.100
11	9	7:10	0.074	0.111	0.091	0.077
12	5	7:13	0.087	0.100	0.118	0.077
12	8	7:13	0.071	0.111	0.080	0.086
14	5	5:12	0.071	0.111	0.091	0.069
15	4	6:7	0.069	0.095	0.083	0.077

of dextrose. In two infants the two hour level was higher than the one hour level, as compared with that obtained in one infant following the dextrose feeding. In eleven infants, the fasting blood sugar level was reached at the end of two hours; in the remaining two, the curve showed a definite downward trend at the end of this period. Maximum absorption occurred at the end of one-half hour in six infants, and at the end of one hour in seven infants. The average value at the end of one-half hour was 0.105 per cent; at the end of one hour, 0.112 per cent, and at the end of two hours, 0.080 per cent. The two hour value with cane sugar feeding was closer to the blood sugar level during fasting than that obtained after feeding any other sugar (table 4).

Lactose Tolerance.—In two infants a maximum value of 140 mg. was obtained. In only one infant was the two hour value higher than the one hour value. In two infants the two hour value was the same as

5. MacLean and Sullivan (footnote 2, twelfth reference).

the one hour value. The other curves were at a distinctly lower level than the curves obtained following the ingestion of dextrose or saccharose. In one, practically no rise occurred. The maximum value was obtained at the end of one-half hour in five infants and at the end

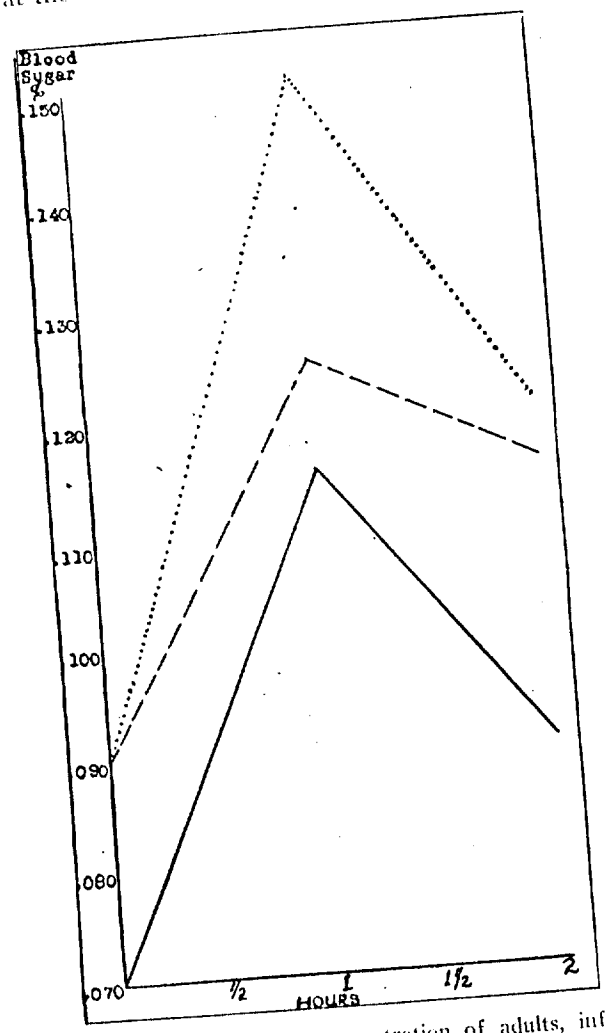

Chart 4.—Curves of blood sugar concentration of adults, infants aged from 5 weeks to 3 years and new-born infants, after the ingestion of dextrose. The dotted line indicates blood sugar concentration of adults; the dash line, the concentration of infants from 5 weeks to 3 years of age (MacLean and Sullivan), and the solid line, the concentration of new-born infants.

of one hour in nine. In nine infants the blood sugar level during fasting was reached at the end of two hours; in the remaining six, there was a definite lag. The average value at the end of one-half hour was 0.1

per cent; at the end of one hour, 0.114 per cent, and at the end of two hours, 0.09 per cent. Apparently, there is a slower rate of absorption for lactose than for dextrose or cane sugar (table 5).

Dextrimaltose Tolerance.—The most irregular curves were obtained following the feeding of dextrimaltose. In four infants practically flat curves were obtained. In one infant, no rise occurred. In three infants

TABLE 4.—*Cane Sugar Tolerance*

Infant	Age in Days	Weight	Blood Sugar Per Cent			
			Starving	½ Hour	1 Hour	2 Hours
1	6	7:1	0.060	0.100	0.111	0.091
2	4	6:1	0.064	0.083	0.091	0.111
3	10	7:6	0.071	0.111	0.118	0.077
4	4	5:1	0.069	0.133	0.125	0.069
5	4	7:0	0.066	0.133	0.154	0.071
6	3	6:15	0.069	0.125	0.133	0.074
7	7	6:12	0.066	0.111	0.111	0.066
8	7	6:6	0.064	0.087	0.111	0.087
9	7	7:2	0.053	0.071	0.080	0.080
10	5	8:1	0.071	0.074	0.133	0.095
11	8	7:14	0.077	0.125	0.100	0.077
12	3	...	0.059	0.125	0.111	0.077
13	2	7:8	0.077	0.111	0.166	0.083
14	5	9:0	0.059	0.118	0.057	0.053
15	3	7:5	0.071	0.091	0.100	0.080

TABLE 5.—*Lactose Tolerance*

Infant	Age in Days	Weight	Blood Sugar Per Cent			
			Starving	½ Hour	1 Hour	2 Hours
1	3	7:4	0.055	0.100	0.105	0.071
2	5	6:2	0.080	0.111	0.111	0.091
3	6	6:14	0.077	0.118	0.105	0.105
4	5	6:12	0.080	0.091	0.105	0.069
5	4	6:11	0.087	0.118	0.095	0.071
6	4	7:8	0.077	0.105	0.125	0.105
7	3	5:5	0.057	0.091	0.080	0.069
8	2	6:0	0.077	0.144	0.111	0.080
9	2	5:10	0.071	0.095	0.144	0.133
10	10	6:4	0.074	0.105	0.125	0.080
11	2	6:13	0.062	0.062	0.100	0.087
12	6	8:1	0.083	0.095	0.118	0.133
13	8	7:5	0.074	0.087	0.091	0.091
14	4	6:13	0.077	0.095	0.105	0.077
15	4	7:10	0.080	0.125	0.166	0.100

a high value of 0.14 per cent was found. The maximum rise occurred at the end of one-half hour in only three infants. In four infants the maximum rise occurred at the end of one hour; in five infants the two hour value was higher than the one-half hour or one hour value; in three infants the blood sugar level during fasting was reached at the end of two hours, and in three infants, although the curve showed a downward trend, the two hour value was still definitely above the blood sugar concentration during fasting. In other words, eight infants

showed a definite lag with dextrimaltose, whereas only six showed it with lactose; only two with cane sugar and three with dextrose. The average value at the end of one-half hour was 0.095 per cent; at the end of one hour, 0.102 per cent, and at the end of two hours, 0.095 per cent (table 6).

The dextrimaltose curve closely parallels that for the breast-fed infants and is much flatter than the curve found in infants fed with other sugars. The amount of breast milk taken by each infant was not controlled, and it is therefore obviously incorrect to compare the curves obtained after a breast feeding with the curves obtained after feeding definite amounts of various sugars. If it is assumed that the breast-fed infants studied received adequate amounts of milk, it must be concluded that the best tolerance is for lactose, as it exists in breast milk. It is unlikely that the flatness of the curve is

TABLE 6.—*Dextrimaltose Tolerance*

Infant	Age in Days	Weight	Blood Sugar Per Cent: Starving	½ Hour	1 Hour	2 Hours
1	10	6:12	0.074	0.100	0.105	0.095
2	10	8:0	0.080	0.105	0.118	0.105
3	?	7:0	0.080	0.095	0.087	0.095
4	7	6:9	0.066	0.077	0.125	0.154
5	7	7:7	0.064	0.095	0.111	0.083
6	4	6:0	0.071	0.125	0.154	0.118
7	4	6:13	0.069	0.105	0.133	0.125
8	7	5:15	0.062	0.082	0.157	0.060
9	8	7:0	0.077	0.111	0.144	0.111
10	6	7:2	0.060	0.062	0.052	0.059
11	5	7:2	0.069	0.118	0.111	0.066
12	4	7:15	0.071	0.091	0.100	0.105
13	4	8:8	0.077	0.091	0.087	0.095
14	2	7:8	0.071	0.087	0.071	0.069
15	1	7:2	0.066	0.083	0.080	0.091

due to delayed absorption since it is generally conceded that breast milk is the ideal food, particularly for the new-born infant. In addition, the presence of protein, fat and salts in breast milk may be a factor in the absorption and assimilation of lactose. This phase of the problem is being studied at the present time, and the results will be reported in a subsequent paper.

It is questionable whether the flatness of the curve following the ingestion of dextrimaltose may be considered due to an increased tolerance for this sugar, as was the case with lactose present in breast milk. It is more likely that dextrimaltose is absorbed more slowly than the other sugars, since with no other sugar did we obtain as large a number of flat and lag curves.

Chart 5 gives the average values for cane sugar, dextrimaltose, lactose and saccharose. With all sugars, the maximum value was obtained at the end of one hour. The curves obtained following the

feeding of lactose, cane sugar and dextrose closely parallel each other, except that the two hour period for cane sugar more closely approximated the blood sugar level during fasting. The average curve for dextrimaltose showed a decided lag. The best absorption occurred with dextrose. The curve obtained after feeding dextrose closely approximated the curves obtained in adults and older children after the ingestion of dextrose, except that it was at a definitely lower level, as is indicated by chart 4.

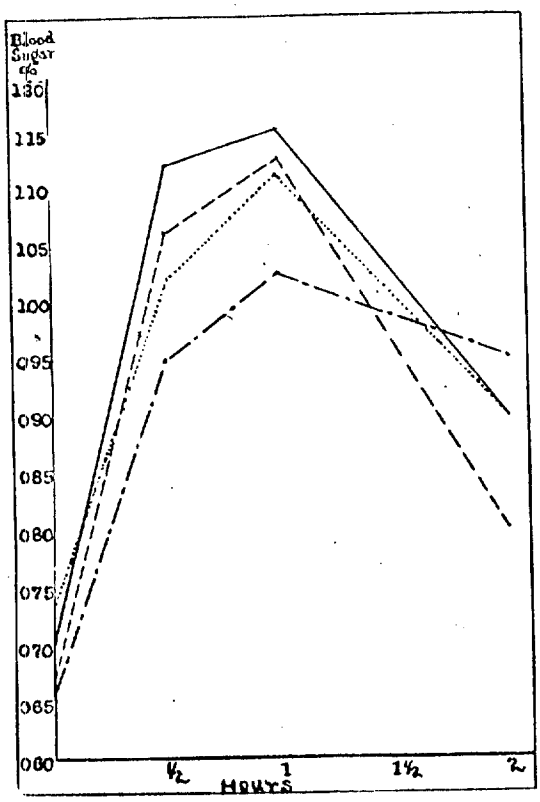

Chart 5.—Average curves for dextrose, saccharose, lactose and dextrimaltose. The solid line indicates course of dextrose; the dash line, saccharose; the dotted line, lactose, and the dot and dash line, dextrimaltose.

SUMMARY

In eighteen infants the blood sugar concentration was followed after a breast feeding during the first forty-eight hours of life; practically no increase in the blood sugar concentration occurred. This was most likely due to an insufficient secretion of breast milk during this period. In seven infants, the blood sugar curve after a breast feeding was studied from the first to the sixth day of life. A definite rise in the blood sugar concentration occurred in from one-half to one hour in practically all

infants after the second day of life. From the curves obtained, it is concluded that the best tolerance is for lactose as it occurs in breast milk.

Sixty infants were fed definite amounts of dextrose, lactose, saccharose and dextrimaltose; each sugar was administered to fifteen infants. The blood sugar curve after a dextrose meal is at a definitely lower level during the neonatal period than at any other period in life. The best rate of absorption occurred after the ingestion of dextrose, saccharose, lactose and dextrimaltose in the order in which they are named. The most irregular curves were obtained with dextrimaltose; with dextrose, saccharose and lactose fairly uniform curves were obtained.

107

Reducing Action of Glucose

—A lowering of the temperature brings about the
ve result as does lowering the pH.
of Solution—Increasing the salt content likewise
action and increases the final quantity of oxidant

of Glucose—The final quantity of oxidant reduced
ortional to the concentration of glucose (within the
sugar determinations), and the greater the concen-
se, the longer is the reaction time.
of Oxidant—Increasing the concentration of the
increases the final amount of oxidant reduced.
similarity is pointed out in the data obtained with
with copper solutions.
ng action of glucose is shown to be greatly in-
prese of NaCN, a fact which offers an explana-
blood sugar values obtained with the Folin micro-
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shes to thank Professor W. M. Clark of the Johns
ity Medical School for his kind assistance, and
his appreciation to Associate Professor Grinnell
Dill, and Dr. H. T. Edwards of Harvard Uni-
helpful advice.

BIBLIOGRAPHY

Biol. Chem., 70, 599 (1926).
d Szanogyi, M., J. Biol. Chem., 100, 695 (1933).
and Hawkins, J. A., J. Biol. Chem., 79, 739 (1928).
The determination of hydrogen ions, Baltimore, 3rd
(1928).
Biol. Chem., 75, 33 (1927).
Biol. Chem., 100, 357 (1933).
Chem., 77, 421 (1928); 81, 231 (1929).
J. Biol. Chem., 68, 759 (1926); 76, 457 (1928); 92, 141

and Van Slyke, D. D., J. Biol. Chem., 81, 459 (1929).
J. Biol. Chem., 84, 79 (1929).

J. Biol. Chem, 110, 1935

THE EFFECT OF THE TYPE OF CARBOHYDRATE ON THE SYNTHESIS OF THE B VITAMINS IN THE DIGESTIVE TRACT OF THE RAT*

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In a previous communication from this laboratory (1) data were presented which emphasized the danger of coprophagy as it affects vitamin B and vitamin G assays. In a later publication (2) further data were submitted which showed that the type of carbohydrate employed in making up vitamin B- and vitamin G-deficient dietaries is an important factor in this consideration.

The experiments herein described were carried out as a continuation of the latter investigation. Through these studies, additional data have been obtained concerning dextrinized corn-starch and sucrose-containing diets; and, in addition, diets containing lactose, glucose, and commercial corn-starch have likewise been investigated.

EXPERIMENTAL

The technique employed in this investigation was for the most part similar to that described in a former communication. In the present studies, a series of diets was used which were similar in composition to those previously reported (2). The composition of these several diets differed only in the type of carbohydrate which they contained and is given in Table I.

Young rats obtained from our breeding colony were used as

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experimental animals. These animals were weaned when 21 days of age and were put directly on experiment. In placing the animals on experiment, efforts were made to distribute litters and sexes uniformly throughout the several groups. Each animal was placed in an individual metal cage, which was provided with a raised screen bottom, having 2 meshes per inch. All animals were provided with ample quantities of the respective diets under consideration. Fresh distilled water was kept before the animals at all times, and a weekly record was made of changes in body weight and of the amount of food consumed.

TABLE I

Components and Percentage Composition of Various Diets Used in This Investigation

Components	Diet 349	Diet 353	Diet 355	Diet 356	Diet 357
Extracted casein	18	18	18	18	18
Salt Mixture 185*	4	4	4	4	4
Agar agar (U.S.P. grade)	2	2	2	2	2
Cod liver oil (Patch's Medicinal)	2	2	2	2	2
Butter fat (filtered)	3	3	3	3	3
Dextrinized corn-starch†	71	0	0	0	0
Sucrose (commercial)	0	71	0	0	0
Lactose (C.P. powder)	0	0	71	0	0
Glucose (technical)	0	0	0	71	0
Corn-starch (commercial)	0	0	0	0	71

* McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 33, 63 (1918).

† Made from commercial corn-starch by moistening the starch with a 0.1 per cent solution of citric acid, autoclaving for 4 hours at 15 pounds pressure, drying, and pulverizing.

The number of animals placed on each of the diets under consideration ranged from seventeen to forty-four animals. In the case of the diet containing dextrinized corn-starch (Diet 349), a total of forty-four animals was used. Twenty-four of these animals (Group 1) received this diet unsupplemented. This group of animals was put on this diet from 17 to 21 days before the remaining animals used in the investigation were placed on experiment. This was done in order that it might be possible to have their feces available as supplements to other vitamin B complex-deficient diets containing types of carbohydrate other than dextrinized corn-

starch. The feces from each of these
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in individual glass containers.

A second group of fifteen animals (
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The third group of animals (Group
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A total of twenty-nine animals wa
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as follows: Twelve animals (Group 4
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daily by all of their own feces, and si
this diet supplemented by 0.25 gm. da
animals of Group 1.

Seventeen animals received the die
the source of carbohydrate (Diet 35
received the diet without additional s
of the animals (Group 8) had their fec
while each of the five remaining anim
0.25 gm. of the feces voided by the a
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A total of twenty animals received
the source of carbohydrate (Diet 35
animals (Group 10) received no fur
second group, containing nine anima
feces returned to the cage daily.
animals (Group 12) received daily a

starch. The feces from each of these twenty-four animals were removed from the pans at weekly intervals, combined, dried, extracted with ether, and pulverized. Each weekly collection of feces was treated in a similar manner and added to the previous supply of pulverized feces. Whenever these pulverized feces were fed as dietary supplements, a daily quantity of 0.25 gm. was fed in individual glass containers.

A second group of fifteen animals (Group 2) was placed on the dextrinized corn-starch diet (Diet 349). Beginning with the 2nd day of the experimental period, the total feces voided by each of these animals were placed in separate containers and returned to the respective cages. This practice was continued daily throughout the entire experimental period.

The third group of animals (Group 3) which received this diet was given no additional supplement during the first 21 days of the experiment. On the 21st day all cages were cleaned thoroughly, and beginning with the 22nd day and continuing throughout the remainder of the experimental period, all the feces voided by each of these animals were returned to their respective cages.

A total of twenty-nine animals was fed the sucrose-containing diet (Diet 353). These animals were divided into three groups as follows: Twelve animals (Group 4) received the diet unsupplemented, eleven animals (Group 5) received this diet supplemented daily by all of their own feces, and six animals (Group 6) received this diet supplemented by 0.25 gm. daily of the feces voided by the animals of Group 1.

Seventeen animals received the diet in which lactose constituted the source of carbohydrate (Diet 355). Seven of these animals received the diet without additional supplement (Group 7). Five of the animals (Group 8) had their feces returned to the cage daily, while each of the five remaining animals (Group 9) received daily 0.25 gm. of the feces voided by the animals of Group 1, as a supplement to the diet.

A total of twenty animals received the diet containing glucose as the source of carbohydrate (Diet 356). A group of five of these animals (Group 10) received no further dietary supplement. A second group, containing nine animals (Group 11), had their own feces returned to the cage daily. Each of the remaining six animals (Group 12) received daily allotments of 0.25 gm. of pul-

verized feces which had been voided by those animals composing Group 1.

Eighteen animals received the diet in which commercial corn-starch constituted the source of carbohydrate (Diet 357). Six of these animals received this diet without further supplementation (Group 13). A second group of six animals (Group 14) had their own feces returned to the cage daily, while each of the remaining six animals (Group 15) received daily 0.25 gm. of the pulverized feces from the animals of Group 1, as a supplement to this diet.

A condensed outline of the plan of these studies, together with the corresponding growth responses, is given in Fig. 1.

DISCUSSION

On examining the data obtained in these studies (Fig. 1) it at once becomes apparent that those groups of animals which had access to feces, voided by other animals while receiving a diet in which dextrinized corn-starch constituted the source of carbohydrate, made a much more favorable growth response than did comparable groups of animals which had received the respective diets unsupplemented. This condition is found to be true in every case, regardless of the type of carbohydrate used in the diet. On the other hand, when groups of animals receiving each of the several diets had access to their own feces, marked beneficial effects toward growth were shown only by those animals which had received the diet containing dextrinized corn-starch (Curve 2). A slightly beneficial effect of coprophagy was also shown by those animals which received the diet containing lactose (Curve 8). The well being of those animals which received diets containing sucrose, glucose, or commercial corn-starch was only very slightly affected by having access to their own feces during the entire experimental period (Curves 5, 11, and 14). In fact, the growth responses made by these groups of animals did not differ greatly from the responses made by those groups of animals which received similar dietaries, but which did not have access to their feces (Curves 4, 10, and 13). When the animals composing the various groups were denied access to their own feces, their deportments were found to be relatively similar regardless of the type of carbohydrate used in the diet (Curves 1, 4, 7, 10, and 13), although less paralysis and lower mortality were observed among the animals which received the dextrin diet.

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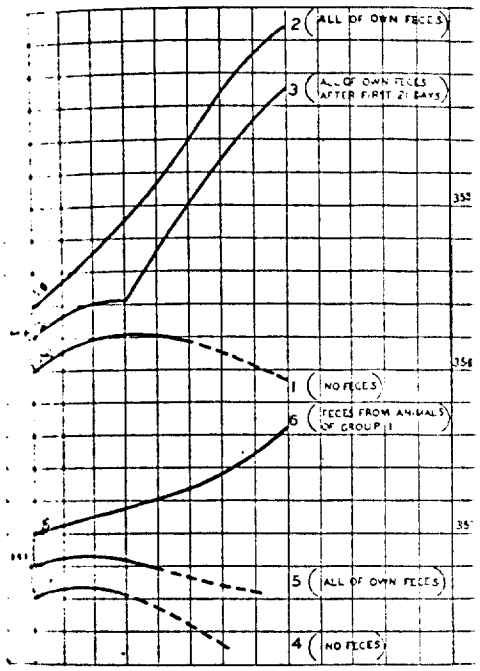

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From the above facts it appeared evident that dextrinized corn-starch was at least one of the dietary factors which determines the vitiating effects of coprophagy. Since no plausible explanation was known concerning the mechanism through which this dietary factor reacted in order to bring about this vitiating effect, some

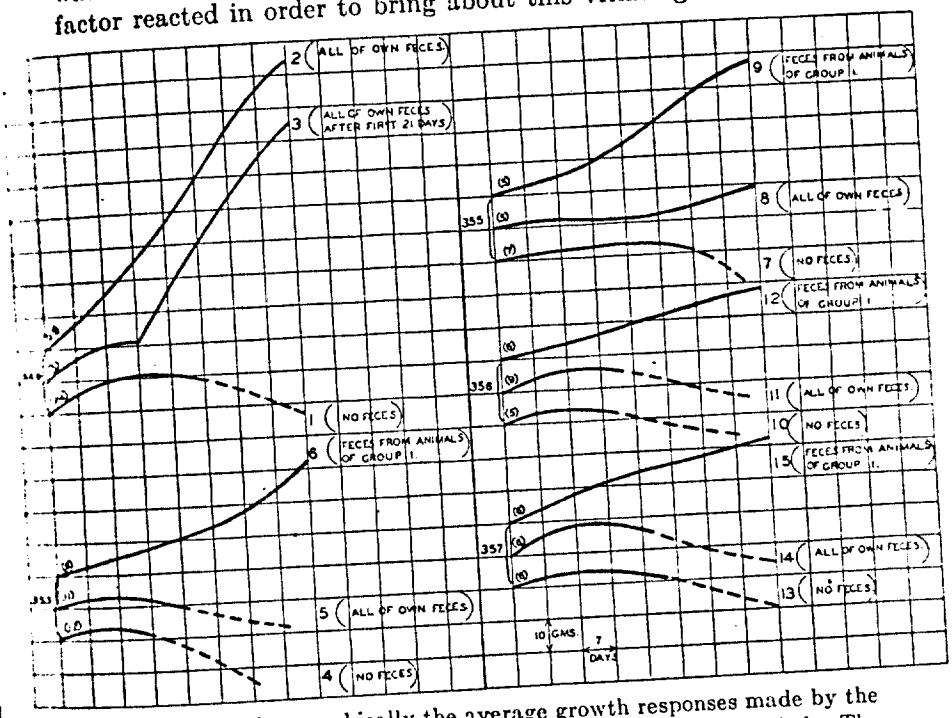

FIG. 1. Showing graphically the average growth responses made by the several groups of animals during the 8 week experimental period. The number preceding the growth curves designates the respective diets, the number in parentheses denotes the number of animals considered, and the numbers following the growth curves designate the respective animal groups. The break in the curves designates the initial death among the animals comprising the respective groups.

time was devoted in an attempt to throw some light on this subject. Any belief that the beneficial effects of the dextrinized starch was due to the retained vitamin or vitamins does not seem tenable when we are confronted with the fact that the untreated starch failed to show any such properties (Curve 14). Naturally, our thoughts turned toward an investigation of the intestinal flora of

experimental animals which were receiving some of the diets mentioned above. In our past experience with vitamin B complex-deficient diets containing dextrinized corn-starch, sucrose, and glucose as sources of carbohydrate, we have observed a very definite contrast in the nature of the feces voided by the animals given dextrin, compared to the feces voided by the animals receiving either the sucrose or the glucose diets. The feces voided by the animals given dextrin were invariably well formed, large bulky pellets, having a dark grayish color, while those voided by animals receiving the sucrose and the glucose diets were usually small black pellets of stringy or irregular form, and of such nature as to indicate a certain degree of constipation. It, therefore, seemed entirely possible that the beneficial effects of coprophagy among rats receiving diets containing dextrinized corn-starch might be the result of the action of certain microorganisms that inhabited the digestive tract of the animals when taken from the breeding colony and placed on experiment. And it also seemed that, if such was the case, the effects of coprophagy might become less pronounced as the experiment progressed. An examination of Curve 2 (Fig. 1) does not show this to be the case. In fact, when experimental animals, which received diets containing the dextrinized starch, were denied access to their feces for the first 21 days of the experimental period, and after this date were fed their own feces daily, the resultant growth responses were comparable to those produced by other animals on a similar regimen but having access to the feces from the beginning of the experiment (Curves 2 and 3). From this it appears that either the bacterial flora of the digestive tract of the rat is not readily altered by changes in the dietary habits of this animal, or else the bacterial flora in the digestive tract of the rat (when placed on experiment) is not an important consideration in this connection.

The bacteriological phase of the problem was attacked from other angles with the hope of ascertaining the etiological factor implicated. This study was conducted solely on experimental rats receiving either the dextrin- or the sucrose-containing diets, since such rats had shown the greatest discrepancies in the feces voided. The most suggestive part of this phase of the study presented itself when feces from groups of rats receiving these two diets (Diets 349 and 353) were suspended in saline solutions and streaked on

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eosin-methylene agar. The results, while not definitely conclusive, were sufficiently indicative to state that cultures made from the feces of rats fed on the dextrin diet resulted in an abundant growth of non-metallic type of colonies, whereas the cultures made from the feces of the rats receiving the sucrose diet gave invariably the typical metallic colonies, and these in relatively small numbers. Such findings, however, did not prove of definite significance.

Since attempts to feed cultures of the various isolations of the non-metallic colonies or organisms to vitamin B-deficient animals as dietary supplements did not meet with success, an attempt was made to locate the particular region of this synthetic activity within the digestive tract of the rat. In order to do this, twenty-three healthy young rats were maintained on the dextrin diet (Diet 349) for 4 weeks. They were then killed and the entire digestive tract removed and cut into eight proportional lengths. The unabsorbed material was removed from each of the corresponding sections separately, dried in a partial vacuum, and fed in proportional amounts to other animals as supplements to a vitamin B complex-deficient diet. The results obtained proved unusually interesting. Growth responses were made only by those vitamin B-deficient animals which received the unabsorbed material from the cecal and the colonic sections of the digestive tract. Material which had been taken aseptically from the cecum of dextrin-fed rats and cultured on a sterile medium (made up of 95 parts of nutrient broth and 5 parts of Diet 349) for 4 days at 37° gave a profuse growth of yeast, while material taken from the cecum of the sucrose-fed rats and treated in a similar manner gave only a mixed culture with a preponderance of the colon type of organism. When the same materials were cultured on a sterile medium containing 95 parts of nutrient broth and 5 parts of Diet 353, under similar conditions, little or no growth of yeast was observed from either culture. These results indicated that this phase of the investigation was a definite problem in itself; therefore, no efforts were made to carry these particular studies beyond this point.

As a result of the above findings we were led to believe that we had finally obtained an answer to the question, why is dextrin an unsatisfactory source of carbohydrate when incorporated into diets used in assaying for the B vitamins? Since the presence of

yeast in the digestive tract had been established, little remained to be assumed as to why coprophagy among experimental rats receiving diets containing dextrinized corn-starch had vitiating effects on vitamin B and vitamin G assays. Our attention had been previously attracted by the unusually large cecum of those animals which had received the dextrinized corn-starch diet compared to the size of the cecum of other animals that had received vitamin B complex-deficient diets which contained either sucrose or glucose as the source of carbohydrate. On autopsy examination of such animals, the cecum of the dextrin-

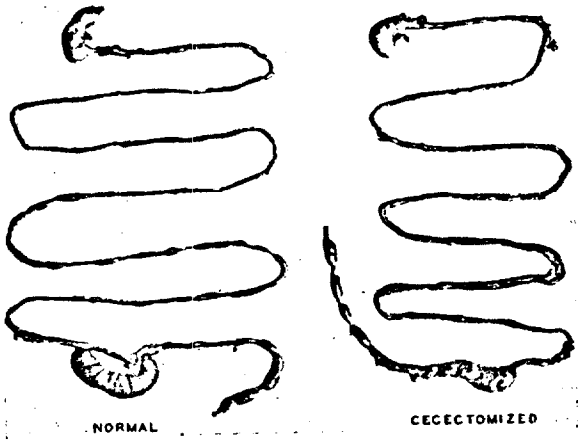

FIG. 2. Showing the digestive tract of the cecectomized rat as it appeared at the end of the experimental period, as well as that of a normal animal of comparable weight, which had been fed a diet containing dextrin as the sole source of carbohydrate.

fed rat was invariably found to be distended and filled with residual dietary material, while the cecum from the sucrose-fed rat or that of the glucose-fed rat was usually contracted and practically void of food material. Since previous tests had shown that the cecal contents of dextrin-fed rats was highly effective in supplementing a vitamin B-deficient diet, it seemed highly probable that the elaboration of the supplementary substances might take place in this organ. To determine if this was actually the case two healthy rats of about 60 days of age were anesthetized and the ceca removed (Fig. 2). Only one of the animals completely recovered from

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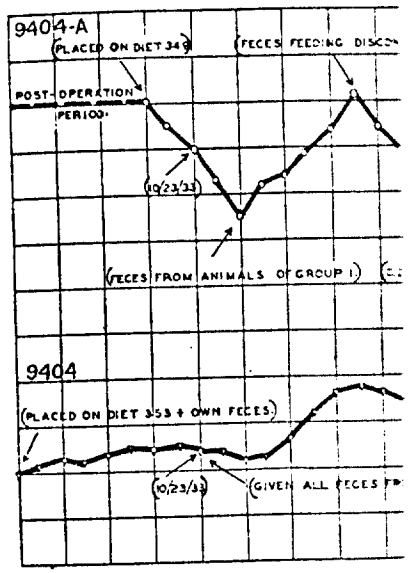

FIG. 3. Showing the responses n
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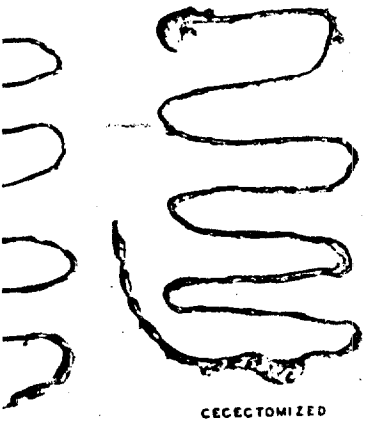

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the operation, the other having died several days after the operation, evidently as a result of intestinal obstruction. The animal that recovered was given a 6 week postoperative period, during the last 4 weeks of which it received our regular breeding colony diet. At the end of the 6 week postoperative period this animal was placed on the dextrinized corn-starch diet (Diet 349), and was maintained on this diet throughout the remainder of the experiment. During the first 28 days, the animal received this diet

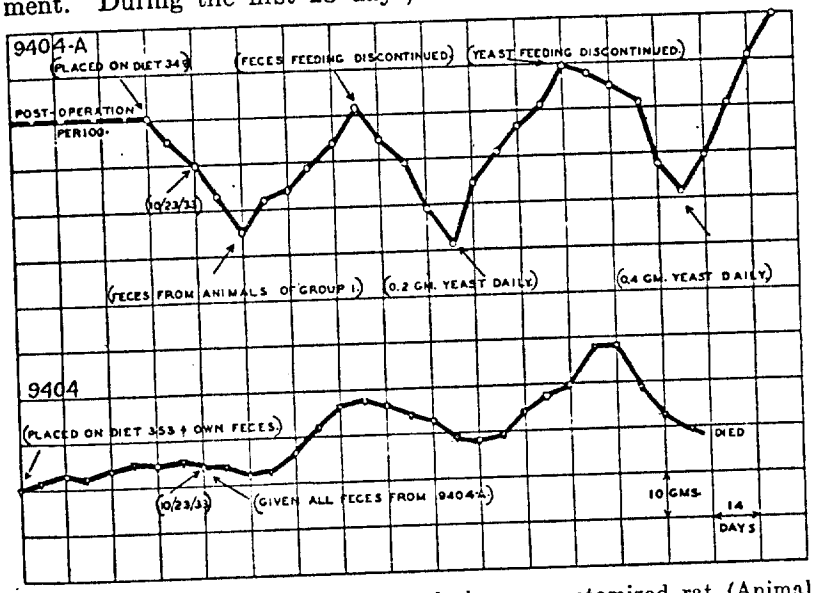

FIG. 3. Showing the responses made by a cecectomized rat (Animal 9404-A) receiving a vitamin B complex-deficient diet as a result of certain dietary supplements, as well as the responses made by another animal (No. 9404) which had access to all feces voided by the cecectomized animal.

without additional supplement, and, as the result, declined in weight to the extent of 24 gm. The animal was then fed a daily supplement of 0.4 gm. of pulverized feces which had been voided by the animals of Group 1. The feeding of feces as a dietary supplement was continued for 5 weeks, during which time the animal gained 26 gm. During the next 4 weeks the basal diet remained unsupplemented, and again the animal lost weight, this time to the extent of 31 gm. This was followed by a 5 week period, during which 0.2 gm. of dry bakers' yeast was fed daily as a dietary sup-

plement. During this yeast feeding period the body weight of the animal increased 38 gm. When this yeast feeding was discontinued, the animal declined in weight, with the resultant loss of 28 gm. during a 5 week period. But when the yeast supplement was again fed, this loss in weight was rapidly regained.

After the cecectomized animal had been on the dextrinized cornstarch diet for a 2 week period, the feces of this animal were collected daily and fed to another but younger animal which received the diet containing sucrose as the carbohydrate (Diet 358). Fig. 3 shows the various changes in regimen of these two animals and also gives the corresponding responses made as a result of such changes.

From an examination of the two growth curves given in Fig. 3, it is shown quite conclusively that Animal 9404 could not maintain growth while subsisting upon a vitamin B complex-deficient diet which was supplemented daily by the feces voided by Animal 9404-A, except when the latter animal was also receiving such dietary supplements. This would indicate that the feces from Animal 9404-A did not contain the required substances necessary to supplement a vitamin B complex-deficient diet, except when the diet of this animal was being supplemented by such dietary essentials. Such had not been the case when the animals of Group 6 had been fed the feces voided by the animals of Group 1 (Fig. 1). Therefore, it seems highly probable that the supplementary value of the feces voided by Animal 9404-A had been greatly reduced as the result of the removal of the cecum.

It is recognized that definite conclusions cannot be safely drawn from the observation on only one animal or a pair of animals. The data obtained in this connection are significant, however, in that they substantiate that which had been formerly obtained; namely, that it is in the cecum of the dextrin-fed rat that the unabsorbed food materials first become capable of supplementing vitamin B complex-deficient diets. Thus, it would appear that the cow is not unique in its ability to synthesize the B vitamins in its digestive tract (3). The cow does have the advantage over the rat in that the vitamins are synthesized higher up in the alimentary canal of the cow (rumen), thus enabling this animal to secure (on a ration deficient in the B vitamins) full benefits of the synthesized products without the necessity of reingestion of feces.

SUM

1. Data are presented which
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2. This peculiar property of
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3. This dietary elaboration a
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4. Since live yeast cells were
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SUMMARY

1. Data are presented which show that the vitiating effect of coprophagy on the assay of the B vitamins is much more serious when the experimental diet contains dextrinized corn-starch as a source of carbohydrate than when the diet contains a similar amount of either sucrose, lactose, glucose, or commercial corn-starch. Lactose, however, appears to occupy somewhat of an intermediate position between the dextrins and sucrose in this respect.

2. This peculiar property of the dextrinized corn-starch is not due to the retained B vitamins, but rather to the formation of these vitamins in the lower part of the digestive tract of the rat, as a result of the incomplete digestion of this particular form of carbohydrate.

3. This dietary elaboration apparently takes place in the cecum of dextrin-fed rats.

4. Since live yeast cells were found to exist in the cecum of such rats in enormous numbers, these microorganisms are believed to be the specific agents of elaboration of the B vitamins.

BIBLIOGRAPHY

1. Guerrant, N. B., and Dutcher, R. A., *J. Biol. Chem.*, **98**, 225 (1932).
2. Guerrant, N. B., and Dutcher, R. A., *Proc. Soc. Exp. Biol. and Med.*, **31**, 796 (1934).
3. Bechdel, S. I., Honeywell, H. E., Dutcher, R. A., and Knutsen, M. H., *J. Biol. Chem.*, **80**, 231 (1928).

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112

THE INFLUENCE OF DIETARY CARBOHYDRATE ON LEVELS OF AMINO ACIDS IN THE FECES OF THE WHITE RAT[1]

By Alfred E. Harper, Mitsuru C. Katayama, and Bohdan Jelinek

Abstract

The effect of various carbohydrates on the levels of amino acids excreted in the feces of rats has been studied. Potato starch, autoclaved potato starch, or dextrin each induced much greater levels of amino acids in the feces than either sucrose or glucose. The amino acids appear to be derived mainly from endogenous nitrogen. However, when rats were fed rations containing unmodified potato starch, undigested dietary protein apparently contributed a significant amount of the amino acids found in the feces. The inclusion of 10% lactose in a ration containing sucrose as the source of carbohydrate caused a statistically significant increase in the levels of lysine and histidine in the feces. The influence of lactose may be related to changes in the intestinal flora. The incorporation of ammonium salts into 9% casein diets had no significant effect on the amounts of amino acids in the feces.

Introduction

The nature of the diet has been shown to influence the quantity of amino acids excreted in the feces. Pearson and Panzer (11) found less valine, histidine, and methionine in the feces of rats which were fed corn oil in their diet than in the feces of rats which were fed diets free of fat. Ross (12) observed that breast-fed infants excreted more free amino acids in the feces than did infants receiving formulas. The present study demonstrated that the carbohydrate component of the diet may influence the quantity of certain essential amino acids excreted in the feces of rats.

Experimental

Male weanling rats of the Wistar strain, weighing between 40 and 50 gm., were housed in individual cages having elevated screen bottoms. Groups, consisting of five to six animals each, were fed the experimental rations for a period of either four or six weeks. The animals were weighed weekly and individual food consumption records were kept.

The 9% casein rations were fed ad libitum but the food consumption of rats fed the rations containing 18% casein was controlled by restricting the amount of ration offered. Equal quantities of food were offered during the first few days, but when the average food intake reached 10 gm. per day, the ration thereafter offered was restricted to this amount. This procedure was followed in order to ensure that the nitrogen intake of the various groups receiving 18% casein would be similar.

During preliminary experiments it had been observed that the food intake of rats receiving, ad libitum, rations containing 18% casein with either sucrose or corn starch as the source of carbohydrate, gradually increased. The male

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animals grew at a rate of 40 to 44 gm. per week, which is considered to be excellent growth. When, on the other hand, the ration contained potato starch, food intake did not increase much above 10 gm. per day and normal growth could not be obtained.

The percentage composition of the basal ration was as follows: casein 9, carbohydrate 82.6, salt mixture* 4, corn oil 4, inositol 0.1, choline chloride 0.2, whole liver powder (Wilson's) 0.1. Vitamins were added so as to provide, per kilogram of ration: thiamine 5 mgm., riboflavin 10 mgm., pyridoxine 5 mgm., nicotinic acid 5 mgm., calcium pantothenate 25 mgm., *p*-aminobenzoic acid 300 mgm., α-tocopherol 25 mgm., menadione 2 mgm., folic acid 2 mgm., biotin 200 μgm., liver oil to provide 60,000 I. U. vitamin A and 12,000 I. U. vitamin D. The various rations fed are indicated in Table I. All supplements were included in the basal diet at the expense of the carbohydrate.

Feces excreted during the first three days of the experiment were discarded. Thereafter, the feces from each rat were collected at least twice weekly from absorbent paper that had been placed beneath the cage. The feces were screened from food particles and hair, and were then air-dried at room temperature, weighed, and ground for the analyses.

The proteins in the feces were hydrolyzed by autoclaving 1 gm. of feces in 20 ml. of 5 *N* hydrochloric acid for 10 hr. at 120° C. The amino acids were determined by the microbiological procedure of Henderson and Snell (3). *Leuconostoc mesenteroides* P-60 was used as the test organism for lysine determinations, *Lactobacillus arabinosus* for valine, and *Streptococcus faecalis* for methionine and histidine. A Cannon titrator (2) with a quinhydrone electrode was used throughout for titrations.

The influence of various carbohydrates on the digestibility of casein was studied. The animals were housed in cages with screen bottoms of $\frac{1}{4}$ in. mesh. The cages were supported by metal funnels. To collect the feces, a fine mesh screen was placed a few inches above the inverted apex of each funnel. The feces were removed every second day and were air-dried for 24 hr. The nitrogen in each sample was determined by the micro-Kjeldahl method (1). The techniques followed were similar to those used by Mitchell (7). The endogenous nitrogen loss for each group, which consisted of three young male rats, was determined by placing the rats on a ration containing 4% egg albumen as the sole source of protein. Each group was then fed a test ration containing 9% casein. In changing from one ration to another, at least three days were allowed to elapse before the feces were again collected and analyzed. The low nitrogen diet had the same general composition as the basal ration except that 4% egg albumen was substituted for 9% casein and the difference was made up by increasing the amount of carbohydrate.

According to Mitchell (7) the nitrogen excreted during the feeding of a low protein or nitrogen-free ration is a measure of the loss of endogenous nitrogen

* *Salt mixture 351 (4) with the following changes: NaF was omitted; $MnSO_4 . 4H_2O$ was increased from 0.35 gm. to 15.2 gm.; 60 gm. $FeSO_4$ was substituted for 20.5 gm. of $FePO_4 . 4H_2O$; 800 gm. of Na_2HPO_4 and 0.52 gm. of $ZnCl_2$ were added.*

which is derived from the intestinal tract and its secretions. The value, calculated as fecal nitrogen excreted per gram of food consumed, is used as a correction factor in the calculation of the "true" digestibility of the protein in an experimental ration. The true digestibility is then calculated according to the formula:

$$\text{True digestibility} = \frac{\text{N intake} - (\text{fecal N} - \text{endogenous fecal N}) \times 100}{\text{N intake}}.$$

Results and Discussion

The growth rates of the rats fed the various rations are reported in Table I. There were no marked growth differences among groups which were fed sucrose, glucose, dextrin, or autoclaved potato starch as the source of carbohydrate. However, when unmodified potato starch was used as the only source of carbohydrate in the diet, the growth rate of the rats was low. The rats on this ration excreted white, bulky feces which were found by microscopic examination to consist largely of undigested starch granules which gave a blue color with iodine. Also, the digestion of dietary protein was shown to be impaired when unmodified potato starch was used as the sole source of carbohydrate.

TABLE I

GROWTH RESPONSE OF RATS FED DIETS CONTAINING VARIOUS CARBOHYDRATES

Expt. No.	Ration No.	% Casein	Ration	Mean weekly gain in weight, gm.	Mean daily food intake, gm.
I	1	18	Potato starch	13.5	9.8
	2		Sucrose	21.9	9.7
	3		Autoclaved potato starch*	22.2	9.9
	4		Glucose	22.2	9.7
	5		Dextrin**	20.3	9.8
II	6	18	Potato starch	8.8	8.9
	7		Potato starch and 20% lactose	17.5	9.6
	8		Sucrose	23.5	9.6
	9		Sucrose and 10% lactose	22.1	9.6
III	10	9	Potato starch and sucrose	13.6	9.5
	11		Potato starch and sucrose + 0.619% N***	12.4	9.0
	12		Autoclaved corn starch*	13.8	8.4
	13		Autoclaved corn starch* + 0.619 N***	8.8	6.8
	14		Corn starch	10.8	8.2
	15		Corn starch + 0.619% N***	9.1	7.7

* *Autoclaved starches were prepared by moistening the starch with water and autoclaving at 120° C. for two hours. The product was dried in the air at room temperature and then ground in a Wiley mill.*

** *Dextrin was prepared by baking thin layers of potato starch in an oven at 145° C. for eight hours with frequent stirring. The product gave a purple coloration on addition of iodine.*

*** *Provided as either ammonium citrate or ammonium bicarbonate.*

TABLE II

EFFECT OF CARBOHYDRATES ON THE DIGESTIBILITY OF CASEIN

Ration	Rat No.	Initial weight, gm.	N intake, mgm.	Fecal N, mgm.	Endogenous fecal N, mgm.	True digestibility, %
Potato starch	4	83	100	40	25	85
	5	84	126	49	29	84
	6	74	94	33	12	78
Autoclaved potato starch	7	80	78	20	14	92
	8	92	101	24	19	95
	9	74	75	17	13	95
Sucrose	10	69	56	6	5	98
	11	95	99	8	11	100
	12	66	81	4	6	100

Apparently the lower rate of growth of rats fed the unmodified potato starch ration was due to the poor utilization of unmodified potato starch and to the coincident decrease in the extent of digestion of the dietary protein.

The inclusion of lactose at a level of 20% in the unmodified potato starch ration improved the growth rate slightly, whereas the incorporation of 10% lactose into the sucrose ration did not cause any observable change in the growth of rats. Mitchell (9), using diets containing various levels of lactose combined with corn starch, found that the growth of rats was practically normal on diets containing 30% or less of lactose. Similarly Outhouse, Smith, Merritt, and White (10) observed that there was no alteration in growth when 25% sucrose or lactose replaced an equivalent amount of corn starch in the basal ration. The beneficial effect obtained when unmodified potato starch was partially replaced by lactose may have been due to the utilization of lactose per se by the rat.

The rate of gain of rats was not improved by supplementing 9% casein rations with ammonium salts. Similar results were obtained by Lardy and Feldott (6) when they incorporated ammonium citrate into rations containing suboptimal amounts of fibrin or soybean oil meal.

The values presented in Table III indicate the effects of various carbohydrates on the progressive weekly excretion of lysine, valine, histidine, and methionine in the feces. Potato starch, autoclaved potato starch, and dextrin each induced much greater excretion of amino acids in the feces than either sucrose or glucose. Mitchell, Hamilton, and Beadles (8) found that sucrose, fructose, and lactose caused greater losses of organic nutrients in the feces than did glucose. They concluded that carbohydrates may have differential effects on the digestibility of the organic nutrients contained in the rations. In the study reported here, a significant loss of dietary protein was induced by raw potato starch, whereas dietary protein was almost completely digested and absorbed when either autoclaved potato starch or sucrose

TABLE III

EFFECT OF VARIOUS CARBOHYDRATES ON THE WEEKLY LEVELS OF AMINO ACIDS IN THE FECES

The amino acid levels are expressed as mgm. per week (mean values ± standard error of the mean)

No.	Ration	Week of expt.	Lysine	Valine	Histidine	Methionine
1	Potato starch	1	48 ± 9.0	50 ± 3.3	18 ± 2.1	17 ± 1.7
		2	79 ± 9.1	71 ± 7.0	28 ± 2.9	22 ± 2.4
		3	98 ± 6.2	101 ± 9.8	37 ± 3.0	33 ± 2.0
		4	100 ± 11.5	100 ± 8.2	35 ± 2.7	31 ± 2.6
		5	112 ± 10.5	103 ± 9.6	35 ± 2.5	39 ± 2.2
2	Sucrose	1	14 ± 1.2	18 ± 1.5	5.0 ± 0.45	5.3 ± 0.45
		2	14 ± 1.0	17 ± 1.0	5.1 ± 0.71	5.5 ± 0.39
		3	21 ± 1.3	25 ± 1.7	5.9 ± 0.77	8.5 ± 0.45
		4	23 ± 1.8	27 ± 1.6	6.3 ± 0.74	9.1 ± 0.77
		5	26 ± 1.8	29 ± 1.4	6.8 ± 0.55	8.0 ± 0.55
3	Autoclaved potato starch	1	48 ± 3.3	52 ± 3.0	13 ± 0.9	14 ± 0.7
		2	67 ± 4.0	73 ± 6.5	19 ± 1.3	19 ± 1.9
		3	71 ± 6.0	83 ± 6.1	21 ± 1.2	24 ± 1.5
		4	66 ± 8.1	76 ± 8.7	21 ± 1.6	21 ± 2.5
		5	80 ± 9.3	81 ± 9.1	30 ± 2.8	24 ± 2.1
4	Glucose	1	17 ± 2.3	32 ± 4.3	12 ± 1.9	7.3 ± 1.32
		2	14 ± 3.4	26 ± 4.2	10 ± 1.4	6.3 ± 1.10
		3	34 ± 2.0	49 ± 2.8	15 ± 1.8	10.8 ± 1.26
		4	20 ± 2.6	27 ± 2.9	9 ± 1.1	6.6 ± 0.92
		5	27 ± 2.4	32 ± 2.2	11 ± 1.0	9.1 ± 0.90
5	Dextrin	1	75 ± 5.4	64 ± 3.4	31 ± 2.8	19 ± 1.3
		2	73 ± 13.9	60 ± 11.6	30 ± 6.4	18 ± 2.6
		3	97 ± 12.0	83 ± 10.8	38 ± 4.6	31 ± 4.0
		4	75 ± 10.2	73 ± 9.9	31 ± 4.0	24 ± 3.4
		5	103 ± 12.5	95 ± 9.2	43 ± 4.5	35 ± 3.5

was used as the source of carbohydrate (Table II). The extent to which dietary protein contributed amino acids in the feces was apparently modified by the type of carbohydrate fed.

The levels of amino acids excreted in the feces were generally lower during the first week of the experiment than during the following weeks. During the first week the quantity of food consumed by the rats was least. Since Mitchell (7) has shown that the endogenous nitrogen excreted in the feces is proportional to the food intake, the smaller quantity of amino acids from endogenous sources could account, in part at least, for the lower levels of amino acids found in the feces during the first week.

That the intestinal secretions and the intestinal microorganisms probably contribute the major portion of the amino acids found in the feces is supported by the data in Table II, which show that endogenous nitrogen makes up a large portion of the nitrogen excreted in the feces. Also, unmodified potato starch and autoclaved potato starch, which induced relatively large excretions of endogenous nitrogen, were found to induce the highest levels of amino acids

in the feces. It thus appears reasonable to conclude that the type of carbohydrate ingested by the rat may influence the amounts of amino acids from endogenous sources which are found in the feces.

The effect of lactose on the fecal excretion of lysine, valine, and histidine is shown in Table IV. The inclusion of 20% of lactose in the potato starch ration apparently did not influence the amino acid content of the feces; however, the inclusion of 10% of lactose in the sucrose ration caused an increase in the amino acid content of the feces. The t test (5) was applied and the increases in the lysine and histidine were found to be statistically significant ($t = 3.73, P < 0.01$, and $t = 2.80, P \leq 0.02$ respectively). The increase in the valine value was not statistically significant ($t = 1.64$, $P > 0.1$). Ross (12, 13) has shown that breast-fed infants excrete greater quantities of free amino acids in the feces than do infants receiving prepared diets. The high lactose content of human milk was mentioned as possibly influencing the amount of free amino acids excreted in the feces. Ross also

TABLE IV

EFFECT OF LACTOSE ON THE LEVELS OF AMINO ACIDS IN THE FECES

The amino acid levels are expressed as mgm. per week
(mean values ± standard error of the mean)

No.	Ration	Lysine	Valine	Histidine
6	Potato starch	67 ± 6.2	88 ± 7.0	38 ± 2.4
7	Potato starch and lactose	73 ± 4.9	88 ± 3.9	41 ± 2.9
8	Sucrose	21 ± 0.9	26 ± 1.3	7.7 ± 0.22
9	Sucrose and lactose	29 ± 2.1	31 ± 2.4	9.0 ± 0.45

found that in the feces of the breast-fed infants the predominant organism was *Lactobacillus bifidus* which comprised almost the whole flora; whereas with the infants on artificial diets the fecal organisms were mixed, and *L. bifidus* was either scanty or absent. Mitchell (9) showed that there was an increase of *Bacillus acidophilus* in the intestinal flora of the rat when 15% of lactose was incorporated into the diet. Thus it seems possible that an alteration of the intestinal flora may be associated with the increased levels of amino acids found in the feces of rats receiving supplementary lactose in the sucrose ration. The slow rate of removal of lactose from the intestinal tract may permit the growth of a greater and more varied flora. However, the laxative effect of lactose, which may cause greater losses of ingested protein, cannot be entirely disregarded.

Since the food intake influences the amount of endogenous nitrogen excreted, the amounts of amino acids excreted per gram of protein ingested are reported

in Table V for the groups receiving supplementary nonprotein nitrogen. On this basis, there was a tendency for an excretion of slightly higher levels of amino acids in the feces when ammonium salts were included in the rations containing either unmodified potato starch or autoclaved corn starch. However, this increase was not found to be statistically significant.

TABLE V

EFFECT OF AMMONIUM SALTS ON THE LEVELS OF AMINO ACIDS IN THE FECES

The results are expressed as mgm. of fecal amino acid per gram of protein ingested (mean values ± standard error of the mean)

No.	Ration	Lysine	Valine	Histidine	Methionine
10	Potato starch	13.7 ± 0.89	13.4 ± 0.63	6.8 ± 0.32	3.0 ± 0.84
11	Potato starch and N	16.1 ± 1.96	15.5 ± 1.41	7.4 ± 0.59	3.9 ± 0.54
12	Autoclaved corn starch	11.8 ± 0.97	12.2 ± 0.67	4.3 ± 0.22	3.5 ± 0.32
13	Autoclaved corn starch and N	13.5 ± 1.08	13.3 ± 0.95	4.8 ± 0.36	3.8 ± 0.28
14	Corn starch	4.8 ± 0.45	5.3 ± 0.40	2.0 ± 0.24	1.3 ± 0.04
15	Corn starch and N	3.7 ± 0.40	4.3 ± 0.23	2.0 ± 0.16	1.1 ± 0.07

References

1. CLARK, E. P. J. Assoc. Offic. Agr. Chemists, 24 : 641. 1941.
2. HENDERSON, L. M., BRICKSON, W. L., and SNELL, E. E. J. Biol. Chem. 172 : 31. 1948.
3. HENDERSON, L. M. and SNELL, E. E. J. Biol. Chem. 172 : 15. 1948.
4. HUBBELL, R. B., MENDEL, L. B., and WAKEMAN, A. J. J. Nutrition, 14 : 273. 1937.
5. JOHNSON, L. P. V. An introduction to applied biometrics. Burgess Publishing Co., Minneapolis. 1950.
6. LARDY, H. A. and FELDOTT, G. J. Biol. Chem. 186 : 85. 1950.
7. MITCHELL, H. H. J. Biol. Chem. 58 : 873. 1923.
8. MITCHELL, H. H., HAMILTON, T. S., and BEADLES, J. R. J. Nutrition, 14 : 435. 1937.
9. MITCHELL, H. S. Am. J. Physiol. 79 : 542. 1927.
10. OUTHOUSE, J., SMITH, J., MERRITT, L., and WHITE, F. R. J. Nutrition, 14 : 579. 1937.
11. PEARSON, P. B. and PANZER, F. J. Nutrition, 38 : 257. 1949.
12. ROSS, C. A. C. Lancet, 258 : 716. 1950.
13. ROSS, C. A. C. Lancet, 261 : 190. 1951.

Jr. Nutr, 51, 1953

113

INFLUENCE OF VARIOUS CARBOHYDRATES ON THE UTILIZATION OF LOW PROTEIN RATIONS BY THE WHITE RAT

II. COMPARISON OF SEVERAL PROTEINS AND CARBOHYDRATES. GROWTH AND LIVER FAT [1]

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In a previous paper, Harper and Katayama ('53) reported that 9% casein diets support better growth of rats when sucrose is replaced, as the source of dietary carbohydrate, by either cornstarch or dextrin. An effect of the type of dietary carbohydrate on liver fat deposition in rats fed low-protein diets has also been reported, and it has been shown that the accumulation of fat in the livers of rats which are fed a 9% casein-sucrose diet containing choline is reduced when sucrose is replaced by dextrin (Litwack, Hankes and Elvehjem, '52; Harper, Monson, Benton and Elvehjem, '53b). Apparently dextrin acts in both instances by permitting more efficient utilization of the protein in these low-protein diets.

Since Litwack, Williams, Chen and Elvehjem ('52) have shown that the xanthine oxidase activity of rat liver is positively correlated with the amount of protein in the diet, and since the growth and liver fat studies cited above indicate that dextrin acts by improving protein utilization, replacement of

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sucrose by dextrin in low-protein diets might be expected to cause an increase in liver xanthine oxidase activity.

In the present paper experimental evidence is presented which extends previous reports on the influence of dietary carbohydrates on both growth and liver fat deposition, and an experiment on the effect of dietary carbohydrate on liver xanthine oxidase activity is included.

EXPERIMENTAL

Male weanling rats of the Sprague-Dawley strain weighing from 40 to 50 gm were used throughout these studies. They were divided into similar groups of 6 animals each and were maintained in individual cages with raised screen bottoms. Unless otherwise noted, the animals were fed ad libitum and each animal was weighed weekly during the experimental periods of from two to 12 weeks.

The composition of the basal diet was as follows: sucrose, 81.6; casein, 9.0; DL-methionine, 0.3; corn oil, 5.0; salts 4 (Hegsted, Mills, Elvehjem and Hart, '41), 4.0; and choline chloride, 0.13%. Vitamins were included to provide, in milligrams per 100 gm of ration, thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 1.0; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12}, 0.002; and inositol, 10.0. Two drops of halibut liver oil, fortified to provide vitamin A, 1,000 I.U.; vitamin D, 10 I.U.; 2-methyl,1-4 naphthoquinone, 0.04 mg and α-tocopherol, 0.8 mg, were administered weekly.

Supplements, including increases in protein, were included in the diet at the expense of carbohydrate. The carbohydrate component of each diet is listed in the tables, which present our experimental results. In experiment 5, which was of only two weeks' duration, 0.1% DL-tryptophan was included in order to insure more uniform growth. Casein was omitted from the diets in experiments in which other proteins were tested, except for experiment 6, in which 0.23% casein was used as a carrier for vitamins. The composition of the amino acid

mixture used in experiment 6 is listed in table 1 and was calculated to be equivalent to 9% casein (12.4% N). The analysis presented by Block and Bolling ('51) was used as the basis for the calculations, except that the amount of L-proline, which was non-essential and costly, was reduced.

The various proteins and carbohydrates, except dextrin, were commercial products. The casein was extracted three times with boiling alcohol. The term "dextrin" used throughout this paper refers to the product obtained by heating moist cornstarch in an autoclave at 15 pounds' pressure for three hours. This material gave a blue color with iodine. Dextrin A was prepared by heating dry cornstarch in an oven at 145°C. for 10 hours. Dextrin M was a commercial product. The last two gave red-violet colors with iodine, indicating the presence of shorter chain molecules.

TABLE 1

Composition of amino acid mixture equivalent to 9% casein

AMINO ACID	% IN DIET	COMMENTS
L-arginine·HCl	0.39	
L-histidine·HCl·H_2O	0.33	
L-lysine·HCl	0.87	
L-tyrosine	0.49	
DL-tryptophan	0.12	D = 3/4 L for rats (Oesterling and Rose, '52)
DL-phenylalanine	0.48	D = L
L-cystine	0.03	
DL-methionine	0.27	D = L
DL-threonine	0.68	D not utilized
DL-serine	0.52	
DL-leucine	1.52	D not utilized
DL-isoleucine	1.09	D not utilized
DL-valine	1.16	D not utilized
L-glutamic acid	1.75	
DL-aspartic acid	0.53	
Glycine	0.16	
DL-alanine	0.25	
L-proline	0.30	

The paired-feeding experiment was conducted under exactly the same physical conditions as the ad libitum trials.

The rats in the sucrose group were allowed to eat ad libitum and each of those fed dextrin was given, every second day, an amount of food equivalent to that consumed by its control in the sucrose group. Spillage was carefully measured and corrected for at each feeding.

At the termination of the experimental period each rat was killed by a blow on the head, was decapitated and bled, and the liver was excised for the determination of fat. Fat was determined by ether extraction of the dried and ground liver (Hawk and Elvehjem, '53).

For xanthine oxidase determinations, the animals were killed as described above. Each liver, immediately after removal, was chilled in ice and blotted free of moisture. A weighed portion of each liver was homogenized with 5 volumes of ice-cold 0.039 M sodium potassium phosphate buffer (pH 7.3). The xanthine oxidase activity of the homogenates was determined by the method of Axelrod and Elvehjem ('41).

Nitrogen was determined by the Kjeldahl method. Mercuric oxide was used as the catalyst.

Histological studies of the livers of animals from experiment 5 will be reported separately.

RESULTS

Each experiment is reported individually because the absolute values for similar groups vary from experiment to experiment even though the relative values are consistent. A part of the variation observed can probably be accounted for by variations in the nitrogen content of different lots of casein, and by variations in the ages of the animals at the end of the different experiments. Two control groups, one fed the sucrose and the other the dextrin basal diet, were included in each experiment to permit accurate comparisons.

Carbohydrates

The results of experiments designed to determine the effect of various carbohydrates on growth and liver fat deposition in rats fed low-casein diets are presented in table 2. In every

Table 1

TABLE 2

Effect of various carbohydrates on growth and liver fat of rats fed low casein diets[1]

EXP. NO.	GROUP NO.	TYPE OF DIET	RATE OF GAIN	LIVER FAT	
				dry weight	wet weight
			gm/wk.	%	%
1	1	9% casein-sucrose	15.5 ± 1.7[2]	25.9 ± 2.1[2]	8.4 ± 0.8[2]
	2	9% casein-dextrin	34.4 ± 0.4	19.2 ± 1.5	6.0 ± 0.5
	3	9% casein-sucrose + 10% lactose	20.9 ± 0.7	24.4 ± 1.6	7.6 ± 0.6
2	4	9% casein-sucrose	11.3 ± 1.6	23.2 ± 1.8	7.3 ± 0.7
	5	11.2% casein-sucrose	22.4 ± 2.0	13.8 ± 0.9	4.1 ± 0.3
	6	13% casein-sucrose	31.1 ± 2.4	9.8 ± 0.5	2.8 ± 0.2
	7	9% casein-dextrin	31.9 ± 1.5	22.6 ± 2.0	7.0 ± 0.7
	8	11% casein-dextrin	39.7 ± 0.7	8.9 ± 1.1	2.8 ± 0.3
	9	9% casein-cerelose	23.1 ± 1.0	15.9 ± 0.4	4.5 ± 0.1
	10	9% casein-sucrose + 10% lactose	15.7 ± 0.9	26.1 ± 2.8	8.5 ± 1.1
	11	9% casein-sucrose 5% eq. gum acacia, cellulose	18.6 ± 1.2	20.3 ± 1.9	6.3 ± 0.7
3	12	9% casein-sucrose	15.0 ± 1.2	32.9 ± 2.3	11.1 ± 1.0
	13	9% casein-sucrose:dextrin 3:1	19.2 ± 1.9	36.0 ± 2.9	12.9 ± 1.3
	14	9% casein-sucrose:dextrin 1:1	24.6 ± 1.4	32.8 ± 4.5	11.4 ± 2.2
	15	9% casein-sucrose:dextrin 1:3	27.7 ± 2.3	30.0 ± 3.3	10.1 ± 1.5
	16	9% casein-dextrin	31.4 ± 2.0	...	...
	17	9% casein-dextrin A[3]	25.2 ± 2.1	...	...
	18	9% casein-dextrin M[3]	25.9 ± 1.0	18.0 ± 1.8	5.3 ± 0.5
	19	9% casein-glucose	17.8 ± 0.8	19.3 ± 0.6	6.0 ± 0.2
4	20	9% casein-sucrose	17.8 ± 1.3	26.8 ± 1.9	8.7 ± 0.8
	21	9% casein-dextrin	34.3 ± 1.8	16.3 ± 1.0	4.8 ± 0.3
	22	9% casein-dextrin:cerelose 3:1	34.4 ± 1.7	17.6 ± 0.5	5.1 ± 0.1
	23	9% casein-dextrin:levulose 3:1	32.6 ± 2.1	22.4 ± 2.7	7.1 ± 1.0
	24	9% casein-dextrin:sucrose 3:1	31.6 ± 1.5	19.1 ± 1.2	5.8 ± 0.4
	25	9% casein-cerelose	22.9 ± 0.9	18.7 ± 1.1	5.6 ± 0.3
	26	9% casein-cerelose:levulose 3:1	26.2 ± 1.5	22.2 ± 2.6	7.0 ± 1.0
	27	9% casein-cerelose:dextrin 3:1	27.8 ± 0.7	17.2 ± 2.1	5.1 ± 0.7
	28	9% casein-cerelose:sucrose 3:1	23.7 ± 1.1	27.1 ± 2.5	8.3 ± 1.0
	29	9% casein-cerelose:sucrose 1:1	21.2 ± 1.6	27.3 ± 2.4	8.2 ± 0.9
5	30	9% casein-sucrose	19.7 ± 1.9	34.1 ± 2.9	10.8 ± 1.2
	31	9% casein-dextrin	36.2 ± 0.6	15.4 ± 1.2	4.1 ± 0.4
	32	9% casein-cerelose	28.0 ± 1.3	15.0 ± 0.4	4.1 ± 0.2
	33	9% casein-levulose	13.8 ± 1.2	29.6 ± 2.6	9.5 ± 1.0

[1] Experiment 1 was of 5 weeks' duration; 2 and 3, 4 weeks'; 4, three weeks' and 5, two weeks'.

[2] Mean ± standard error of the mean for 6 rats.

[3] See text.

experiment with 9% casein diets the groups fed dextrin grew at between double and triple the rate of those fed sucrose (groups 1 and 2, 4 and 7, 12 and 16, 20 and 21, 30 and 31). In experiment 2, among the sucrose groups, only group 6, which received 13% casein, grew as well as the 9% casein-dextrin control (group 7). When only 25% of the carbohydrate was supplied as sucrose (group 15), the rate of growth was reduced slightly below that of the dextrin group. Dextrins A and M, the shorter chain products, did not support quite as good growth (groups 17 and 18) as the dextrin used routinely.

When cerelose (glucose monohydrate) was used as the carbohydrate (groups 9, 25, 32), it supported a rate of growth intermediate between that observed with sucrose and that observed with dextrin. The inclusion of 10% lactose (groups 3 and 10) or 10% bulk (group 11) in the basal ration increased growth slightly. The use of fructose to supply 25% of the carbohydrate with dextrin or cerelose (groups 23 and 26) had little effect on growth but when it was fed as the sole source of carbohydrate growth was slightly depressed (experiment 5).

In every experiment liver fat deposition [2] was highest (25 to 39%) in groups which were fed the sucrose basal diet (groups 1, 4, 12, 20, 30) and in every case except one (group 7) least fat (16 to 22%) accumulated in the livers of animals fed dextrin. Even in the experiment in which the exception occurred with 9% casein, a difference between the sucrose and the dextrin groups was observed with 11% casein. Liver fat depositions in the glucose and the dextrin groups were usually similar. Fructose, like sucrose, induced greater liver fat deposition. In experiment 4, in which only 25% of the carbohydrate was supplied as fructose (groups 23 and 26), liver fat was slightly elevated over that of the comparable groups which received no fructose. In experiment 5, when all of the carbohydrate was provided as fructose, liver fat deposition was as high as that of the sucrose group.

[2] All liver fat values cited in the text are based on the dry weight of liver.

TABLE 3

Growth and liver fat of rats receiving sucrose or dextrin in various low-protein diets[1]

EXP. NO.	GROUP NO.	TYPE OF DIET	RATE OF GAIN	LIVER FAT	
			gm/wk.	%	%
6	34	9% casein-sucrose	19.1 ± 1.8[2]	26.8 ± 3.0[2]	8.4 ± 1.1[2]
	35	9% casein-dextrin	36.5 ± 1.4	18.2 ± 1.4	5.3 ± 0.5
	36	Amino acids = 9% casein-sucrose[3]	20.6 ± 0.8	23.4 ± 1.0	7.2 ± 0.3
	37	Amino acids = 9% casein-dextrin[3]	29.6 ± 1.6	18.7 ± 1.8	5.4 ± 0.5
	38	15% wheat gluten + 0.7% lysine-sucrose	19.0 ± 2.1	18.2 ± 1.3	5.5 ± 0.4
	39	15% wheat gluten + 0.7% lysine-dextrin	35.5 ± 2.2	15.8 ± 1.1	4.4 ± 0.3
	40	9% egg albumin-sucrose	13.7 ± 1.2	23.9 ± 2.5	7.4 ± 1.0
	41	9% egg albumin-dextrin	24.0 ± 1.4	26.6 ± 4.3	8.5 ± 1.7
7	42	9% casein-sucrose	15.0 ± 1.2	39.9 ± 3.9	14.7 ± 2.7
	43	9% casein-dextrin	31.3 ± 3.0	24.9 ± 2.6	7.8 ± 0.9
	44	9% casein + 6% gelatin-sucrose	17.5 ± 2.0	16.3 ± 2.2	4.8 ± 0.7
	45	9% casein + 6% zein-sucrose	22.8 ± 1.5	27.3 ± 2.3	8.8 ± 0.9
	46	9% egg albumin-sucrose	13.8 ± 0.7	28.7 ± 2.2	9.2 ± 0.9
	47	9% egg albumin + 0.5% lysine-sucrose	11.9 ± 1.3	33.7 ± 2.5	11.8 ± 1.1
	48	9% fibrin-sucrose	14.3 ± 0.8	16.1 ± 1.0	4.9 ± 0.3
	49	9% fibrin-dextrin	32.2 ± 3.2	17.4 ± 1.0	5.2 ± 0.5

[1] Experiment 6 was of 4 weeks' duration; 7, three weeks'.
[2] Mean ± standard error of the mean for 6 rats.
[3] See table 1.

Proteins

The results of experiments designed to compare the effects of sucrose and dextrin on the growth and liver fat deposition of rats fed some other proteins are presented in table 3. Growth differences between groups of rats fed sucrose and those fed dextrin were observed with three other proteins: wheat gluten supplemented with lysine (groups 38 and 39), egg albumin (groups 40 and 41) and fibrin (groups 48 and 49). A growth difference was also observed when 9% casein was replaced by its equivalent in amino acids (groups 36 and 37). In each instance dextrin supported much better growth than sucrose.

TABLE 4

Influence of paired feeding on growth and liver fat of rats receiving sucrose or dextrin in 9% casein diets[1]

	GAIN/WK.	DAILY FOOD INTAKE	LIVER FAT	
			Dry wt.	Wet wt.
	gm	*gm*	%	%
9% casein-sucrose	15.0 ± 1.2[2]	8.8 ± 0.6[2]	32.9 ± 2.3[2]	11.1 ± 1.0[2]
9% casein-dextrin	16.5 ± 1.3	8.8 ± 0.6	16.3 ± 2.1	4.8 ± 0.7

[1] Experiment was continued 4 weeks.
[2] Mean ± standard error of the mean for 6 rats.

The effect of dextrin in lowering liver fat was demonstrable only with the casein and the amino acid diets. With the wheat gluten and the fibrin diets relatively low liver fat was found, regardless of the type of dietary carbohydrate. With the albumin diet, high values were obtained even in the presence of dextrin (groups 40 and 41) and the addition of lysine to the albumin-sucrose diet (group 47) did not affect liver fat deposition.

The addition of gelatin or zein (groups 44 and 45) to the casein diet brought about reductions in liver fat. Zein was less effective than gelatin. The results given in table 2 (groups 4 to 8) show that a reduction in liver fat also occurred when the casein in the diet was increased.

In table 4 the results of a paired-feeding experiment are presented. In this experiment, in which the food intake of the dextrin group was kept down to that of the sucrose group, only a slight growth difference between the two groups was observed; however, less fat was found in the livers of the animals fed dextrin.

The results of a 12-week experiment are presented in table 5. The values for liver fat at 4 weeks were taken from group 18, which was fed dextrin, and from the sucrose group of the paired-feeding experiment, experiments which were run concurrently. The growth rate of rats receiving the 9% casein-sucrose ration increased, while that of animals fed dextrin decreased, over the 12-week period. Although the liver fat

TABLE 5

Influence of time on growth and liver fat of rats receiving sucrose or dextrin in 9% casein diets

	GROWTH		LIVER FAT (DRY WT.)	
	4 wks.	12 wks.	4 wks.	12 wks.
	gm/wk.	*gm/wk.*	%	%
9% casein-sucrose	14.5 ± 0.9 [1]	19.1 ± 0.8 [1]	32.9 ± 2.3 [1]	14.4 ± 1.0 [1]
9% casein-dextrin	31.4 ± 2.0	25.6 ± 0.9	18.0 ± 1.8	14.1 ± 0.8

[1] Mean ± standard error of the mean for 6 rats.

of the sucrose animals was high (32.9%) after 4 weeks, by 12 weeks it had dropped to 14%, the same as that of the dextrin group.

Values for the xanthine oxidase activity of liver homogenates from animals fed the sucrose and the dextrin diets were 30.0 ± 5.7 and 50.5 ± 2.5 µl of oxygen per gram of liver per hour, respectively. Although the values for both groups are relatively low (the normal value for rats of comparable age fed 18% casein is 100 to 130), a substantial difference between the two groups was observed.

DISCUSSION

Growth

The results of the growth study extend the observations of Harper and Katayama ('53). Since many factors (strain

of rats, sex ratio of groups, vitamin and mineral content of the diets, environment) were altered in the latter of the two studies, the differences observed are not surprising. In the present experiments a somewhat greater growth rate was observed throughout than in the previous work, and slightly more casein was required in the sucrose diet to support growth equal to that obtained with 9% casein and dextrin. The relative values, however, are in good agreement, and it is evident that dextrin permits much better growth of rats fed low-casein diets ad libitum than does sucrose.

The results obtained with wheat gluten, fibrin and albumin (table 2) indicate that this phenomenon is not restricted to low-casein diets. The excellent growth obtained with 15% wheat gluten supplemented with lysine when dextrin was used as the carbohydrate is of interest in view of recent reports of the value of amino acid supplementation of wheat proteins (Sure, '52, '53) and rice proteins (Pecora and Hundley, '51). Since Pecora ('53) has also shown that antibiotics stimulate the growth of animals fed rice proteins supplemented with lysine and threonine, it would be of interest to know whether animals fed wheat proteins might respond similarly.

The intermediate growth observed with cerelose as the carbohydrate suggests that it too permits better utilization of protein than does sucrose. Similar results have been observed in studies with chicks (Monson, Dietrich and Elvehjem, '50). The behavior of fructose paralleled that of sucrose.

The results of the paired-feeding experiment indicate that the more efficient utilization of low-protein diets containing dextrin (Harper and Katayama, '53) is associated with increased food consumption. However, failure to demonstrate the effect of dextrin on growth in such an experiment may be associated with the non-physiological conditions of alternate starvation and immoderate eating.

The 12-week study (table 4) shows that, although a substantial weight difference between rats fed sucrose and those fed dextrin was evident even after 12 weeks, the rates of

growth were beginning to converge. This convergence parellels the decreased protein requirement of maturing animals.

It has been suggested previously that at least part of the growth response is related to the rate of digestion and absorption of the carbohydrate and protein components of the diets. The lower growth obtained when shorter-chain dextrins were fed (groups 17 and 18) suggests that the size of the molecule of the carbohydrate in the diet is of significance. The growth reduction observed when casein in the dextrin diet was replaced by amino acids suggests that the rate of release or absorption of amino acids from casein is also important. However, since dextrin supported much better growth than sucrose even when casein was replaced by amino acids, it is apparent that intact protein is not essential for this effect. Concurrent absorption of carbohydrate and amino acids, which probably occurs over a longer period of time with dextrin in the diet, may reduce the loss of amino acids through deamination (Geiger, '51). Since it is well-known that dextrin and sucrose produce different types of intestinal flora, the effect observed may be due, at least in part, to these changes.

The growth rates of the groups fed amino acids are of interest in their own right, in view of the observations of Maddy and Swift ('52) on the failure of amino acids to support as good growth as intact casein. With sucrose as the dietary carbohydrate, amino acids simulating 9% casein supported as good growth as did casein. With dextrin as the dietary carbohydrate, the growth of the amino acid group was somewhat less than that of the comparable casein group; nevertheless the average growth of the rats (29.6 gm/wk.) which received amino acids equivalent to only 9% casein was equal to that obtained by Maddy and Swift ('52), using sucrose with 23.25% amino acids plus an antibiotic. The only protein supplied in the amino acid rations used in this study was 0.23% casein, which was used as a carrier for the vitamins. Whether the good growth of rats fed the amino acid diets was merely the result of ideal experimental conditions

at the time, or whether it may be attributable to some factor related to the small amount of casein, remains to be determined. Since the casein could scarcely supply a significant amount of amino acids, the amino acid analysis of casein presented by Block and Bolling ('51) is evidently quite accurate in terms of biological utilization.

Liver fat

The observation that less fat was deposited in the livers of rats fed 9% casein diets when sucrose was replaced by dextrin confirms previous observations (Litwack et al., '52; Harper et al., '53b). The reduction in liver fat observed when cerelose was fed and the high liver fat when fructose was fed have not been reported previously. Since a reduction also occurred when more protein was added to the casein-sucrose basal diet, the accumulation of liver fat is apparently caused by the low level of protein. However, the suggestion that sucrose and fructose may be metabolized in a manner that favors fat formation (see Lamb, '50, for references) merits further investigation. Nevertheless, the reduction in liver fat deposition observed when sucrose was replaced by cerelose or dextrin, like the improvement in growth discussed earlier, can probably be attributed to improved utilization of dietary protein.

The exception (experiment 2) in which dextrin caused no reduction in liver fat with 9% casein, although it did with 11% casein, indicates that the level of dietary casein necessary for the demonstration of this effect may be a critical factor. Threonine, which also causes a reduction in liver fat when added to the basal diet (Singal et al., '53a; Harper et al., '53a), is less effective with lower levels of dietary protein (unpublished results). Possibly the failure of Sauberlich ('53) to demonstrate an effect of the type of dietary carbohydrate on liver fat deposition may have been due to the low protein of the diet employed.

The lack of effect of the type of dietary carbohydrate on liver fat deposition when other proteins were fed may indi-

cate that a specific amino acid balance is required before such an effect can be shown. Reference has already been made to the specific effect of threonine. The higher protein content of the wheat gluten diets may account for the failure to obtain high liver fat deposition with either of these; the high nitrogen content (15.0%) and the high threonine content (7.3%) of fibrin may account for the similar results with this protein. The low nitrogen content of egg albumin (12.2%) may have contributed to the failure of dextrin to lower liver fat deposition when this protein was fed. Even the addition to the albumin diet of lysine, which causes a reduction of liver fat in rats fed lysine-deficient amino acid diets (Singal et al., '53b), was without effect.

Although the threonine content of zein is higher than that of gelatin (3.0% and 1.9%, respectively), a greater reduction in liver fat was observed in rats fed 6% gelatin than in those fed 6% zein. Since dietary threonine is very effective in reducing the liver fat of animals fed such diets, these results support the observation that the threonine in zein is not readily available (Geiger, Courtney and Geiger, '52).

The results of the 12-week experiment (table 4) indicate that the fat accumulation occurs during the period of most rapid growth. It coincides with the period of highest protein requirement and decreases as the animals mature and their amino acid requirements are reduced. Unpublished work of the authors indicates that diets of much lower protein content are required to cause high liver fat in more mature rats.

The results of the xanthine oxidase determinations indicate that when sucrose is replaced by dextrin, the protein of 9% casein diets is more efficiently utilized. Thus the sparing effect of dextrin can be shown whether xanthine oxidase, growth or liver fat deposition is used as the criterion.

SUMMARY

The influence of the type of dietary carbohydrate on growth, liver fat deposition and liver xanthine oxidase activity of rats fed *low*-protein diets has been studied.

Dextrin supported better growth of rats than sucrose, whether the protein in the diet was supplied as casein, egg albumin or wheat gluten or whether a mixture of amino acids simulating casein was used. With low-casein diets cerelose supported growth which was intermediate, while with fructose growth was slightly below that obtained with sucrose.

The liver fat deposition of rats fed low-casein diets or amino acid diets of similar composition was reduced when the sucrose in the diet was replaced by dextrin. This effect of dextrin on liver fat deposition could not be demonstrated when other proteins were fed.

Xanthine oxidase activity was higher in the livers of rats fed low-casein diets in which sucrose was replaced by dextrin.

The implications of the results have been discussed and it has been suggested that each of these observations can be explained as a result of improved utilization of dietary protein when the sucrose in low-protein diets is replaced by dextrin.

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LITERATURE CITED

AXELROD, A. E., AND C. A. ELVEHJEM 1941 The xanthine oxidase content of rat liver in riboflavin deficiency. J. Biol. Chem., *140:* 725.

BLOCK, R. J., AND D. BOLLING 1951 The Amino Acid Composition of Proteins and Foods. Charles C Thomas, Publisher, Springfield, Ill., 2nd ed.

GEIGER, E. 1951 Extra caloric function of dietary components in relation to protein utilization. Fed. Proc., *10:* 670.

GEIGER, E., G. W. COURTNEY AND L. E. GEIGER 1952 The physiological availability of some essential amino acids present in zein, in the acid and in the enzymatic hydrolysate of zein. Arch. Biochem. Biophys., *41:* 74.

HARPER, A. E., AND M. C. KATAYAMA 1953 The influence of various carbohydrates on the utilization of low protein rations by the white rat. I. Comparison of sucrose and cornstarch in 9% casein rations. J. Nutrition, *49:* 261.

HARPER, A. E., W. J. MONSON, D. A. BENTON AND C. A. ELVEHJEM 1953a The influence of protein and certain amino acids, particularly threonine, on the deposition of fat in the liver of the rat. Ibid., *50:* 383.

HARPER, A. E., W. J. MONSON, D. A. BENTON AND C. A. ELVEHJEM 1953b Influence of carbohydrates and proteins on liver fat deposition in rats. Fed. Proc., *12:* 416.

HAWK, E. A., AND C. A. ELVEHJEM 1953 The effects of vitamin B_{12} and B_{12f} on growth, kidney hemorrhage and liver fat in rats fed purified diets. J. Nutrition, *49:* 495.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in the nutrition of chicks. J. Biol. Chem., *138:* 459.

LAMB, A. R. 1950 On the relative nutritional efficiency of sucrose and glucose in the albino rat. J. Nutrition, *41:* 545.

LITWACK, G., L. V. HANKES AND C. A. ELVEHJEM 1952 The effect of factors other than choline on liver fat deposition. Proc. Soc. Exp. Biol. Med., *81:* 441.

LITWACK, G., J. N. WILLIAMS, JR., L. CHEN AND C. A. ELVEHJEM 1952 A study of the relationship of liver xanthine oxidase to quality of dietary protein. J. Nutrition, *47:* 299.

MADDY, K. H., AND R. W. SWIFT 1952 Growth and metabolism studies with rats fed rations containing purified amino acids. Ibid., *47:* 243.

MONSON, W. J., L. S. DIETRICH AND C. A. ELVEHJEM 1950 Studies on the effects of different carbohydrates on chick growth. Proc. Soc. Exp. Biol. Med., *75:* 256.

OESTERLING, M. J., AND W. C. ROSE 1952 Tryptophan requirement for growth and utilization of its optical isomers. J. Biol. Chem., *196:* 33.

PECORA, L. J. 1953 Effect of antibiotics on rats fed amino acid-supplemented rice diets. J. Nutrition, *49:* 621.

PECORA, L. J., AND J. M. HUNDLEY 1951 Nutritional improvement of white polished rice by the addition of lysine and threonine. Ibid., *44:* 101.

SAUBERLICH, H. E. 1953 Relationship of amino acids and proteins to the production of fatty livers in rats. Fed. Proc., *12:* 263.

SINGAL, S. A., S. J. HAZAN, V. P. SYDENSTRICKER AND J. M. LITTLEJOHN 1953a The production of fatty livers in rats on threonine- and lysine-deficient diets. J. Biol. Chem., *200:* 867.

——— 1953b The lipotropic action of threonine and related substances in the rat. Ibid., *200:* 883.

SURE, B. 1952 Influence of lysine, valine and threonine additions on the efficiency of the proteins in whole wheat. Arch. Biochem. Biophys., *39:* 463.

——— 1953 Improving the nutritive value of cereal grains. I. Improvement in the efficiency of the proteins in milled wheat flour with lysine, valine and threonine and an extract from condensed fish solubles. J. Nutrition, *50:* 235.

Br. J. Pharmac. Chemother. (1967), 29, 16–24.

THE INFLUENCE OF MOLECULAR WEIGHT AND STRUCTURE ON THE VASCULAR PERMEABILITY RESPONSES INDUCED BY GLUCOSE POLYMERS IN RAT SKIN

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An intraperitoneal injection of dextran into rats produces a generalized anaphylactoid reaction characterized by hyperaemia, pruritus and oedema of the extremities (Voorhees, Baker & Pulaski, 1951). Dextrans of average molecular weight between 25,000 and 2,000,000 are the most effective in producing this response, whereas low molecular weight fractions of 4,000 are ineffective (Halpern, Briot & Neveu, 1954; Harris & West, 1963, 1964). However, when injected intradermally, these low molecular weight polymers produce a slight increase in vascular permeability (Bonaccorsi & West, 1963).

In 1961 Harris and West found that some rats of the Wistar albino strain failed to react to an intraperitoneal dose of clinical dextran (Intradex) and these genetically resistant rats were subsequently designated as non-reactors (Harris & West, 1963; Harris, Kalmus & West, 1963). Intradermal administration of dextrans of various molecular weights also failed to increase vascular permeability in these rats (Bonaccorsi & West, 1963), unless given in very high dosage (Harris & Luscombe, 1965). The present work was undertaken in order to investigate in more detail the effects of molecular weight and chemical structure on the activity of dextrans and other polyglucoses in increasing vascular permeability in the skin of both reactor and non-reactor rats. Also, glucose and several other sugars have been tested as antagonists to the responses produced by these agents, as Poyser & West (1965) have shown that simple carbohydrates inhibit the local vascular changes produced by clinical dextran in rat skin.

METHODS

Preliminary screening

Male rats (body weight about 200 g) of the hooded Lister and Wistar strains were used. The hooded Listers were all known to be reactors but the Wistar rats were tested by the screening procedure described by Harris & West (1963) to separate them into reactors and non-reactors.

Tests on vascular permeability

These were carried out using the method of Bonaccorsi & West (1963). After the intravenous injection of azoron blue dye (7 mg/kg), each rat received eight to ten intradermal injections into the shaved ventral abdominal skin. Each agent was administered in a volume of 0.1 ml. After 30

min, the rats were killed, and the reaction to each injection was assessed by measuring the mean diameter of the blue area on the inner surface of the skin. In each experiment three groups of three reactor or non-reactor rats were used and the results averaged for each type. The experimental design was similar to that described by Poyser & West (1965) and the activity of polysaccharides increasing vascular permeability in reactor rats was expressed on a percentage scale, clinical dextran (Intradex) of average molecular weight 140,000 being assigned a value of 100.

Agents used

Polyglucoses tested for their ability to increase vascular permeability included a range of dextran fractions of average molecular weight 4,000 to 2,000,000. These were prepared by acid hydrolysis and fractional precipitation of a native dextran produced by *Leuconostoc mesenteroides*, strain NRRL-B512, and were supplied by Fisons, Glaxo and Pharmacia. The sample of clinical dextran used (Intradex, Glaxo) was prepared in the same manner and from the same strain of organism. Other polymers of glucose tested were a synthetic polyglucose (Ricketts, 1954; Ricketts & Rowe, 1955), luteose prepared from a culture of *Penicillium luteum* (Anderson, Haworth, Raistrick & Stacey, 1939) and a sample of a highly branched dextran obtained from the Birmingham strain of *Octacoccus arabinosaceous* (Barker, Bourne, Bruce, Neely & Stacey, 1954; Rowe, 1957). These were kindly supplied by Dr. C. R. Ricketts of the M.R.C. Industrial Injuries and Burns Research Unit, Birmingham. A sample of dextrin (Astra) obtained from potato starch and of average molecular weight 6,000 was also used.

Inhibition by sugars

This was carried out using the method of Poyser & West (1965). Doses of glucose producing 50% inhibition (the ID50 values) of the responses produced by the polysaccharides were obtained. Each value shown is the mean and standard error of three experiments, each using three rats.

RESULTS

Activity of dextrans of various molecular weights

Dextrans of average molecular weight 10,000 to 200,000 were approximately equiactive in increasing vascular permeability in the skin of hooded Lister and Wistar reactor rats (Fig. 1). Above 200,000 activity slowly declined as molecular weight increased whereas below 10,000 there was a sharp fall in activity, dextrans of average molecular weight 4,000 to 6,000 being only one-tenth as active as Intradex (molecular weight 140,000).

Activity of other polyglucoses

The activity of a synthetic polyglucose of average molecular weight 3,000 was similar to that of dextran of molecular weight 4,000 (Table 1). Dextrin (average molecular weight 6,000) was three to four times more active than the dextran of the same molecular weight, whereas luteose of average molecular weight 14,000 was one-third to one-half as active as the equivalent dextran. The sample of highly branched dextran (Birmingham strain) was two to three times more active than Intradex (B512 strain).

Activity of dextrans in non-reactors

The responses produced by a dose of 1 mg of dextrans of average molecular weight 10,000 and above were much larger in reactors than non-reactors, whereas samples of average molecular weight 5,000 to 6,000 produced similar effects in both types of rat (Fig. 2). Therefore as molecular weight increased above the region of 6,000 to 10,000 there was increased activity in reactor rats but decreased activity in non-reactors.

Br. J. Pharmac. Chemother. (1967), 29, 16–24

115

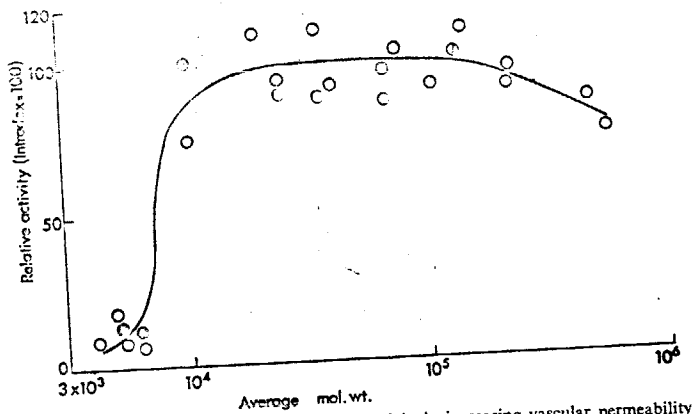

Fig. 1. Activities of dextrans of various molecular weight in increasing vascular permeability in the skin of hooded Lister (○), and Wistar reactor (●) rats. Activity (ordinate) is expressed on a percentage scale, clinical dextran (Intradex) of average molecular weight 140,000 being assigned a value of 100. Abscissa with log scale.

TABLE 1

ACTIVITIES OF POLYGLUCOSES OF VARIOUS MOLECULAR WEIGHTS IN INCREASING VASCULAR PERMEABILITY IN THE SKIN OF HOODED LISTER AND WISTAR REACTOR RATS

Activity is expressed on a percentage scale, clinical dextran (Intradex) of average molecular weight 140,000 being assigned a value of 100

Polyglucose	Average molecular weight	Activity	
		Hooded Lister	Wistar
Synthetic polyglucose	3,000	8	17
Dextran (B512)	4,000	8	11
Dextran (B512)	5,000	16	14
Dextran (B512	6,000	8	12
Dextrin	6,000	32	34
Dextran (B512)	10,000	74	100
Luteose	14,000	27	53
Dextran (B512)	20,000	110	90
Dextran (B512	40,000	91	87
Dextran (B512)	70,000	96	86
Dextran (B512, Intradex)	140,000	100	100
Dextran (Birmingham)	150,000	374	205
[illegible] (B512)	250,000	91	94
[illegible] (B512)	500,000	84	—
[illegible] (B512)	2,000,000	32	—

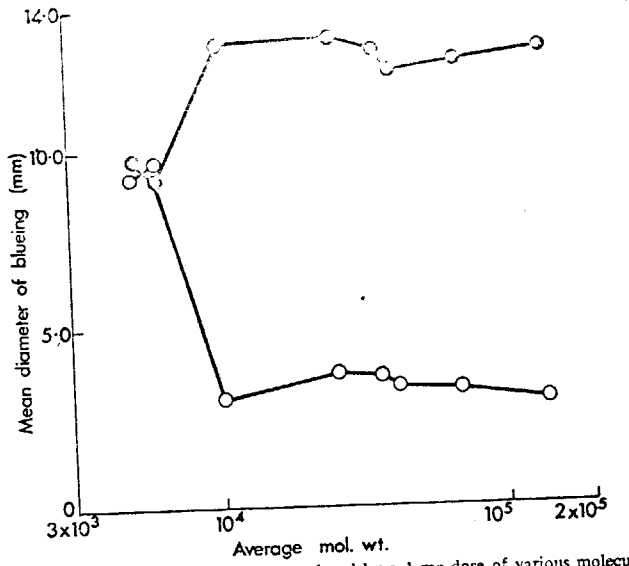

Fig. 2. The increase in vascular permeability produced by a 1 mg dose of various molecular weight dextrans in reactors (●) and non-reactors (○). Responses (ordinate) are expressed as the mean diameter of blueing in excess of that produced by the saline control. Abscissa with log scale.

Activity of other polyglucoses in non-reactors

Molecular weight also appeared to be a determinant factor regarding the activity of other polyglucoses in non-reactor rats. Low molecular weight polymers (synthetic polyglucose and dextrin) were equiactive in both types of rat whereas polyglucoses of higher molecular weight such as luteose and dextran (Birmingham strain) were much less effective in non-reactors (Table 2), the change again occurring in the 6,000 to 10,000 region.

TABLE 2

ACTIVITIES OF POLYGLUCOSES IN INCREASING VASCULAR PERMEABILITY IN THE SKIN OF WISTAR NON-REACTOR RATS

Values are quoted as the dose in non-reactors divided by the dose to produce a similar response in reactors

Polyglucose	Average molecular weight	Dose in non-reactors / Dose in reactors
Synthetic polyglucose	3,000	1·13
Dextran (B512)	6,000	0·78
Dextrin	6,000	1·75
Dextran (B512)	10,000	450
Luteose	14,000	395
Dextran (B512, Intradex)	140,000	450
Dextran (Birmingham)	150,000	435

Inhibition by glucose

Equiactive doses of the polysaccharides were used and it was observed that the doses of glucose required to produce 50% inhibition of the response (the ID50 values) were not significantly different ($P<0.05$) when tested against each of the dextrans and other polyglucoses of average molecular weight 10,000 to 2,000,000 (Table 3). However, larger amounts of glucose were required to antagonize the responses produced by low molecular weight polymers such as dextrans of average molecular weight 4,000 to 6,000, synthetic polyglucose and dextrin. When tested against polysaccharides active in non-reactor rats, the ID50 values for glucose did not significantly differ ($P<0.05$) from those obtained in reactors (Table 4).

TABLE 3

INHIBITION BY INTRADERMAL GLUCOSE OF THE INCREASE IN VASCULAR PERMEABILITY IN RAT SKIN PRODUCED BY EQUIACTIVE INTRADERMAL DOSES OF POLYGLUCOSES OF VARIOUS MOLECULAR WEIGHTS

Inhibition was measured as the dose required to produce 50% reduction in response. ID50s are means and standard errors of three experiments, using hooded Lister rats

Polyglucose	Average molecular weight	Polyglucose dose (μg)	ID50 Glucose (μg)
Synthetic polyglucose	3,000	1,000	1,317 ± 92·8
Dextran (B512)	4,000	1,200	418 ± 38·0
Dextran (B512)	5,000	1,000	577 ± 46·3
Dextran (B512)	6,000	1,250	400 ± 42·3
Dextrin	6,000	300	355 ± 13·2
Dextran (B512)	10,000	100	172 ± 2·3
Luteose	14,000	375	173 ± 3·5
Dextran (B512)	20,000	100	182 ± 3·7
Dextran (B512)	40,000	100	193 ± 8·8
Dextran (B512)	70,000	100	178 ± 5·2
Dextran (B512, Intradex)	140,000	100	183 ± 24·5
Dextran (Birmingham)	150,000	27	213 ± 6·0
Dextran (B512)	250,000	100	196 ± 12·5
Dextran (B512)	500,000	100	207 ± 6·7
Dextran (B512)	2,000,000	300	132 ± 23·9

TABLE 4

INHIBITION BY INTRADERMAL GLUCOSE OF THE INCREASE IN VASCULAR PERMEABILITY PRODUCED BY EQUIACTIVE INTRADERMAL DOSES OF LOW MOLECULAR WEIGHT POLYGLUCOSES IN REACTOR AND NON-REACTOR WISTAR RATS

Inhibition was measured as the dose required to produce 50% reduction in response. ID50s are means and standard errors of three experiments

Polyglucose	Average molecular weight	Polyglucose dose (μg)	ID50 Glucose (μg) Reactors	ID50 Glucose (μg) Non-reactors
Synthetic polyglucose	3,000	1,000	1,400 ± 150·3	1,375 ± 125·3
Dextran	6,000	1,000	310 ± 28·1	320 ± 10·1
Dextrin	6,000	300	315 ± 14·0	310 ± 25·1

Inhibition by other sugars

The range of sugars used was as previously described by Poyser & West (1965). A dose of 500 μg of each sugar was tested against Intradex (dextran B512), dextran (Birmingham strain) and luteose. For the two low molecular weight polymers, synthetic

polyglucose and dextrin, the dose of sugar was increased in proportion to the ID50 values for glucose. Varying degrees of inhibition were obtained by the different sugars under test and the results were similar to those reported by Poyser & West (1965) for other carbohydrate polymers (Table 5). In all instances where inhibition occurred it was of a similar order against each of the polysaccharides tested.

TABLE 5

PERCENTAGE INHIBITION BY VARIOUS SUGARS OF THE INCREASE IN VASCULAR PERMEABILITY IN RAT SKIN PRODUCED BY EQUIACTIVE DOSES OF VARIOUS POLYGLUCOSES

The dose of each sugar used was 500 μg against luteose and the two dextrans, whereas 970 μg were used against dextrin and 3,500 μg against synthetic polyglucose (these values being in proportion to the ID50 values for glucose). Hooded Lister rats were used

Sugar	Percentage inhibition of response due to polyglucose: Synthetic polyglucose	Dextrin	Luteose	Dextran (Birmingham)	Dextran (Intradex)
Trehalose	100	96	93	100	100
Maltose	100	90	98	92	100
Cellobiose	95	85	100	100	96
Gentiobiose	87	100	100	90	100
Lactose	12	7	0	0	11
Sucrose	64	55	45	35	40
D-Glucose	100	100	100	100	100
D-Mannose	100	92	100	100	100
D-Galactose	30	5	11	7	8
D-Fructose	100	88	100	100	100
L-Sorbose	100	97	100	100	100
D-Ribose	39	18	14	22	12
D-Arabinose	100	97	90	96	95
D-Xylose	94	88	98	100	100
D-Lyxose	100	99	100	94	91
6-Deoxy-L-mannose	0	0	2	0	7
2-Deoxy-D-glucose	98	96	100	100	91
DL-Glyceraldehyde	96	85	78	82	100
Dihydroxyacetone	0	1	0	3	7

DISCUSSION

A molecular weight of 10,000 appears to be a critical value regarding the activity of polyglucoses in increasing vascular permeability in rat skin. For example, dextrans (B512 strain) of average molecular weight 4,000 to 6,000 are equiactive in both reactor and non-reactor rats, but when the molecular weight increases to 10,000 and above there is increase in activity in reactors but a decrease in activity in non-reactors. Two other polyglucoses of average molecular weight below 10,000—namely, synthetic polyglucose and dextrin—also produce similar effects in both types of rat, whereas luteose and dextran (Birmingham strain) of higher molecular weight are much more active in reactors than non-reactors.

Harris & West (1963, 1964) reported that low molecular weight fractions of dextran failed to produce an anaphylactoid response when injected intraperitoneally into reactor rats and that all the fractions of dextran that they tested were ineffective in non-reactors. However, Ankier & West (1964a and b) observed that large doses of dextrin will produce

an anaphylactoid reaction when injected intraperitoneally or intravenously in both types of rat. The use of local injections in the present study has revealed that, whereas dextrin is three to four times more active than the same molecular weight dextran (6,000), each is equally effective in increasing vascular permeability in the skin of reactor and non-reactor rats. The higher activity of dextrin may be due to a difference in molecular structure or to the fact that the activity curve for dextrans is very steep at the molecular weight range under consideration, and therefore any slight variance in weight average or distribution will greatly affect activity.

Recently we have been able to show that large doses of low molecular weight dextran (5,000 to 6,000) will produce an anaphylactoid reaction in both reactor and non-reactor rats when injected intraperitoneally or intravenously (unpublished observations). Therefore rats designated by Harris & West (1963) as non-reactors because of their failure to respond to intraperitoneal injections of clinical dextran will react to low molecular weight polymers of glucose in the same way as reactors.

All the polyglucoses found to increase vascular permeability in the present work were branched chain structures. Furthermore, the highly branched dextran obtained from the Birmingham strain of *Betacoccus arabinosaceous* was observed to be two to three times more active than the less branched clinical dextran of the same molecular weight (Intradex). These findings provide further evidence to support the suggestion made by Poyser & West (1965) that activity may be related to the extent of branching within the molecule. This suggestion was based on the observation that yeast mannan, a highly branched polymer of the sugar mannose, was several times more active than dextran (Intradex) of similar molecular weight. However, the two polymers used for this comparison were composed of different sugars, whereas in the present study a similar conclusion has been reached using two polyglucoses. These effects of branching on the activity of polysaccharides in the rat agree with the clinical observations of Tarrow & Pulaski (1953), who found that the more highly branched dextrans were associated with a greater incidence of allergic reactions in man than the less branched dextran that they tested. This suggests a similarity between these effects in the rat and allergic reactions to dextran in man.

Senti (personal communication to Wales, Marshall & Weissberg, 1953) reported that the degree of branching in NRRL-B512 dextrans was independent of the molecular weight, whereas Granath (1958) indicated that branching diminishes rapidly as molecular weight approached 10,000. This may explain the rapid fall off in activity of dextrans of molecular weight below this figure and also why dextrans of average molecular weight 10,000 to 200,000 are equiactive. The decrease in activity with high molecular weight polymers may be due to a failure to penetrate to the site of action or to structural factors such as an increase in the size of branch chains associated with high molecular weight (Wales, Marshall & Weissberg, 1953).

Luteose has a fairly high degree of branching but it is less active than dextran (B512) of the equivalent molecular weight. Unlike dextran the predominant glucosidic link in luteose is β-1:6 and also chemical evidence suggests that the branch chains are interconnected to form a closed chain structure (Anderson *et al.*, 1939). Luteose will therefore, tend to have a more rigid configuration compared with the flexible structure [illegible] (Granath, 1958). These properties may account for the low activity of this [illegible] of glucose.

Several sugars are equally effective in inhibiting the responses of all the polyglucoses under test. Also, the amount of glucose required to antagonize the effects of each of the low molecular weight polysaccharides in non-reactor rats is similar to the amount required in reactors. This suggests that a similar mechanism is involved for each agent in both types of rat. The stereospecificity of the receptor system involved in the action of these polyglucoses is similar to that described by Poyser & West (1965) for other carbohydrate polymers such as ovomucoid and yeast mannan. It is interesting to note that larger amounts of glucose are required to antagonize the responses produced by equiactive doses of low molecular weight polysaccharides. This would suggest that decreased activity of these polyglucoses is not due to a decreased affinity for the receptor site.

SUMMARY

1. Dextran fractions of average molecular weight 10,000 to 200,000 are more effective in increasing vascular permeability in rat skin than those of lower molecular weight.

2. Whereas a synthetic polyglucose was equiactive, dextrin was more active and luteose less active than the corresponding molecular weight dextrans. A highly branched dextran was two to three times more active than the less branched clinical sample.

3. Dextrin, synthetic polyglucose and dextrans of average molecular weight below 10,000 were equiactive in both rats which react (reactors) and those that fail to react (non-reactors) to intraperitoneal injections of clinical dextran. However, luteose and dextrans of higher molecular weight were much more active in reactors than non-reactors.

4. Although structural factors such as branching are important, a molecular weight of 10,000 appears to be a critical value regarding the local vascular changes produced by polyglucoses in rat skin.

5. The responses to all the polyglucoses were each antagonized by glucose and several other sugars suggesting that a common site of action may be involved.

We are grateful to Dr. C. R. Ricketts for his help and interest in this work and for supplying samples of synthetic polyglucose, luteose and dextran of the Birmingham strain. We also thank Dr. K. J. Child and Dr. S. Ball of Glaxo Laboratories Ltd., Dr. Shirvell Price of Pharmacia Ltd., and Fisons Pharmaceuticals Ltd., for generous gifts of various dextran fractions.

REFERENCES

ANDERSON, C. G., HAWORTH, W. N., RAISTRICK, H. & STACEY, M. (1939). Polysaccharides synthesized by micro-organisms. IV. The molecular constitution of luteose. *Biochem. J.*, 33, 272–279.

ANKIER, S. I. & WEST, G. B. (1964a). The anaphylactoid reaction in rats. *J. Pharm. Pharmac.*, 16, 129–130.

ANKIER, S. I. & WEST, G. B. (1964b). Further studies on the inflammatory anaphylactoid reaction in rats. *Int. Archs Allergy appl. Immun.*, 25, 114–126.

BARKER, S. A., BOURNE, E. J., BRUCE, G. T., NEELY, W. B. & STACEY, M. (1954). Immunopolysaccharides. Part II. Structure of a *Betacoccus arabinosaceous* dextran. *J. chem. Soc.*, 2395–2399.

BONACCORSI, A. & WEST, G. B. (1963). Absence of capillary permeability response in rats to dextran and egg-white. *J. Pharm. Pharmac.*, 15, 372–376.

GRANATH, K. A. (1958). Solution properties of branched dextrans. *J. Colloid Sci.*, 13, 308–328.

HELPERN, B. N., BRIOT, M. & SIMIER, TH. (1954). Dextran et anaphylatoxine. *C. r. Séanc. Soc. Biol.*, 148, 50–52.

HARRIS, J. M., KALMUS, H. & WEST, G. B. (1963). Genetical control of the anaphylactoid reaction in rats. *Genet. Res.*, 4, 346–355.

HARRIS, J. M. & LUSCOMBE, D. K. (1965). Carbohydrates and inflammation—the local effects of dextran, ovomucoid and glycogen in dextran-resistant rats. *Int. Archs Allergy appl. Immun.*, 28, 50–59.
HARRIS, J. M. & WEST, G. B. (1961). Anaphylactoid reaction in rats. *Nature, Lond.*, 191, 399–400.
HARRIS, J. M. & WEST, G. B. (1963). Rats resistant to the dextran anaphylactoid reaction. *Br. J. Pharmac. Chemother.*, 20, 550–562.
HARRIS, J. M. & WEST, G. B. (1964). A new link between the anaphylactoid reaction in rats and human allergy. *Int. Archs Allergy appl. Immun.*, 25, 46–57.
POYSER, R. H. & WEST, G. B. (1965). Changes in vascular permeability produced in rats by dextran, ovomucoid and yeast cell wall polysaccharides. *Br. J. Pharmac. Chemother.*, 25, 602–609.
RICKETTS, C. R. (1954). The preparation and structure of a synthetic polyglucose. *J. chem. Soc.*, 4031–4032.
RICKETTS, C. R. & ROWE, C. E. (1955). Preparation and structure of synthetic hexose polymers. *J. chem. Soc.*, 3809–3813.
ROWE, C. E. (1957). The relationship between specific optical rotation and branching of dextran. *Chemy. Ind.*, 816–817.
TARROW, A. B. & PULASKI, E. J. (1953). Reactions in man from infusion with dextran. *Anesthesiology*, 14, 359–366.
VOORHEES, A. B., BAKER, H. J. & PULASKI, E. J. (1951). Reactions of albino rats to injections of dextran. *Proc. Soc. exp. Biol. Med.*, 76, 254–256.
WALES, M., MARSHALL, P. A. & WEISSBERG, S. G. (1953). Intrinsic viscosity-molecular weight relationships for dextran. *J. Polym. Sci.*, 10, 229–240.

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SOME DATA ON TWO PURIFIED KININOGENS FROM HUMAN PLASMA

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Work on the purification of the substrate in plasma for kinin-forming enzymes, kininogen, has been carried out in several laboratories. Habermann (1963) thus purified kininogen from bovine serum and Suzuki, Mizushima, Sato & Iwanaga (1965) purified a kininogen from bovine plasma. Habermann (1965, 1966) also studied the structure of the kinin-yielding polypeptides of his purified bovine kininogen (peptic kinin-yielding polypeptides). Pierce & Webster (1966) isolated two kininogens from human plasma. These kininogens were shown to be glycoproteins with molecular weights of about 50,000, as is also the case with the bovine kininogen (Habermann, 1963).

Jacobsen (1966b) found that two different substrates for plasma kinin-forming enzymes could be separated from plasmas of several mammals. These substrates had different molecular dimensions and they differed in their reaction patterns towards various kinin-forming enzymes. The present paper reports the results of further purification with some characterization of these two substrates. The substrate with the larger molecular dimension has been designated substrate 1, and the other and smaller one has been designated substrate 2. The methods employed have been DEAE anion exchange chromatography, gel filtration, disc gel electrophoresis and sucrose gradient ultracentrifugation.

METHODS

Plasma. Citrated human plasma was obtained by using siliconized equipment throughout, as described by Jacobsen (1966b).

Initial purification procedures for kininogens (anion exchange chromatography and gel filtration). The separation procedures previously described (Jacobsen, 1966a, 1966b) have been modified and expanded, so as to include disc gel electrophoresis (see below). The initial procedure was a separation of fractions containing the two kininogens by chromatography of human citrated plasma on a DEAE anion exchanger. Each of the substrates was subsequently exposed to further purification procedures (see below and Table 1). For initial chromatography, plasma (25 ml.) was mixed with 0.05 M Tris-HCl buffer (pH 8) containing 0.25 M sodium chloride (12.5 ml.) and the mixture applied to a DEAE-Sephadex A 50 (A. B. Pharmacia, Uppsala, Sweden) column (18 × 1.8 cm) equilibrated with 0.05 M Tris-HCl buffer (pH 8) containing 0.2 M sodium chloride. Substrate 1 is retained on the column while substrate 2 is eluted with the equilibrating buffer. Substrate 1 could then be eluted with a 0.05 M Tris-HCl buffer (pH 8) containing 0.35 M sodium chloride. The effluent containing substrate 2 was treated with "massive contact exposure" in order to eliminate the plasma kallikrein activity (Jacobsen, 1966b), and then applied to a DEAE-Sephadex A 50 column (10 × 1 cm) equilibrated with 0.05 M Tris-HCl buffer (pH 8) containing 0.1 M sodium chloride. Substrate 2 was eluted from this column with a buffer containing 0.25 M sodium chloride. Buffer concentrations in the samples were always adjusted to that of the buffer used for equilibration in the following purification procedure.

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124

INFLUENCE OF FRUCTOSE AND OTHER CARBOHYDRATES ON THE NIACIN REQUIREMENT OF THE RAT

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Krehl *et al.* (1) have shown that certain types of carbohydrates alter the growth of rats on niacin-deficient rations. Starch, dextrin, glucose, and maltose increased growth, whereas sucrose decreased it, indicating that these carbohydrates modified the niacin requirement of the rat. These results might be explained by assuming that the type of carbohydrate influenced the amount of niacin synthesized by the intestinal flora. The evidence for this view was presented and discussed by these investigators (1). There are, however, many other possibilities which might explain these differences.

The experiments to be reported here were designed to test the possibility that varying amounts of niacin may be required in the metabolism of different sugar units.

EXPERIMENTAL

The basal diet consisted of casein ("vitamin-free") 9, gelatin (U. S. P.) 3, L-cystine 0.15, carbohydrate 81, corn oil 3, and salts 4 (2). Vitamins were incorporated in the diet in the following amounts: thiamine 0.2, riboflavin 0.3, pyridoxine 0.25, calcium pantothenate 2, choline 100, inositol 10, 2-methylnaphthoquinone 0.1, biotin 0.01, and pteroylglutamic acid 0.1 mg. per cent. 2000 and 400 U. S. P. units of vitamins A and D respectively were given as a supplement to each rat twice weekly and 3 mg. of α-tocopherol per rat were given once each week. Weanling rats develop a niacin deficiency in 7 to 14 days if sucrose is used in this ration.

The various experimental diets were made by substituting in the basal diet the following carbohydrates: levulose, 97 per cent practical grade or, in most instances, C.P. quality (Pfanstiehl); glucose, C.P. anhydrous (Merck); sucrose, commercial granulated; lactose, C.P. (Pfanstiehl); D-galactose (Pfanstiehl); white dextrin, N. F. V (Merck); corn-starch, (Argo); and, maltose (Nutritional Biochemicals). The starch, dextrin, sucrose, levulose, and glucose were free of detectable amounts (3) of niacin. The other carbohydrates were not assayed. Weanling male rats of National Institutes of Health (Sprague-Dawley) or Osborne and Mendel strains, weighing 40 to 50 gm., were maintained on the various diets in

individual suspended wire mesh cages. Litter mates were distributed to the various experimental groups by age and weight. Individual body weights were recorded three times weekly.

The niacin content of liver and thigh muscles was obtained by using the entire liver and the entire thigh muscles of one leg. The assay was made according to the microbiological method of Snell and Wright (3), slightly modified. Urinary N^1-methylnicotinamide determinations were made as described by Huff and Perlzweig (4).

TABLE I

Growth of Rats As Influenced by Various Carbohydrates and Niacin

No. of rats	Carbohydrate	Growth* per wk. per rat	
		No niacin added	2 mg. per cent niacin added
		gm.	*gm.*
10	Fructose	1.5 ± 0.7†	12.0
10	Sucrose	3.3 ± 0.94	17.2
5	½ glucose, ½ fructose	6.8 ± 1.1	22.0
15	Glucose	9.4 ± 0.71	23.0
5	Dextrin	13.5 ± 1.2	21.6
10	Starch	12.9 ± 1.04	22.7
5	Maltose	10.2 ± 1.27	28.6
5	½ glucose, ½ galactose‡	18.0 ± 1.14	19.3
5	½ " ½ lactose§	2.3 ± 1.17	12.0

* The growth periods were 4 weeks on the unsupplemented diet and an additional week on the niacin-supplemented diet, except for the last two groups in which 3 weeks growth on the unsupplemented diet was used.

† Probable error of the mean result.

‡ All of the rats on this diet developed bilateral cataracts.

§ Lactose and galactose, alone, were also used in these experiments.

Results

Effect of Various Carbohydrates on Growth—When diets containing the various carbohydrates were fed, wide differences in growth were observed (Table I). Each of the carbohydrates, except the glucose-galactose mixture, produced a deficiency of niacin as judged by growth of the same animals before and after niacin was added to the diet. The marked sparing action of the glucose-galactose mixture has not been described previously, nor has it been studied further here.

The most severe deficiencies were produced by fructose and sucrose. Glucose, a glucose-fructose mixture, and maltose produced somewhat less severe deficiencies. In other experiments (see Fig. 1 and Table II) glucose produced growth somewhat superior to that shown in Table I. Starch and

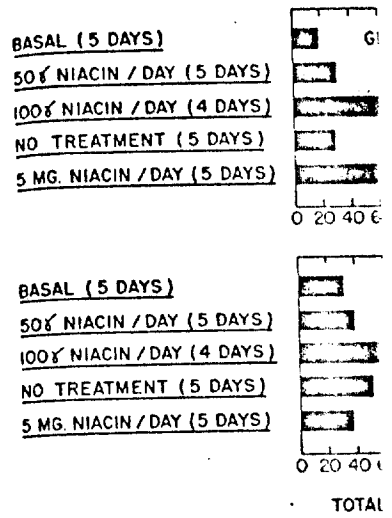

FIG. 1. Growth on various carb
taneously. The growth periods r
been on their respective diets for
representative sample of their gro

Amount of Dietary N

Niacin	Fructose	
	No. of rats	Mean
mg. per cent		*gm. per wk. per rat*
0	14	2.4
0.5	10	4.5
1.0	13	10.2
1.5	9	12.8
2.0	9	12.8
2.5	9	11.8
3.0		
10.0	14	12.6
	Glucose	
0	10	13.4
0.5	10	15.8
1.0	10	18.4
1.5	10	17.0
2.0	10	17.8
3.0	9	18.3

Growth periods 4 weeks in all

re mesh cages. Litter mates were distributed to
l groups by age and weight. Individual body
ree times weekly.
ver and thigh muscles was obtained by using the
thigh muscles of one leg. The assay was made
biological method of Snell and Wright (3), slightly
ethylnicotinamide determinations were made as
rlzweig (4).

TABLE I

fluenced by Various Carbohydrates and Niacin

bohydrate	Growth* per wk. per rat	
	No niacin added	2 mg. per cent niacin added
	gm.	*gm.*
	1.5 ± 0.7†	12.0
	3.3 ± 0.94	17.2
½ fructose	6.8 ± 1.1	22.0
	9.4 ± 0.71	23.0
	13.5 ± 1.2	21.6
	12.9 ± 1.04	22.7
	10.2 ± 1.27	28.6
½ galactose‡	18.0 ± 1.14	19.3
½ lactose§	2.3 ± 1.17	12.0

4 weeks on the unsupplemented diet and an addi-
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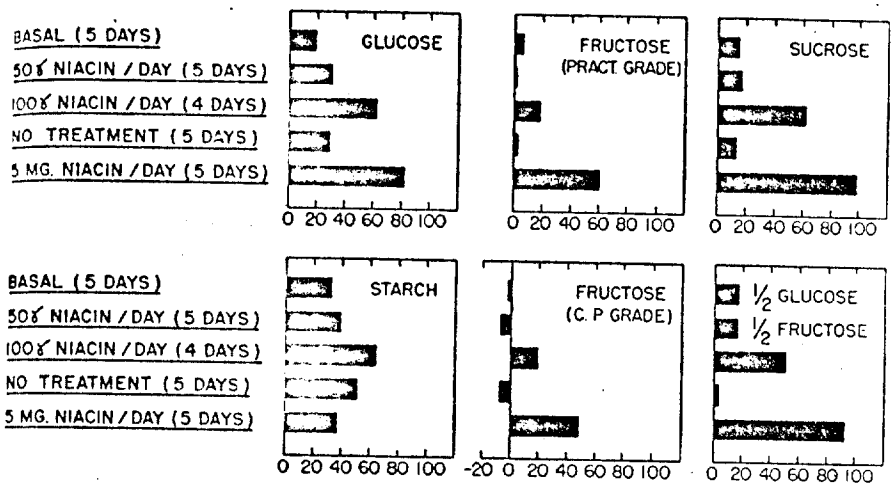

FIG. 1. Growth on various carbohydrates and test doses of niacin given subcutaneously. The growth periods represented above were begun after the rats had been on their respective diets for 3 weeks. The basal 5 day period, however, is a representative sample of their growth during the preceding 3 weeks.

TABLE II

Amount of Dietary Niacin Required for Maximal Growth

Niacin	Fructose			Sucrose		
	No. of rats	Mean	Probable error	No. of rats	Mean	Probable error
mg. per cent		*gm. per wk. per rat*			*gm. per wk. per rat*	
0	14	2.4	±0.59	14	3.1	±0.39
0.5	10	4.5	±0.52	10	6.6	±0.63
1.0	13	10.2	±0.75	19	13.3	±0.42
1.5	9	12.8	±0.94	10	15.4	±0.60
2.0	9	12.8	±0.62	10	15.2	±0.81
2.5	9	11.8	±0.56			
3.0				10	15.4	±0.63
10.0	14	12.6	±0.66	10	14.0	±0.74
	Glucose			Starch		
0	10	13.4	±0.57	14	14.1	±0.81
0.5	10	15.8	±0.93	15	17.1	±0.90
1.0	10	18.4	±0.69	10	16.4	±0.80
1.5	10	17.0	±1.00	5	18.6	±1.46
2.0	10	17.8	±0.76	10	16.3	±1.0
3.0	9	18.3	±0.95	10	17.6	±1.14

Growth periods 4 weeks in all groups.

dextrin gave relatively good growth and seemed to produce a rather mild deficiency. Even here, however, there was still a definite requirement for niacin as judged by the response to added niacin. A glucose-lactose mixture seemed to produce a severe deficiency, but the results were complicated by the toxic effects of the high lactose concentration. The toxic effect of high lactose concentrations in low fat diets is well known (5). The results reported here with sucrose, glucose, dextrin, and starch confirm those of Krehl *et al.* (1). The influence of fructose in increasing niacin requirements has not been reported previously.

It is of interest to compare the growth performance of the rats on sucrose and on a mixture of glucose and fructose which simulated sucrose. The rats on sucrose gained 3.3 gm. per week, while those on the glucose-fructose mixture gained 6.8 gm. per week, a difference of doubtful statistical significance. In another experiment (Fig. 1) the rats gained almost equally (4.6 and 4.3 gm. per week) on these two diets. In both experiments the rats were markedly deficient in niacin as judged by their growth response to it. The data would seem to exclude the possibility that a failure of the sucrose inversion mechanism is an important factor in these animals.

Amount of Niacin Necessary to Produce Growth—It seemed quite possible that the fructose content of sucrose was the factor responsible for the action of the latter in increasing the niacin requirement. This possibility was investigated by comparing the minimal amount of niacin necessary to produce maximal growth with fructose, sucrose, glucose, and starch (Table II). It can be seen that the growth peak occurred with 1.5 mg. per cent of added niacin in the fructose and sucrose diets. 1 mg. per cent of niacin gave maximal growth with glucose, while only 0.5 mg. per cent was necessary for starch.

The differences between growth in the absence of niacin are strikingly brought out in Table II. In this series of animals, glucose gave about 4 times as much growth as sucrose and about 5 times as much as fructose. It should also be noted that the maximal growth potential on these diets is different with the various sugars. Glucose and starch showed a considerably higher growth maximum than either sucrose or fructose. It is evident therefore that factors other than niacin limit the efficiency of utilization of fructose *in this type of diet.* Using another type of diet containing adequate protein, Bachmann *et al.* (6) observed no difference in growth rate between fructose and glucose.

The high niacin requirement with fructose and sucrose might be explained by assuming an increased metabolic need for niacin. Some evidence might be obtained relative to these possibilities by determining the relative tissue concentrations of niacin in these various groups of rats.

Niacin Content of Liver and Muscles—After 4 weeks on the various diets

most of the rats reported in T
of the liver and hamstring mus

Amount of Niacin in Tissues As I

Niacin in diet	No. of rats	Niacin: Liver
	Fructose	
mg. per cent		*γ per gm.*
0	10	144 (131–150)
0.5	5	138 (125–155)
1.0	10	151 (131–180)
1.5	5	140 (134–150)
2.0	5	161 (152–180)
2.5	5	159 (130–190)
3.0	5	172 (160–195)
10.0	5	187 (158–215)
	Glucose	
0	10	133 (118–150)
0.5	10	146 (124–180)
1.0	10	155 (142–190)
1.5	10	146 (130–162)
2.0	10	155 (130–180)
3.0	9	150 (140–165)

The values in parentheses indicate

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Mu —After 4 weeks on the various diets

most of the rats reported in Table II were sacrificed and the niacin content of the liver and hamstring muscles was determined (Table III). Rats sub-

TABLE III

Amount of Niacin in Tissues As Influenced by Amount of Dietary Niacin and Various Carbohydrates

Niacin in diet	Fructose			Sucrose		
	No. of rats	Niacin		No. of rats	Niacin	
		Liver	Muscle		Liver	Muscle
mg. per cent		*γ per gm.*	*γ per gm.*		*γ per gm.*	*γ per gm.*
0	10	144 (131–150)	35 (33–38)	14	125 (112–140)	45 (32–55)
0.5	5	138 (125–155)	42 (35–45)	10	114 (100–120)	35 (25–44)
1.0	10	151 (131–180)	42 (38–47)	15	135 (110–155)	37 (25–50)
1.5	5	140 (134–150)	55 (47–60)	10	150 (130–200)	72 (54–96)
2.0	5	161 (152–180)	67 (60–70)	10	151 (130–163)	73 (55–79)
2.5	5	159 (130–190)	79 (75–85)			
3.0	5	172 (160–195)	78 (75–85)	10	185 (164–220)	96 (68–120)
10.0	5	187 (158–215)	75 (71–85)	5	170 (160–180)	69 (65–80)
	Glucose			Starch		
0	10	133 (118–150)	36 (32–40)	14	131 (120–150)	35 (30–49)
0.5	10	146 (124–180)	77 (69–90)	15	138 (106–175)	39 (33–48)
1.0	10	155 (142–190)	60 (52–66)	10	148 (132–183)	64 (55–67)
1.5	10	146 (130–162)	81 (50–120)	5	113 (103–120)	64 (62–67)
2.0	10	155 (130–180)	74 (47–95)	10	142 (130–150)	71 (68–80)
3.0	9	150 (140–165)	75 (60–82)	10	139 (130–150)	74 (65–85)

The values in parentheses indicate the range.

sisting on the niacin-free diets showed low tissue niacin, irrespective of the carbohydrate in the diet. These levels were comparable to those obtained previously in niacin-deficient rats (7) and to those reported by Singal *et al.* (8).

The results obtained from the liver analyses were somewhat difficult to interpret. None of the levels of niacin fed produced "normal" (*i.e.* about 180 γ per gm.) liver concentration in the glucose and starch groups, nor was there a well defined maximum when these various groups were compared. On the other hand the fructose and sucrose groups showed approximately normal levels of liver niacin when 3.0 mg. per cent of dietary niacin was present.

The reason for the failure of the rats receiving glucose and starch to attain normal niacin levels in the liver is not known. It may have been related to the presence of fatty liver. Fatty liver seemed to be more prominent in rats receiving glucose or starch. Careful quantitative, comparative observations were not made, however. Krehl *et al.* (9) found that fatty liver may interfere with the storage of niacin in this organ.

The results were somewhat more consistent in the muscle assays. If one arbitrarily adopts 60 γ per gm. as the lower limit of normal, then 2.0 and 1.5 mg. per cent of dietary niacin produced normal levels with fructose and sucrose respectively, while only 0.5 and 1.0 mg. per cent were necessary for glucose and starch respectively.

One further point can be made if the data in Table III are compared with the growth data in Table II. Since the niacin levels in tissue were similarly depressed in all of the niacin-free diets, it is evident that rats can grow at a rate of 12 to 15 gm. per week in the presence of these low niacin concentrations when metabolizing glucose or starch, but hardly at all when metabolizing fructose or sucrose. This difference is even more apparent in the group receiving 0.5 mg. per cent of niacin, since the rats receiving starch grew maximally with low tissue niacin levels, while those on fructose and sucrose grew only about 30 per cent of the maximum, with similar levels of tissue niacin. It would seem therefore that the concentration of tissue niacin must be more nearly normal before fructose and sucrose can be metabolized efficiently.

Results with Subcutaneous Test Doses of Niacin—One difficulty that was inherent in the type of experiment reported above was that, although the amounts of niacin added to the diet were exactly equivalent in the various groups, the food intake varied considerably. This produced a constant and comparable intake of niacin per gm. of carbohydrate, but the total daily niacin intake varied with the quantity of diet consumed. It seemed clear that the principal factor influencing food intake in these rats was the amount of added niacin. Nevertheless such factors as food texture and palatability, which have been reported to influence food intake in rats (10), might have introduced some error.

Accordingly, another experiment was designed with fixed doses of niacin administered subcutaneously. Six matched groups of five rats each were

placed on diets without niac
were maintained on these di
total growth of each group o
(basal 5 days). Then 50 γ
daily for 5 days. This was
sucrose, glucose-fructose mix
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and all growth rates declined.
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those on fructose, promptly
indicate that 100 γ of niacin

Excretion of N¹-Methylnicotina

Four rats in each group.

Niacin	
	Sucros
mg. per cent	γ
0	38
0.5	20
1.0	31
10.0	361

of niacin in the rats receivin
groups.

It should be emphasized th
irrespective of body weight.
sented in Fig. 1 were 51 and 41
gm. in the sucrose and glucos
66 gm. for the starch and glu
fructose groups receiving 50 γ
dose of niacin per unit of body
though the latter responded to

Excretion of N¹-Methylnicoti
appreciable quantities of most
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placed on diets without niacin, as indicated in Fig. 1. The weanling rats were maintained on these diets for 3 weeks. At the end of this period the total growth of each group of five rats in the following 5 days was recorded (basal 5 days). Then 50 γ of niacin were given subcutaneously to each rat daily for 5 days. This was not sufficient to cause a growth response in the sucrose, glucose-fructose mixture, or the fructose groups, but did cause a response in the starch and glucose groups. The amount of niacin was then increased to 100 γ daily. This produced a definite, though submaximal, response in all groups. The response of the fructose groups was much less than that of the others. At the end of this period niacin was discontinued and all growth rates declined. The groups on glucose and starch continued to grow above the basal level, however, while the other groups, especially those on fructose, promptly declined below the basal level. This would indicate that 100 γ of niacin per day were sufficient to permit some storage

TABLE IV

Excretion of N^1-Methylnicotinamide As Influenced by Dietary Niacin and Certain Carbohydrates

Four rats in each group.

Niacin	Per 100 gm. of rat per day		
	Sucrose	Fructose	Starch
mg. per cent	γ	γ	γ
0	38	38	30
0.5	20	42	35
1.0	31	40	64
10.0	361	420	420

of niacin in the rats receiving dextrose and starch but not in the other groups.

It should be emphasized that the doses of niacin used here were given irrespective of body weight. The average body weights at the time represented in Fig. 1 were 51 and 41 gm. per rat in the fructose groups, 61 and 60 gm. in the sucrose and glucose-fructose groups, as compared with 92 and 66 gm. for the starch and glucose groups. Consequently, the rats in the fructose groups receiving 50 γ of niacin actually received about twice the dose of niacin per unit of body weight as did those in the starch group, although the latter responded to this dose, while the former did not.

Excretion of N^1-Methylnicotinamide—It is known that rats will excrete appreciable quantities of most vitamins only when they have a slight excess over their metabolic needs. It seemed possible therefore that additional information might be acquired by determining the level of niacin necessary to produce an increased excretion of N^1-methylnicotinamide (the principal

derivative of niacin in the urine of rats (11)). Preliminary experiments indicated that 2.0 to 3.0 mg. per cent of dietary niacin were necessary to produce appreciable excretion in rats on sucrose diets, whereas less was required with starch diets.

Accordingly, small groups of rats were placed on various diets, as indicated in Table IV. Rats receiving none and 0.5 mg. per cent of added niacin showed similarly low excretion in all groups. However, 1.0 mg. per cent of dietary niacin produced an appreciable rise in excretion in the starch group but no comparable rise in the fructose and sucrose groups. 10 mg. per cent of added niacin produced a marked rise in excretion in all groups, as expected.

These data on N^1-methylnicotinamide have limited statistical significance because of the small number of animals and determinations. They were not extended further since the results agreed with data previously obtained.

DISCUSSION

The data presented here seem to establish the fact that a relatively large amount of dietary niacin is required when fructose is the sole dietary carbohydrate. It appears fairly certain that the high niacin requirement with sucrose is due principally to its fructose content. Also it is probable from the studies reported by Krehl *et al.* (1) and from the data reported here that glucose in any of its forms results in a lower requirement for niacin.

The mechanism by which these differences in niacin requirement arise is not clear, however. Since fructose and sucrose supported less rapid growth than glucose when fed in the type of diet used in this study, it is possible that these sugars have a "toxic" effect in high concentrations, just as lactose and galactose have. Whether this would alter the requirement for niacin is not known.

It is believed that the evidence presented in this communication supports the idea that more niacin is actually needed in the metabolism of fructose, since rats receiving fructose grew well only when the concentration of tissue niacin approached normal, while rats receiving glucose grew well in the presence of reduced niacin levels. Furthermore, the data depicted in Fig. 1 indicate that more niacin is required to promote growth in niacin-deficient rats receiving fructose than in those receiving glucose.

From what is known concerning the pathway of enzymatic utilization of glucose and fructose, one would not expect any difference in niacin requirements. Nevertheless, many differences in the way the body handles these two sugars are known. Their absorption from the intestine is different (12). Their effects on the respiratory quotient and blood lactic acid are different (13, 14). Less deposition of body fat occurs in animals receiving fructose compared with glucose (6). The existence of separate

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BIBLIO

1. Krehl, W. A., Sarma, P. S., Teply, L.
(1946).
2. Osborne, T. B., and Mendel, L. B., *J.*
3. Snell, E. E., and Wright, L. D., *J. Bi*
4. Huff, J. W., and Perlzweig, W. A., *J.*
5. Schantz, E. J., Elvehjem, C. A., and
38).
6. Bachmann, G., Haldi, J., Wynn, W., a
7. Hundley, J. M., *J. Nutr.*, **34**, 253 (19
8. Singal, S. A., Sydenstricker, V. P., a
1069 (1948).
9. Krehl, W. A., Henderson, L. M., de la
Chem., **166**, 531 (1946).
10. Carlson, A. J., and Hoelzel, F., *Scienc*
11. Rosen, F., Huff, J. W., and Perlzweig,
12. Cori, C. F., *J. Biol. Chem.*, **66**, 691 (1
13. Blatherwick, N. R., Bradshaw, P. J.,
Chem., **136**, 615 (1940).
14. Bachmann, G., and Haldi, J., *J. Nutr.*
15. Cori, G. T., and Slein, M. W., *Federati*

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gluco- and fructokinases has been demonstrated (15). Many other differences are known, but which of these, if any, are responsible for the altered niacin requirement is not evident.

An influence of fructose on the niacin requirement would seem rather unusual. No other instance has been reported, to the best of our knowledge, in which the type of sugar unit metabolized increased the requirement for a vitamin.

SUMMARY

The niacin requirement of rats was greater on diets containing fructose or sucrose and less on diets containing dextrin or starch than on a diet in which the carbohydrate was glucose.

Rats using fructose grew well only when the levels of tissue niacin approached normal. Similar rats metabolizing glucose grew well in the presence of decreased concentrations of tissue niacin.

The data suggest the possibility that more niacin is consumed in the metabolism of fructose than of glucose.

Possible mechanisms by which dietary carbohydrates might alter the niacin requirement are mentioned.

BIBLIOGRAPHY

1. Krehl, W. A., Sarma, P. S., Teply, L. J., and Elvehjem, C. A., *J. Nutr.*, **31**, 85 (1946).
2. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, **32**, 369 (1917).
3. Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, **139**, 675 (1941).
4. Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, **167**, 157 (1947).
5. Schantz, E. J., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, **122**, 381 (1937–38).
6. Bachmann, G., Haldi, J., Wynn, W., and Ensor, C., *J. Nutr.*, **16**, 229 (1938).
7. Hundley, J. M., *J. Nutr.*, **34**, 253 (1947).
8. Singal, S. A., Sydenstricker, V. P., and Littlejohn, J. M., *J. Biol. Chem.*, **176**, 1069 (1948).
9. Kreul, W. A., Henderson, L. M., de la Huerga, J., and Elvehjem, C. A., *J. Biol. Chem.*, **166**, 531 (1946).
10. Carlson, A. J., and Hoelzel, F., *Science*, **109**, 63 (1949).
11. Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, **163**, 343 (1946).
12. Cori, C. F., *J. Biol. Chem.*, **66**, 691 (1925).
13. Blatherwick, N. R., Bradshaw, P. J., Ewing, M. E., and Sawyer, S. D., *J. Biol. Chem.*, **136**, 615 (1940).
14. Bachmann, G., and Haldi, J., *J. Nutr.*, **13**, 157 (1937).
15. Cori, G. T., and Slein, M. W., *Federation Proc.*, **6**, 246 (1947).

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130

THE INTESTINAL MICROFLORA OF HENS AS INFLUENCED BY VARIOUS CARBOHYDRATES IN A BIOTIN-DEFICIENT RATION[1,2]

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The chicken is an animal of great economic importance and is widely used in the assay of certain vitamins, but little is known about its intestinal flora. The first study of the microbial flora of the chicken's intestinal tract was conducted by Kern (1897). His investigations embraced not only the chicken and other gallinaceous birds, but many of the common wild birds. Other work on the microflora of chickens has been reported by Rahner (1901), King (1905), Gage (1911), Menes and Rochlin (1929), and Emmel (1930). All of these workers agreed that *Escherichia coli* and *Aerobacter aerogenes* made up the greatest proportion of the intestinal flora. Other types of microorganisms commonly found in the chicken's intestines by these workers were lactobacilli, micrococci, pseudomonads, bacilli, sarcinae, clostridia (in two instances), and yeasts. Unfortunately, none of these studies was quantitative, and some were not too exact from a qualitative standpoint.

The type of dietary carbohydrate has a marked effect on the intestinal flora of animals. Early studies on the subject are well reviewed by Rettger and Cheplin (1921), who presented further evidence concerning the influence of carbohydrate upon the intestinal microflora. Later studies have confirmed and enlarged upon these earlier reports (Evenson, 1947). From the standpoint of vitamin synthesis, the type of carbohydrate in the diet is an important factor. Elvehjem and Krehl (1947) have reviewed this subject, hence an account of specific reports on the role of carbohydrates upon intestinal biosynthesis of vitamins will be unnecessary. Elvehjem and Krehl state, "One might place the carbohydrates in the following decreasing order in their favorable effect on vitamin requirement: dextrin, starch, lactose, glucose, sucrose, although this varies with different vitamins."

The work presented in this paper was carried out over a period of six months in connection with a nutritional investigation (Couch *et al.*, 1948) that was performed to determine the effects of different carbohydrates upon the intestinal synthesis of biotin by chickens. The microbiological work had as its objective the determination of the influence of various carbohydrates upon the numbers and kinds of microorganisms in the intestinal tract.

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METHODS

Animals. The animals employed were Single Comb White Leghorn pullets which had been reared on the experiment station range. Twenty-four pullets were placed in individual battery laying cages equipped with raised screen bottoms in order to minimize coprophagy. An all-mash laying ration was fed these hens 4 weeks prior to the start of the experiment. The pullets were divided into six groups of four each, and placed on six different diets, five of which were composed of purified ingredients (Couch *et al.*, 1948). The five synthetic rations were numbered B31, B32, B33, B34, and B35. They contained as their sole carbohydrate sucrose, dextrin, lactose plus sucrose, sucrose, and whey plus sucrose (the weight of the whey was adjusted to give the same percentage of lactose as in B33), respectively. Ration B34 contained added biotin and was considered nutritionally adequate; the other diets were biotin-deficient. The sixth group of birds was fed the same all-mash laying ration that they had received during the pre-experimental 4-week period.

Concurrent with the bacteriological work, Couch and collaborators made a nutritional study of the pullets. They kept an account of the egg production and hatchability, the abnormalities of the embryos and hatched chicks, the weekly weight gains of the hens, and the biotin content of the yolks and whites of some of the eggs produced.

Microbiological determinations. Fecal droppings for bacteriological analyses were collected on large pieces of clean waxed paper which were placed beneath the raised screen bottoms. As soon as a dropping was deposited upon the paper, it was removed with an alcohol-flamed spatula and put in a sterile wide-mouthed bottle. All fecal samples were collected between the hours of 7:45 and 9:30 A.M.

As soon as four to six samples were obtained (usually within a 0.5-hour period), the feces were prepared for analyses. One-half gram of each sample was weighed to the nearest milligram on a small piece of clean waxed paper and placed in a 49.5-ml (sterile tap water) 6-ounce dilution bottle containing a half-inch layer of glass beads. These initial 1:100 dilutions were kept on ice for a short time until preparations were completed for their microbiological analyses. The first dilutions were vigorously shaken until all visible clumps were dispersed. Serial decimal dilutions were then made in sterile tap water up to a 10^{-8} or 10^{-10} dilution and inoculations made into the following media: (1) 0.5 per cent tryptone, 0.3 per cent yeast extract, 0.5 per cent glucose, 1.5 per cent agar for aerobic plate counts; (2) "Thioglycolate medium" (Baltimore Biological Laboratories) for total dilution counts (steamed and cooled prior to inoculation); (3) carrot-liver extract ("CL") agar shake tubes (Garey *et al.*, 1941) for an indication of numbers of lactic acid bacteria; (4) "SF" broth of Hajna and Perry (1943) for dilution counts of enterococci; (5) Difco eosin methylene blue agar ("EMB") for coliform plate counts; and (6) potato-glucose agar acidified to pH 3.5 with citric acid (according to *Standard Methods for the Examination of Dairly Products*, 1941) for the enumeration of yeasts. Cultures prepared with media (1), (2), (3), and

(5) were incubated at 37 C; those with medium (4) were incubated at 45 C; and medium (6) cultures were incubated at room temperature. Cultures prepared with media (3) and (4) were incubated for 3 days, those with medium (6) for 5 days, and cultures made with other media were incubated for 2 days before counts were made. Five tubes per dilution were used for all dilution counts, and the most probable numbers of organisms in these samples were determined from the table in *Standard Methods for the Examination of Water and Sewage* (1946). All plates and shake tubes were run in duplicate. Dry weight determinations were made of each sample so that the final count could be expressed on a dry weight basis; this eliminated some of the variations encountered.

Occasional gram stains were made from smears of the 1:100 dilution and examined. No direct counts were made, however, since it was felt that such counts would contribute little to the objective of this investigation.

At the conclusion of the 6-month nutritional study it was possible to sacrifice the animals in order to obtain information on the distribution of the microorganisms within different segments of the intestinal tract. Two hens from each ration group were sacrificed by decapitation, and a posterior ventral incision was made so that the entire intestinal tract could be laid out. Representative 0.5-g samples were removed from the duodenum (a mixture of ascending and descending loops), ileum (from the middle 6 inches), cecal pouches, and colon. These samples were handled in the manner already described for fecal droppings, except that dry weight determinations were not made because many of the samples were insufficient in amount for such a purpose after the aliquot for microbiological procedures had been obtained.

Statistical analysis. Because of extreme variation in the results secured, the data on fecal dropping counts were analyzed statistically according to Snedecor (1946) and Torrie (1948). The data secured from samples taken at different levels of the intestinal tract were believed inadequate for statistical treatment.

For convenience in handling each individual count was converted to its respective logarithm to the base 10 (the mantissa was carried out to the second place). An analysis of variance for counts obtained on each medium was made, and the F value was determined. Since the coliform flora of hens on ration B31 was so obviously different from that of hens on the other five rations, EMB counts of feces from these four pullets were omitted from the analysis of variance in order to allow a more valid test of significance between such counts from hens on the remaining rations. The following statistics were then calculated: (1) arithmetic mean ($\bar{x}$); (2) standard deviation (s); (3) standard error of the mean ($s_{\bar{x}}$); (4) coefficient of variation (*C. V.*); and (5) least significant difference (*LSD*) where the F value was significant at the 1 or 5 per cent level of probability.[4]

In plotting the data graphically, it was decided to use modal values of fecal

[4] Note that all statistics are computed from logarithms, hence their antilogarithms will not be exactly similar to like statistics calculated from natural numbers of individual microbial counts. However, this more convenient method will result in comparable values, i.e., the change will be proportionate (Torrie, 1948).

counts rather than arithmetic means. It was believed that modes would give a more representative picture for comparative purposes since extreme values would be eliminated.

RESULTS

Fecal droppings. The enumerative results of this portion of the experiment may be seen in table 1 and figure 1. Note the similarity of the agar plate and Brewer's dilution counts on feces of birds on the six rations. Differences in fecal counts between hens on the various diets appear to be most prominent in carrot-liver agar shake (CL), SF dilution, eosin methylene blue agar plate (EMB), and yeast counts. The two lactose-containing diets (B33 and B35) appear to stimulate the development of lactic acid and coliform bacteria as indicated by their CL and EMB counts. Ration B31, which contained sucrose as its sole carbohydrate, was very depressing to coliforms. Enterococci appeared to be favored most by the grain and B35 rations. Dextrin (ration B32) favored intestinal coliforms but was somewhat depressing to yeasts.

Analyses of variance for the counts on feces of hens made with the six media indicate that differences between counts from birds on the six rations (five for coliforms) are highly significant (the probability, P, of assuming a significant difference is 0.01 or greater, or, 1:100) in the case of CL, SF, and yeast counts; the differences were only significant ($P = 0.05$) for fecal counts of coliform organisms. Examination of table 2 reveals a high degree of variation between individual counts in any one ration group (within rations variation) for coliform determinations, as indicated by the high value (.246) for s. Also, the $C.\ V.$ for coliforms is very high (55.09 per cent), which would tend to throw some doubt upon the validity of the EMB F value. The remaining $C.\ V.$ values were fairly similar; $s_{\bar{x}}$ values were comparable except in the case of coliform counts.

From results of LSD calculations, CL counts on feces from hens on rations B33 and B35 were highly significantly different from the rest, but not from each other; no significant difference was found to exist between the four low-count CL enumerations. Enterococcus counts revealed two rations which yielded droppings of comparatively high counts: B35 and grain. Fecal SF counts from hens on these two rations were not significantly different from each other or from samples of B34-fed hens. However, they were highly significantly different from SF counts on feces of hens on ration B31, and significantly different from B32- and B33-fed pullets. The LSD of SF counts between rations B31 and B34 was significant, but not between B32- and B34-fed birds. Despite the wide differences shown in fecal coliform counts, the F value was significant only at the 5 per cent level of probability. Since EMB counts of samples from animals fed ration B31 were left out of the analysis of variance for coliforms, LSD's between B31-fed chickens and any of the other ration groups could not be calculated. However, its difference of greater than 2.5 logarithms from the next lowest group (grain) would indicate that these counts differ highly significantly from coliform counts obtained from hens on any one of the other five diets; most of the $LSD_{.01}$'s for coliforms ranged from 2.2 to 2.3 + logarithm units. The two high-count coliform groups (B32 and B33) gave an LSD value which was not

TABLE 1

Summary of microbial counts of fecal droppings from hens

MEDIUM	RATION	AVERAGE	MODE	NO. OF SAMPLES
CL		(10^5)*	(10^5)	
	B31	6,675.0	4,160.0	18
	B32	3,684.0	3,199.7	17
	B33	109,014.4	128,777.0	13
	B34	2,936.6	2,548.5	17
	B35	52,225.8	49,084.0	13
	Grain	6,504.6	3,134.3	17
Brewer's dilutions		(10^6)	(10^6)	
	B31	2,573.0	1,550.8	16
	B32	1,559.2	2,012.4	16
	B33	1,453.1	929.5	12
	B34	1,603.2	897.7	15
	B35	4,812.7	1,604.0	11
	Grain	1,395.4	1,138.4	18
SF		(10^4)	(10^4)	
	B31	107.1	123.5	7
	B32	534.7	293.6	8
	B33	819.2	561.7	7
	B34	1,747.9	787.6	9
	B35	6,758.4	2,651.5	7
	Grain	4,348.6	2,469.3	8
Agar plate		(10^5)	(10^5)	
	B31	4,082.1	1,875.0	20
	B32	2,331.0	2,003.0	18
	B33	2,287.6	1,873.7	14
	B34	1,840.1	2,532.8	17
	B35	5,755.0	3,042.1	13
	Grain	10,127.4	5,150.0	19
EMB		(10^4)	(10^4)	
	B31	0.06	0.01	20
	B32	4,078.4	1,210.2	18
	B33	2,592.1	839.9	14
	B34	1,328.0	6.5	17
	B35	397.6	64.8	12
	Grain	78.9	3.5	18
Acidified potato-glucose agar		(10^4)	(10^3)	
	B31	2,785.0	2,785.0	7
	B32	382.8	16.6	7
	B33	3,994.4	3,163.1	6
	B34	4,896.0	3,847.0	7
	B35	5,160.5	3,386.6	6
	Grain	99.7	169.6	7

* All counts are expressed in numbers per gram of dry weight; the average moisture content was about 80 per cent.

significant. Neither B32- nor B33-fed birds yielded fecal droppings which were found to differ significantly in numbers of coliforms from those of chickens fed

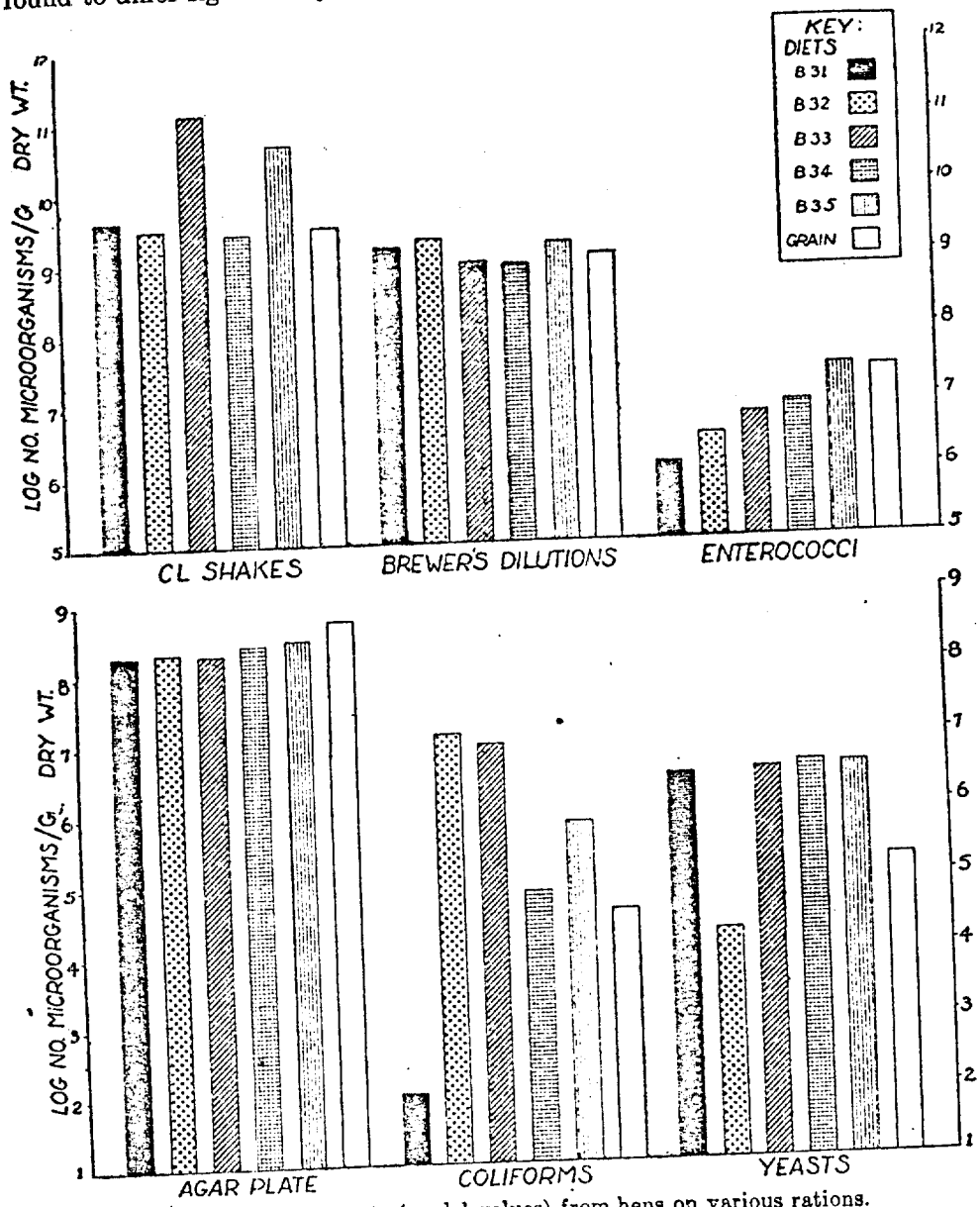

Figure 1. Fecal counts (modal values) from hens on various rations.

B35, but their *LSD*'s were significant when compared with B-34 hens. The following comparisons resulted in the finding of no significant differences in numbers of fecal coliforms: B34 and grain; B35 and grain; and B34 and B35. The grain and B32 rations were found to be least stimulatory to the growth of

yeasts. *LSD* calculations revealed that the difference between yeast counts of feces from hens fed the latter diets was not significant, and that fecal yeast counts on hens fed rations B31, B33, B34, and B35 were not significantly different from one another. However, the feces of chickens on any of the latter four rations had average yeast counts which differed significantly from those fed B32 or grain.

Difficulty was encountered in isolation of lactobacilli from the fecal samples, but enterococci could be isolated with ease from CL shakes and SF dilution tubes. Numerous gram stains of representative colonies appearing in CL tubes revealed cells morphologically similar to lactobacilli along with large cocci. Therefore, it was believed that CL counts, particularly at the higher dilutions, rep-

TABLE 2

Summary of statistical analyses of enumerative data from fecal droppings of hens on various rations

RATION	TYPE OF COUNT					
	Coliforms	Yeasts	Agar plate	Brewer's	Enterococci	CL agar shakes
B31	—	6.27[1]	8.10	8.78	5.76	9.35
B32	5.35	3.84	8.06	8.97	5.96	9.33
B33	5.55	6.35	7.88	8.59	6.16	10.58
B34	3.39	6.49	7.97	8.71	6.66	9.03
B35	4.74	6.55	8.33	9.06	7.42	10.21
Grain	3.56	4.40	8.30	8.44	7.07	9.33
STATISTICS[2]						
$\bar{x}$	4.46	5.61	8.11	8.75	6.51	9.58
s	2.46	1.02	0.73	0.84	0.88	0.86
$s_{\bar{x}}$	0.28	0.16	0.07	0.09	0.13	0.09
$C.V.$	55.09%	18.20%	8.94%	9.64%	13.57%	8.99%
F	2.725*	9.404**	0.944	1.124	4.040**	7.095**

[1] Average logarithm of the logarithm for each individual count.

[2] $\bar{x}$ = arithmetic mean; s = standard deviation; $s_{\bar{x}}$ = standard error of the mean; $C.V.$ = coeficient of variation; F = F value (*–F significant at the 5 per cent point; **–F significant at the 1 per cent point).

resented a fairly accurate enumeration of lactic-acid-producing bacteria in the feces. When colonies appearing in such shake tubes were picked and transferred into CL broth, the gram-positive, rod-shaped bacteria rarely "came up" after incubation for as long as one week at 37 C. The cocci grew in nearly all cases. The few lactobacilli which were isolated were anaerobic to microaerophilic, and reduced litmus, produced acid, and coagulated skim milk within 24 to 36 hours at 37 C. Because of the difficulty in isolating and maintaining these lactobacilli, no further study was made of them. The enterococci were not studied to any great extent, but those encountered in the feces of young chicks have been partially characterized and found to be *Streptococcus faecalis* (Johansson and Sarles, 1948).

Coliform bacteria appeared to be predominately of the *E. coli* and inter-

TABLE 3

Summary of microbial counts at four levels of the intestinal tract

MEDIUM	LEVEL OF INTESTINE				
	Ration	Colon	Cecum	Ileum	Duodenum
CL		(10^6)	(10^6)	(10^6)	(10^6)
	B31	220	3,950	36.2	11.5
	B32	650	5,150	113.0	4.9
	B33	626	8,500	31.2	12.0
	B34	280	905	35.0	10.4
	B35	1,567	2,100	17.7	4.7
	Grain	1,725	14,000	12.5	0.4
Brewer's dilutions		(10^6)	(10^5)	(10^6)	(10^6)
	B31	192	2,950	28.0	79.0
	B32	721	6,350	185.0	9.3
	B33	54	177	24.9	12.0
	B34	262	560	9.0	0.2
	B35	2,315	1,140	1.2	0.9
	Grain	250	4,770	1.9	0.3
SF		(10^4)	(10^4)	(10^4)	(10^4)
	B31	1,100.0	239.0	280.0	0.02
	B32	2,300.0	350.0	300.0	18.7
	B33	—	—	—	—
	B34	—	—	—	—
	B35	57.0	4.1	80.0	17.5
	Grain	2.8	259.0	4.8	3.3
Agar plate		(10^5)	(10^5)	(10^5)	(10^5)
	B31	665	242	157.0	37.0
	B32	4,800	4,620	5,070.0	80.0
	B33	11	25	1.2	0.5
	B34	688	119	160.0	36.0
	B35	450	191	31.1	6.8
	Grain	90	490	145.0	4.0
EMB		(10^4)	(10^4)	(10^4)	(10^4)
	B31	0.008	0.55	0.008	0*
	B32	37.59	578.5	6.2	0.24
	B33	1.97	23.61	0.001	0.008
	B34	0.008	0.04	0.01	0.01
	B35	214.0	50.0	0.007	0.005
	Grain	0*	0.68	0*	0*
Acidified potato-glucose agar		(10^3)	(10^3)	(10^3)	(10^3)
	B31	80	15.0	94	8.8
	B32	2,220	287.0	4,600	59.0
	B33	103	16.0	10	10.0
	B34	4,200	137.0	53	149.0
	B35	460	10.7	111	19.0
	Grain	5	8.2	1	0.05

Each count is the average of two samples and is given on a fresh weight basis.

* Count of 0 means no colonies at the 10^{-2} dilution.

mediate types; only an occasional typical *A. aerogenes* colony was observed on EMB plates from any fecal sample.

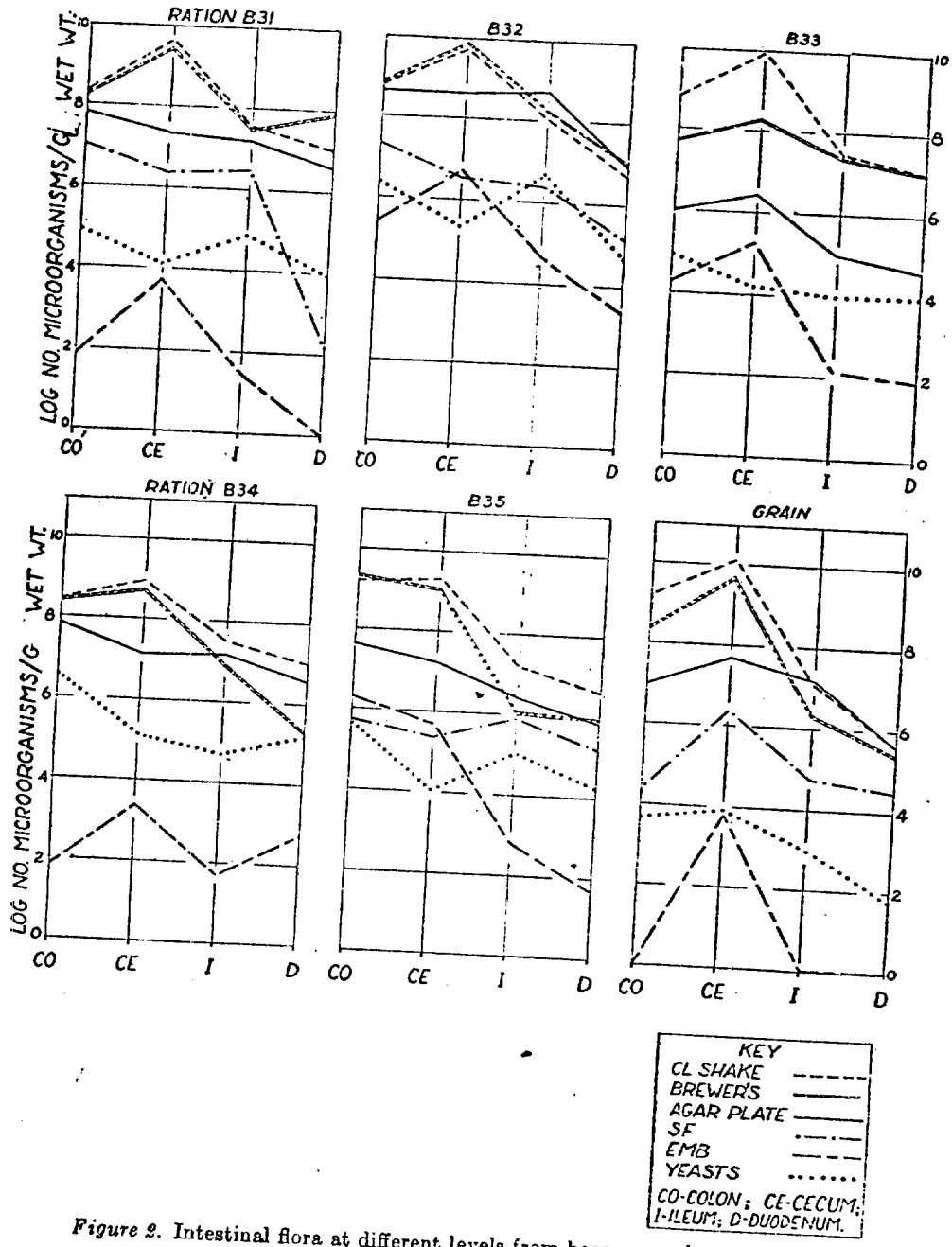

Figure 2. Intestinal flora at different levels from hens on various rations.

Seventy yeast cultures were isolated from the acidified potato-glucose agar plates. All grew well at 37 C, 14 produced a coral red pigment, 13 fermented dextrin, and 2 fermented lactose. However, those isolates able to ferment

lactose or dextrin did not necessairly come from fecal samples of birds consuming a lactose or dextrin diet.

It is believed that Brewer's dilution counts gave a fair indication of the anaerobic flora in fecal samples. Frequently, some of the highest dilution tubes evidenced growth indicative of a pure culture. Pleomorphic, gram-positive rods were isolated from such tubes but grew with difficulty and soon were lost.

Intestinal contents. Inspection of table 3 and figure 2 reveals an over-all increase in numbers of bacteria from the duodenum to the colon, and points to the cecum as the seat of the greatest concentration of microorganisms. In every case, CL counts reached a peak in the cecum and abruptly decreased between the cecum and duodenum. A difference in CL counts of intestinal contents between the lactose-fed hens and those from hens on the other rations was not apparent despite the fact that marked differences had been noted in the fecal droppings from these birds. However, on the basis of two samples for each ration, a difference is difficult to show, and might not be statistically significant even if it appeared to be obvious. Brewer's dilution counts paralleled CL counts at a lower level except in birds fed B32; in them Brewer's counts were slightly higher than CL counts.

Enterococcus counts were not obtained from hens on rations B33 and B34 because of incubator troubles. Only in birds on diet B31 was there a striking drop in numbers of enterococci from the colon to the duodenum. This decrease is but slight in the tract of hens fed B32; birds on B35 or grain showed no such decrease. Agar plate counts generally followed those obtained with CL and Brewer's media, but at a lower level, and with less differences between segments of the tract.

Coliforms increased in numbers going down the intestinal tract (from the duodenum to the cecum) except in hens on the "complete" synthetic ration (B34); in these birds coliforms were present in almost as great numbers in the duodenum as in the cecum, but the total numbers were comparatively low. No coliforms were detected in the duodenum of hens on B31, nor in the colon, ileum, or duodenum of hens on grain. Hens on dextrin (B32) maintained the richest flora of coliforms at all levels, whereas B31- and grain-fed birds possessed meager populations of these organisms.

Yeasts were found at all levels of the intestinal tract of hens on each ration. Only a very slight increase was found in samples from the duodenum to the colon. It is interesting to observe that pullets fed the dextrin ration had considerably more yeasts in their intestines than were found in fecal dropping samples. Only in hens fed the all-mash diet did yeast counts drop below 10^4 per gram at any level.

DISCUSSION

Fecal droppings. Several differences between the fecal flora of hens on the six rations might be considered important: (1) the extremely low numbers of coliforms in samples from hens on the sucrose-containing ration B31; (2) the relatively low numbers of yeasts in droppings from birds on the dextrin ration (B32);

and (3) the very high carrot-liver extract agar shake tube counts on fecal samples of pullets on rations B33 (lactose + sucrose) and B35 (whey + sucrose). Other differences were statistically significant, e.g., enterococcus counts and those between coliform counts of hens on rations other than B31 (sucrose).

The factor which interfered most with this study was that of variation between individual counts from hens on any one ration; even between counts from the same hen from week to week the variation was considerable. For example, the lowest coliform count obtained from hens on ration B32 was less than 100 per gram, whereas the highest was $49{,}200 \times 10^4$ per gram, dry weight (average $= 4{,}078.4 \times 10^4$; mode $= 1{,}210.2 \times 10^4$). Thus, statistical treatment of these data was necessary. The reason for such extreme variation is not known. At first it was believed that differences in water content of the fecal samples might increase the variation; hence all counts were expressed on the dry weight basis. This reduced the variation somewhat. A further step was instituted, and counts were determined on the basis of the dry organic matter content of the samples. This failed to alter appreciably the variation. A more likely explanation lies in the source of the fecal dropping taken for analysis. Cecal pouch contents are usually evacuated in the morning shortly after sunrise, and in the evening shortly before sunset. Therefore, a cecal dropping, which has a characteristic appearance, was not uncommon at the time of collecting (around 8:00 A.M.). The cecum appears to be the site of the greatest concentration of intestinal microorganisms. Therefore, a cecal sample, or a mixed cecal sample, would be expected to deviate in its microbial content from that of a usual colon sample. It is questionable that this can account for all of the variation observed. Other factors probably are involved, e.g., the time of day, time elapsed since last feeding, individual variation between birds under identical conditions, and experimental error.

Least significant differences, which were calculated only from counts on those media showing significant F values, give an indication as to the degree of significant difference that can be detected with the various media employed. For instance, at the 5 per cent point, the difference in coliform counts between samples from hens fed the six diets had to be approximately log 1.8 (nearly a 100-fold difference) to be considered significant. It was found, thus, that unless a difference is anywhere from 8-fold (for CL counts) to 100-fold (for EMB counts), it cannot be detected by the methods employed in this study.

The manner in which the data for fecal droppings were handled does not permit an inspection for trends. Only one definite trend was observed: a gradual increase in numbers of yeasts in feces from hens on ration B32 (dextrin) at the close of the study. At one point during this increase a tremendous development of red-pigmented yeasts was noticed, almost to the exclusion of the more common white varieties. However, the predominance of pigmented yeasts soon subsided to a point at which their occurrence was more sporadic, as found in the other samples, but the yeast flora of these dextrin-fed hens then remained at the higher level (note the high level of yeasts in the various levels of the tracts of these birds).

The results of Couch *et al.* (1948) suggested that the only birds receiving sufficient biotin, either by ingestion or biosynthesis, were those on rations B32, B34, and grain. Therefore, it must be concluded that the carbohydrate, dextrin, exerted a pronounced stimulatory effect upon microbial synthesis of biotin. The dextrin-fed hens possessed very high numbers of coliform organisms in their fecal droppings; coliforms are notably good biotin synthesizers (Landy and Dicken 1941; Landy *et al.*, 1942; Thompson, 1942; Burkholder and McVeigh, 1942). Also, these chickens were found to have relatively small numbers of fecal lactic acid bacteria, which are known to have high requirements for B vitamins. One might therefore conclude that dextrin encouraged the development of a biotin-synthesizing intestinal flora and discouraged the proliferation of a biotin-utilizing flora.

The stimulatory nature of dextrin on the synthesis of B vitamins in other animals is well known, but sucrose and lactose have usually been found to be of no particular value in establishing a vitamin-synthesizing flora in various experimental animals on purified rations (Elvehjem and Krehl, 1947). Hens fed lactose (B33) or whey (B35) also had a rich fecal coliform flora. However, they were found also to have large numbers of lactic acid bacteria. A competitive microbial equilibrium for biotin might have existed in these hens. In the case of whey, the minerals as well as lactose might have exerted some influence upon biotin-synthesizing bacteria (Daniel and Harvey, 1947). Sucrose was extremely depressing to coliform organisms; the majority of determinations resulted in the finding of fewer than 100 coliforms per gram in fecal droppings from these hens. It is difficult to understand why sucrose-fermenting coliforms such as *A. aerogenes* and *E. coli* var. *communior* did not establish themselves in these birds.

Pullets on the grain and B34 rations appeared to have a more balanced fecal flora, i.e., no one group of microorganisms was strikingly high or low in numbers.

There is already fair evidence of biotin synthesis in animals (McElroy and Jukes, 1940; Wegner *et al.*, 1941; Nielsen *et al.*, 1942; Mitchell and Isbell, 1942; McGregor *et al.*, 1947); and in humans (Gardner *et al.*, 1943, 1945, 1946; Oppel, 1942). It can be assumed, then, that the hens on the purified biotin-deficient diet containing dextrin as the sole carbohydrate were able to synthesize and assimilate adequate amounts of biotin; the source of such biotin is apparently from metabolic activities of intestinal microorganisms, perhaps those belonging to the coliform group.

One unknown quantity is that of strain difference among biotin-synthesizing microorganisms. Chickens kept on the two lactose-containing diets may have had few strains of coliforms able to synthesize appreciable amounts of biotin. Chemical and biological antagonisms should be considered also when evaluating the over-all influence of the intestinal flora on the nutrition of the host.

The carrot-liver extract agar of Garey *et al.* (1941) was by no means selective. However, lactic acid bacteria were present in such large numbers that, in effect, a lactic count was obtained as the result of dilution. The difficulty in isolation of lactobacilli might be indicative of large numbers of the anaerobic type, *Lacto-*

bacillus bifidus. It is not known whether the Brewer's medium dilution counts indicated actual numbers of anaerobic bacteria in the samples except that anaerobes could be isolated from this medium. Agar plates incubated aerobically also provided little qualitative information; colonies of sarcinae, micrococci, actinomycetes, bacilli, coliforms, and other types of microorganisms were encountered; it is doubtful that lactobacilli developed in these plates as prepared.

Intestinal contents. A brief survey of these data reveals increasingly greater numbers of intestinal microorganisms from the duodenum to the cecum with a slight decrease from the latter segment to the colon. This agrees with the early work on the intestinal flora of chickens and other birds (Rahner, 1901; King, 1905; Gage, 1911).

The duodenum of all hens studied contained very few coliform organisms. In hens on rations B31 or grain no coliforms were found in the 10^{-2} dilution of duodenum contents (recorded as zero). Yeasts were found in relatively large numbers in the duodenum of all birds except those consuming the grain mash, and increased the least of any of the organisms involved in this study from the duodenum to the colon. Enterococci also were encountered in relatively high numbers in the duodenum; these results agree to some extent with those of Kendall and Haner (1924), who claimed that *Micrococcus ovalis* (*Streptococcus faecalis*) is present in the largest numbers in the duodenum of the human intestine. Agar plate, Brewer's dilution, and CL counts indicated approximately the same concentration of microorganisms in the duodenum.

The flora of the ileum was very similar to that of the duodenum except that it appeared to contain slightly greater numbers of bacteria. No coliforms were found in the ileum of hens on the grain diet.

Colon counts were somewhat lower than cecal counts and slightly higher than ileum counts. The importance of the colon in the chicken in relation to intestinal synthesis is uncertain. It is a very small segment of the intestinal tract wherein fecal material resides for but a brief time before evacuation. Vitamins elaborated in this segment may be very incompletely absorbed by the host before being passed out of the tract. On the other hand, the cecum, which in the avian species has two large lobes, is a reservoir which is emptied usually twice a day; it may thus be of great importance in the synthesis and absorption of biotin as well as other B vitamins. Cecal counts were found to be the highest of any segment of the tract in this work. This is particularly true in the case of CL, Brewer's dilution, and coliform counts. Coliforms were found only in the ceca of birds on grain. The latter finding may have a bearing on the role of the cecum in intestinal synthesis because of the ability of coliforms to synthesize a wide variety of B vitamins, including biotin.

Birds on diet B32 (dextrin) generally had the greatest numbers of microorganisms at all levels of the intestinal tract as well as the least "spread" between the different counts. The latter might be indicative of a more heterogeneous flora in these birds.

It was hoped that the study of the microflora at different levels of the intestines

of these hens would yield results that might lend themselves, in part at least, to an explanation of the findings of the biochemists (Couch *et al.*, 1948). It is believed that the high concentration of bacteria in the cecum points to this organ as the focus of intestinal B vitamin synthesis, and we might assume that biotin was synthesized in the ceca of hens on the dextrin-containing ration. Several good studies on the rat indicate, in fact, that the cecum is the site of the greatest synthesis of B vitamins: Guerrant *et al.*, 1935; Mitchell and Isbell, 1942; Taylor *et al.*, 1942; Day *et al.*, 1943; McGregor *et al.*, 1947. The ingenious method of Mitchell and Isbell revealed the rat's cecum to absorb the greatest proportion of the various B vitamins synthesized therein by bacteria. Is the chicken similar to the rat in this respect?

Digestive enzymes might be intimately concerned with the stimulatory nature of dextrin and the depressing effect of sucrose and lactose toward biosynthesis of vitamins. Dextrin may be incompletely attacked by digestive enzymes, thus permitting a residual amount of fermentable carbohydrate to reach the cecum; this carbohydrate may be vital for vigorous vitamin synthesis. Sucrose and lactose, which are relatively soluble disaccharides, may be completely assimilated by the time peristalsis brings the ingesta into the cecum, and the consequent lack of carbohydrate may decrease the vitamin-synthesizing activities of cecal organisms.

SUMMARY AND CONCLUSIONS

The type of dietary carbohydrate was found to influence the microflora of fecal droppings from laying pullets ingesting a purified biotin-deficient ration. Dextrin was found to stimulate the development of considerable numbers of coliform bacteria. Lactose-containing diets likewise encouraged a fecal coliform flora, but lactic acid bacteria proliferated extensively in the intestines of hens fed such a diet. The most marked effect noted in case of the sucrose diet was a depressing action on fecal coliforms.

In general, intestinal microorganisms were found to increase in numbers from the duodenum to the cecum; yeasts and enterococci increased the least. Birds on the dextrin-containing diet appeared to have the greatest numbers of microorganisms at all levels of the intestinal tract as well as the least "spread" between the different counts. The cecum was found to be the site of the greatest concentration of intestinal microorganisms.

An analysis of variance was successfully applied to the data from fecal dropping samples. This revealed the presence of certain differences that were further detected by least significant difference calculations.

REFERENCES

Burkholder, P. R., and McVeigh, I. 1942 Synthesis of vitamins by intestinal bacteria. Proc. Natl. Acad. Sci. U. S., 28, 285–289.

Couch, J. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G. 1948 Relation of carbohydrate to intestinal synthesis of biotin and hatchability in mature fowl. J. Nutrition, 35, 67–72.

Daniel, F. K., and Harvey, E. H. 1947 Some observations on the nutritional value of dialyzed whey solids. J. Nutrition, 33, 429–436.

DAY, H. G., WAKIM, K. G., KRIDER, M. M., AND O'BANION, E. E. 1943 Effects of cecectomy, succinylsulfathiazole, and *p*-aminobenzoic acid on vitamin K synthesis in the intestinal tract of rats. J. Nutrition, **26**, 585–600.

ELVEHJEM, C. A., AND KREHL, W. H. 1947 Imbalance and dietary interrelationships in nutrition. J. Am. Med. Assoc., **135**, 279–287.

EMMEL, M. W. 1930 Bacterial flora in feces of the normal fowl. J. Infectious Diseases, **46**, 293–297.

EVENSON, ADELAIDE E. 1947 The intestinal flora of laboratory animals and its modification by diet and drugs. Unpublished Ph.D. thesis, University of Wisconsin.

GAGE G. E. 1911 A study showing bacteria and animal organisms determined in the feces and intestinal mucosa of healthy chickens. Maryland Agr. Expt. Sta., Bull. **153**, 201–226.

GARDNER, JOSEPHINE, NEAL, A. L., PETERSON, W. H., AND PARSONS, H. R. 1943 Biotin, pantothenic acid and riboflavin balances of young women on a milk diet. J. Am. Dietet. Assoc., **19**, 683–684.

GARDNER, JOSEPHINE, PARSONS, H. T., AND PETERSON, W. H. 1945 Human biotin metabolism on various levels of biotin intake. Arch. Biochem., **8**, 339–348.

GARDNER, JOSEPHINE, PARSONS, H. T., AND PETERSON, W. H. 1946 Human utilization of biotin from various diets. Am. J. Med. Sci., **211**, 198–204.

GAREY, J. C., FOSTER, E. M., AND FRAZIER, W. C. 1941 The bacteriology of brick cheese. I. Growth and activity of starter bacteria during manufacture. J. Dairy Sci., **24**, 1015–1025.

GUERRANT, N. B., DUTCHER, R. A., AND TOMEY, L. F. 1935 The effect of the type of carbohydrate on the synthesis of the B vitamins in the digestive tract of the rat. J. Biol. Chem., **110**, 233–243.

HAJNA, A. A., AND PERRY, C. A. 1943 Comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am. J. Pub. Health, **33**, 550–556.

JOHANSSON, K. R., AND SARLES, W. B. 1948 Bacterial population changes in the ceca of young chickens infected with *Eimeria tenella*. J. Bact., **56**, 635–647.

KENDALL, A. I., AND HANER, R. C. 1924 *Micrococcus ovalis*. LXX. Studies in bacterial metabolism. J. Infectious Diseases, **35**, 67–76.

KERN, H. 1897 Beitrag zur Kenntnis der im Darme und Magen der Vogel vorkommenden Bacterien. Arbeiten Bact. Inst. Tech. Hochschule Karlsruhe, **1**, 379–532.

KING, W. E. 1905 The bacterial flora of the intestinal mucosa and conjunctiva of the normal chicken. J. Am. Med., **10**, 400–404.

LANDY, M., AND DICKEN, D. M. 1941 Biotin synthesis by microorganisms. Proc. Soc. Exptl. Biol. Med., **46**, 449–452.

LANDY, M., DICKEN, D. M., BICKING, M. M., AND MITCHELL, W. R. 1942 Use of avidin in studies on biotin requirement of microorganisms. Proc. Soc. Exptl. Biol. Med., **49**, 441–444.

MCELROY, L. W., AND JUKES, T. H. 1940 Formation of the anti egg-white-injury factor (biotin) in the rumen of the cow. Proc. Soc. Exptl. Biol. Med., **45**, 296–297.

MCGREGOR, MARGARET A., PARSONS, H. T., AND PETERSON, W. H. 1947 Biotin balance in the albino rat. J. Nutrition, **33**, 517–527.

MENES, E., AND ROCHLIN, E. 1929 Darmmikroflora des Hausgeflugels. Zentr. Bakt. Parasitenk., I, Orig., **113**, 321–324.

MITCHELL, H. K., AND ISBELL, E. R. 1942 Intestinal bacterial synthesis as a source of B vitamins for the rat. Univ. Texas Pub. No. 4237, 125–134.

NIELSEN, E., SHULL, G. M., AND PETERSON, W. H. 1942 Response of bacteria, yeast and rats to peroxide-treated biotin. Intestinal synthesis of biotin in the rat. J. Nutrition, **24**, 523–533.

OPPEL, T. W. 1942 Studies of biotin metabolism in man. Am. J. Med. Sci., **204**, 856–875.

RAHNER, R. 1901 Bakteriologische Mitteilungen uber die Darmbakterien der Huhner. Zentr. Bakt. Parasitenk., I, Orig., **30**, 239–244.

REITGER, L. F., AND CHEPLIN, H. A. 1921 A treatise on the transformation of the intestinal flora with special reference to the implantation of *Bacillus acidophilus*. Yale University Press, New Haven, Conn.

SNEDECOR, G. W. 1946 Statistical methods applied to experiments in agriculture and biology. 4th ed. Iowa State Coll. Press, Ames, Iowa.

Standard methods for the examination of dairy products. 1941 8th ed. Am. Pub. Health Assoc., New York, N. Y.

Standard methods for the examination of water and sewage. 1946 9th ed. Am. Pub. Health Assoc., New York, N. Y.

TAYLOR, A., PENNINGTON, D., AND THACKER, J. 1942 The vitamin requirements of cecectomized rats. Univ. Texas Pub. No. 4237, 135–144.

THOMPSON, R. C. 1942 Synthesis of B vitamins by bacteria in pure culture. Univ. Texas Pub. No. 4237, 87–96.

TORRIE, J. H. 1948 *Personal communication.*

WEGNER, M. I., BOOTH, A. N., ELVEHJEM, C. A., AND HART, E. B. 1941 Rumen synthesis of the vitamin B complex on natural rations. Proc. Soc. Exptl. Biol. Med., 47, 90–94.

GRAPHY

ysiol., 25, 167 (1941).
1933).
m., 125, 115 (1938).
chem. J., 24, 993 (1930).
Compt.-rend. trav. Lab. Carlsberg, 12, 1

Compt.-rend. trav. Lab. Carlsberg, Série

1 (1939).
pt.-rend. trav. Lab. Carlsberg, 19, 1 (1933)
938).
9, 77 (1916).
938).
).
arlsberg, Série chim., 22, 487 (1938).
H., Kassell, B., and Ryan, F. J., J. Am.

em., 125, 435 (1938).
r, M. Proc. Am. Soc. Biol. Chem., J. Biol.

., J. Biol. Chem., 125, 415 (1938).
., 145, 365 (1942).
l. Chem., 151, 635 (1943).
., 31, 292 (1937).
Chem., 251, 14 (1938).
. Physiol., 18, 307 (1934).

J Biol Chem, 166, 1946

143

RELATION OF AMINO ACID IMBALANCE TO NIACIN-TRYPTOPHANE DEFICIENCY IN GROWING RATS*

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The growth inhibition which results from the inclusion of corn in a low protein, niacin-deficient ration (1) and the apparent dual rôle of niacin and tryptophane in counteracting this growth depression have resulted in considerable study on the mechanism of these relationships. It has been indicated that intestinal microorganisms may play a significant part in this deficiency syndrome, since the deleterious effect of corn can be greatly modified by the use of carbohydrates previously shown to favor intestinal synthesis. The suggestion has also been made that corn might contain a substance structurally analogous to niacin which would compete with this vitamin (2). The observation that tryptophane administration results in the increased excretion of niacin and niacin derivatives (3) indicates that the similar growth-promoting activity of niacin and tryptophane may be due to the fact that tryptophane functions as a metabolic precursor of niacin. The fact that niacin synthesis is greater on a synthetic ration than when corn is added, despite the fact that the former ration contains slightly less tryptophane than the latter (1), does not lend credence to the explanation that tryptophane acts as a precursor of niacin under these conditions. It has been demonstrated, however, that niacin may improve the animal's production or utilization of tryptophane (4). If the synthesis

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The 3-acetylpyridine hydrochloride and the pyridine-3-sulfonic acid used in these experiments were supplied by Dr. F. M. Strong, Biochemistry Department, and Dr. S. M. McElvain, Chemistry Department, University of Wisconsin, respectively.

(3)

of niacin is impaired or if niacin is supplied in inadequate amounts, the dietary requirement for tryptophane is increased.

Considerable evidence is at hand to show that the chief protein of corn (*i.e.*, zein), other proteins such as gelatin, and acid-hydrolyzed proteins, free of tryptophane, create a syndrome exactly analogous to that produced by corn (5). These results indicate that the growth depressions caused by corn and the non-corn-containing rations which contain tryptophane-free proteins have a common basis; namely, an imbalance of amino acids.

Attempts have been made in the present study to extend our information concerning the deleterious action of corn grits and the counteraction by various materials and to elucidate the nature of the amino acid imbalance conducive to the growth failure with rations which contain added protein or protein hydrolysates free of tryptophane.

EXPERIMENTAL

The composition of the diets used is given in Table I. The vitamins were added in the following amounts per 100 gm. of ration: thiamine 0.2 mg., riboflavin 0.3 mg., pyridoxine 0.25 mg., calcium pantothenate 2.0 mg., choline 100 mg., 2 methyl-1,4-naphthoquinone 0.1 mg., inositol 10 mg., and biotin 0.01 mg. Vitamins A and D were supplied as halibut liver oil, 2 drops every week, together with α-tocopherol, 1 mg. per rat per week. Unless otherwise indicated, the basal rations contained practically no nicotinic acid. Weanling male rats of the Sprague-Dawley strain were used in all cases and each group contained three rats.

In an effort to determine whether an insoluble sulfonamide would inhibit the effect of niacin in corn grits rations, sulfasuxidine was added with and without niacin. Synthetic *Lactobacillus casei* factor was also added at a level of 25 γ per 100 gm. of ration, since a deficiency of this factor is known to occur in the rat in the presence of sulfonamides (6). The results for Groups 1 and 2 in Table II show that sulfasuxidine does not counteract the beneficial action of niacin. In a similar experiment, *streptothricin* was added to the corn grits ration at a level of 25,000 units per 100 gm. of ration and it likewise failed to impair the beneficial action of niacin. If it is assumed that niacin favors the intestinal synthesis of tryptophane it appears that the above drugs did not impair the activity of organisms responsible for this synthesis. This is not surprising in view of the fact that these drugs have been shown to alter the synthesis of some but not all factors.

In an experiment to test the value of natural foodstuffs in counteracting the action of corn grits two rations were devised containing peanuts and brewers' yeast respectively. These rations were prepared by reducing the casein content so that the tryptophane level was approximately the same as

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Compositio

Ration constituent	1
Sucrose	78
Dextrin	
Casein*	15
Gelatin	
Zein	
Fibrin hydrolysate	
Casein "	
Egg albumin hydrolysate	
Peanuts (ground)	
Brewers' yeast	
Corn grits	
" oil	3
Salts IV†	4
l(−)-Cystine	0.2

* Crude casein extracted three times
† Phillips, P. H., and Hart, E. B., *J*

therefore, according to the metho
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that in the usual corn grits ration. In this manner the effectiveness of these supplements could be tested, based on their niacin content, which for the amounts used was about 1.5 mg. per cent for both the peanut and brewers' yeast rations. The results shown in Table II, Groups 3 and 4, indicate the beneficial value of these supplements in the corn grits diet.

In a report on the nutritive value of the Mexican tortilla (7), it was indicated that this food, made from corn, provided a major portion of the Mexican dietary. Pellagra, while not unknown in Mexico, does not appear extensively as a result of this diet. Tortillas were prepared,

TABLE I

Composition of Basal Ration

Ration constituent	Ration No.								
	1	2	3	4	5	6	7	8	9
Sucrose	78	84		38	43		82	82	82
Dextrin			84			73			
Casein*	15	9	9	7	8	9	9	9	9
Gelatin						6			
Zein						3			
Fibrin hydrolysate						2	2		
Casein "								2	
Egg albumin hydrolysate									2
Peanuts (ground)				10					
Brewers' yeast					4				
Corn grits				40	40				
" oil	3	3	3	2	2	3	3	3	3
Salts IV†	4	4	4	3	3	4	4	4	4
l(−)-Cystine	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

* Crude casein extracted three times with boiling 95 per cent ethanol.

† Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 109, 657 (1935).

therefore, according to the method described by Cravioto (7), dried, and added to the basal ration in the usual manner. It can be seen that dried tortillas inhibited growth just as severely as corn grits (Table II, Group 6) and that niacin completely counteracted this inhibition. Since the most significant difference between the Mexican tortilla and whole yellow corn is the much higher calcium content of the tortilla, the yellow corn ration was supplemented with 0.5 per cent $CaCO_3$. Marked growth inhibition was again evident (Table II, Group 5), which was corrected with niacin.

In a previous report (1) it was shown that, of the various cereals tested, only corn inhibited growth under the conditions used. Additional experiments with cow peas, which are used extensively in the South, and with

TABLE II

Growth Results on Various Diets

Group No.	Basal ration (Table I)*	Type of ration† and supplement to basal ration	Gain per wk. for 4 wks., 3 rats per group‡
			gm.
1	1	Corn grits 40%	9
2	1	" " 40 % + 2% sulfasuxidine	4 (+N, 29)
3	4	(" " 40%, peanut)	31
4	5	(" " 40%, brewers' yeast)	29
5	1	Yellow corn, 40% + $CaCO_3$, 0.5%	7 (+N, 30)
6	1	Dried tortillas 40%	9 (+N, 27)
7	1	Cow peas 40%	28
8	1	Corn bran 40%	30
9	1	" grits 40% + 5% corn germ	28
10	1	" " 40% + 10% " "	25
11	1	" " 40% + 3% dried pig stomach	27
12	1	" " 40% + 3-CN pyridine 1.5 mg. %	7 (+N, 32)
13	1	" " 40% + indole 125 mg. %	4 (+T, 33)
14	1	" " 40% + anthranilic acid 100 mg. %	7 (+T, 33)
15	1	" " 40% + indole-3-acetic acid 100 mg. %	4 (+T, 31)
16	2	(Sucrose)	15 (+N, 18)
17	3	(Dextrin)	27
18	2	(Sucrose) + pyridine-3-sulfonic acid 0.2%	9 (+N, 15)
19	2	" + 3-acetylpyridine · HCl 0.2%	17
20	6	(Dextrin-casein 9%, gelatin 6%, zein 3%, fibrin hydrolysate 2%)	11 (+N, 32) (+T, 29)
21	3	(Dextrin) + glycine 1%	25
22	3	" + *l*(−)-leucine 1%	24
23	3	" + *l*(+)-glutamic acid 1%	23
24	3	" + glycine, *l*(−)-leucine, and *l*(+)-glutamic acid 1% each	27 (+N, 27) (+T, 24)
25	2	(Sucrose) + glycine 1.5%, *l*(−)-leucine 1%	8 (+N, 18)
26	2	" + *l*(+)-glutamic acid 1.5%, *l*(−)-leucine 1%	12 (+N, 21)
27	2	(Sucrose) + glycine 1.5%, *l*(−)-leucine 1.0%, *l*(−) tyrosine 0.25%, *dl*-phenylalanine 0.5%, *dl*-valine 0.25%	5 (+N, 13)
28	2	(Sucrose) + *l*(+)-glutamic acid 1.5%, *l*(−)-tyrosine 0.25%, *dl*-phenylalanine 0.5%, *dl*-valine 0.25%	11 (+N, 16)

* Supplements of corn grits, yellow corn, dried tortillas, cow peas, and corn bran were added at the expense of the entire basal ration indicated. Other supplements were added at the expense of sucrose, except 3-CN pyridine, indole, anthranilic acid, indole-3-acetic acid; pyridine-3-sulfonic acid, and 3-acetylpyridine hydrochloride which were added to the entire basal ration.

† Parentheses indicate the type of ration.

‡ The letters N and T in parentheses indicate oral supplementation with 0.25 mg. of niacin and 10 mg. of *dl*-tryptophane per rat per day respectively. The accompanying figures are the average weekly gains during the 4th and 5th weeks when supplements were given. The figures outside of the parentheses represent average weekly gains during the first 3 weeks. A control rat from each group was continued on the deficient or niacin-low ration.

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basal ration	Gain per wk. for 4 wks., 3 rats per group:
	gm.
	9
ne	4 (+N, 29)
	31
	29
	7 (+N, 30)
	9 (+N, 27)
	28
	30
	28
	25
omach	27
1.5 mg. %	7 (+N, 32)
%	4 (+T, 33)
100 mg. %	7 (+T, 33)
acid 100 mg. %	4 (+T, 31)
	15 (+N, 18)
	27
id 0.2%	9 (+N, 15)
0.2%	17
ein 3%, fibrin	11 (+N, 32)
	(+T, 29)
	25
	24
%	23
and *l*(+)-glu-	27 (+N, 27)
	(+T, 24)
ucine 1%	8 (+N, 18)
5%, *l*(−)-leu-	12 (+N, 21)
ine 1.0%, *l*(−)	5 (+N, 13)
0.5%, *dl*-valine	
%, *l*(−)-tyrosine	11 (+N, 16)
-valine 0.25%	

d tortillas, cow peas, and corn bran tion indicated. Other supplements 3-CN pyridine, indole, anthranilic acid, and 3-acetylpyridine hydro- ation.

e oral supplementation with 0.25 rat per day respectively. The ac- ns during the 4th and 5th weeks side of the parentheses represent A ol rat from each group was

corn bran show that these materials fed at a level comparable to corn grits produce no growth inhibition (Table II, Groups 7 and 8).

The excellent nutritive quality of corn germ is evident by the fact that when it was included in the corn grits ration at a level as low as 5 per cent (at the expense of sucrose) good growth was obtained. In considering the somewhat surprising effectiveness of such low levels of corn germ it is worth while to note the narrow range in which the level of tryptophane is active in counteracting growth inhibition. For example when wheat gluten and gelatin were used as the protein source (1) and the tryptophane content was 120 mg. per cent, poor growth resulted, but the same ration allowed good growth when the tryptophane level was increased to only 145 mg. per cent. The effectiveness of corn germ may be due in part to the fact that it contributes several other of the amino acids which may be present in the corn grits ration in borderline amounts, although its protective action is perhaps better explained by its higher niacin (*i.e.* about 40 to 45 γ per gm.) and tryptophane content (see Table II, Groups 9 and 10). Dried pig stomach, a good source of both niacin and tryptophane, gave excellent protection against corn grits (see Table II, Group 11).

Certain compounds related to niacin or tryptophane were tested for their ability to counteract the growth deficit caused by corn grits. It has been shown that 3-CN pyridine is unable to cure canine blacktongue (8) and it is clear that it has no niacin activity in the rat, although when niacin was given after 3 weeks to rats receiving 3-CN pyridine a marked growth response was observed (Table II, Group 12). Indole and indole-3-acetic acid were tested for tryptophane activity in counteracting the deleterious effect of corn and found to be completely inactive. No competitive activity of these compounds against tryptophane was observed since added *dl*-tryptophane, at a level of 10 mg. per rat per day, resulted in excellent growth. Anthranilic acid was tested in a similar manner because it is known to be active for certain bacteria (9), but it too was inactive, although it did not prevent the beneficial action of tryptophane. (The results are given in Table II, Groups 13 to 15).

Woolley reported the antiniacin activity of certain compounds related to niacin and showed that 3-acetylpyridine given orally in single test doses created a niacin deficiency in the mouse (10) which was counteracted by large doses of niacin. In a similar experiment with pyridine-3-sulfonic acid, no niacin inhibition was observed even at very high levels of this compound (11).

In the present study 3-acetylpyridine as the hydrochloride was incorporated in the ration at a level of 0.2 per cent with no untoward effects (compare Group 19 with Group 16, Table II). Whether this difference is due to species variation or the somewhat different manner in which the

compound was given is uncertain. When pyridine-3-sulfonic acid was included in the same ration at a level of 0.2 per cent (9 per cent casein, sucrose basal) some growth retardation was observed which was counteracted by niacin (Table II, Group 18). In earlier experiments this compound had been fed to rats at levels up to 3 per cent with no harmful effects. However, at that time we were using a ration containing 20 per cent casein. This difference in behavior of the sulfonamide may be largely due to the difference in the protein level of the rations. Woolley has shown, for example, that the antiniacin action of 3-acetylpyridine can be counteracted with tryptophane (2).

In a continuation of the previous study with non-corn, low protein rations (5) experiments were conducted to study the growth inhibition caused by the addition of proteins or protein hydrolysates free of tryptophane. In verification of previous results (5), dextrin produced better growth than sucrose when the basal ration contained 9 per cent casein (Table II, Groups 16 and 17). However, the presence of dextrin did not protect the rat from the growth inhibition which resulted when tryptophane-free proteins or protein hydrolysates, such as a combination of 6 per cent gelatin, 3 per cent zein, and 2 per cent acid-hydrolyzed fibrin, were added (Table II, Group 20), despite the fact that the nitrogen content was increased to a level corresponding to about 20 per cent protein.

That the growth inhibition induced by acid-hydrolyzed proteins such as fibrin, egg albumin, and casein at levels of 2 per cent in a 9 per cent casein ration can be immediately counteracted by niacin, even after 3 weeks on the inhibitory regimen, is evident in Fig. 1, growth Curves B, C, and D. The control rats on these rations which received no niacin grew very little. It is also clear that when niacin is added the slopes of the growth curves are the same as those obtained with rations containing no supplement of acid-hydrolyzed protein (Fig. 1, Group A). The addition of niacin to the latter ration stimulates growth only slightly.

Inasmuch as one or more of the amino acids, glycine, leucine, and glutamic acid, are present in rather large amounts in the tryptophane-low proteins, zein and gelatin, and in the tryptophane-free acid-hydrolyzed proteins, fibrin and casein, these amino acids were added separately and collectively at a level of 1 per cent each to the 9 per cent casein-dextrin ration. No growth inhibition could be observed from these amino acids under the conditions employed (Table II, Groups 21 to 24). When sucrose was used as the carbohydrate, however, and glycine and $l(-)$-leucine added together at a level of 1.5 and 1 per cent respectively, a growth inhibition was observed which was corrected by the addition of niacin at a level of 250 γ per rat per day. A combination of glutamic acid and leucine at levels of 1.5 and 1 per cent respectively produced slightly less growth than that

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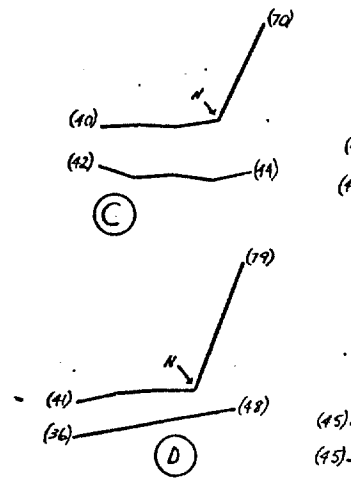

FIG. 1. Growth responses in rat
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obtained with the 9 per cent casein-sucrose basal, but the addition of niacin resulted in marked growth stimulation. The addition of 0.25 per cent $l(-)$-tyrosine, 0.5 per cent *dl*-phenylalanine, and 0.25 per cent *dl*-valine to the above combinations of glycine and $l(-)$-leucine seemed to depress growth still more and the addition of niacin was much less effective in

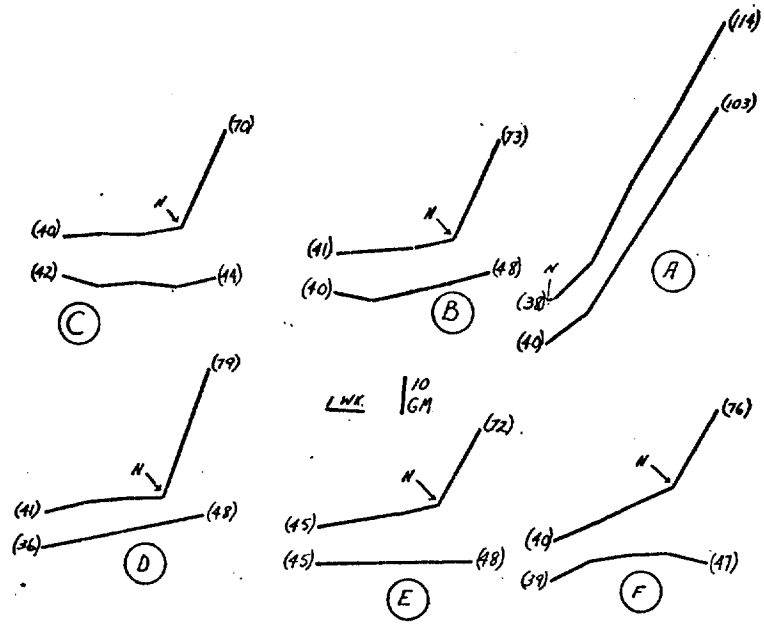

FIG. 1. Growth responses in rats on various diets when supplemented with niacin (*N*) at the indicated point in the growth curve. Group A, 9 per cent casein (sucrose) basal with and without niacin; Group B, 9 per cent casein (sucrose) basal plus 2 per cent acid-hydrolyzed fibrin; Group C, 9 per cent casein (sucrose) basal plus 2 per cent acid-hydrolyzed egg albumin; Group D, 9 per cent casein (sucrose) basal plus 2 per cent acid-hydrolyzed casein; Group E, 9 per cent casein (sucrose) basal plus 6 per cent gelatin; and Group F, 9 per cent casein (sucrose) basal plus 2 per cent glycine.

counteracting this inhibition (for the results consult Table II, Groups 25 to 28).

The growth inhibition caused by 6 per cent gelatin and the counteraction by niacin are shown in Fig. 1, Group E. That glycine is intimately concerned in the amino acid imbalance involved in these studies is indicated by the fact that the addition of 2 per cent glycine to the sucrose-casein ration tends to retard growth (Fig. 1, Group F) and niacin counteracts this retardation. It should be noted, however, that glycine caused very little, if any, growth inhibition when dextrin was used as the carbohydrate.

One of the observations made upon autopsy of rats which received corn

without added niacin was the fact that the livers appeared fatty and friable. When the fat content of these livers was determined, it was found that the animals which received corn had, as a rule, more liver fat than the animals receiving added niacin. On analysis of the livers from the deficient animals for niacin (12) it was found that they contained less of this vitamin than the control animals. That the level of choline has a bearing on the problem of liver fat is evident from the fact (Table III)

TABLE III

Niacin and Fat Content of Rat Livers on Various Diets

Basal ration (Table I)	Supplement to basal ration*	Liver niacin				Liver fat	
		−N†	+N†	−T‡	+T‡	−N†	+N†
		γ per gm.	*γ per gm.*	*γ per gm.*	*γ per gm.*	*per cent*	*per cent*
1	(Sucrose basal) (choline 100 mg. %)	160	165			3.0	2.8
1	+ 40% corn grits	110	164			8.4	3.2
1	+ 40% yellow corn (choline 60 mg. % in ration)	116	146			19	3.3
1	+ 40% corn grits + indole-3-acetic acid			80	136		
1	+ 40% " " + 3-CN pyridine	84	108				
1	+ 40% " " (choline 10 mg. % in ration)	84	120			25	20
1	+ 40% corn grits (choline 30 mg. % in ration)	86	86			22	18
1	+ 40% corn grits (choline 60 mg. % in ration)	110	126			17	9.7
2	(Sucrose) + 3% gelatin	98	105		159		
2	" + 3% zein	77	108				
2	" + 2% acid-hydrolyzed fibrin	97	108		141		

* The parentheses indicate the type of ration.

† −N and +N refer to no niacin in the ration and added niacin at a level of 1.5 mg. per cent.

‡ −T and +T refer to no added tryptophane or added tryptophane at a level of 50 mg. per cent.

that, as the choline content of the ration is decreased, the liver fat markedly increased, even though the animals still received a favorable growth response when niacin was added. However, the niacin content of the liver remained low as long as liver fat was high.

It was interesting to note (Table III) that the niacin content of the livers from deficient animals which received the growth-inhibiting proteins, gelatin and zein, or acid-hydrolyzed fibrin was low and was not greatly increased until the addition of niacin. When tryptophane was added, however, the content of niacin in the liver markedly increased. This would indicate that tryptophane may play a direct rôle in niacin synthesis (3).

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III

t Livers on Various Diets

	Liver niacin				Liver fat	
	−N†	+N†	−T‡	+T‡	−N†	+N†
	γ per gm.	γ per gm.	γ per gm.	γ per gm.	per cent	per cent
	60	165			3.0	2.8
	10	164			8.4	3.2
% in	116	146			19	3.3
acid			80	136		
	84	108				
% in	84	120			25	20
% in	86	86			22	18
in	110	126			17	9.7
	98	105		159		
	77	108				
rin	97	108		141		

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DISCUSSION

The evidence so far obtained indicates that the action of corn in niacin-low synthetic rations is a function of the character of the predominant protein present, zein, and that this, in turn, is due to an amino acid imbalance in this protein. It seems that this imbalance is made apparent through growth depression under any set of circumstances which provides too little tryptophane and niacin. That an imbalance of amino acids results in an increased tryptophane requirement, and therefore poor growth, is evident by the fact that rats will grow reasonably well on rations which contain the same amount of tryptophane but which also contain a well balanced protein. This increased requirement could, however, be related to a greatly reduced supply of niacin, through inadequate synthesis, which results in decreased tryptophane utilization or production.

It is clear that the kind of carbohydrate used in the ration influences the results obtained. The very much better growth obtained with dextrin as compared with sucrose even on the basal ration probably indicates one of three things: (1) dextrin is stimulating the synthesis of additional factors needed by the rat; (2) dextrin not only provides desirable conditions in the intestinal tract for bacterial activity but, because of its slow hydrolysis and absorption, favors a more gradual absorption and better utilization of other accompanying nutrients; and (3) dextrin may carry some unidentified factor *per se*. A combination of the first two postulations seems more reasonable.

Albanese and Irby (13) early reported the inferiority of essential amino acids for growth as compared with protein hydrolysates and implicated the unnatural forms of certain amino acids for this growth failure. Kinsey and Grant (14) indicated that high levels of glycine were toxic and Hier *et al.* (15) also showed that gelatin and the amino acids, glycine, phenylalanine, and proline, resulted in growth inhibition. The levels used in these instances were undoubtedly toxic since adequate amounts of niacin were included in their rations.

Although glycine of the amino acids tested seems to inhibit growth the most, this cannot be the only amino acid involved, since acid-hydrolyzed casein and zein, both of which inhibit growth, contain little or no glycine. This does not preclude the possibility that more than one amino acid may be involved in the imbalance of amino acids in such circumstances.

SUMMARY

Natural materials, such as peanuts, brewers' yeast, corn germ, corn bran, and dried pig stomach, used as supplements to the corn grits ration under controlled conditions, adequately prevent growth inhibition.

Compounds such as 3-CN pyridine, indole, indole-3-acetic acid, and anthranilic acid did not replace either niacin or tryptophane in counter-

acting the deleterious action of corn grits, but neither did they compete with niacin or tryptophane to reduce their beneficial action.

Some inhibition of growth, which was corrected by niacin, was observed by pyridine-3-sulfonic acid in rations containing a low level of protein but not at a high protein level.

An amino acid imbalance in zein and other tryptophane-low proteins creates a growth depression on niacin-low diets, and, of the specific amino acids so far tested, glycine seems to be one of the amino acids most responsible. This effect is dependent to some extent on the type of carbohydrate used, since glycine exhibits no inhibition when dextrin is used in place of sucrose. In all cases, dextrin rations produced markedly superior growth to sucrose rations.

BIBLIOGRAPHY

1. Krehl, W. A., Sarma, P. S., Teply, L. J., and Elvehjem, C. A., *J. Nutr.*, **31**, 85 (1946).
2. Woolley, D. W., *J. Biol. Chem.*, **162**, 179 (1946).
3. Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, **163**, 343 (1946).
4. Krehl, W. A., de la Huerga, J., and Elvehjem, C. A., *J. Biol. Chem.*, **164**, 551 (1946).
5. Krehl, W. A., Sarma, P. S., and Elvehjem, C. A., *J. Biol. Chem.*, **162**, 403 (1946).
6. Nielsen, E., and Elvehjem, C. A., *J. Biol. Chem.*, **145**, 713 (1942).
7. Cravioto, R. O., Anderson, R. K., Lockhart, E. E., Miranda, F. P., and Harris, R. S., *Science*, **102**, 91 (1945).
8. Woolley, D. W., Strong, F. M., Madden, R. J., and Elvehjem, C. A., *J. Biol. Chem.*, **124**, 715 (1938).
9. Snell, E. E., *Arch. Biochem.*, **2**, 389 (1943).
10. Woolley, D. W., *J. Biol. Chem.*, **157**, 455 (1945).
11. Woolley, D. W., and White, A. G. C., *Proc. Soc. Exp. Biol. and Med.*, **52**, 106 (1943).
12. Krehl, W. A., Strong, F. M., and Elvehjem, C. A., *Ind. and Eng. Chem., Anal. Ed.*, **15**, 471 (1943).
13. Albanese, A. A., and Irby, V., *Science*, **98**, 286 (1943).
14. Kinsey, V. E., and Grant, W. M., *Science*, **99**, 303 (1944).
15. Hier, S. W., Graham, C. E., and Klein, D., *Proc. Soc. Exp. Biol. and Med.*, **56**, 187 (1944).

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By KARL DITTMER, W

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the Fisher-Hirschfelder-Taylor atomic models of the analogues. The greater effective volume of trichloromethyl over methyl tends to maintain the benzene rings in a more nearly rigid configuration by virtue of steric blocking of rotation of the benzene rings. Secondly, by virtue of an inductive effect through the resonating ring system, the chlorine atoms would tend to increase the activity of the p,p'-hydroxyl groups. This latter effect may not markedly influence activity, however, since the trichloromethyl group is not attached directly to the ring but is 'insulated' by one aliphatic carbon atom. Much more effective in increasing estrogenic potency would be the influence of the trichloromethyl group on the p,p'-hydroxyl hydrogens by inductive action through space via the field effect.

In conclusion it must be pointed out that the distance between the active hydrogens of DHDT can never achieve the optimum (14.5 Å) and one would not anticipate activity approaching that of estradiol or diethyl stilbestrol.

Summary. 1. DDT and 2,2'-bis-(p-methoxyphenyl)-1,1,1-trichloroethane possess no estrogenic activity in ovariectomized rats in total doses up to 45 mg. 2. 2,2'-bis-(p-Hydroxyphenyl)-1,1,1-trichloroethane possesses seven times the estrogenic activity of its non-halogenated congener 2,2'-bis-(p-hydroxyphenyl)-ethane and this increase in potency may be related to field and steric effects exerted by the trichloromethyl group.

1. Burlington, H., and Lindeman, V. F., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 48.
2. Schueler, F. W., *Science*, 1946, v103, 221.
3. Keasling, H. H., and Schueler, F. W., *J. Am. Pharm. Assn.* (Sci. Ed.), 1950, vXXXIX, 87.
4. Preliminary Report, *Fed. Proc.*, 1952, v11, 345, pt. 1.
5. ———, *Fed. Proc.*, 1952, v11, 20, pt. 1.
6. Ter Meer, *Ber.*, 1874, v7, 1201.
7. Allen, E., and Doisey, E. A., *J. Am. Med. Assn.*, 1923, v81, 819.
8. Solmssen, U. V., *Chem. Rev.*, 1945, v37, 481.

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Effect of Factors other than Choline on Liver Fat Deposition.* (19904)

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The effects of certain amino acids on the metabolic relationship between tryptophan and niacin have been studied extensively (1-6). Recently we were surprised to observe fat deposition in the livers of animals receiving similar diets to those used in the above studies. Since the basal ration contained 0.1% choline and since methionine failed to prevent the occurrence of fatty livers, it appeared that other factors in the ration were involved.

In this paper, we wish to present experiments which demonstrate certain effects of proteins, amino acids and carbohydrate on liver fat deposition.

Experimental. Three-week-old male Sprague-Dawley rats kept in individual cages

TABLE I. Basal Ration for Fat Deposition Studies.

Component	Level in diet (per 100 g ration)
Casein	9 g
L-cystine	.2
Sucrose or dextrin	81.9
Corn oil	5
Salts IV (7)	4
Thiamin · HCl	.2 mg
Riboflavin	.3
Pyridoxine · HCl	.25
Ca pantothenate	2
Choline chloride	100
Inositol	10
Biotin	.01
Folic acid	.02

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TABLE II. Effect of Various Supplements to Sucrose Basal Ration on Liver Fat and Growth Rate.

Supplement	No. of animals	50 mg DL-tryptophan	1.5 mg niacin	% fat in rat livers — Wet basis Avg	Wet basis Range	Dry basis Avg	Dry basis Range	Avg growth rate (g/wk) Avg	Range
0	9			8.8	(4.4-13.3)	20.4	(12.7-28.4)	14.4	(10.4-20.8)
0	9	+		9.3	(6.6-15.9)	23	(13.5-37.8)	19.8	(15.2-26.4)
0	9		+	13.4	(9.5-19.9)	32.3	(24.4-41.7)	16.4	(8.8-20.8)
6% gelatin	6			2.9	(2.2- 3.8)	8.3	(6.2-10)	3.2	(1 - 6.6)
	6	+		4.8	(3 - 9.6)	11.9	(8.4-15.2)	25.6	(19.2-35)
	6		+	3.7	(3 - 4.4)	11.1	(8.9-14)	25.6	(14.2-38.8)
.16% threonine	9			3.2	(2.3- 4.7)	8.7	(6.5-10.1)	4.4	(.8- 7.6)
	9	+		6.6	(3.8-13.5)	17.5	(11.3-26.6)	23	(14.8-29)
	9		+	7.3	(5.1-15.1)	19.7	(15.1-35.5)	20	(12.2-35.2)
.31% threonine	6			2.4	(2.3- 2.5)	6.9	(6.4- 7.4)	3.2	(2.2- 4)
	6	+		6.2	(5.9- 6.9)	16.1	(15 -18.1)	22	(21.8-22.4)
	6		+	6.5	(5.7- 7.9)	17.2	(15 -19.9)	22.8	(19.2-26.2)

were fed *ad libitum* and were weighed at weekly intervals. The basal diet to which supplements were made at the expense of the carbohydrate is shown in Table I. This basal diet contained cystine in all cases except where indicated. The fat-soluble vitamins were administered orally in the form of 2 drops of halibut liver oil each week. The animals were sacrificed at the end of a 5-week period and the livers of the animals were removed and analyzed for fat content using essentially the procedure outlined by the Association of Official Agricultural Chemists(8). The livers were dried at 50°C in a vacuum oven for moisture determination, and the fat content was determined on duplicate samples of the dried livers. Samples were ground with sand and extracted with ether for 16 hours on Goldfisch extractors. After evaporation of the ether the fat samples were heated in a 100°C oven for 15 minutes, cooled in a desiccator, and weighed to constant values.

Results. The data in Table II show the effect of various supplements when added to sucrose rations containing 9% casein with 0.2% L cystine. When either 50 mg % DL tryptophan or 1.5 mg % niacin was added to this basal ration the growth rate increased but no protective effect upon liver fat deposition was noted. When 6% gelatin was added to the basal ration, however, a decided depression of liver fat together with a reduced growth rate occurred. Either tryptophan or niacin, added with the 6% gelatin supplement, stimulated growth over the controls while the liver fat was still significantly lowered. From this experiment, it appeared that the factors controlling growth and liver fat deposition were not the same. When 0.15% DL threonine was added to the basal ration, growth was inhibited as much as that caused by 6% gelatin and the liver fat was lowered to the same level. When the indicated amounts of either tryptophan or niacin were added to the threonine supplement, growth was increased markedly, but no depression of liver fat occurred. The growth depression was undoubtedly due to an amino acid imbalance(4). Either niacin or tryptophan alleviated this growth depression but an increase in liver fat still occurred.

Griffith and Nawrocki(9) have reported intensification of a choline deficiency when threonine was added with cystine to a low casein diet. The influence of threonine was believed to be due to a direct stimulation of growth or metabolism rather than an antagonistic action towards labile methyl. This seems to be the case since 0.1% choline was present in the basal ration under our conditions. The growth depression which we observed is probably due to a tryptophan-niacin deficiency, yet when either of these factors was added to bring about a growth response fatty livers occurred. When the level of threonine was doubled as in the last experiment in Table II in the presence of either tryptophan or niacin, growth was restored and liver lipids were reduced, but not to the extent brought about by the gelatin supplement

on Liver Fat and Growth R

livers — Dry basis Avg	Range	Avg growth r (g/wk) Avg	Range
.4	(12.7-28.4)	14.4	(10.4-2
	(13.5-37.8)	19.8	(15.2-2
.3	(24.4-41.7)	16.4	(8.8-2
.3	(6.2-10)	3.2	(1 - 6.
1.9	(8.4-15.2)	25.6	(19.2-35
1.1	(8.9-14)	25.6	(14.2-38.
.7	(6.5-10.1)	4.4	(.8- 7.
7.5	(11.3-26.6)	23	(14.8-29
9.7	(15.1-35.5)	20	(12.2-35.
6.9	(6.4- 7.4)	3.2	(2.2- 4
6.1	(15 -18.1)	22	(21.8-22.
7.2	(15 -19.9)	22.8	(19.2-26.

l significantly lowered. From
it appeared that the factors
wth and liver fat deposition
me. When 0.15% DL threo-
o the basal ration, growth was
uch that caused by 6%
live at was lowered to the
en the indicated amounts of
n or niacin were added to the
ement, growth was increased
no depression of liver fat oc-
owth depression was undoubt-
n amino acid imbalance(4).
r tryptophan alleviated this
on but an increase in liver fat

Nawrocki(9) have reported
of a choline deficiency when
added with cystine to a low
e influence of threonine was
ue to a direct stimulation of
olism rather than an antag-
owards labile methyl. This
case since 0.1% choline was
basal ration under our condi-
wth depression which we ob
ly due to a tryptophan-niacin
when either of these factors
ring about a growth response,
urred. When the level of
oubled as in the last experi-
II in the presence of either
growth was restored and
reduced, but not to the ex-
out by the gelatin supplement

TABLE III. Effect of Various Supplements to Dextrin Basal Ration on Liver Fat and Growth Rate.

Supplements	No. of animals	50 mg DL-tryptophan	1.5 mg niacin	% fat in rat livers — Wet basis Avg	Range	Dry basis Avg	Range	Avg growth rate (g/wk) Avg	Range
	6			4.9	(3.7-6)	13.9	(11.2-17.6)	21.8	(14 -26.8)
	6	+		3.9	(3.2-5.2)	11.4	(9.4-14.7)	17.8	(9.2-26.2)
	6		+	5.9	(5.3-6.5)	16.3	(14.2-18.5)	22.4	(16.2-26)
6% gelatin	6			3.1	(2.5-4.1)	9.3	(7.2-12.5)	7.6	(6 - 9)
	6	+		4.2	(3.5-4.7)	12.9	(11.2-14.1)	22.8	(14.8-30.4)
	6		+	3.2	(2.4-4.4)	9.6	(8 -12.2)	22	(12.8-32.6)
.18% threonine	3			3.9	(3.3-5)	8.9	(7.7-10.7)	11	(5.4-15.6)
+.24% phenyl-	3	+		3.1	(2.4-4.1)	8.9	(7.2-11.3)	31.4	(10.4-32.4)
alanine	3		+	4.5	(4.1-5.3)	13.5	(12.6-15.2)	23.6	(18.8-26)
2% acid-hydro-	3			4.3	(2.9-6.4)	12.8	(8.8-18.8)	15	(13.6-17.6)
lized casein+	3	+		5.1	(4 -5.8)	15.1	(12.2-17.6)	30	(27.6-33.8)
1.5% glycine	3		+	5.6	(5.4-5.7)	16.8	(16.5-17.1)	23.4	(19.2-27.8)

alone. It is clear that gelatin exerts an effect beyond the action of threonine.

In Table III similar data are given when dextrin rather than sucrose was the carbohydrate. The controls on the dextrin basal showed a higher growth rate even without additions of niacin or tryptophan. At the same time it can be noted that the deposition of liver fat was not as severe as in the sucrose group. The addition of either tryptophan or niacin in the indicated quantities failed to promote increased growth over the controls. When 6% gelatin was added to the basal diet, a drop in liver lipids and growth rate was observed. Supplements of either tryptophan or niacin together with gelatin completely overcame the apparent growth antagonism precipitated by the gelatin and maintained the liver fat at low levels. When 0.18% DL threonine and 0.24% DL phenylalanine were supplemented to the basal ration, a similar effect was noted. The addition of either tryptophan or niacin restored the growth without affecting the liver fat. In the final experiment in Table III, 2% acid-hydrolyzed casein and 1.5% glycine were added together to the basal ration. The growth was depressed but liver fat was only slightly altered if at all with respect to the controls. Tryptophan or niacin increased growth and liver fat deposition slightly. The dextrin basal rations supported growth rates of control animals which were much greater than the sucrose controls as has been previously observed in this laboratory by Teply and coworkers(10). On the other hand, it can be observed that the lipid contents of the livers of the dextrin controls were lower. Gelatin exerted a greater effect when sucrose was the carbohydrate.

McHenry(11) cited the roles of biotin, thiamin and carbohydrate in the production of fatty livers. More recently Harper and Katayama(12) have studied the utilization of sucrose or corn starch for growth when added to low casein diets. They showed that corn starch supported better growth than sucrose in a 9% casein basal ration supplemented with methionine. This was also true when methionine was not added to the basal diet. One can surmise that the differences between the 2 carbohydrates may be in the rate of passage of the diet through the gut. A greater availability of the amino acids in the casein might be suspected if the ration passed through the intestine more slowly. The observations by Mulford and Griffith(13), and Salmon(14) that cystine supplementation aggravates renal damage and fatty infiltration of the livers of rats receiving 8% casein diets deficient in choline; and Griffith and Nawrocki(9) that further intensification of the choline deficiency occurred when threonine was added with cystine, prompted us to carry out a third set of experiments which are reported in Table IV. We decided to omit cystine from the basal ration and test the effects of certain protein and amino acid supplements. The most striking effect of the cystine omission may be seen in the control group where the growth was about 50% lower

TABLE IV. Effect of Increasing Protein and Single Amino Acids Supplemented to Sucrose Basal Ration Without Cystine.

Supplement, %	No. of animals	% fat of dry liver		Avg growth rate (g/wk)	
		Avg	Range	Avg	Range
0	6	29	(13.9-35.2)	7.6	(5.8-11.6)
3 Casein	6	11.5	(6.9-17)	15.6	(12.6-18.8)
6	6	10.2	(7 -15.2)	22.6	(16.2-24.8)
3 Gelatin	6	17.2	(9.7-25.8)	10.4	(8.8-12.2)
6	6	13.9	(9 -18.4)	10	(6.2-13.8)
.3 L Proline	6	23.6	(14.8-35.8)	6.6	(1.8- 9.2)
.6	6	24.5	(17 -25.4)	7.2	(4.8-10.4)
.6 DL Alanine	6	23.1	(15.6-28.1)	5.6	(4.2- 8.4)
.3 DL Methionine	3	26.7	(23.6-29.6)	14.9	(10 -17.6)

than similar controls in Table II where cystine was included. It is likely that the sulfur amino acids were limiting for growth in the 9% casein rations(4). Larger amounts of casein added to the basal diet increased growth and decreased liver fat. This, however, was not true in the case of gelatin. Increasing levels of gelatin failed to stimulate growth proportionately to the level added to the diet, but did decrease liver fat proportionately. L proline was chosen as an amino acid supplement because of its high concentration in gelatin and DL alanine was chosen because it showed a slight effect upon the reduction of liver lipids in preliminary trials. The supplements of 0.3% L proline and 0.6% DL alanine are comparable and each decreases the liver fat slightly while the growth in either group is similar to the controls. This observation strengthens the earlier conclusion that those factors controlling the liver fat deposition are not necessarily the same as those controlling growth. To show this more clearly, 0.3% DL methionine was added to the basal ration which resulted in a restoration in the growth equivalent to that exerted by cystine in the control group presented in Table II. While the addition of methionine to the basal ration without cystine increased growth 100% over the controls, it exerted no effect upon the liver fat deposition. It appears that 0.3% DL methionine improved growth without antagonizing liver fat deposition as is often the case when added cystine is present(9,13-15).

The fatty liver which we have obtained under our conditions seems to be one which may be influenced by at least 3 factors: protein, amino acids and carbohydrate. There has been much conjecture as McHenry(11) has pointed out, upon the role of the pancreas in preventing fatty livers. Some observations suggest the essential factor in the pancreas to be the hormone, lipocaic, while other workers claim to have ruled out the hormone action and assert the idea that the proteolytic enzymes from pancreatic juice are necessary for the complete digestion of dietary protein which then reduces fat deposition by some mechanism; perhaps one which involves one or more amino acids specifically. It seems likely from our data that, under our conditions, carbohydrate may effect liver fat and growth by controlling the rate of absorption of the necessary amino acids from the gut by affecting the rate of passage of the diet through the gut or affecting the microorganisms for production of necessary vitamins, or both. Whole protein, such as casein or gelatin, apparently supplies necessary amino acids for controlling liver fat deposition. While certain individual amino acids exert a slight effect on the reduction of liver lipids, it appears that combinations of these amino acids are necessary to bring about a greater reduction in liver fat deposition. (Harper, Monson, Elvehjem: work in progress.)

Summary. A number of factors under our experimental conditions influence fat deposition in the liver: the availability of niacin to the animal; the type of carbohydrate used in the ration and type and level of protein used. Individual amino acids and mixtures of amino acids which support optimal growth conditions do not produce minimum levels of fat

ds Supplemented to Sucros-

Avg growth rate (g/wk)	
Avg	Range
7.6	(5.8-11.6)
15.6	(12.6-18.8)
22.6	(16.2-24.8)
10.4	(8.8-12.2)
10	(6.2-13.8)
6.6	(1.8- 9.2)
7.2	(4.8-10.4)
5.6	(4.2- 8.4)
14.9	(10 :17.6)

carbohydrate. There has
cture as McHenry(11) has
the role of the pancreas in
livers. Some observations
tial factor in the pancreas
, lipocaic, while other work-
ruled out the hormone action
ea that the proteolytic en-
eatic ' 'e are necessary for
estio f dietary protein
es fat deposition by some
ps one which involves one
ids specifically. It seems
ata that, under our condi-
may effect liver fat and
ing the rate of absorption
mino acids from the gut by
of passage of the diet
affecting the microorgan-
of necessary vitamins, or
n, such as casein or gelatin,
necessary amino acids for
deposition. While certain
ids exert a slight effect on
er lipids, it appears that
se amino acids are neces-
a greater reduction in liver
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ons influence fat deposi-
availability of niacin to
of carbohydrate used in
and level of protein used.
ds and mixtures of amino
of growth condi-
minimum levels of fat

in the liver.

1. Krehl, W. A., Henderson, L. M., de la Huerga, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1946, v166, 531.
2. Henderson, L. M., Deodhar, T., Krehl, W. A., and Elvehjem, C. A., *J. Biol. Chem.*, 1947, v170, 261.
3. Hankes, L. V., Henderson, L. M., Brickson, W. L., and Elvehjem, C. A., *J. Biol. Chem.*, 1948, v174, 873.
4. Hankes, L. V., Henderson, L. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, v180, 1027.
5. Hankes, L. V., Lyman, R. L., and Elvehjem, C. A., *J. Biol. Chem.*, 1950, v187, 547.
6. Lyman, R. L., and Elvehjem, C. A., *J. Nutr.*, 1951, v45, 101.
7. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B. *J. Biol. Chem.*, 1941, v138, 459.
8. *Methods of Analysis*, Association of Official Agricultural Chemists, 6th Ed., 1945, p. 422.
9. Griffith, W. H., and Nawrocki, M. F., *Fed. Proc.*, 1948, v7, 228.
10. Teply, L. J., Krehl, W. A., and Elvehjem, C. A., *Am. J. Physiol.*, 1947, v148, 91.
11. McHenry, E. W., *Biological Symposia*, J. Cattell Press, Lancaster, Pa., 1941, p. 177.
12. Harper, A. E., and Katayama, M. K., *J. Nutr.*, in press.
13. Mulford, D. J., and Griffith, W. H., *J. Nutr.*, 1942, v23, 91.
14. Salmon, W. D., *J. Nutr.*, 1947, v33, 155.
15. Tyner, E. P., Lewis, H. B., and Eckstein, H. C., *J. Biol. Chem.*, 1950, v187, 651.

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Fecal Electrolytes and Nitrogen During Cortisone or ACTH Therapy.*† (19905)

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In view of the established effects of cortisone, ACTH, and DOCA on the excretion of certain body constituents through the kidney and the skin(1-6), the possible influence of two of these agents on the composition of stools has been studied in a series of patients. Compound E, 50 to 1000 mg per day, or ACTH, 10 to 100 mg per day administered for 3 to 50 or more days, did not produce any characteristic alteration in the output of electrolytes and nitrogen. Collection of control data to establish this fact has incidentally provided information about the amounts of sodium, potassium, chloride and nitrogen excreted in feces during maintenance of a miscellaneous group of subjects on certain types of milk diets.

Materials and methods. Stools have been collected from hospitalized children and adults while on one of the following dietary regimens supplemented by vitamins and iron: a) essentially complete sodium restriction, *i.e.*, low-sodium milk fortified with carbohydrate and milk protein, providing an adequate nitrogen and caloric intake, or b) a whole milk diet similarly modified. Patients with gastrointestinal disorders were excluded, but the available clinical material made it impossible to limit the study to any one category of patients with respect to age or disease. This study is therefore based upon analyses of stools from a miscelleneous group of children and adults ill with rheumatic fever, diabetes mellitus, rheumatoid arthritis, leukemia, muscular disorders, *et cetera*. The daily intake of formula usually exceeded two liters but the seriously ill patients at times took less than the prescribed amount.

The *output* of sodium, potassium, chloride and of nitrogen in formed stools has been measured in control studies as well as during periods of therapy with cortisone or ACTH. Water was taken as desired. The beginning and end of a stool collection period, usually

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167

EFFECT OF THE COMPOSITION OF THE DIET ON THE RIBOFLAVIN REQUIREMENT OF THE RAT[1]

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In a previous report (Mannering, Lipton and Elvehjem, '41) data were presented to show that the fat content of the diet had a marked effect on the riboflavin requirement of the rat. The feeding of riboflavin-low dextrin rations in which a large proportion of the carbohydrate had been isocalorically replaced by fat increased the amount of riboflavin needed by rats for growth and enabled the production of deficiency symptoms of greater severity than are usually observed. Animals fed these high fat rations developed a spastic paralysis of the hind quarters which could be prevented or cured by the administration of riboflavin. Shaw and Phillips ('41) have substantiated the above work and have been able to correlate the paralysis with degenerative neural changes.

The increased riboflavin requirement resulting from the substitution of fat for dextrin might be explained on the basis that an increased cellular demand for the vitamin occurs during the metabolism of fat, or, as seems more likely from the experiments that are to follow, that such an alteration in dietary constitution results in a decreased synthesis of available riboflavin by intestinal bacteria. Several papers have appeared in the literature emphasizing the role of dextrin in promoting the intestinal synthesis of certain vitamins. The coprophagy studies of Guerrant, Dutcher and Tomey ('35) and Guerrant, Dutcher and Brown ('37) demonstrated that the feces of dextrin-fed rats contained greater quantities of undifferentiated B complex than the feces from rats fed sucrose, lactose, glucose, or cornstarch. Guerrant and Dutcher ('34) studied the effects of various dietary constituents on the requirement of rats for thiamine and vitamin G (the heat stable portion of the

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8

B complex). They concluded that fat and protein had no effect, that higher levels of salts increased, and that agar, cellulose and dextrin decreased the amount of vitamin G needed in the diet. Although riboflavin was not considered independently of vitamin G, these studies strongly suggest that the amount of riboflavin required in the diet by the rat may depend in part on the amount of riboflavin supplied from an enteric source, and that this, in turn, can be greatly influenced by certain dietary ingredients, particularly dextrinized cornstarch.

The present investigation was undertaken to obtain further information regarding the relationship between dietary fat, dextrin, and riboflavin, and was later extended to include a study of the effect of other carbohydrates and of fat on the requirement for and fecal excretion of riboflavin.

EXPERIMENTAL AND RESULTS

Effect of substituting fat for dextrin

The composition of the riboflavin-low diets used is presented in table 1. Rations K-25 and K-27, containing 25 and 40% of fat, respectively, are modifications of high carbohydrate ration K-24 made by isodynamically replacing dextrin with lard. Ration K-26 differs from ration K-25 only in that a fat of vegetable origin[3] was substituted for lard. All rations used in this series and those following were kept under refrigeration. The dextrin was prepared by making a paste of commercial cornstarch and water, autoclaving for 3 hours at 15 pounds pressure, drying, and grinding.

Three-week-old male albino rats weighing 35 to 40 gm. were partially depleted of their riboflavin reserves for 2 weeks on ration K-24 before being divided into four groups of twelve animals receiving the above diets and varying amounts of riboflavin (0, 3, 6, 9, and 12 μg. per day). The riboflavin was fed in ½ ml. of a 25% ethanol solution which was pipetted into small dishes. The animals were housed in individual cages having ¼ inch mesh screen bottoms. Food consumption records were kept throughout the supplementation period. Spilled food was collected on paper towels placed beneath the cages and weighed. The results obtained during the 7 weeks following the depletion period are summarized in table 2.

Examination of the table reveals that at each level of riboflavin intake the growth of the animals receiving the high dextrin ration (7% fat) was superior to that of rats fed the 40% fat diet. The growth of animals fed the 25% fat rations was intermediate. No important dif-

[3] Crisco.

TABLE 1
Riboflavin-low rations.

CONSTITUENTS	K-24	K-25	K-26	K-27	K-28	K-29	K-30*	K-31*	K-32*	K-33*	K-34*	K-35*	K-36*	K-37*	K-38*	K-39*
Dextrin, gm.	71	37.5	37.5	21.5					72	22.5						

TABLE 1

Riboflavin low rations.

CONSTITUENTS	K-24	K-25	K-26	K-27	K-28	K-29	K-30*	K-31*	K-32*	K-33*	K-34*	K-35*	K-36*	K-37*	K-38*	K-39*
Dextrin, gm.	71	37.5	37.5	21.5					73	22.5						
Sucrose, gm.					71	21.5	73	22.5			33		73	22.5		
Lactose, gm.											40	22.5				
Cornstarch, gm.															73	
Extracted dextrin,[1] gm.																73
Cellulose,[2] gm.													10	10		
Lard, gm.		14		22		22		22.5		22.5		22.5		22.5		
Crisco, gm.			14													
Corn oil, gm.	2	2	2	2	2	2	5	5	5	5	5	5	5	5	5	5
Washed butterfat,[3] gm.	3	3	3	3	3	3										
Cod liver oil, gm.	2	2	2	2	2	2										
Casein,[4] gm.	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
Salts IV,[5] gm.	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Liver extract[6]	(4)	(4)	(4)	(4)	(4)	(4)										
Thiamine, mg.	.3	.3	.3	.3	.3	.3										
Pyridoxine, mg.	.4	.4	.4	.4	.4	.4										
Pantothenic acid, mg.	.5	.5	.5	.5	.5	.5										
Nicotinic acid, mg.	5	5	5	5	5	5										
Choline, mg.	100	100	100	100	100	100										

All rats fed the above rations received 2 drops of haliver oil per week.

[1] Extracted continuously for 3 days with hot 95% ethanol.

[2] Cell-U flour.

[3] Washed 3 times with hot water.

[4] Borden's purified casein, supplied under the trade name "Labco".

[5] Hegsted et al. ('41).

[6] Riboflavin-free liver extract (Wagner et al., '40). Mixed into the ration in amounts equivalent to 4% of the original liver powder.

* Rats receiving these rations were given a daily oral supplement of the following vitamins (μg.) dissolved in ½ ml. of a 25% ethanol solution: thiamine, 40; pyridoxine, 50; pantothenic acid, 150; nicotinic acid, 500; and choline, 10,000.

ferences could be shown between the groups receiving lard and Crisco. If the gains in weight of the rats fed each of the rations are plotted against the daily riboflavin dosage (omitted here to conserve space), it can be shown that increasing the fat in the diet to 40% at the expense of dextrin had an effect of increasing the riboflavin requirement about 4 to 6 μg. per day. Similarly, the feeding of rations containing 25% fat increased the requirement by about 2 μg. per day. The economy of food utilization, calculated as grams gained per 100 Cal. consumed, is closely correlated with the responses in growth made by the rats receiving the different rations and varying amounts of riboflavin.

TABLE 2

Growth of rats receiving rations containing different percentages of fat and graded amounts of riboflavin. (Each figure represents the average response of 3 rats.)

RATION NO.	TYPE OF RATION	DAILY RIBOFLAVIN INTAKE IN MICROGRAMS	GRAMS GAINED IN 7 WEEKS [1]	GRAMS GAINED PER CALORIE FED × 100 (7 WEEKS)
K-24	High dextrin	0	12	1.1
		3	72	5.7
		6	102	6.9
		9	148	7.1
		12	170	8.0
	Average		101	5.8
K-25	25% lard	0	10	.7
		3	41	4.0
		6	71	5.4
		9	88	6.6
		12	121	7.1
	Average		66	4.8
K-26	25% Crisco	0	8	.9
		3	39	4.1
		6	61	5.7
		9	98	6.6
		12	116	6.6
	Average		65	4.8
K-27	40% lard	0	4	.4
		3	16	2.1
		6	53	4.8
		9	84	5.9
		12	98	6.9
	Average		51	4.1

[1] Total grams gained during 7 weeks following 2-week depletion period on riboflavin low ration K-24.

That a 40% level of fat in the diet does not restrict the growth of rats when adequate riboflavin is supplied was demonstrated by raising two groups of four weanling male rats on rations K-24 and K-27 plus 100 μg. of riboflavin per day. At the end of 6 weeks the animals fed the high dextrin ration showed an average weight increase of 184 gm. while those receiving the high fat diet had gained an average of 189 gm.

It was conceivable that in riboflavin deficient rats the deleterious effect of fat might be due to an impairment of fat absorption. In order to test this possibility fat balance studies were conducted during the third and fourth weeks following the depletion period on rats receiving the high dextrin (K-24) and high fat (K-27) rations. Fat retention was excellent in all animals regardless of the type of ration fed or of the riboflavin intake. The average retention of the fat of ration K-24 was 96% (95 to 99) and of ration K-27, 98% (97 to 99).

The total fat content of the livers of all the rats was determined in an attempt to detect a disturbance in fat metabolism or transport. Liver fat on a dry basis varied between 1.6 and 5.9%. The variations could not be associated with either the amount of riboflavin or the type of ration fed.

Effect of substituting fat for sucrose

As mentioned previously, the effect of dietary fat in increasing the riboflavin requirement might be due not to the fat itself but to the replacement of dextrin, a carbohydrate known to stimulate the synthesis of vitamins by intestinal bacteria. Sucrose apparently lacks a similar stimulatory property. If the problem is essentially one of bacterial synthesis, the use of sucrose rather than dextrin as the source of carbohydrates should reduce growth, and the substitution of fat for sucrose should not result in a further reduction. With this in mind the following experiment was designed.

Groups of sixteen male weanling rats were partially depleted of their riboflavin reserves by feeding for 2 weeks the following riboflavin-low rations (table 1): K-24 (high dextrin), K-27 (40% fat, dextrin), K-28 (high sucrose), and K-29 (40% fat, sucrose). The animals within each group were then given varying amounts of riboflavin, namely, 0, 6, 12, 24, and 36 μg. per day. The growth responses of the rats during the 6-week experimental period including the first 2 weeks of depletion are given in table 3.

The superiority in growth of rats receiving the high dextrin ration over animals fed the dextrin-lard ration is again substantiated. This same relationship cannot be shown when sucrose is employed as the

source of carbohydrate. This is in agreement with the observations of Potter, Axelrod and Elvehjem ('42) who were unable to show any effect of fat on the riboflavin requirement of dogs fed sucrose rations. The growth of rats fed the high sucrose ration was not significantly greater than that of animals fed either of the high fat diets. Rats fed the lard-dextrin ration, K-27, grew no better than those receiving the sucrose or lard-sucrose diets although this diet contains considerable amounts of dextrin. It would appear that fat reduces or eliminates the riboflavin sparing effect of dextrin when these two constituents are included in the same ration.

TABLE 3

Growth of rats receiving different riboflavin-low diets and various levels of riboflavin. (Total grams gained in 6 weeks, including 2-week depletion period.)

RATION NO.	TYPE OF RATION	MICROGRAMS OF RIBOFLAVIN/DAY [1]				
		0 (4)	6 (3)	12 (3)	24 (3)	36 (3)
K-24	Dextrin	33	69	88	159	159
K-27	Lard-dextrin	4	40	75	116	124
K-28	Sucrose	21	43	89	121	149
K-29	Lard-sucrose	12	46	75	128	153

[1] Number of rats indicated by numbers in parentheses.

The comparison of the sucrose and high-fat sucrose rations was repeated by raising two groups of five young male rats on rations K-28 and K-29 plus a suboptimal daily dose of 6 μg. of riboflavin. The preliminary depletion period was omitted. Food consumption records were kept throughout the 7-week experimental period. The rats fed the high-sucrose ration gained an average of 82 gm. and those receiving the 40% fat-sucrose diet showed a weight increase of 76 gm. The utilization of the two rations was comparable, 5.9 and 5.4 gm. gained per 100 Cal. consumed of rations K-28 and K-29, respectively.

Effect of various carbohydrates and of fat on the requirement for and fecal excretion of riboflavin

The above work suggests that the beneficial effect of dextrin is due to its stimulation of the production of available riboflavin by the intestinal flora of the rat. If this concept is correct, a positive correlation might be found between growth and the amount of riboflavin excreted in the feces or contained in the cecum. The size of the cecum might also be related to the amount of riboflavin synthesized. The following experiment was designed to determine whether or not such correlations could be made.

A total of 78 three-week-old male rats were fed isocalorically equivalent rations differing only in the kind of carbohydrate or in the amount of lard present. The following types of diets were employed (table 1): high sucrose (K-30), 40% fat, sucrose (K-31), high dextrin (K-32), 40% fat, dextrin (K-33), 40% lactose, sucrose (K-34), 40% fat, lactose (K-35), high-sucrose to which 10 parts of cellulose per 100 parts of ration had been added (K-36), 40% fat, sucrose, cellulose (K-37), high commercial cornstarch from which the dextrin had been prepared (K-38), and high alcohol-extracted dextrin (K-39). The last ration was fed to determine whether or not alcohol extraction would remove any hypothetical substance responsible for the stimulatory effect of dextrin. All animals received 6 μg. of riboflavin per day throughout the 6-week test period in order to insure consistent yet suboptimal growth. Half of the rats received the fullers' earth-treated butanol liver extract equivalent to 4% of the original liver powder, but because these animals responded no differently than did non-supplemented animals of the same group, no differentiation is made between the rats within each group. The rations containing high levels of fat were prepared daily and fed in clean food dishes to prevent rancidity.

At the end of 6 weeks the feeding of riboflavin was discontinued. The rats were maintained for an additional 9 days and throughout the last week of this period the feces were collected daily and stored in the refrigerator. The animals were then killed and the cecal contents collected quantitatively. The feces and cecal contents were dried in vacuo at 55°C., weighed, and saved for later riboflavin analysis.

The riboflavin content of the feces of individual rats was determined in duplicate using essentially a combination of the fluorometric methods of Hodson and Norris ('39) and Conner and Straub ('41) as outlined by Andrews ('43). Because of the small quantities of material available, the cecal contents of the animals of each group were combined for analysis. Several samples of feces were assayed by the bacteriological method of Snell and Strong ('39) as well as by the fluorometric method. The values obtained by the former procedure were erratic and as much as two or three times higher than those obtained by fluorometric analysis. This discrepancy was eliminated, however, by employing the Strong and Carpenter ('42) modification of the bacteriological assay which involves the removal of fatty stimulatory substances by means of ether extraction. The average riboflavin content of twenty samples of feces as measured by the fluorometric and bacteriological procedures was, respectively, 27.6 and 26.3 μg. per gram.

Lamoreux and Schumacher ('40) have reported that the riboflavin content of chicken excreta increases as much as 100% in 24 hours on standing at room temperature. That a similar synthesis of riboflavin did not occur in the rat feces during the collection intervals was shown indirectly by comparing the riboflavin content of the formed fecal material from the lower colon, obtained when the animals were autopsied, with that of the feces that had been collected throughout the preceding week. The colonic "feces" of eight animals taken at random from the various groups contained an average of 30.1 μg. per gram while the dropped feces from the same rats had a comparable riboflavin content of 32.9 μg. per gram. Active riboflavin synthesis probably does not occur in rat feces on standing because of their relatively low moisture content.

TABLE 4

Effect of various carbohydrates and of fat on growth and on the fecal excretion of riboflavin. All rats received 6 μg. of riboflavin per day except during period of feces collection.

RATION NO.	TYPE OF RATION	NO. OF RATS	GAIN IN 6 WKS.	RIBOFLAVIN/GM. OF FECES	RIBOFLAVIN EXCRETED IN FECES/WEEK	RIBOFLAVIN EXCRETED IN FECES/WK./100 GM. RAT	RIBOFLAVIN/GM. OF CECAL CONTENTS	CECAL CONTENTS/100 GM. OF RAT
			gm.	*μg.*	*μg.*	*μg.*	*μg.*	*gm.*
K-30	Sucrose	14	80	25.0	23.2	19.3	22.0	.15
K-31	Lard-sucrose	8	75	23.5	24.5	21.7	19.0	.16
K-32	Dextrin	8	108	34.3	72.4	50.3	25.6	.25
K-33	Lard-dextrin	8	68	24.3	24.3	22.8	19.5	.20
K-34	Lactose-sucrose	6	89	45.3	89.0	67.5	41.8	.48
K-35	Lard-lactose	6	87	23.4	50.6	38.8	25.6	.35
K-36	Cellulose-sucrose	6	83	6.7	28.3	23.1	9.7	.26
K-37	Lard-cellulose-sucrose	6	81	6.4	30.2	24.9	8.2	.24
K-38	Cornstarch	8	98	30.5	43.9	32.5	21.7	.14
K-39	Alcohol extracted dextrin	8	97	29.5	58.4	42.3	24.7	.31

It was obviously important to determine whether or not significant quantities of riboflavin were being contributed by the variable constituents of the diets. Lard, sucrose, cornstarch and lactose were found to contain less than 0.1 μg. of riboflavin per gram as measured by the fluorometric method, amounts that could not seriously affect the results of this experiment. The results are given in table 4.

The growth of rats receiving the high dextrin, cornstarch, and alcohol-extracted dextrin diets was superior to that of animals fed any of the other rations. Again the substitution of lard for a large part of the dextrin of the diet had a deleterious effect on growth while a similar

replacement of sucrose by lard was without effect. The inclusion of cellulose in the sucrose and lard-sucrose rations did not improve growth appreciably. The replacement of a large part of the sucrose of ration K-30 with lactose resulted in a slight increase in growth.

With the exception of the lactose-fed groups, growth is roughly proportional to the amount of riboflavin excreted per week in the feces. Similarly, but to a less marked degree, those groups of rats which exhibited the best growth, produced feces containing the greatest amount of riboflavin per gram. About the same amount of fecal riboflavin was excreted weekly by the animals receiving the high sucrose, fat-sucrose, fat-dextrin, and the cellulose diets. Although the riboflavin content of the feces of animals fed the cellulose diets was very low, the large amount of feces voided made the total excretion comparable to that of rats fed the high-sucrose diet without cellulose.

The feeding of the lactose-sucrose diet resulted in the greatest fecal production of riboflavin. According to the above correlation made between growth and the amount of riboflavin found in the feces, one might have expected the growth of the lactose-fed rats to have been correspondingly above that of the other animals, but this was not the case. It is possible, however, that when lactose was fed, the suboptimal riboflavin intake was not the primary growth-limiting factor. The data of Boutwell, Geyer, Elvehjem and Hart ('43) show that on a diet containing 48% lactose and an abundance of all the known vitamins, rats do not grow as well as when other carbohydrates are employed. When lard was substituted for the sucrose and part of the lactose of lactose-sucrose ration, K-34, the total fecal riboflavin was greatly reduced. Likewise the replacement of a substantial part of the dextrin of ration K-32 with lard resulted in a decreased fecal riboflavin output. The relatively low degree of intestinal synthesis of riboflavin which results when the high-sucrose (K-30) and sucrose-cellulose (K-36) rations are fed was not further decreased by the inclusion of high levels of fat in these diets.

With certain exceptions, the total amount of riboflavin excreted in the feces is related to the size of the cecum as measured by the dry weight of the cecal contents. The ceca of lactose-fed rats were greatly distended and contained whitish material, presumably lactose. The feeding of dextrin rations K-30 and K-39 also resulted in large ceca. It was in these animals that the greatest synthesis of riboflavin occurred. On the other hand, the feeding of cornstarch was responsible for abundant fecal production of riboflavin even though large ceca were not found. Conversely, the intestinal synthesis of riboflavin in

cellulose-fed rats was not great although the cecal size per 100 gm. of these animals was about the same as that of the rats receiving the high-dextrin ration. Apparently the amount of riboflavin excreted in the feces is as dependent upon the type as upon the quantity of material in the cecum. Although somewhat lower in most instances, the riboflavin values of the cecal contents are a reflection of those of the feces.

Effect of a high dietary intake of fat on the deficiency symptoms and survival of riboflavin deficient rats

It has been shown previously (Mannering et al., '41) that rats receiving a high fat-dextrin diet deficient in riboflavin develop a spastic paralysis of the hind quarters, a condition that does not occur with any marked severity when a high dextrin ration is fed. This same relationship of paralysis to fat intake can be shown when sucrose rather than dextrin is employed as the source of carbohydrate.

One group of rats weighing between 65 and 75 gm. received the basal high-sucrose ration K-30 and another, the lard-sucrose ration K-31. Animals of this size were employed in order to produce a chronic rather than the more acute state of deficiency which results when smaller rats are used, a higher incidence of spastic paralysis resulting when the deficiency is prolonged over many weeks. Animals were allowed to continue on experiment for 133 days throughout which time no riboflavin was administered. The high fat ration was prepared and fed daily to prevent rancidity.

Of six rats fed the high-sucrose diet three were still alive after 133 days. The average survival time was 118 days (83–133 days). Although several of these rats showed minor symptoms of spasticity before dying, in no instance could the condition be considered severe. None of the seven rats fed the lard-sucrose diet survived the 133-day period and the average survival time was 81 days (50–126 days). All of these rats developed severe paralysis.

These results were confirmed in a similar experiment. Eight of twelve rats fed the high carbohydrate diet survived the 133-day period, the average survival time being 113 days (85–133 days), as compared to 91 days (66–125 days) for twelve animals receiving the high fat diet. Again severe spasticity occurred only in rats fed the high lard ration. Three of the rats of each group were given 2 μg. of biotin per day (biotin liver concentrate) and three others received 10 mg. of inositol per day. Neither of these substances had any influence on survival or the deficiency symptoms. That the extremely emaciated condition of the

deficient rats was not responsible for the paralysis was shown by maintaining the weights of six rats receiving the high fat diet plus 100 μg. of riboflavin per day equal to the weights of six of the above rats fed the same ration without riboflavin by limiting the food intake of the former. Although emaciated, the starved animals receiving abundant riboflavin remained outwardly in excellent condition.

In addition to aggravating the production of spastic paralysis and decreasing the survival time the feeding of a high fat diet results in a more severe dermatitis and alopecia than is obtained when a high sucrose diet is fed. Denuded and incrusted areas are particularly noted about the face and limbs. Animals in the advanced stages of the deficiency have been cured by feeding 100 μg. of riboflavin per day. By the end of 2 or 3 weeks the spasticity completely disappears. Large clumps of a mixture of hair, skin and ration are pulled off by the rat leaving practically the entire surface of the animal bare. Gradually the hair grows in and the rat appears normal in all respects except that a few scars remain, especially about the face where incrustation is usually severe.

A condition known as "spectacle eye", characterized by a local alopecia immediately surrounding the eyelids of rats, has been related to biotin (Nielson and Elvehjem, '41) and to inositol (Pavcek and Baum, '41) deficiency. Chick, Macrae and Worden ('40) have noted a similar loss of hair in riboflavin deficient rats. A majority of the animals in our experiments developed this peculiar hair loss about the eyes which could not be prevented by the daily administration of 2 μg. of biotin or 10 mg. of inositol. These rats were commonly observed in the act of rubbing their eyes which probably accounts for the observed "spectacle eye".

The reddish accumulation of porphyrin on the paws, nose and whiskers of riboflavin deficient rats described by Chick et al. ('40) was observed in all but a small percentage of the above animals. The number of rats showing cataract was two out of eighteen fed the high carbohydrate diet and six out of nineteen receiving the high fat ration.

DISCUSSION

It is apparent from the foregoing experiments that dextrin and cornstarch reduce the dietary riboflavin requirement of the rat and that sucrose, cellulose, or fat do not share this property. Dextrin and cornstarch probably increase the amount of available riboflavin synthesized in the intestine by providing the intestinal microorganisms with a favorable medium for such synthesis, by increasing the number of

microorganisms, or by changing the flora to a type capable of producing greater quantities of the vitamin. The incomplete digestion of dextrin or cornstarch allows some of the carbohydrate to reach the lower regions of the tract where microorganisms are ordinarily found in abundance. Sucrose is more readily digested and absorbed than dextrin or cornstarch and would not be expected to reach this site of increased bacterial activity in any great quantity. This concept is in keeping with that suggested by Guerrant et al. ('35). Fat and cellulose, though capable of reaching the lower tract, are apparently of little value in promoting the synthesis of available riboflavin. In fact, fat may inhibit such synthesis.

The position of lactose in sparing riboflavin is uncertain, allowing growth intermediate between that obtained on the sucrose and dextrin diets. Morgan, Cook and Davison ('38) have reported that the feeding of lactose reduces the riboflavin requirement below that of animals fed either sucrose or cornstarch and were able to show that the ceca of lactose-fed rats contained appreciable amounts of riboflavin. Contrary to our observations, their data do not indicate that cornstarch supports better growth than does sucrose when the riboflavin intake is limiting.

Numerous investigators in the past have noted that the feeding of vitamin-low rations containing certain constituents results in greater growth than can be accounted for by the vitamin content of these constituents, and they have attempted to correlate such unusual growth with an increased vitamin content of the feces or of the material found in various sections of the digestive tract. Most of this work has not been of a strictly quantitative nature due largely to inadequacy of vitamin determination methods and to the inability to properly differentiate between the individual components of the B complex.

The idea has prevailed that a measurement of the vitamins in the tract and in the feces could somehow be used as a relative measure of the amount of the vitamin made available to the animal through bacterial synthesis. That such a relationship must necessarily exist is untenable on theoretical grounds. It can be successfully argued that the vitamin content of the feces or of other intestinal material may have little to do with the amount of the vitamin absorbed by the host. For example, the riboflavin may be synthesized in a region of the intestine from which it cannot be absorbed. Selye ('43) has studied the absorption of riboflavin from the various sections of the tract, separated by ligatures, using nephrectomized rats. He concludes that in the small intestine, riboflavin is both absorbed and excreted, while in the cecum and colon, injected riboflavin is rapidly destroyed with little,

if any, absorption of the vitamin. However, in his experiments very large concentrations of riboflavin were employed, and only amounts of the vitamin that could be detected grossly were considered. Whether or not minute amounts of riboflavin, such as are involved under normal conditions, can be absorbed from the cecum and colon still remains a question. That the cecum does actually contribute to the supply of the rat's B vitamins has been demonstrated in the cecectomy studies of Taylor, Pennington and Thacker ('42). The cecum has been emphasized as the most probable site of bacterial synthesis largely because of the abundance of microorganisms found there. However, other sections of the tract cannot be ignored in this respect. Porter and Rettger ('40) find, contrary to much prevailing opinion, that the upper intestine of the rat contains appreciable numbers of viable bacteria.

Secondly, the vitamin may exist as an integral part of the bacteria, unavailable to the animal. Abdel-Salaam and Leong ('38) have demonstrated that the thiamine synthesized by the mixed flora taken from the ceca of rats and grown in vitro is contained in the bacterial cells rather than in the surrounding medium, and Mitchell and Isbell ('42) find that the greater proportion of the vitamins found in the cecal contents of rats is contained in the bacterial cells. However, it has been shown that when bacteria, capable of synthesizing vitamins, including those species normally found in the tract, are grown in pure cultures, the vitamins synthesized are found largely in the medium (Burkholder and McVeigh, '42, Thompson, '42, Rodgers, '42). The vitamins elaborated are a result of both bacterial excretion and autolysis. This difference between the amount of free vitamin obtainable when mixed and when pure cultures were used is understandable if, in the mixed cultures, bacteria requiring or destroying an external source of the vitamin are present as well as those capable of its synthesis, in which case, the former would utilize the vitamin released by the latter. Comparable symbiotic relationships may be expected to exist in the cecum and other parts of the tract containing a mixed flora.

The contents of the tract may be considered to be in constant state of activity, bacteria constantly dividing, dying, disintegrating, and giving up their contents for digestion and absorption. Organisms may be excreting vitamins directly into the intestinal media to be absorbed with varying degrees of rapidity by either the host or by still other bacteria requiring the vitamin. The state of the intestinal contents at any given instant, then, will be the result of the relative rates at which the many possible processes proceed. A small quantity of either total or available vitamin in the intestinal material or feces might be inter-

preted to mean that large amounts of the vitamin had been formed and absorbed as well as that a small quantity had been produced.

In view of the above considerations, it is understandable that in our experiments, growth could not be unconditionally correlated with the fecal riboflavin excretion. Perhaps it is more surprising than otherwise that the correlation was as good as it was, the only exception being that of the groups receiving lactose.

It is important that investigators conducting vitamin balance studies recognize that riboflavin is produced in significant quantities by bacteria in the tract and that the amount of riboflavin in the feces is largely independent of the amount fed in the diet. Sure and Ford ('42) overlooked this fact and considered the fecal excretion of riboflavin to be unabsorbed vitamin of dietary origin. Vitamin balance studies such as those of Mitchell and Isbell ('42) do not give accurate values for the amounts of the B complex obtained by the rat from an enteric source because the degree of destruction of these vitamins in the tissues is not known.

Though little difference can be shown between the growth of riboflavin deficient rats receiving sucrose and high fat-sucrose diets, fat aggravates the production of spastic paralysis, suggesting a role of riboflavin in nerve metabolism. Nielson and Elvehjem ('42) have reported a similar spasticity in biotin deficient rats receiving abundant riboflavin. The two conditions are not identical, however, in that biotin failed to prevent the paralysis resulting from the lack of riboflavin.

While studying the sparing action of fat on thiamine, Evans, Lepkovsky and Murphy ('34) observed that rats continued to grow on a 55% fat ration deficient in thiamine but adequate in vitamin G, and that if vitamin G was discontinued, the animals developed what were described as typical symptoms of beriberi. They concluded that vitamin G prevented these symptoms, and in effect, actually spared thiamine. It now seems more likely that these workers had observed the paralysis due to riboflavin deficiency rather than the symptoms of thiamine avitaminosis.

SUMMARY

When the riboflavin intake is sub-optimal, rats receiving a diet rich in dextrin or cornstarch show greater weight increases than do animals receiving diets characterized by their content of sucrose, cellulose, lactose, or lard. Lactose is not entirely without effect in this respect.

The total fecal output of riboflavin of rats receiving various riboflavin-low diets has been determined, and in general, those carbo-

hydrates which decrease the amount of dietary riboflavin needed by the rat for growth are also responsible for the greatest quantities of riboflavin in the feces. However, the fecal excretion of riboflavin was greatest when lactose was fed, although the growth-promoting effect of lactose was not great.

The relationship between growth, cecal size, intestinal synthesis, and the quantity of riboflavin in the cecum and feces is discussed.

The feeding of high levels of dietary fat to riboflavin deficient rats results in a spastic paralysis of the hind quarters, a condition not noted with any degree of severity when riboflavin-low rations of high carbohydrate content are employed. Riboflavin deficient rats survive for shorter periods of time when fed a high fat ration than when maintained on a high carbohydrate diet.

LITERATURE CITED

ABDEL-SALAAM, A., AND P. C. LEONG 1938 Synthesis of vitamin B_1 by intestinal bacteria of the rat. Biochem. J., vol. 32, p. 958.

ANDREWS, J. S. 1943 A collaborative study of riboflavin assay methods. Cereal Chem., vol. 20, p. 3.

BOUTWELL, R. K., R. P. GEYER, C. A. ELVEHJEM AND E. B. HART 1943 Further studies on the comparative value of butter fat, vegetable oils, and oleomargarines. J. Nutrition, vol. 26, p. 601.

BURKHOLDER, P. R., AND I. MCVEIGH 1942 Synthesis of vitamins by intestinal bacteria. Proc. Nat. Acad. Sci., vol. 28, p. 285.

CHICK, H., T. F. MACRAE AND A. N. WORDEN 1940 Relation of skin lesions in the rat to deficiency in the diet of different B_2-vitamins. Biochem. J., vol. 34, p. 580.

CONNER, R. T., AND G. J. STRAUB 1941 Combined determination of riboflavin and thiamin in food products. Ind. Eng. Chem. (Anal. Ed.), vol. 13, p. 385.

EVANS, H. M., S. LEPKOVSKY AND E. A. MURPHY 1934 The sparing action of fat on vitamin B. VI. The influence of the levels of protein and vitamin G. J. Biol. Chem., vol. 107, p. 429.

GUERRANT, N. B., AND R. A. DUTCHER 1934 Some effects of the composition of the diet on the vitamin B and vitamin G requirement of the growing rat. J. Nutrition, vol. 8, p. 397.

GUERRANT, N. B., R. A. DUTCHER AND R. A. BROWN 1937 Further studies concerning the formation of the B-vitamins in the digestive tract of the rat. J. Nutrition, vol. 13, p. 305.

GUERRANT, N. B., R. A. DUTCHER AND L. F. TOMEY 1935 The effect of the type of carbohydrate on the synthesis of the B-vitamins in the digestive tract of the rat. J. Biol. Chem., vol. 110, p. 233.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in the nutrition of chicks. J. Biol. Chem., vol. 138, p. 459.

HODSON, A. Z., AND L. C. NORRIS 1939 A fluorometric method for determining the riboflavin content of foodstuffs. J. Biol. Chem., vol. 131, p. 621.

LAMOREUX, W. F., AND A. E. SCHUMACHER 1940 Is riboflavin synthesized in the feces of fowl? Poul. Sci., vol. 19, p. 418.

MANNERING, G. J., M. A. LIPTON AND C. A. ELVEHJEM 1941 Relation of dietary fat to riboflavin requirement of growing rats. Proc. Soc. Exp. Biol. and Med., vol. 46, p. 100.

MITCHELL, H. K., AND E. R. ISBELL 1942 Intestinal bacterial synthesis as a source of B vitamins for the rat. The University of Texas Publication, no. 4237, p. 125.

MORGAN, A. F., B. B. COOK AND H. G. DAVISON 1938 Vitamin B_2 deficiencies as affected by dietary carbohydrate. J. Nutrition, vol. 15, p. 27.

NIELSEN, E., AND C. A. ELVEHJEM 1941 Cure of spectacle eye condition in rats with biotin concentrates. Proc. Soc. Exp. Biol. and Med., vol. 48, p. 349.

——— 1942 Cure of paralysis in rats with biotin concentrates and crystalline biotin. J. Biol. Chem., vol. 144, p. 405.

PAVCEK, P. L., AND H. M. BAUM 1941 Inositol and spectacled eye in rats. Science, vol. 93, p. 502.

PORTER, J. R., AND L. F. RETTGER 1940 Influence of diet on the distribution of bacteria in the stomach, small intestine and cecum of the white rat. J. Infectious Diseases, vol. 66, p. 104.

POTTER, R. L., A. E. AXELROD AND C. A. ELVEHJEM 1942 The riboflavin requirement of the dog. J. Nutrition, vol. 24, p. 449.

RODGERS, N. E. 1942 The synthesis of riboflavin by microorganisms. A thesis submitted in partial fulfillment of the requirements for the degree of doctor of philosophy. Univ. of Wisconsin.

SELYE, H. 1943 The role played by the gastrointestinal tract in the absorption and excretion of riboflavin. J. Nutrition, vol. 25, p. 137.

SHAW, J. H., AND P. H. PHILLIPS 1941 The pathology of riboflavin deficiency in the rat. J. Nutrition, vol. 22, p. 345.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.), vol. 11, p. 346.

STRONG, F. M., AND L. E. CARPENTER 1942 Preparation of samples for microbiological determination of riboflavin. Ind. Eng. Chem. (Anal. Ed.), vol. 14, p. 909.

SURE, B., AND Z. W. FORD, JR. 1942 Vitamin interrelationships. II. Thiamine and riboflavin interrelationships in metabolism. J. Biol. Chem., vol. 146, p. 241.

TAYLOR, A., D. PENNINGTON AND J. THACKER 1942 The vitamin requirements of cecectomized rats. The University of Texas Publication, no. 4237, p. 135.

THOMPSON, R. C. 1942 Synthesis of B vitamins by bacteria in pure culture. The University of Texas Publication, no. 4237, p. 87.

WAGNER, J. R., A. E. AXELROD, M. A. LIPTON AND C. A. ELVEHJEM 1940 A rat assay method for the determination of riboflavin. J. Biol. Chem., vol. 136, p. 357.

Proc. Soc Exp Biol Med, 75, 1950

179

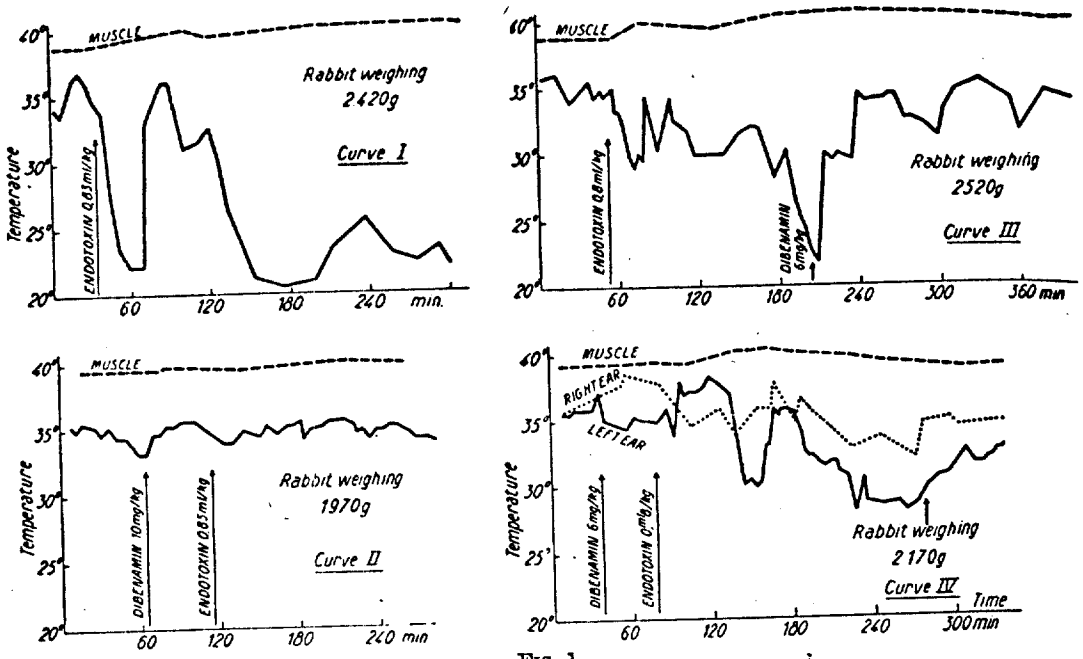

FIG. 1.

Effects of injections of dibenamin before and after the administration of typhoid endotoxin on the skin temperature of the rabbit's ear.

Curve 1—Skin temperature after an intravenous injection of typhoid endotoxin (1 m.l.d.).

Curve 2—Effect of an injection of dibenamin before the administration of the toxin.

Curve 3—Effect of an injection of dibenamin after the administration of the toxin.

Curve 4—Effect of 2 injections of dibenamin, one before and one after the administration of the toxin (the left ear has been dienervated four days before the experiment).

In the 4 curves, the internal temperature is indicated by a dotted line.

can be decreased by previous administration of Dibenamin. However, to maintain this effect, it is necessary to follow with another injection of Dibenamin (3 mg per kg) (Curve 4).

Summary and conclusions. The injection *S. typhosa* endotoxin appears to stimulate the liberation of adrenalin or adrenalin-like substances, bringing about peripheral vasoconstriction. Dibenamin, a sympatho-adrenal blocking substance, antagonizes this effect.

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Studies on the Effect of Different Carbohydrates on Chick Growth.* (18164)

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Nutritional differences among sucrose, dextrin, lactose, and cerelose have been reported by several workers. Elvehjem(1,2) has reviewed much of the work done on carbo-

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TABLE I. Effect of Supplements on Growth of Chicks Receiving Different Carbohydrates.

Treatment	4-week % wt gain (basal groups = 100%)*			
	Dextrin	Sucrose	Lactose	Cerelose
Basal	279 g = 100% (36)	206 g = 100% (36)	149 g = 100% (32)	259 g = 100% (12)
Folic acid deficient	35% (12)	22% (17)	53% (11)	—
Basal +				
0.1 μg vit. B_{12}/day inj.	119 (12)	113 (12)	107 (12)	—
1.0 μg "	110 (12)	118 (11)	134 (8)	—
0.6 μg vit. B_{12}/100 g ration	113 (12)	—	127 (12)	—
reticulogen (1 U.S.P. unit/day inj.)	114 (12)	121 (12)	142 (9)	—
fish solubles (3%)	110 (12)	107 (12)	143 (11)	—
sulfasuxidine (2%)	100 (12)	81.5 (12)	86.5 (9)	—
sulfasuxidine (2%) + cellulose (10%)	88 (12)	86 (12)	74 (11)	—
cellulose (10%)	91 (12)	84 (12)	—	—
2 × B vitamins	98 (12)	100 (12)	—	—
Basal (50%) + 50% dextrin	—	117 (12)	174 (12)	—

* Figures in parentheses indicate the number of chicks surviving. Those with numbers greater than 12 indicate that two or three experiments were run.

hydrate differences in experimental animals. Only a small amount of work has been done on the effect of different carbohydrates in rations for the chick. Luckey *et al.*(3) found that the folic acid requirement of the chick varies with the type of carbohydrate. Couch *et al.*(4) showed that the biotin content of eggs from hens receiving sucrose as the carbohydrate is much lower than that of eggs from hens on dextrin rations. Sarma *et al.*(5) presented differences in vitamin B_6 synthesis with different carbohydrates.

It is the purpose of this paper to compare the nutritional value of sucrose, dextrin, lactose, and cerelose and to study the effect of folic acid, vit. B_{12}, and other growth factors on these carbohydrates as they affect chick growth.

Experimental. Straight run (New Hampshire ♂♂ X Single Comb White Leghorn ♀♀) cross bred chicks, which were the progeny of hens fed diet B-1 described previously(6) were used in all studies. The chicks were housed in electrically heated batteries with raised screen floors. Feed and water were supplied *ad libitum*. The chicks were wing-banded and weighed at 1 day of age. Weights were recorded at weekly intervals. Each group contained 12 birds. All chicks were divided into groups according to weight and immediately placed on test for four weeks. They all received a semi-purified ration containing carbohydrate 61 g, alcohol extracted casein 18 g, gelatin 10 g, salts V(7) 6 g, soybean oil 5 g, L-cystine 0.3 g, thiamine hydrochloride 0.3 mg, riboflavin 0.6 mg, nicotinic acid 5.0 mg, pyridoxine hydrochloride 0.4 mg, calcium pantothenate 2.0 mg, choline chloride 150 mg, biotin 0.03 mg, inositol 100 mg, 2-methyl-1,4-naphthoquinone 0.05 mg, α-tocopherol 0.3 mg, and folic acid 0.2 mg (except where indicated). Fortified haliver oil (60,000 U.S.P. units of vit. A, 6,000 U.S.P. units of vit. D_3 per g) was given by dropper (2 drops per bird per week). All supplements were added at the expense of the carbohydrate. Table I was compiled by cal-

1. Elvehjem, C. A., *J. Am. Dietet. Assoc.*, 1946, v22, 959.

2. Elvehjem, C. A., *Federation Proceedings*, 1948, v7, 410.

3. Luckey, T. D., Moore, P. R., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1946, v62, 307.

4. Couch, J. R., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *J. Nutrition*, 1948, v35, 57.

5. Sarma, P. S., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.*, 1946, v165, 55.

6. Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Poultry Sci.*, 1948, v27, 443.

7. Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem*, 1943, v148, 163.

culating the per cent gain or retardation of each group relative to that group's basal control. The basal groups are shown as 100%, and the weight in grams is the average of the three experiments run.

Results and discussion. Table I shows that the chicks receiving the basal ration containing dextrin grew 73 g more than those receiving sucrose. The chicks receiving cerelose grew 53 g more than the sucrose-fed birds, and the birds receiving lactose grew 57 g less than the sucrose-fed birds. The feathering and general appearance of the birds receiving dextrin, cerelose, or sucrose were excellent. By the end of the first week, the chicks on the lactose diets developed severe diarrhea which lasted throughout the test period. Their general appearance was very poor, which may have been due partly to the fact that it was impossible to keep their cages clean and dry. These birds showed no decrease in food consumption, and they consumed about twice the amount of water consumed by the other birds, indicating the extent of the diarrhea.

When folic acid was omitted from the basal rations, there was a very significant decrease in growth in all cases. Although the birds receiving lactose showed the smallest percentage decrease, the birds receiving dextrin showed the best growth. This confirms work done by Luckey *et al.*(3) who showed that the chick has a smaller requirement for folic acid on rations containing dextrin than on rations containing sucrose.

When chicks receiving the basal rations were injected with a low level of vit. B_{12}, slight responses were obtained with all carbohydrates tested. However, when a higher level of vit. B_{12} was injected or fed orally, the response obtained with lactose was greater than the responses obtained with the other carbohydrates. Reticulogen and fish solubles also gave a better response with chicks on a lactose diet than with chicks on diets containing sucrose or dextrin. This may indicate that chicks on a lactose diet have a higher requirement for unknown growth factors which have been reported to be present in these preparations.

We have shown previously(8) that sulfasuxidine affects the growth of chicks as well as the liver storage and caecal content of folic acid. When sulfasuxidine was fed to chicks on different carbohydrates, the growth of the birds receiving sucrose and lactose was retarded in contrast to no effect in the case of the dextrin-fed birds. This indicates that the intestinal flora established on a ration containing dextrin as the carbohydrate constituent is affected in a different way or to a different extent by sulfasuxidine than the flora of chicks fed sucrose or lactose.

Since growth responses had been reported by Lepp *et al.*(9) with cellulose when 18% casein was fed, 10% cellulose was tried but gave a growth depression in all cases. A combination of sulfasuxidine and cellulose did not produce any response. Groups of chickens were fed twice the level of folic acid and other B vitamins. This was done to determine whether the difference in folic acid requirements on dextrin and sucrose diets, or intestinal synthesis of other known factors, was the cause of better growth with dextrin. No further growth responses were observed. In no case was it possible to obtain as good growth with chicks fed the sucrose ration as with chicks fed the dextrin ration.

It was interesting to find when diets with a carbohydrate consisting of 50% dextrin and either 50% sucrose or lactose were fed; the chicks exhibited significantly better growth. The response was especially striking in the lactose group in which a weight gain of 115 g and complete disappearance of the diarrhea were observed.

Johansson *et al.*(10) showed that the flora of hens on a dextrin diet contain 10 times the number of coliforms as the flora of hens on a sucrose plus biotin diet. It may be that 50% dextrin is enough to change the intestinal flora to the apparently more favorable condition and in the case of the lactose

8. Dietrich, L. S., Monson, W. J., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., in press.

9. Lepp, A., Harper, A. E., and Elvehjem, C. A., *Poultry Sci.*, 1949, v28, 372.

10. Johansson, K. R., Shapiro, S. K., and Sarles, W. B., *J. Bacteriol.*, 1947, v54, 35.

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Sucrose	11
Dextrin	14

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May 13, 1950.

1. Hargraves, M.,
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TABLE II. Excretion Time of Chicks as Affected by Different Carbohydrates.*

Carbohydrate	2 wk minutes	4 wk Group 1 minutes	4 wk Group 2 minutes
Lactose	77 ± 7	86 ± 14	78 ± 6
Sucrose	115 ± 22	133 ± 6	149 ± 17
Dextrin	140 ± 8	177 ± 5	181 ± 11

* Includes stand. error. $\sqrt{\frac{d^2}{n(n-1)}}$.

group to also eliminate entirely the dehydration caused by lactose.

It was thought that a clearer understanding of the intestinal effects would be had by determining the time it takes for food to pass through the digestive tract of the chicken. At 2 and 4 weeks, 5 chicks per group were put in individual cages and given feed and water *ad libitum*. Each bird was given a number zero capsule filled with carmine 40, which is not absorbed by the intestine. The length of time taken for red to first appear in the feces was taken as the excretion time. The results are shown in Table II. The excretion time of the dextrin birds was 20% longer than the sucrose birds. These data lend support to the theory that carbohydrate differences may be explained on the basis of intestinal synthesis or efficiency of carbohydrate breakdown in the gut. It would seem that dextrin allows more time for synthesis to take place, since chicks fed this ration retain the food longer. This extra time may allow additional synthesis of some unknown chick growth factor or factors.

Summary. When the rate of growth of chicks is used to measure the nutritional efficiency of carbohydrates they fall in the following order: dextrin, cerelose, sucrose, and lactose. Cellulose, sulfasuxidine, reticulogen, fish solubles, or vit. B_{12} do not change the significant differences observed when these carbohydrates are fed. However, the lactose-fed birds do give the greatest response to reticulogen, fish solubles and the higher levels of vit. B_{12}. The excretion times of chickens fed these different carbohydrates are in the following decreasing order: dextrin, sucrose, and lactose. The possibility of these nutritional differences being explained by the synthesis of known and/or unknown factors is discussed.

We are indebted to Merck and Co., Inc., Rahway, N. J., for crystalline vit. B_{12}, and crystalline vitamins; to the Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y., for synthetic folic acid; to Abbott Laboratories, North Chicago, Ill., for haliver oil; to Wilson and Co., Inc., Chicago, Ill., for gelatin; and to E. I. duPont de Nemours and Co., Inc., New Brunswick, N. J., for crystalline vit. D_3.

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Experimental Production of the L. E. Phenomenon in the Skin of Man.* (18165)

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The L.E. cell, first described by Hargraves, Richmond and Morton(1), was originally observed in incubated marrow aspirates of patients suffering with acute disseminated lupus erythematosus. Sundberg and Lick(2) found occasional L.E. cells in the peripheral blood in acute disseminated lupus erythematosus although again leukocytic concentration in the test tube was employed. Haserick and Bortz

* Presented before the Joint Meeting of the North Central Region College of American Pathologists and the Michigan Pathological Society, Detroit, Mich., May 13, 1950.

1. Hargraves, M., Richmond, H., and Morton, R., *Proc. Staff Meet. Mayo Clinic*, 1948, v23, 25.

2. Sundberg, R. D., and Lick, N., *J. Invest. Derm.*, 1949, v12, 83.

J. Nutr., 53, 1954

180

THE EFFECT OF LEVEL OF DIETARY PROTEIN ON THE GROWTH OF CHICKS FED PURIFIED DIETS CONTAINING SUCROSE OR DEXTRIN [1]

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INTRODUCTION

Monson, Dietrich and Elvehjem ('50) reported that chicks raised on a complete purified diet containing dextrin (autoclaved cornstarch) grew more rapidly than those fed a similar diet with sucrose as the carbohydrate. The growth of chicks receiving the sucrose diet was improved when certain natural materials, such as 4% of primary yeast, were included in the diet (Dietrich et al., '52) but no improvement was observed when the levels of the known vitamins were increased (Monson et al., '52).

Since additional casein had been shown to improve the growth of chicks fed a diet containing 18% casein, 10% gelatin and cerelose (Lepp et al., '49), and since Harper and Katayama ('53) had observed that a diet containing 9% casein with cornstarch as the carbohydrate supported better growth of rats than did a sucrose diet containing the same level of protein, it was considered important to determine the effect

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We are indebted to Merck and Co., Rahway, N. J. for some of the crystalline vitamins and for crystalline penicillin; to Commercial Solvents Corporation, Terre Haute, Ind., for bacitracin; to Wilson and Co., Inc., Chicago, Ill., for gelatin; to Abbott Laboratories, North Chicago, Ill., for haliver oil; and to E. I. DuPont de Nemours and Co., Inc., New Brunswick, N. J., for crystalline vitamin D_3.

of additional dietary protein on the growth of chicks fed the sucrose diet of Monson et al. ('50).

The effects of protein and amino acid supplements and of antibiotics on the growth of chicks fed a purified diet containing sucrose or dextrin as the carbohydrate are reported in this paper.

EXPERIMENTAL

Straight run (New Hampshire ♂♂ × Single Comb White Leghorn ♀♀) cross-bred chicks, which were the progeny of hens fed diet B-1 described previously (Robblee et al., '48) were used in all studies. The chicks were wing-banded and weighed at one day of age and were separated into similar groups of 15 birds each on the basis of their initial weights. They were housed in electrically heated brooder-type batteries with raised screen floors and were fed ad libitum. Individual weights were recorded weekly during the experimental periods of 4 weeks.

The purified basal ration was composed of carbohydrate, 60.2 gm; alcohol extracted casein, 18 gm; gelatin, 10 gm; salts V (Briggs et al., '43), 6 gm; soybean oil, 5 gm; L-cystine, 0.5 gm; thiamine hydrochloride, 0.6 mg; riboflavin, 1.2 mg; nicotinic acid, 10 mg; pyridoxine hydrochloride, 0.8 mg; calcium pantothenate, 4.0 mg; choline chloride, 170 mg; biotin, 0.06 mg; inositol, 100 mg; folic acid, 0.5 mg; vitamin B_{12}, 0.005 mg; para-aminobenzoic acid, 10 mg; 2-methyl-1,4-naphthoquinone, 0.05 mg; and α-tocopherol acetate, 1 mg. Fortified halibut liver oil (60,000 U.S.P units of vitamin A, 6,000 U.S.P. units of vitamin D_3 per gram) was given by dropper (two drops per bird per week). All birds also received as a supplement two drops of freshly prepared thiamine hydrochloride (10 mg/ml) at the end of the first and second weeks. All supplements were provided at the expense of the carbohydrate.

RESULTS

The results of 4 representative experiments, of the total of 9 experiments conducted in the first series, are presented

in table 1. These show the effects of different levels of casein and different supplements on the growth of chicks fed diets containing sucrose or dextrin as the source of carbohydrate. The 4-week weights of the chicks are tabulated separately for each experiment in order that each group may be compared with its own control. This is considered necessary because considerable variation is observed from experiment to experiment among groups receiving similar diets.

In experiment 1 various levels of casein were fed to groups of chicks receiving either sucrose or dextrin diets. When the level of casein in the diets was 18% or less, each group that received dextrin as the carbohydrate grew more rapidly than its sucrose control. The greatest absolute difference in growth was observed between the groups fed 18% casein diets. When the casein content of the diets was increased to 22% no appreciable difference in growth occurred. A similar growth response was obtained when the basal (18% casein-sucrose) diet was supplemented with 2% beef extract. In experiments 4 and 7 and in one experiment not reported in detail, in which sucrose and dextrin diets containing 18 and 25% casein were compared, similar results were obtained.

In experiment 1, and in a similar experiment not included in the table, groups that were fed 18% casein-sucrose diets supplemented with a mixture of 6 essential amino acids (see table 1) grew as well as the best of the groups fed dextrin diets. In experiment 2, although the amino acids improved growth somewhat, none of the sucrose groups grew as well as the dextrin control. In one experiment which is not reported, a much larger supplement of animo acids caused a growth depression. Similar cases have been reported (Almquist, '52), suggesting that a large excess of amino acids may exert deleterious effects.

Experiments 2 and 3 represent other situations that have been encountered during the course of this investigation. In experiment 2, although the group fed the 22% casein-sucrose diet grew better than that receiving the 18% casein-sucrose basal diet, it did not grow as well as the groups fed the dextrin

TABLE 1

Effect of level of dietary protein and type of dietary carbohydrate on chick growth

TYPE OF DIET		EXPERIMENT							
		1		2		3		4	
Casein	Supplement	Sucrose	Dextrin	Sucrose	Dextrin	Sucrose	Dextrin	Dextrin	Sucrose
%		*gm* [1]	*gm* [1]	*gm* [1]	*gm* [1]	*gm* [1]	*gm* [1]	*gm* [1]	*gm* [1]
13		199 ± 15	217 ± 16						
15		306 ± 17	335 ± 18						
18		342 ± 20	374 ± 18	299 ± 12	366 ± 13	350 ± 12	355 ± 9	300 ± 13	350 ± 14
22		369 ± 10	377 ± 10	327 ± 13	365 ± 11				
25						354 ± 12		364 ± 10	357 ± 14
18	2% Beef extract	376 ± 11		329 ± 17	364 ± 9	369 ± 16			
18	4% Beef extract					362 ± 14		354 ± 8	
18	Amino acid mix [2]	373 ± 11		317 ± 14					
18	Antibiotics [3]							378 ± 9	364 ± 9
25	Antibiotics [3]							384 ± 13	

[1] Values represent mean ± standard error of the mean of the 4-week weights for a group of 12 to 15 chicks.

[2] L-lysine·HCl, 0.1%; DL-threonine, 0.1%; DL-tryptophan, 0.15%; DL-valine, 0.1%; L-histidine·HCl·H_2O, 0.1%; and DL-methionine, 0.15% of the diet.

[3] Bacitracin, 2.5 mg + penicillin, 10 mg per 100 gram of ration.

diets. In this experiment the growth of the group receiving the sucrose basal was less than that of the comparable groups in experiments 1 and 3. This situation also occurred in two of the unreported experiments in which groups fed 22% or 25% casein-sucrose diets were included. In experiment 3 and in one unreported case in which the chicks receiving the sucrose basal diet grew extremely well, no response to either protein or dextrin occurred.

TABLE 2

Effect of supplements of different proteins on growth of chicks

DIET			EXPERIMENT 5
Casein	Carbohydrate	Supplement	Wt. at 4 weeks [1]
%			gm
18	Sucrose		293 ± 11
25	Sucrose		317 ± 12
30	Sucrose		336 ± 16
18	Sucrose	10% Fibrin	327 ± 13
18	Sucrose	10% Wheat gluten	339 ± 13
18	Dextrin		332 ± 16

[1] Mean ± standard error of mean for a group of 15 chicks.

Some growth response was observed (exp. 4) when an antibiotic mixture was fed with the dextrin basal diet and with the 25% casein-sucrose diet but the increase in growth was much smaller than that observed in groups receiving only 18% casein in sucrose diets.

The results presented in table 2 show that the growth of chicks fed 18% casein-sucrose diets was increased to that of groups fed 18% casein-dextrin diets when the protein content of the sucrose diet was increased by the addition of other proteins. In this experiment, as in experiments 1 and 4 (table 1), growth of the chicks receiving sucrose diets with higher levels of protein was as good as that of those fed the 18% casein-dextrin diet.

The results of three experiments in which chicks were fed sucrose diets containing different levels of casein are reported in table 3. The average 4-week weights for the males

and the females are presented as well as the combined averages. In each experiment growth (based on the group averages) was appreciably improved when the level of casein was increased from 18% to 25%. The results with the intermediate level of protein are less consistent but appear to follow this trend. When, however, the separate averages for males and females are compared, in only one experiment (exp. 7), in which the growth of the basal group was very poor, did the male birds show a response to the higher levels of casein.

TABLE 3

Effect of level of dietary casein and sex on growth of chicks fed sucrose diets

EXP. NO.	CASEIN LEVEL	WEIGHT AT 4 WEEKS [1]		
		Males	Females	Group
	%	gm	gm	gm
6	18	368 ± 22 (7)[2]	334 ± 28 (7)	351 ± 18
	22	388 ± 29 (7)	301 ± 23 (6)	347 ± 22
	25	366 ± 21 (9)	389 ± 23 (5)	374 ± 16
7	18	272 ± 6 (23)	260 ± 7 (20)	266 ± 5
	22	290 ± 14 (6)	277 ± 10 (8)	283 ± 8
	25	307 ± 16 (7)	293 ± 10 (5)	301 ± 10
8[3]	18	318 ± 8 (7)	275 ± 6 (23)	286 ± 6
	22	328 ± 14 (11)	314 ± 8 (19)	319 ± 7
	25	309 ± 11 (13)	307 ± 9 (17)	308 ± 6

[1] Mean ± standard error of the mean.
[2] Figures in parentheses indicate the number of chicks in the group.
[3] Experiment 8 was of 25 days' duration.

In each experiment when the level of casein in the diet was increased from 18% to 25% the growth of the females was improved, the minimum increase in growth being 32 gm. A similar trend may be noted with the intermediate level of casein except in experiment 6.

DISCUSSION

The observation that chicks grew more rapidly when sucrose in purified diets containing 13%, 15% or 18% casein was replaced with dextrin parallels results obtained with rats

fed similar diets of lower protein content (Harper and Katayama, '53; Harper et al., '53). Since more protein was required in the sucrose than in the dextrin diets to support equivalent growth of chicks it would seem that the protein of purified diets containing up to 18% casein is better utilized, particularly by female chicks of the breed used, when the carbohydrate is provided as dextrin.

Although the results in this study were not as consistent as those reported previously with rats, in 10 of the 12 experiments a growth response was obtained when the casein content of the sucrose diets was increased above 18%. No growth response was obtained when the casein content of the comparable dextrin diets was increased. In three experiments the effects of different levels of casein were compared only in sucrose diets (table 3) so for these no direct comparison with groups receiving dextrin is possible. In 4 of the remaining experiments the rate of growth of chicks fed sucrose diets containing additional casein equalled that of those fed 18% casein and dextrin, and in three others, although an increase was noted it was not as great as that obtained when sucrose was replaced with dextrin. Of the two other experiments it should be pointed out that in one case growth was not improved either by additional protein or by dextrin.

Certain factors that may contribute to the greater variation in the chick experiments have been noted. Differences in the protein content of different lots of casein, for example, could account for some of the variation between experiments. This could not affect the results within an experiment because the same lot of casein was used for all diets in a single experiment. It might, however, account for the extremely good growth of all of the groups in occasional experiments, e.g., experiment 3.

Some experimental variation may be caused by differences in the response of different lots of chicks. Since males responded to additional protein only when the overall growth rate was very low (table 3), differences in the sex ratios of groups in the earlier experiments (exps. 1 to 5), in which

no sex differentiation was attempted, could reduce the magnitude of the average growth differences. In no instance, however, could this cause an apparent growth difference where none existed.

One point should be mentioned with regard to the standard errors reported in the tables. In many of the dextrin groups and better sucrose groups the variance is magnified by the presence of one or two excessively heavy birds while in the negative control groups (18% casein-sucrose) the variance is frequently exaggerated by the presence of a few birds considerably below the average. This situation which cannot be compensated for in the calculations tends to make the differences appear less significant.

The variability in the average final weights of the 18% casein-sucrose groups (273–354 gm) from experiment to experiment compared to the more consistent growth of the 18% casein-dextrin groups (332–387 gm), together with the failure from time to time of amino acid supplements and additional casein to induce a rate of gain of the sucrose groups equal to that of the dextrin controls, suggests that other factors, either physiological or nutritional, may be of importance. Monson et al. ('52) have reported that the growth of chicks fed an 18% casein-sucrose diet was improved by a supplement of 4% of primary yeast. Since the growth responses obtained with the amino acid mixture and with beef extract in the present study as well as that with yeast in the earlier study were similar in magnitude and variability to that obtained when the casein content of the sucrose diet was increased above 18%, and since each of these supplements provided additional amino acids (either free or as protein), the growth responses obtained with them appear to be due, in part at least, to the amino acids they contain. The results of experiment 5 (table 2), in which the growth of chicks fed the 18% casein-sucrose diet was improved when supplements of fibrin and wheat gluten were provided, also support this conclusion.

The results obtained with antibiotic supplements provide further support for this interpretation. Although a growth

response was obtained in each case when antibiotics were provided, the response to antibiotics was of doubtful significance in groups fed either the 25% casein-sucrose diet or the 18% casein-dextrin diet. Since, in groups that received only 18% casein with sucrose, a highly significant growth response was obtained, it would appear that both dextrin and antibiotics exert a protein-sparing action. Evidence for a protein-sparing action of antibiotics has been presented by Machlin et al. ('52) but Biely et al. ('52) and Scott et al. ('52) were unable to demonstrate such an action under their experimental conditions.

Both Monson et al. ('50) and Stokstad et al. ('53) have shown that sucrose diets pass more rapidly than dextrin diets through the gastro-intestinal tract of chicks. In work with rats Geiger ('51) has suggested that carbohydrate fed with protein reduces the rate of deamination of amino acids in the liver, and Harper and Katayama ('53) have suggested that the improvement in the growth of rats fed low protein diets in which sucrose was replaced with dextrin may have a similar basis. Womack et al. ('53) and Marshall and Womack ('54) have demonstrated increased nitrogen retention in rats fed low protein diets in which sucrose was replaced with dextrin. These factors might well contribute to improvement in the utilization of dietary protein in chicks fed dextrin diets.

Whether all of the improvement in growth due to dextrin can be accounted for when these interrelationships have been studied in greater detail or whether certain unknown growth factors may also be required, remains to be established. Despite the fact that recent developments in nutrition have made it possible to improve greatly the growth of chicks fed highly purified diets, there are still many reports showing that growth can be further improved by including certain crude materials in such diets (see Menge et al., '52, for references). When the growth rate of chicks is increased by providing the most complete diet at present possible, the balance of the

major dietary constituents assumes greater significance. Supplements of natural materials may, under such conditions, increase the rate of growth in two ways other than by providing unknown growth factors. Such supplements may provide additional amino acids and thus improve the entire amino acid balance of the diet (a point emphasized by the work of Grau and Kamei, '50; and Almquist, '52) or they may permit improved utilization of dietary protein in a manner similar to that postulated for dextrin.

SUMMARY

A previous report that chicks fed purified diets containing dextrin as the carbohydrate grow at a faster rate than those fed similar diets containing sucrose has been extended. The effects of different levels of dietary casein, of certain protein-containing supplements and of an antibiotic mixture included in such diets have been studied.

With the exception of groups that received antibiotics the best rate of growth occurred consistently in groups fed a diet containing 18% casein and dextrin. Chicks receiving dextrin grew more rapidly than those receiving sucrose when the level of casein was 18% or less. However, when the casein content of the sucrose diets was increased above 18% or when amino acids or supplements of other proteins were provided with 18% casein in sucrose diets, growth approached that of chicks receiving 18% casein with dextrin. The rate of growth of chicks receiving either sucrose or dextrin with 18% to 25% casein was increased when a mixture of penicillin and bacitracin was included in the diets, the effect of the antibiotics being much less in the presence of dextrin and higher levels of casein.

The effect of dextrin in improving growth was most evident in female chicks and it is suggested that dextrin exerts this effect largely by permitting better utilization of dietary protein.

LITERATURE CITED

ALMQUIST, H. J. 1952 Amino acid requirements of chickens and turkeys — a review. Poultry Sci., *31:* 966.

BIELY, J., B. E. MARCH AND D. F. SMITH 1952 The effect of feeding antibiotics on the carbohydrate and protein requirements of the chick. Ibid., *31:* 863.

BRIGGS, G. M., JR., T. D. LUCKEY, C. A. ELVEHJEM AND E. B. HART 1943 Studies on two chemically unidentified water-soluble vitamins necessary for the chick. J. Biol. Chem., *148:* 163.

DIETRICH, L. S., W. J. MONSON AND C. A. ELVEHJEM 1952 Studies on an unidentified growth factor for chicks and hyperthyroid rats fed purified rations. Arch. Biochem. Biophys., *38:* 91.

GEIGER, E. 1951 Extra caloric function of dietary components in relation to protein utilization. Fed. Proc., *10:* 670.

GRAU, C. R., AND M. KAMEI 1950 Amino acid imbalance and the growth requirements for lysine and methionine. J. Nutrition, *41:* 89.

HARPER, A. E., AND M. C. KATAYAMA 1953 The influence of various carbohydrates on the utilization of low protein rations by the white rat. I. Comparison of sucrose and cornstarch in 9% casein rations. Ibid., *49:* 261.

HARPER, A. E., W. J. MONSON, D. A. ARATA, D. A. BENTON AND C. A. ELVEHJEM 1953 Influence of various carbohydrates on the utilization of low protein rations by the white rat. II. Comparison of several proteins and carbohydrates. Growth and liver fat. Ibid., *51:* 523.

LEPP, A., A. E. HARPER AND C. A. ELVEHJEM 1949 Cellulose in purified diets for chicks. Poultry Sci., *28:* 372.

MACHLIN, L. J., C. A. DENTON, W. L. KELLOG AND H. R. BIRD 1952 Effect of dietary antibiotic upon feed efficiency and protein requirement of growing chickens. Ibid., *31:* 106.

MARSHALL, M. W., AND M. WOMACK 1954 Influence of carbohydrates, nitrogen source and prior state of nutrition on nitrogen balance and liver composition in the adult rat. J. Nutrition, *52:* 51.

MENGE, H., C. F. COMBS, PENG-TUNG HSU AND M. S. SHORB 1952 Unidentified growth factors required by chicks and poults. Poultry Sci., *31:* 237.

MONSON, W. J., L. S. DIETRICH AND C. A. ELVEHJEM 1950 Studies on the effect of different carbohydrates on chick growth. Proc. Soc. Exp. Biol. Med., *75:* 256.

——— 1952 The effect of different carbohydrates and antibiotics on the growth of chicks and the storage of vitamins. J. Nutrition, *46:* 411.

ROBBLEE, A. R., C. A. NICHOL, W. W. CRAVENS, C. A. ELVEHJEM AND J. C. HALPIN 1948 The effect of hen diet and chick diet on the need of chicks for an unidentified growth factor. Poultry Sci., *27:* 443.

SCOTT, H. M., E. A. GOFFI AND W. A. GLISTA 1952 The protein requirements of the chick as influenced by aureomycin. Ibid., *31:* 751.

STOKSTAD, E. L. R., T. H. JUKES AND W. L. WILLIAMS 1953 The growth promoting effect of aureomycin on various types of diet. Ibid., *32:* 1054.

WOMACK, M., M. W. MARSHALL AND A. B. PARKS 1953 Some factors affecting nitrogen balance in the adult rat. J. Nutrition, *51:* 117.

THE EFFECT OF PARTLY SYNTHETIC DIETS ON THE DENTAL CARIES INCIDENCE IN SYRIAN HAMSTERS

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SEVERAL experimental studies in the past few years have revealed that the Syrian hamster (*Cricetus auratus*) is sufficiently susceptible to tooth decay and sufficiently different from the albino rat in its reaction to various caries-inducing procedures to make it a useful animal for various kinds of dental caries investigations, particularly for certain kinds of studies which have been difficult to make with the rat.

Arnold[1] first demonstrated that molar lesions develop in these animals when they are fed a soft or finely ground carbohydrate diet. The carious process in hamsters was as marked in speed of development and in extent of lesions as the carious destruction encountered in the commonly studied experimental caries of the white rat. Furthermore, lesions in the hamster developed without the occurrence of cusp fractures, which Rosebury, Karshan, and Foley[2] and others described and attributed to the feeding of coarse, hard particle diets such as those developed by Shibata[3] and by Hoppert, Webber, and Cauniff[4] and which have been generally accepted as essential to the development of occlusal caries in the white rat. Carious lesions have been encountered in areas of the rat molar which were free of fractures, but these seem to occur less frequently and when found were smaller and slower in their development. The hamster, however, appears to be more susceptible to caries-inducing influences which are not dependent upon preceding fracture of the cusps.

Keyes and Dale, who described the morphological structure of the hamster dentition,[5] confirmed Arnold's findings and gave detailed descriptions of several typical carious lesions developed in the molars of this animal.[6] With the hamster a number of studies have been conducted upon dental caries-producing and caries-inhibiting diets.[7-13] In earlier reports from this laboratory, some of the first work of Keyes and his confreres was confirmed[14] and descriptions were given of the oral bacteria found associated with dental caries in the hamster.[15] These reports were based on data obtained from a portion of the animals used in a series of experiments designed to explore in a preliminary fashion the influence of certain partly synthetic diets upon the incidence of dental caries. This series, involving a total of 894 hamsters† distributed into 18 different dietary groups and including the animals used in the previous studies (except those on commercial rations), provides the material for the present report.

Since many types of bacteria, including those which have been considered important in the etiology of tooth decay, are known to require certain vitamins

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†Of the 894 hamsters which survived the test period, 212 were used in preliminary experiments while the principal study included 682 animals as reported earlier.[11]

or vitamin-like factors for growth and metabolic activity, it was considered possible that a decrease in the amount of tooth decay might result from a deficiency in one or more of these substances in the substrate available as food to bacteria in the oral cavity. Contrary effects may also be present, as has been demonstrated for vitamin K,[16] which by its presence seems to inhibit acid formation by the mixed flora of the saliva. That certain qualitative and quantitative changes in the flora might be expected to occur when certain nutrient substances essential for bacterial metabolism are eliminated from the diet was considered, and such findings have been reported previously.[15] This study, then, concerns itself with the dental caries incidence in hamsters which were fed upon various, partly synthetic diets, each of the orally administered diets being deficient in a simple vitamin which was supplied by parenteral injection.

EXPERIMENTAL METHODS

Stock Colony Diets

The animals used in preliminary experiments were from a stock colony, originally maintained in the laboratory but subsequently kept by an animal supplier, which was fed a commercially prepared food in pellet form.* Each cage was continuously supplied with water and with a supply of food pellets in excess of the amount consumed. The young hamsters removed from the mothers' cages at 21 to 25 days of age, were kept in ⅓ inch mesh wire bottom cages and placed upon the experimental diets. Two lots of the commercially prepared pellets were used as the basal ration of the breeding colony. The first lot contained 20 ppm (parts per million) of fluorine,† according to the manufacturer's analysis. The second lot, stated to contain 7 ppm, was found upon analysis in the laboratory‡ to contain 17 ppm fluorine.

Progeny from this colony, when fed for 100 days on the experimental diets, developed considerably less caries (Table I) than had been found by Arnold who reported 79 per cent of the molars to be carious after 90 to 95 days on fine corn meal diets. It was assumed that previous exposure to fluorides may have been responsible, at least in part, for the unexpectedly low incidence of caries in these experiments, and arrangements were made with the animal supplier to have the colony fed on grains, leafy vegetables, and carrots until a commercially prepared ration low in fluorides could be obtained. On request two manufacturers submitted food samples of supposedly low fluoride content. One of these was found to contain 17 ppm of fluorine, and the other, 1.5 ppm. The latter, in dry meal form, conformed, in preparation and in analysis except for lowered fluoride content, to the standard laboratory animal food prepared by that manufacturer.§ The stock colonies of an additional supplier of hamsters as well as the one previously engaged were fed on this stock ration throughout the remainder of these studies. Weanling hamsters from these stocks, approximately 21 days old when received, were fed for 5 days in the laboratory on the same low-fluoride diet before being placed on experimental diets.

*Rockland Rat Diet, prepared by Arcady Farms Milling Co., Chicago, Ill.

†Fluorine when expressed as parts per million in this paper refers to the fluoride ion.

‡The authors are indebted for the fluoride analyses to Dr. Delbert A. Greenwood, Department of Pharmacology and the American Meat Institute Research Laboratory, University of Chicago.

§Purina Dog Chow Checkers, prepared by Ralston Purina Co., St. Louis, Mo.

TABLE I

DENTAL CARIES IN HAMSTERS IN PRELIMINARY TESTS OF THREE EXPERIMENTAL DIETS

STOCK RATION* FED TO THE BREEDING COLONY		EXPERIMENTAL DIETS FED TO TEST ANIMALS†	NUMBER OF ANIMALS TESTED	NUMBER OF MOLARS EXAMINED	CARIOUS MOLARS DETERMINED BY GROSS OBSERVATION	
RATION LOTS	FLUORINE CONTENT (PPM)				NO.	%
A	20	I	28	336	84	25
B	17	I	5	60	35	58
A	20	II	3	36	4	11
B	17	II	3	36	21	60
A	20	III	3	36	6	17
B	17	III	10	120	67	56

*Commercial Rockland Rat Diet pellets were used. Lot A of this preparation used in the early period of the study (June to Sept., 1944) contained 20 ppm fluorine according to assay figures supplied by the producer, the Arcady Farms Milling Co., Chicago, Ill. Lot B, from the same supplier, was used somewhat later (after Oct., 1944) and was reported to contain 7 ppm, but on analysis at the University was found to have an average fluorine content of 17 ppm.

†These preliminary diets correspond with three of those used later and listed in Table III under the same numbers. All animals were fed 100 days, abiotized, and their molars then examined.

Experimental Diets

Originally, it was planned to prepare the experimental diets, including extraction of casein, in the laboratory but this proved to be both expensive and time consuming. A commercial, vitamin B test diet* was used as the basal ration. Its composition was as follows:

Sucrose	68 per cent
Vitamin free casein	18 per cent
Vegetable oil	10 per cent
U.S.P. salt mixture No. 2	4 per cent

When it was occasionally necessary to prepare the diet in the laboratory, a vitamin-free casein preparation was obtained from the same commercial supplier.

Vitamins A and D, obtained from another commercial source,† were added to the other vitamin supplements in amounts equal to 2 per cent cod liver oil in the basal diet fed to all the animals and hence were not subjected to test in the study.

The complete vitamin supplement was made up as follows:

Thiamine hydrochloride	50 mg.
Riboflavin	50 mg.
Pyridoxine hydrochloride	50 mg.
Calcium pantothenate	50 mg.
Nicotinic acid	75 mg.
Para-aminobenzoic acid	1 Gm.
Inositol	1 Gm.
Choline chloride	3 Gm.
Biotin	1 ml. (25 μg.)
Alpha-tocopherol (pure)	0.7 ml.
2-methyl naphthoquinone	30 mg.
Vitamin A and D preparation	6 ml.

All vitamins used were pure and in crystalline form; solutions and suspensions of them were kept in the refrigerator.

*Prepared by the S.M.A. Corporation, Chicago, Ill. (now the S.M.A. Division of Wyeth Incorporated, Philadelphia, Pa.).

†Vi-delta, prepared by Lederle Laboratories, Inc. (now Lederle Laboratories Division, American Cyanamid Co., New York, N. Y.)

The effects of the different vitamin B components were tested by elimination. The composition of the 17 different test diets used is shown in Table II. The appropriate supplements were pooled and mixed with 1.0 kg. of vitamin B test diet. This mixture constituted the complete control diet (Diet I). All other diets except Diets VIII and XIV were prepared in the same manner but that one or more vitamins were omitted from the supplement. The missing

TABLE II

COMPOSITION OF EXPERIMENTAL DIETS

DIET NUMBER	BASAL DIET[1] (WITHOUT VITAMINS)	DELETIONS FROM VITAMIN SUPPLEMENT[2]	PARENTERAL SUPPLEMENTATION
I	Complete	None	None
II	Complete	Riboflavin	2 mg. riboflavin in saline (steam sterilized)
III	Complete	Calcium pantothenate	2 mg. calcium pantothenate in saline (steam sterilized)
IV	Complete	Thiamine hydrochloride	2 mg. thiamine hydrochloride in saline (Berkefeld filtered)
V	Complete	Pyridoxine hydrochloride	2 mg. pyridoxine hydrochloride in saline (steam sterilized)
VI	Complete	Nicotinic acid	3 mg. nicotinic acid in saline (steam sterilized)
VII	Complete	Inositol	4 mg. inositol in saline (steam sterilized)
VIII	Complete, but used O.M.W. salt mix instead	None	None
IX	Complete	Para-aminobenzoic acid	4 mg. para-aminobenzoic acid in saline (steam sterilized). Warmed to 90° C. to dissolve crystals; cooled and injected
IXa	Complete, but in addition a saturated solution of sulfadiazine was provided as drinking water	Para-aminobenzoic acid	Same as for Diet IX
X	Complete	2 methyl naphthoquinone (vitamin K)	2 mg. vitamin K in sterile saline; prepared by grinding in sterile mortar and pestle
XI	Complete	Choline chloride	4 mg. choline chloride in saline (steam sterilized)
XII	Complete	Biotin	2.5 mg. biotin in sterile buffered saline
XIII	Complete	Alpha-tocopherol (vitamin E)	5 mg. vitamin E in sterile mineral oil
XIV	Same as Diet VIII but dextrin substituted for sucrose	None	None
XV	Complete	Nicotinic acid and inositol	3 mg. nicotinic acid and 4 mg. inositol in saline (steam sterilized)
XVI	Complete	Thiamine hydrochloride and riboflavin	2 mg. thiamine hydrochloride in saline (filtered) and 2 mg. riboflavin in saline (steam sterilized)

[1] Consists of sucrose, vitamin-free casein, hydrogenated vegetable oil (Crisco), U. S. P. salt mix No. 3.

[2] Vitamin supplement: thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, nicotinic acid, para-aminobenzoic acid, inositol, choline chloride, biotin, alpha-tocopherol, 2-methyl naphthoquinone, and a vitamin A and D preparation (Vi-delta).

vitamin in solution or suspension was supplied in adequate amounts by parenteral injections, twice weekly.

In the beginning the injection was subcutaneous on the abdominal surface, but the animals getting riboflavin by this method developed sterile abscesses. Subsequently intraperitoneal injections were given. There was no evidence of peritoneal inflammation following administration by this route. The biotin solution was strongly acidic and on injection seemed to shock some of the animals temporarily, but mixing it with equal quantities of sterile buffered saline seemed to lessen this effect to some extent.

To test the possibility that the U.S.P. No. 2 salt mixture might not be adequate for hamsters, Diet VIII was devised using the salt mixture of Osborne, Mendel, and Wakeman (O. M. W.). This diet was prepared with vitamin-free casein, sucrose, a hydrogenated vegetable oil,* salt mixture, and vitamin supplement in essentially the same proportions as for Diet I. Diet VIII was also used as a control for Diet XIV in which sucrose was replaced by dextrin, a polysaccharide which is less readily attacked by bacteria of the oral cavity, in an attempt to evaluate the function of carbohydrate in the experimental diet. The basic composition of Diet VIII consisted of the following:

Sucrose	60 per cent
Vitamin-free casein	25 per cent
Vegetable oil	10 per cent
Salt mixture (O.M.W.)	4 per cent
NaCl	1 per cent

The salt mixture was composed of these substances:

$CaCO_3$	543.0 Gm.
KH_2PO_4	212.0 Gm.
KCl	112.0 Gm.
NaCl	69.0 Gm.
$FePO_4 \cdot H_2O$	20.5 Gm.
$MgCO_3$	25.0 Gm.
$MgSO_4$	16.0 Gm.
$CuSO_4$	0.9 Gm.
$MnSO_4$	0.35 Gm.
$Al_2(SO_4)_3 \cdot K_2SO_4$	0.17 Gm.
KI	0.08 Gm.

The same vitamin supplement used for Diets VIII and XIV was used for the rest of the experimental diets as well.

Procedures

Observations of the general state of health of the animals were made frequently and weights were determined about twice a week. At the end of the 100-day experimental period, the hamsters were sacrificed, the jaws of each animal were removed and stored in 70 per cent alcohol. After several days, when the remaining soft tissues had hardened enough to be easily removed, the cleaned jaws were observed with a binocular microscope (magnification of ×15 and ×20) for carious lesions. The incidence of these molar lesions was evaluated,

*Crisco, prepared by Procter & Gamble, Co., Cincinnati, Ohio.

both by the percentage of molars found carious and by a method of scoring almost identical with that of Keyes,[17] wherein the number as well as the size of cavities could be expressed in a caries index for each dietary group.

Preliminary tests were made with 9 small groups of hamsters (Table III). These tests were repeated and extended as shown in Table IV. In the preliminary tests the highest percentage of carious lesions occurred in animals on Diet IX, not containing para-aminobenzoic acid, although this substance was given parenterally. In anticipation of a similarly high incidence in the later experiments, two groups of hamsters were placed on the diet deficient in para-aminobenzoic acid, one of them having as its water supply a saturated aqueous solution of sulfadiazine (Diet IXa). (Table IV.)

TABLE III

PRELIMINARY TESTS. PERCENTAGE OF CARIOUS MOLARS IN HAMSTERS FED FOR 100 DAYS ON DIETS VARYING IN VITAMIN B CONTENT

DIET NUMBER	DIET DESCRIPTON		NUMBER AND SEX OF HAMSTERS	PERCENT OF CARIOUS MOLARS		
	BASAL DIET	VITAMIN SUPPLEMENT		MANDIBULAR	MAXILLARY	TOTAL
I	Sucrose casein vegetable oil salt mix	Complete vitamin Supplement	26 ♂♂ 22 ♀♀	20 51	26 41	23 47
II	Same as Diet I	Complete, except for riboflavin	10 ♂♂ 12 ♀♀	41 32	48 30	45 30
III	Same as Diet I	Complete, except for calcium pantothenate	14 ♂♂ 13 ♀♀	49 41	48 20	48 30
IV	Same as Diet I	Complete, except for thiamine hydrochloride	9 ♂♂ 13 ♀♀	55 47	55 24	55 36
V	Same as Diet I	Complete, except for pyridoxine hydrochloride	7 ♂♂ 7 ♀♀	86 57	90 52	88 55
VI	Same as Diet I	Complete, except for nicotinic acid	3 ♂♂ 3 ♀♀	80 72	88 88	83 81
VII	Same as Diet I	Complete, except for inositol	5 ♂♂ 3 ♀♀	30 28	63 33	46 30
VIII	Same as Diet I but O.M.W. salt mix instead	Complete vitamin supplement	6 ♂♂ 1 ♀	66 66	72 66	69 66
IX	Same as Diet I	Complete, except for para-amino benzoic acid	3 ♂♂ 3 ♀♀	100 100	100 100	100 100

Contrary to findings in preliminary tests it became apparent early in the course of the experiment that animals on Diets VI and VII would have a relatively low dental caries incidence. Accordingly, Diet XV was prepared with both nicotinic acid and inositol deleted; similarly, Diet XVI was made to lack both thiamine and riboflavin. In both cases, however, these dietary double deficiencies were rectified in regard to the hamsters' own nutrition by intraperitoneal injections of the missing vitamins.

TABLE IV

EVALUATION OF DENTAL CARIES IN THE HAMSTERS USED IN THE PRINCIPAL STUDY

DIET	HAMSTERS	CARIOUS MOLARS			CARIES INDEX FOR EACH GROUP
		TOTAL NUMBER	AVERAGE NUMBER PER ANIMAL	PER CENT	
I	17 ♂♂	61	3.59	29.9	3.88
	31 ♀♀	124	4.00	33.3	4.10
	48 total	185	3.85	32.1	4.21
II	17 ♂♂	109	6.41	53.4	4.17
	18 ♀♀	89	4.94	41.2	2.32
	35 total	198	5.66	47.1	3.36
III	4 ♂♂	21	5.25	43.7	9.83
	12 ♀♀	46	3.83	31.9	4.18
	16 total	67	4.19	34.8	5.59
IV	13 ♂♂	92	7.08	59.0	8.05
	6 ♀♀	39	6.50	54.2	7.11
	19 total	131	6.89	57.4	7.75
V	12 ♂♂	83	6.92	57.6	5.57
	8 ♀♀	31	3.88	32.3	3.75
	20 total	114	5.70	47.5	4.84
VI	7 ♂♂	16	2.30	19.0	3.64
	21 ♀♀	51	2.43	20.2	1.59
	28 total	67	2.39	19.9	2.10
VII	23 ♂♂	123	5.35	44.5	3.63
	17 ♀♀	79	4.65	38.7	3.99
	40 total	202	5.05	42.0	3.79
VIII	19 ♂♂	80	4.21	35.1	5.95
	30 ♀♀	173	5.77	48.0	7.27
	49 total	253	5.16	43.0	6.76
IX	25 ♂♂	91	3.64	30.4	3.07
	39 ♀♀	192	4.92	41.0	3.69
	64 total	283	4.42	36.9	3.45
X	15 ♂♂	158	9.20	76.6	16.62
	26 ♀♀	240	9.23	76.6	12.34
	41 total	378	9.22	76.6	13.91
XI	24 ♂♂	234	9.75	84.5	12.15
	25 ♀♀	199	7.96	66.1	8.35
	49 total	433	8.84	73.8	10.21
XII	4 ♂♂	18	4.50	37.5	3.70
	15 ♀♀	53	3.53	29.5	3.58
	19 total	71	3.74	31.1	3.61
XIII	24 ♂♂	181	7.54	62.9	3.84
	21 ♀♀	102	1.86	10.4	2.08
	45 total	283	6.29	52.2	3.02
XIV	15 ♂♂	52	3.47	28.8	3.71
	14 ♀♀	34	2.43	20.3	2.41
	29 total	86	2.97	24.7	3.09
XV	13 ♂♂	109	8.38	69.7	16.79
	33 ♀♀	323	9.79	81.5	1.91
	46 total	432	9.39	78.0	6.09
XVI	27 ♂♂	152	5.63	46.8	3.46
	19 ♀♀	133	7.00	58.3	3.76
	46 total	285	6.19	51.6	3.41
Special "Purina" meal	15 ♂♂	32	1.47	12.2	1.39
	20 ♀♀	7	0.35	2.9	0.05
	35 total	29	0.83	6.9	0.62
IXa (with sulfadiazine water)	22 ♂♂	139	6.32	52.7	3.55
	31 ♀♀	209	6.42	56.4	3.02
	53 total	348	6.57	54.7	2.82

CARIES INDEX FOR EACH GROUP
3.88
4.40
4.21
4.47
2.32
3.36
9.83
4.18
5.59
8.05
7.11
7.75
5.57
3.75
4.84
3.64
1.59
2.10
3.63
3.99
3.79
5.95
7.27
6.76
3.07
3.69
3.45
16.62
12.34
13.91
12.13
8.35
10.21
3.70
3.58
3.61
3.84
2.08
3.02
3.71
2.44
3.03
16.70
1.91
6.09
3.16
3.76
3.44
1.39
0.05
0.62
2.55
3.02
2.82

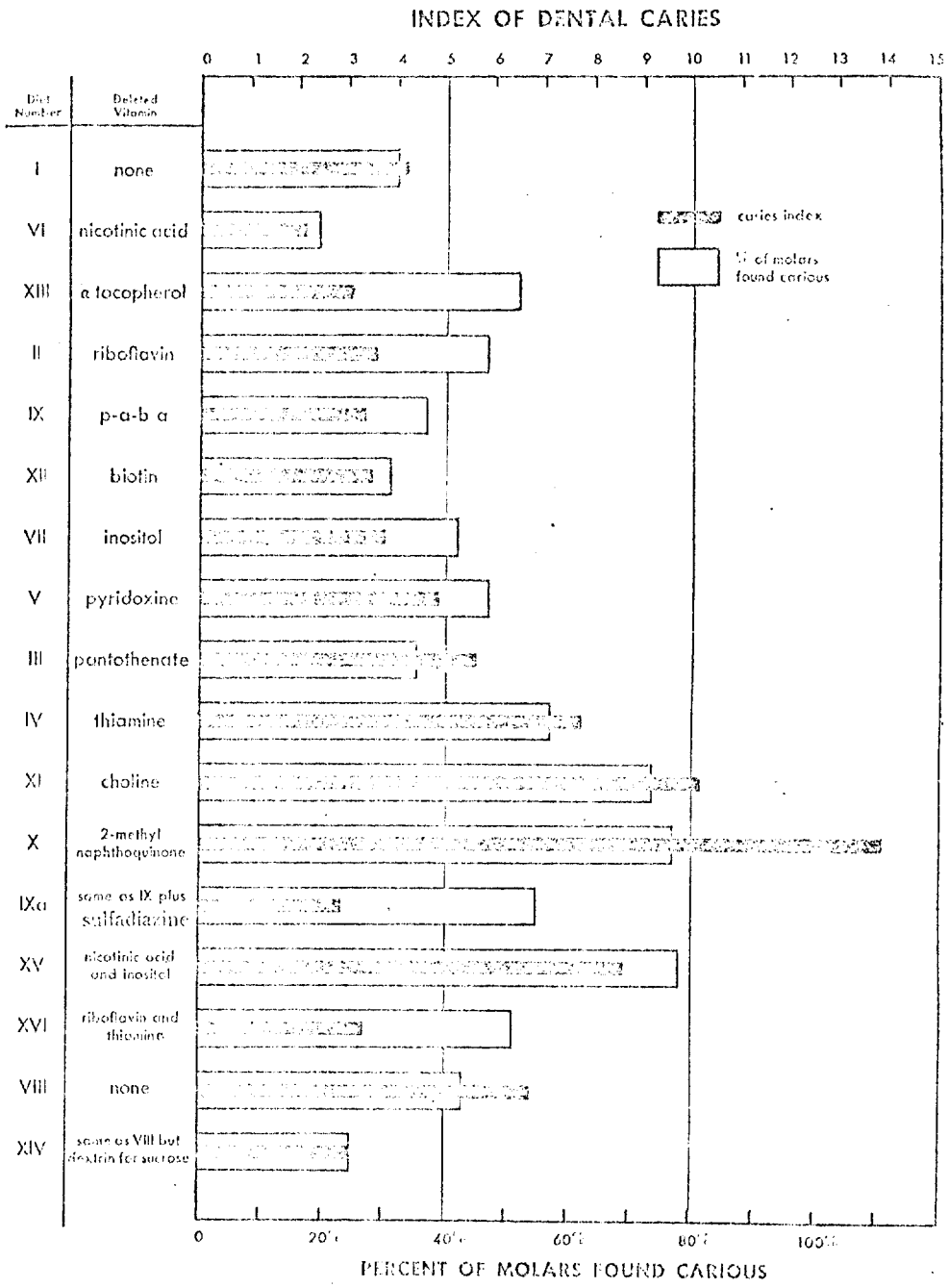

Fig. 1.—Graphic representation of the dental caries incidence for the various dietary groups of hamsters used in the principal study expressed in percentage of molars found to be carious and also by means of the caries index.

RESULTS

The distribution by diet of 682 hamsters that survived the experimental feeding period of 100 days is shown in Table IV. Also shown are the totals, averages, and percentages of the molars becoming carious as well as the caries index for each experimental group. The sexes, having been distributed among the dietary groups at random, showed a difference in susceptibility to caries. As had been reported earlier,[14] the males were somewhat more susceptible as a whole, but this fact did not alter appreciably the percentage of carious molars found nor the caries index of any of the dietary groups with one exception.

TABLE V

NUTRITIONAL REQUIREMENTS OF THE HAMSTER
(AS REPORTED IN THE LITERATURE)

VITAMINS INVESTIGATED	DEFICIENCY SIGNS AND SYMPTOMS	VITAMINS CONSIDERED ESSENTIAL AND NONESSENTIAL (NUMBERS INDICATE REFERENCES)	
		DAILY MAINTENANCE DOSE	+ ESSENTIAL − NON-ESSENTIAL
Thiamine	Polyneuritis in 12 days	3 μg. orally (25)	+(25), +(26), +(28)
Riboflavin	Perioral dermatitis and diarrhea; weight loss and death in 20 days	4 μg. orally (25)	+(25), +(26), +(28)
Pyridoxine	Acrodynia-like perioral dermatitis in 12 days; loss of weight; muscular weakness, and death in 24 days	3 μg. (25) 50 μg. (30)	+(25), +(26) +(28), +(30)
Pantothenic acid	Perioral red crusting; loss of weight and death in 20 days	parenterally, 15 μg. (25)	+(25), +(26), +(28)
Nicotinic acid	Rough, stringy fur, alopecia, stomatitis, weight loss, and death (25) Vitamin necessary only for successful lactation (28)	100 μg. (25)	+(25), −(26), −(28)
Para-amino-benzoic acid	None, if biotin is given	Undetermined	+?(26), −(28)
Inositol	None, if biotin is given. Only necessary for delivery of living young	Undetermined	+?(26), −(28)
Choline	None; only necessary for successful lactation	None	−(28)
Biotin	Cheilosis and poor growth, death 3 to 5 weeks (26)	1 μg. (26)	+(26), −(28)
Vitamin C	None	None	−(26), −(27)
Vitamin D	Typical rickets, but diet must be low in phosphorus as well as dificient in vitamin D	Undetermined	+(29)
Vitamin E	Gradual growth diminishment, terminal incoordination, excitation and shock with death	Therapeutic dose: 5 mg.	+(28)
Vitamin K	Arrested growth; hemorrhage in peritoneum and sinuses. Spontaneous recovery after 3 to 7 weeks possibly due to bacterial synthesis of vitamin K in intestines	Undetermined	+(28)

A graphic representation of the caries incidence for the various dietary groups is presented in Fig. 1. The most marked effects were observed when choline or vitamin K were removed from the diet. In these two groups of

hamsters the caries indices were, respectively, about 2.5 and 3.0 times as great as that of the control group. In the case of Diet X, lacking vitamin K, 76.6 per cent of the molar teeth of 15 male and 26 female animals studied had carious lesions, and an index of 13.91 as compared to the control Diets I and VIII whereon 32.1 per cent and 43.0 per cent, respectively, of the molars showed caries and the caries index was 4.21 and 6.76, respectively. The only dietary group which showed any marked decrease in dental caries was the one deficient in nicotinic acid. In this group the caries index was reduced to about one half that of the control group.

When a deficiency of para-aminobenzoic acid in the diet was supplemented by sulfadiazine in the water (designated as Diet IXa) the number of molars affected remained high but the caries index decreased. This may raise the question whether the drug inhibited the bacteria responsible for continuing the carious process in the dentin. A similar effect was noted when vitamin E was omitted from the diet (designated as Diet XIII) and, to a lesser degree when riboflavin and thiamine were both deleted (as in Diet XVI). On the other hand, the double deficiency of nicotinic acid and inositol seemed to increase the caries index considerably. Since these results are rather inconclusive, further careful study of much larger groups with double and multiple deficiencies seems desirable.

Substitution of dextrin for sucrose in the basic diet resulted in a decrease in the caries index by about 50 per cent.

The group of hamsters fed on the commercial meal which was used for the stock colony had exceedingly little dental caries, despite the fact that this feed was low in fluorides, the abundant presence of which is considered to be caries inhibitory. No evidence has been obtained to indicate whether this result may be due either to absence of a stimulating factor or presence of an inhibitory factor other than fluorides.

The experimental diets were not entirely adequate for growth as may be seen by comparing the weight curves for animals on the commercial, low-fluoride diet with those for hamsters in the two control groups, Diets I and VIII (Fig. 2). The animals were also somewhat abnormal in general appearance, in part because of the matting of their fur by the fat in the food with which the animals would make body contact. In several dietary groups a whitening of the usually brown dorsal fur was noted in the early part of the experimental period. This effect was observed particularly in animals on Diet III which received their calcium pantothenate by injection, and also in some on Diet II, but it was not a constant finding in these groups nor in any other. These apparent nutritional deficiencies cleared up spontaneously in each case, as if a missing factor had been eventually supplied. Such a nutritional sequence suggests the possibility of a change in the intestinal flora or in the synthesizing ability of some bacteria composing the flora.

DISCUSSION

Even a cursory review of the literature on dental decay reveals that considerable indirect evidence has been amassed to indicate some etiological relation

ship between the dietary intake and the oral bacterial flora on the one hand and dental caries on the other. It has been shown repeatedly in man that there is some quantitative correlation of certain oral acidogenic and aciduric bacteria with amount of tooth decay.[18, 19, 20] Similar findings in the albino rat have been reported by one of us[21, 22, 23] in a systematic study of the oral bacterial flora at various stages of dental caries development in rat molars. One of the reports on the hamster study in this laboratory[15] showed that lactobacilli and aciduric streptococci tend to increase considerably in number with an increase in the dental caries incidence. Although some relationship between the presence of certain oral bacteria and tooth decay seems evident, bacteria per se are not to be considered as independent entities in the dental caries process. They act rather as potential etiological agents, active only when there are present suitable substrates which can provide the substances essential for bacterial growth and acid production.

In planning the exploratory experiments here reported, it was considered plausible that if certain growth accessory substances essential for bacterial nutrition could be substantially reduced in quantity in the oral environment, some of the bacteria composing the oral flora presumably would be unable to attain their optimal growth or to produce their normal amount of organic acid. Such a reduction in acid formation in the depths of the molar fossae and sulci could conceivably lead to a reduction in the amount of dental caries.

It has been conjectured that vitamin B components as well as other vitamins are apparently essential growth factors for some oral lactobacilli and for some time it has been known that the presence of such vitamins in media in the form of yeast extract would enhance the growth of almost all such microorganisms.

The experiments here reported may be looked upon as a study of bacterial growth in vivo using the oral cavity of the hamster as the immediate environment. They represent tests of the effects of thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, para-aminobenzoic acid, choline, biotin, 2-methyl naphthoquinone, and alpha-tocopherol upon the caries-producing capacity of the normal, mixed oral flora, that is, tests to determine, if possible, which vitamin constituents of the diet are essential for bacterial acid production in the mouth. The degree of acid production occurring on susceptible tooth surfaces might be measured grossly by the amount of decay in the hamster's molars. However, since most, if not all, of these nutritional accessory substances are required in the metabolic processes of the hamster (Table V), whenever one of them was omitted from the diet it was provided by parenteral injection. Unless the injected vitamin was subsequently concentrated in the oral secretions, or the nutritional needs of the bacteria were otherwise supplied, this procedure should have produced effects attributable to reduced availability of the test substances to the bacterial flora of the tooth surfaces.

The findings as summarized in Table IV and Fig. 1, although not marked, seem to show some measurable effects of reduced vitamin content upon the ex-

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tent of caries activity. Omission from the diet of both nicotinic acid and inositol, of thiamine, of choline, or of 2-methyl naphthoquinone (vitamin K) resulted in increased caries. The role of vitamin K in the dental caries process is not very clear. Acid formation in the saliva has been found to be reduced by the presence of synthetic vitamin K and a few related quinone compounds[31] while clinical studies seem to show that 2-methyl-1, 4-naphthoquinone (synthetic vitamin K) used in chewing gum inhibits dental caries in man.[32]

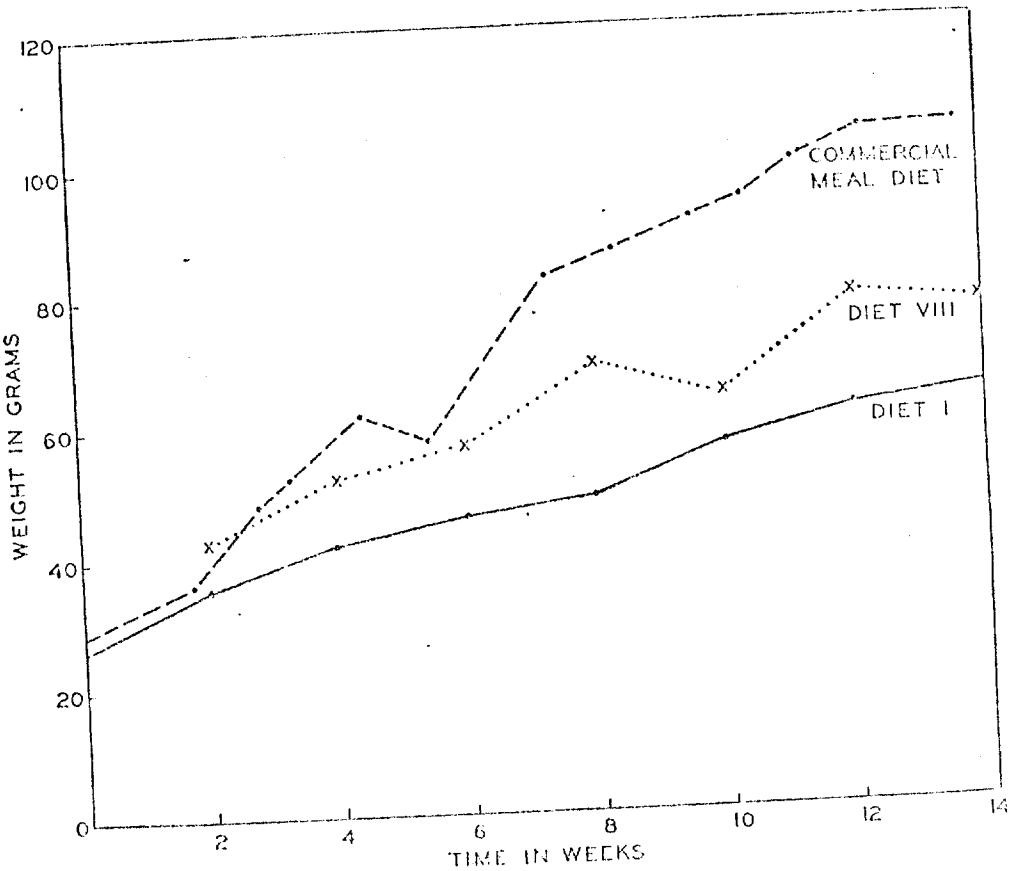

Fig. 2.—Comparative growth curves of hamsters fed experimental diets complete in vitamin supplements and of those fed the low-fluoride commercial meal diets.

Choline chloride, recognized as a growth factor in some animals, has not been found essential to the nutrition of the hamster except for successful lactation[28] nor has it been reported to be an essential growth factor for oral bacteria associated with dental caries. Its apparent effect on the development of caries in the hamster is not clear and should be further investigated.

Although hamsters fed the diet deficient in thiamine showed somewhat more tooth decay than control animals, thiamine according to the in vitro studies of Weisberger and Johnson[27] was found to be an essential growth factor for the

lactobacillus strain studied by them. On the other hand, Koser and Fisher,[23] in a study of 26 strains of oral lactobacilli, found that thiamine was needed by only about one third of the cultures.

When nicotinic acid was absent from the diet (Diet VI), the amount of dental caries was less than half that found in the control Diets I and VIII. In this connection it is interesting to note that Weisberger and Johnson also showed nicotinic acid to be essential for growth of their oral strain of lactobacillus. Likewise, Koser and Fisher reported nicotinic acid was needed by all 26 of their oral strains studied. This would seem to be a significant finding if it were known that all microorganisms associated with dental caries required nicotinic acid and if other dietary deficient groups of animals showed correlative trends. Inositol is not known to be a growth factor for oral bacteria and its absence from the diet seemed not to influence the amount of dental decay in the hamsters. Yet, when the double deficiency of nicotinic acid and inositol is considered, the amount of dental caries was found to be as high as when thiamine or choline were removed from the diet.

The caries index was decreased in animals fed the diet deficient in riboflavin (Diet II) although the number of molar teeth with carious lesions was larger than in the control groups on the complete diet. The same result was obtained when riboflavin deficiency was combined with a deficiency in thiamine (Diet XVI). These results combined with the findings that the caries index was twice as high in animals on a diet which contained riboflavin but was deficient in thiamine (Diet IV) suggest that riboflavin is utilized by the caries-producing flora of the hamster and that its presence is conducive to caries development. Riboflavin was found to be required by the majority of oral lactobacilli isolated from patients with dental caries as observed by Koser and Fisher[23] in their in vitro study. But by contrast, the observation of Weisberger and Johnson[24] indicated that riboflavin was not essential to the growth of the one strain of oral lactobacillus investigated. It must be borne in mind, however, that the oral cavity of rodents as well as of man contains a very complex bacterial flora, that more than one bacterium may be involved in caries production, and that the over-all nutritional requirements of the caries-associated flora may well be different from that of one isolated microorganism observed in vitro.

The amount of dental caries found in hamsters maintained on diets deficient in pyridoxine, pantothenate, para-aminobenzoic acid, and biotin did not seem to differ to a significant degree from that found in animals on the two control diets.

Absence of alpha-tocopherol (vitamin E) did not inhibit the initiation of carious lesions, but apparently had a retarding effect on the progression of such lesions as indicated by the low caries index. In several groups the total number of molar teeth becoming carious (Diets II, IXa, XIII, and XVI) was high in comparison with the total quantity of caries as indicated by the caries index. This suggests the possibility of a kind of bacterial ecological succession in the production of the lesion. Hence, the absence of certain vitamins essential to microorganisms which are important only in the later stages of the dental caries

process may be the explanation for the lessened amount of dentin destruction in these groups. Earlier observations in this laboratory[22, 23] support such a sequence of bacterial succession. The flora of the enamel surfaces of the molar teeth of rats exhibited a progressive increase in the proportion of lactobacilli as well as in alpha streptococci while caries was developing, whereas the aerobic flora of large dentinal lesions consisted almost entirely of streptococci. Moreover, bacterial cultivation from hamsters with carious lesions[15] also tended to portray a similar picture in so far as animals with a low caries index showed lactobacilli to predominate, but where the carious destruction was very great, streptococci outnumbered the lactobacilli cultivated.

Assuming the possibility of reducing the oral flora, sulfadiazine was added to the drinking water of hamsters on a diet lacking para-aminobenzoic acid (Diet IXa). It must be considered that this drug might also exhibit antagonistic action on the intestinal bacteria, inhibiting the synthesis of para-aminobenzoic acid as well as other growth accessory substances which might eventually find their way to the oral cavity. The findings reported here show a higher, rather than a lower, percentage of carious molars although the caries index is not very different from that in para-aminobenzoic acid-deficient animals not receiving the sulfa drug (Diet IX).

Hamsters maintained on Diet XIV, in which dextrin was substituted for sucrose, showed half as much carious destruction as the animals receiving sucrose (Diet VIII). This seemed significant in view of the almost invariable in vitro finding that the polysaccharide, dextrin, is much less readily utilized by oral bacteria for acid production than is the disaccharide, sucrose. Similarly, it has been shown by Schweigert, Shaw, Phillips and Elvehjem[24] that replacing 75 per cent of the sucrose with fine dextrin reduced both the incidence and extent of caries in the cotton rat.

As previously noted, this study was planned as a series of exploratory dietary tests. Most of the results have been equivocal or uncertain. There is some indication, however, that caries is increased in the absence of either choline, thiamine, or vitamin K (2-methyl naphthoquinone). Although deficiencies in nicotinic acid and inositol, separately, did not result in increased caries, there was a considerable increase when both were removed from the diet. Deletion of nicotinic acid alone seemed to decrease the caries. Interpretation of the results is confused by a number of factors. For example, the normal bacterial flora in these rodents is extremely complex and has never been completely studied. When such animals are maintained on special diets, the substrate of the oral cavity changes, the presence of carious lesions in the teeth again changes the oral environment and consequently the oral flora. Additionally, coprophagy could be only minimized, not eliminated. To further complicate matters, it should be mentioned that among bacteria deprived of certain vitamins essential for their growth, there may exist certain variants capable of synthesizing the missing vitamin. Under conditions of laboratory cultivation it has been demonstrated that a strain of propionibacterium (belonging to the same tribe as lactobacilli) could be "trained" to synthesize appreciable quantities of thiamine.[25, 26]

Moreover, in a report by Koser and Wright[37] it was found that from stock cultures of dysentery bacilli which require nicotinamide for growth variants were obtained capable of growth without the vitamin. It was therefore suggested that apparently nicotinamide and coenzyme or some substance physiologically equivalent must be synthesized by these variants. If such adaptations by bacteria can occur in laboratory cultivation, there is reason to suspect that it may also occur in such a natural environment as the oral cavity.

SUMMARY

Based on the assumption that certain oral bacteria are important in the development of dental caries, this investigation concerned itself with the environment of the oral cavity in the Syrian hamster. The factors considered were: (A) the nutritional substrate provided by the ingested food, (B) the bacterial flora subsisting on this substrate, and (C) the molar teeth subjected to carious destruction presumably by the action of the bacteria. Partly synthetic experimental diets containing 11 vitamins in measured amounts, with 1 or 2 of these vitamins being omitted from each test diet, were fed to 682 hamsters distributed into 18 different dietary groups for a 100-day period. During this time, the animals were provided with adequate amounts of the missing vitamins semiweekly by intraperitoneal injection for their own nutrition. As a plausible measure of the degree to which certain oral bacteria were retarded or accelerated in their growth and metabolic activities, the extent of the carious lesions in the hamsters' molar teeth were evaluated.

The trends observed in this study were the following:

1. When vitamin K was absent in the diet, hamsters showed the greatest amount of dental caries.

2. The animals on a diet deficient in choline chloride developed about twice as much caries as animals in the control groups.

3. With the omission of nicotinic acid, the amount of caries was reduced to about one-half that of the control animals.

4. Animals receiving neither nicotinic acid nor inositol showed a higher degree of dental caries than the control animals.

5. Absence of vitamin E did not seem to prevent lesions from occurring but apparently retarded their progress.

6. Substitution of dextrin for sucrose caused a 50 per cent decrease in molar destruction by dental caries.

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REFERENCES

1. Arnold, F. A.: The Production of Carious Lesions in Molar Teeth of Hamsters (*C. auratus*). *Pub. Health Rep.* 57: 1599, 1942.
2. Rosebury, T., Kursban, M., and Foley, G.: Studies in the Rat of Susceptibility to Dental Caries. III. The Experimental Production of Typical Dental Fissure Caries and Other Lesions in Rats, and Preliminary Studies of Their Etiology, *J. D. Res.* 13: 379, 1933; IV. Further Studies on the Etiology of Fissure Caries, *J. A. D. A.* 21: 1599, 1934.

3. Shibata, M.: On the Experimental Dental Caries of Molars of the White Rat, *Jap. Exper. Med.* 7: 247, 1929.

4. Hoppert, C. A., Webber, P. A., and Cauniff, T. L.: The Production of Dental Caries in Rats. *Science* 74: 77, 1931; *J. D. Res.* 12: 161, 1932.

5. Keyes, P. H., and Dale, P. P.: Dentition of the Syrian Hamster, *J. D. Res.* 23: 208, (Abstr.) 1944; A Preliminary Survey of the Pouches and Dentition of the Syrian Hamster, *J. D. Res.* 23: 427, 1944.

6. Keyes, P. H.: The Character and Distribution of Carious Lesions in the Molar Teeth of Syrian Hamsters, *J. D. Res.* 25: 165 (Abstr.) 1946; Dental Caries in the Syrian Hamster. I. The Character and Distribution of Lesions, *J. D. Res.* 25: 41, 1946.

7. Dale, P. P., Lazansky, J. P., and Keyes P. H.: Experimental Dental Caries in the Syrian Hamster, *J. D. Res.* 23: 209 (Abstr.) 1944; Preliminary Observations on the Production and Inhibition of Dental Caries in Syrian Hamsters, *J. D. Res.* 23: 445, 1944.

8. Keyes, P. H.: Dental Caries in the Syrian Hamster. II. A Preliminary Study of the Effect of Three Different Rations on Caries Activity, *J. Nutrition* 32: 525, 1946.

9. Nuckolls, J., Frisbie, H. E., and Killian B.: Controlled Study of the Character and Occurrence of Carious Lesions in the Molar Teeth of the Syrian Hamsters Under Different Dietary Conditions, *J. D. Res.* 26: 475 (Abstr.) 1947.

10. Granados, H., Glavind, J., and Dam, H.: Observations on Experimental Dental Caries. II. The Effect of Two Similar Diets Having the Same Initial pH and the Same Growth-promoting Action, *J. D. Res.* 27: 759 (Abstr.) 1948; *J. D. Res.* 28: 77, 1949.

11. Goldberg, H. J. V., Keyes, P. H., Gilda, J. E., and Shourie K. L.: The Effect of Vitamin D on Caries in the Syrian Hamster *J. D. Res.* 27: 739 (Abstr.) 1948.

12. Mitchell D. F., and Shafer, W. G., The Effects of a Caries-Producing Diet Initiated at Various Stages of Pre- and Post-Natal Development of the Hamster, *J. D. Res.* 27: 761 (Abstr.) 1948; *J. D. Res.* 28: 424, 1949.

13. Shafer, W. G.: The Caries-Producing Capacity of Starch, Glucose, and Sucrose Diets in the Syrian Hamster, *Science* 110: 113, 1949.

14. Orland, F. J.: A Study of the Syrian Hamster, Its Molars, and Their Lesions, *J. D. Res.* 25: 445, 1946.

15. Orland, F. J.: The Oral Bacterial Flora as Related to Dental Caries in the Syrian Hamster, *J. D. Res.* 25: 455, 1946.

16. Fancher, O. E., Calandra, J. C., and Fosdick, L. S.: The Effect of Vitamins on Acid Formation in Saliva, *J. D. Res.* 23: 23, 1944.

17. Keyes, P. H.: A Method of Recording and Scoring Gross Carious Lesions in the Molar Teeth of Syrian Hamsters, *J. D. Res.* 23: 439, 1944.

18. Bunting, R. W., Nickerson, G., and Hard, D. G.: Further Studies of the Relation of *Bacillus acidophilus* to Dental Caries, *D. Cosmos* 68: 931, 1926.

19. Jay, P., Hadley, F. P., Bunting, R. W., and Koehne M.: Observations on Relationship of *Lactobacillus acidophilus* to Dental Caries in Children During Experimental Feeding of Candy, *J. A. D. A.* 23: 846, 1936.

20. Snyder, M. L.: The Occurrence of Lactobacilli and Other Acidogenic Organisms in the Salivas of Selected Caries-Free Children, *J. D. Res.* 18: 497, 1939.

21. Harrison, R. W., Bacteriological Studies on Experimental Dental Caries in the Rat. I. Bacterial Flora of Normal, Non-carious Teeth, *J. Infect. Dis.* 67: 91, 1940.

22. Harrison, R. W.: II. Changes in Tooth Surface Flora Associated With Development and Inhibition of Dental Caries, *J. Infect. Dis.* 67: 97, 1940.

23. Harrison, R. W.: III. Flora of Advanced Carious Lesions, *J. Infect. Dis.* 67: 106, 1940.

24. Weisberger, D., and Johnson, F. G.: Nutritional Requirements for an Oral Strain of *Lactobacillus acidophilus*, *J. D. Res.* 25: 35, 1946.

25. Routh, J. I., and Houchin, O. B.: Some Nutritional Requirements of the Hamster, *Federation Proc.* 1: 191 (Abstr.) 1942.

26. Cooperman, J. M., Waisman, H. A., and Elvehjem, C. A.: Nutrition of the Golden Hamster, *Proc. Soc. Exper. Biol. & Med.* 52: 250, 1943.

27. Clausen, D. F., and Clark, W. G.: Vitamin C Requirements of the Syrian Hamster, *Nature, London* 152: 300, 1943.

28. Hamilton, J. W., and Hogan, A. G.: Nutritional Requirements of the Syrian Hamster, *J. Nutrition* 27: 213, 1944.

29. Jones, J. H.: Experimental Rickets in the Hamster, *J. Nutrition* 30: 143, 1945.

30. Schwartzman, G., and Strauss, L.: Vitamin B₁ Deficiency in the Syrian Hamster, *Federation Proc.* 8: 309 (Abstr.) 1949.

31. Calandra, J. C., Fancher, O. E., and Fosdick, L. S.: The Effect of Synthetic Vitamin K and Related Compounds on the Rate of Acid Formation in Saliva, *J. D. Res.* 23: 37, 1944.
32. Burrill, D. Y., Calandra, J. C., Tildea, E. B., and Fosdick, L. S.: The Effect of 2-Methyl-1, 4-Naphthoquinone on the Incidence of Dental Caries, *J. D. Res.* 24: 273, 1945.
33. Koser, S. A., and Fisher, B. J.: Personal communication, 1949.
34. Schweigert, B. S., Shaw, J. H., Phillips, P. H. and Elvehjem, C. A.: Dental Caries in the Cotton Rat. III. Effect of Different Dietary Carbohydrates on Incidence and Extent of Dental Caries, *J. Nutrition* 29: 405, 1945.
35. Wood, H. G., Andersen, A. A., and Werkman, C. H.: Nutrition of the Propionic Acid Bacteria, *J. Bact.* 36: 201, 1938.
36. Silverman, M., and Werkman, C. H.: Adaptation of the Propionic Acid Bacteria to Vitamin B₁ Synthesis Including a Method of Assay, *J. Bact.* 38: 25, 1939.
37. Koser, S. A., and Wright, M. H.: Experimental Variation of Nicotinamide Requirement of Dysentery Bacilli, *J. Bact.* 46: 239, 1943.

9 Lab & Clin Med 28: 66-70 Oct 1942

208

A COMPARATIVE STUDY OF DEXTROSE AND DEXTRIN TOLERANCE IN PATIENTS WITH CHRONIC ULCERATIVE COLITIS*

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ABSORPTION studies in chronic ulcerative colitis have been confined mainly to dextrose, and it seemed of interest to us to study the intestinal absorption and the blood sugar response of another carbohydrate. For this study, a mixture of polysaccharides (dextrins and maltose) produced by the partial breakdown of starch was selected. For control purposes dextrose absorption was studied simultaneously. Tolerance curves served as a measure of the absorption process.

Low or flat blood sugar curves for oral dextrose tolerance tests have been frequently found by us (Z. B. and R. C.P.)[1] in patients with chronic ulcerative colitis. In one group of 58 standard oral, one dose, two-hour dextrose tolerance tests, 23 (39.6 per cent) tests were of the low or flat type. Groen,[2] using a simplified technique of intestinal intubation, ascribed the decreased absorption of dextrose to a defect in the absorptive capacity of the intestinal wall and not to an unusually rapid passage of the intestinal contents.

METHOD OF STUDY

Twenty-three patients with chronic ulcerative colitis attending an outpatient clinic were studied. All the patients had had the disease for periods of one or more years.

Both the oral dextrose tolerance test and the oral dextrin tolerance test were carried out on the same patient. These tests were alternated in successive patients. The tests were carried out from six to ten days of each other, with no change in diet, so that each patient served as his own control.

The patients reported in the morning without breakfast. They were weighed and a fasting venous blood sample was obtained. One gram per kilogram of body weight of dextrose or dextrin† in 150 to 200 c.c. water was given, and blood samples were taken at one-, two- and three-hour intervals, and in some instances at four and five hours. Urine was also collected and examined for dextrose at these intervals. The venous blood was analyzed for true dextrose content by the Shaffer-Somogyi[3] method. The results, therefore,

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†Since pure dextrin was found unsuitable because of unpalatability and difficulty of making acceptable solutions, "Dexin" (Burroughs Wellcome & Co.), containing 75 per cent dextrins and 24 per cent maltose, 0.75 per cent moisture, and 0.25 per cent mineral matter, was used. Since the predominant constituent of this polysaccharide and disaccharide combination consists of dextrin, we have used the term *dextrin* in the text to denote this dextrin-maltose preparation.

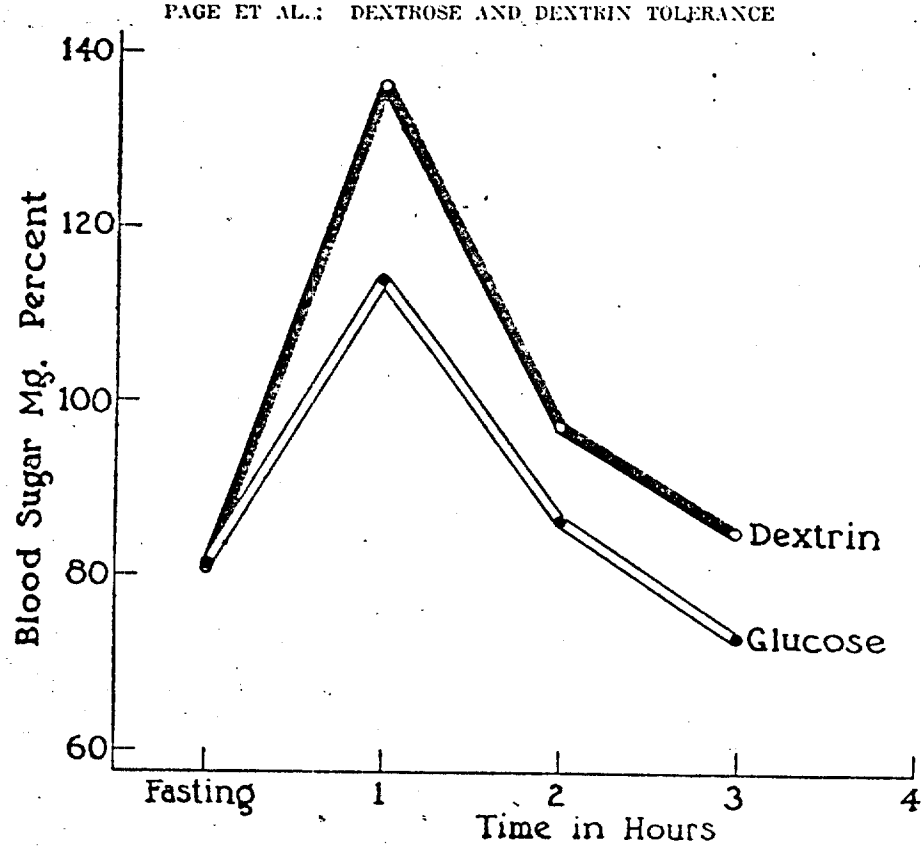

Fig. 1.—Graph showing mean values of comparative results of oral dextrose and dextrin tolerance tests on 23 patients with chronic ulcerative colitis, as given in Table I.

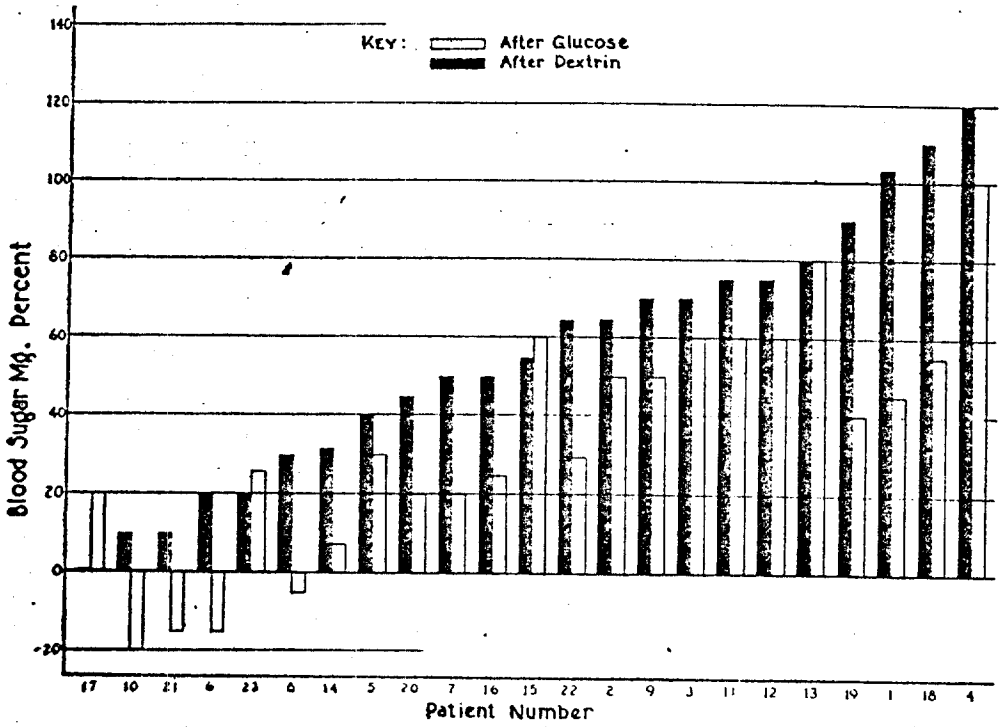

Fig. 2.—Bar graph showing comparison of increase or decrease in blood dextrose level from fasting level one hour after oral dextrose and dextrin tolerance tests in the same patients.

represent dextrose content with only little interference from other reducing substances (vitamin C, glutathione, methionine, cysteine, etc.).

RESULTS OF STUDY

The results are given in Table I and are presented graphically in Figs. 1 and 2. It will be observed that after dextrose administration the blood sugar at the end of the first hour had risen sharply to an average value of 115 mg. per 100 c.c., an increase of approximately 42 per cent over the average fasting level of 81 mg. per 100 c.c. However, at the end of the second hour it had dropped sharply to 86, being then only 7 per cent above the fasting level. At the end of the third hour it had fallen to 73, or 10 per cent below the fasting level. At the end of the fourth hour the lowest value was reached, 69, or 15 per cent below the fasting level. The four determinations available at the fifth hour showed that blood sugar was starting to recover, now being 75, or 7 per cent below the fasting level.

TABLE I

PATIENT NO.	GLUCOSE						DEXTRIN					
	TRUE BLOOD GLUCOSE (MILLIGRAMS PER 100 C.C.)—SHAFFER-SOMOGYI METHOD											
	FASTING LEVEL	1 HR.	2 HR.	3 HR.	4 HR.	5 HR.	FASTING LEVEL	1 HR.	2 HR.	3 HR.	4 HR.	5 HR.
1	70	115	110	85	80		105	208	190	135	110	
2	80	130	95	90	80	85	95	160	145	95	65	80
3	90	150	140	95	80	95	70	140	145	105	75	60
4	80	180	110	70			80	200	135	70		
5	85	115	100	55	55		70	110	65	60		
6	95	80	75	80			80	100	85	90		
7	65	85	60	90			60	110	100	90		
8	105	100	95	60			110	140	105	85		
9	80	130	135	115	85		80	150	160	100		
10	90	70	45	70			70	80	60	60		
11	70	130	70	60			85	160	80	70		
12	95	155	95	80			75	150	70	60		
13	70	150	75	60	70	75	70	150	85	65	65	
14	90	98	65	75	80		80	113	75	75		
15	70	130	65	60	60		80	135	85	75		
16	105	130	80	70			85	135	90	65		
17	65	85	55	45	55		70	70	75	75	60	
18	80	135	100	50			70	180	105	50		
19	55	95	80	45	45	45	65	155	85	140		
20	80	100	95	90	65		115	160	115	95	85	
21	100	85	60	85			80	90	50	85		
22	70	100	90	70			80	145	125	130		
23	70	95	90	80	70		75	95	105	85	75	
Means	80.9	114.9	86.3	73.0	68.8	75.0	80.4	136.4	97.2	85.2	76.4	70.0

After dextrin administration the blood sugar showed a similar but much greater rise by the end of the first hour. From the fasting level of 80 mg. per 100 c.c., the blood sugar rose to 136 at the end of the first hour, an increase of 70 per cent. By the end of the second hour the drop had not reached as low a level as with dextrose, the figure being 97, or 21 per cent above fasting. At the end of the third hour the blood sugar was 85, or still 6 per cent above the fasting level. The seven available observations at the end of the fourth hour gave 76.4, or a drop of 5 per cent below fasting level. The two fifth-hour observations averaged 70, or 13 per cent below fasting level.

Urinalyses on the entire group of patients using quantitative Benedict's test revealed reducing substances in only one patient (No. 8). The fasting specimen was negative, and in the first-hour specimen after oral dextrose the reducing substances were present to the amount of 1.65 Gm., after the second hour 1.13 Gm., and after the third hour 0.45 Gm. In the dextrin test the fasting specimen was negative. After one hour the urine contained 0.59 Gm. of reducing substance, after the second hour 0.45 Gm., and after the third hour 0.19 Gm.

DISCUSSION OF RESULTS

The foregoing observations apparently indicate that at all points the blood sugar levels were higher after the dextrins than after the dextrose. However, it is well known that because of the many variables involved, blood sugar curves undergo wide fluctuations. Therefore, a statistical analysis of the results was made in order to determine whether the greater hyperglycemia following the dextrin administration was real or whether it was only an accident due to chance.

Statistical Analysis.—The dextrin and the dextrose curves were compared by calculating the statistic "t" for the differences in the blood sugar means at the one-hour, two-hour, and three-hour periods. The respective "t" values found were 5.568, 3.846, and 2.756, and the corresponding probabilities, as given by the tables of Fisher and Yates,[4] were $P = <0.001$, $P = <0.001$ and $P = >0.001$ but <0.01. Since a probability of $P = 0.001$ indicates one chance in 1,000 and a probability of $P = 0.01$ indicates one chance in 100, one may conclude the difference is real and that dextrin administration produced higher blood sugar values than did dextrose administration.

Since 100 mg. of the dextrin mixture are theoretically capable of giving approximately 108 mg. of dextrose if completely hydrolyzed, an approximate correction was made by reducing the mean difference by 8 per cent and again calculating the "t" values. This procedure did not appreciably alter the results.

One hundred milligrams of the dextrin preparation used in this study contained 75 mg. of dextrin, $(C_6H_{10}O_5)_n \cdot H_2O$, which if completely hydrolyzed would yield 83.3 mg. of dextrose (using the factor of 0.9, since nine parts of dextrin give ten parts of dextrose upon hydrolysis),[5] and 24 mg. of anhydrous maltose ($C_{12}H_{22}O_{11}$), which would yield 25.2 mg. of dextrose (using the factor of 0.95) if completely hydrolyzed, making a total of 108.5 mg. of dextrose. Actually the maltose is present in the hydrated state ($C_{12}H_{22}O_{11} \cdot H_2O$) so that 24 mg., when hydrolyzed, yields 24 mg. of dextrose (1.2 mg. less than with anhydrous maltose). Using this figure in the calculation, 100 mg. of the dextrin-maltose mixture would yield 107.3 mg. of dextrose instead of 108.5 mg. We have compromised between these two values and assume that 100 mg. of the dextrin mixture would be converted into 108 mg. of dextrose, or an 8 per cent increase over the original weight.

The possibility exists that a comparison of the areas under the blood dextrose curves may present a more comprehensive picture than comparisons made separately at three different points. Accordingly, the areas under the 46 curves were measured with a polar planimeter. Areas below a base line passing through the fasting level were considered negative. These data were subjected to an analysis of variance following the standard procedure, such

as outlined by Bliss and Marks.[6] The variance ascribable to differences in patients was about 25 per cent of the total. This points to the necessity of using a considerable number of patients and emphasizes the wisdom of having each patient serve as his own control. The "t" value of 6.305 ($P = <0.001$) again shows that dextrins were significantly more effective in raising the blood sugar level than was dextrose.

It is impossible to state with certainty the reason for dextrin causing a higher and more prolonged rise in the blood sugar level than with dextrose itself. It is generally held that dextrins in the intestine undergo enzymic hydrolysis to maltose and then to dextrose, which is absorbed in the usual manner. The time factor of this hydrolysis may be the reason for the prolonged rise of blood dextrose after dextrin. Two other possibilities also present themselves: better absorption of the dextrose derived from dextrin, or a slower storage of the dextrose as glycogen by liver and muscle, although no evidence exists as to why such dextrose should behave different from pure dextrose. It is also recognized that the dextrins may on hydrolysis yield some slowly metabolized reducing substance which could react with the Shaffer-Somogyi reagents. No information is available on this point.

SUMMARY AND CONCLUSIONS

1. Twenty-three patients with chronic ulcerative colitis were studied as to the oral dextrose and oral dextrin tolerance tests.

2. One hour after dextrose administration the true blood dextrose had risen 42 per cent over the average fasting level while after the dextrin administration the blood dextrose had in the same time increased 70 per cent.

3. Two hours after dextrose the blood dextrose level was 7 per cent above the fasting level while the level in the dextrin group was 21 per cent above the fasting level.

4. Three hours after dextrose the blood dextrose level was 10 per cent below the fasting level while in the dextrin group it was 6 per cent above the fasting level.

5. A dextrin preparation maintained significantly higher blood dextrose levels for three hours than did dextrose in a group of patients with chronic ulcerative colitis. These findings have led to the assumption that in the patients studied dextrin was better absorbed from the intestine than was dextrose.

REFERENCES

1. Bercovitz, Z., and Page, R. C.: Metabolic and Vitamin Studies in Chronic Ulcerative Colitis. In press.
2. Groen, J.: The Absorption of Glucose From the Small Intestine in Deficiency Disease, New England J. Med. 218: 247, 1938.
3. Shaffer, P. A., and Somogyi, M.: Copper-Iodometric Reagents for Sugar Determination, J. Biol. Chem. 100: 695, 1933.
4. Fisher, R. A., and Yates, F.: Statistical Tables, Edinburgh and London, 1938, Oliver & Boyd.
5. Browne, C. A., and Zerban, F. W.: Physical and Chemical Methods of Sugar Analysis, ed. 3, New York, 1941, John Wiley & Sons, page 1006.
6. Bliss, C. E., and Marks, H. P.: The Biological Assay of Insulin. I. Some General Considerations Directed to Increasing the Precision of the Curve Relating Dosage and Graded Response, Quart. J. Pharm. & Pharmacol. 12: 82, 1939.

238

DENTAL CARIES IN THE COTTON RAT[1]

III. EFFECT OF DIFFERENT DIETARY CARBOHYDRATES ON THE INCIDENCE AND EXTENT OF DENTAL CARIES

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Shaw et al. ('44, '44 a) have reported a high incidence and extent of carious lesions in the molars of cotton rats fed purified rations high in sucrose. In direct contrast, a low incidence and extent of caries were observed in those cotton rats fed natural rations or purified rations where the sucrose was replaced by coarse or fine dextrin. Since such a definite difference was obtained by the replacement of the sucrose by dextrin, an investigation was carried out to determine the effect of several different carbohydrates in purified rations on the incidence and extent of carious lesions. The results of these studies and the growth data obtained from these experiments and those previously reported on dental caries are presented in this paper.

EXPERIMENTAL

The cotton rats used were obtained from our stock colony. Those on the carbohydrate experiments were weaned at a weight of 15–20 gm. rather than at a weight of 20–25 gm. as was done in the previous work. This change was made since it appeared that the animals were able to adapt themselves more readily to the experimental conditions at the earlier age. As nearly as possible males and females were equally divided within each experimental group. Similarly, members of each litter were distributed equally between all groups in any experiment. After the animals had been on experiment for 14 weeks, they were sacrificed for the observation of the incidence and extent of tooth de-

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cay. The techniques of observation and evaluation of the carious lesions were the same as described in the earlier work (Shaw et al., '44, '44 a).

The percentage composition of the sucrose basal ration 801 is as follows: sucrose 73, casein 18, salts IV 4, corn oil 5; and adequate quantities of the B vitamins (McIntire, Schweigert and Elvehjem, '44) were provided. Each rat was given one drop of halibut liver oil per week. Ration 802 was prepared by replacing 6% of the sucrose in ration 801 with casein, which brought the protein level to 24%.

The other carbohydrate diets were prepared in a similar manner by replacing the sucrose with either maltose,[2] glucose,[3] lactose,[4] dextrimaltose[5] (both with and without added NaCl), fructose,[6] fine dextrin, coarse dextrin or soluble starch.[7] The water of crystallization of glucose (cerelose) and maltose was taken into account in the preparation of these rations. The addition of 4% of 1:20 liver extract had been shown previously to increase the growth rate (McIntire et al., '44), and not to alter the incidence or extent of dental caries (Shaw et al., '44). Therefore 4% of 1:20 liver extract was added, at the expense of the entire ration, to all the rations used in the carbohydrate experiments.

TABLE 1

Comparison of the rate of growth of males and females over a 6-week period.

RATION	MALES		FEMALES		PER CENT DIFFERENCE A-B/B × 100
	No. of animals	Grams gain per wk. (A)	No. of animals	Grams gain per wk. (B)	
802	14	6.9	18	5.6	23
802 + 4% 1:20 L.E.	20	9.0	11	7.2	25

In table 1 a summary of the data on the growth rate of males and females obtained from thirty-two rats fed the sucrose basal ration 802 and from thirty-one animals fed ration 802 + 4% 1:20 liver extract is presented. It is obvious that the males consistently grew at a more rapid rate. In order to express the growth data as one figure for each group the values for the females were increased by 25%. Since only a limited number of animals were available for experiments, it was not possible to use animals of one sex in any single experiment.

[2] C.P. hydrate, Pfanstiehl Chemical Company.
[3] Cerelose, Corn Products Refining Company.
[4] U.S.P., Merck and Company.
[5] Mead Johnson and Company.
[6] Levulose C.P. special, Pfanstiehl Chemical Company.
[7] Reagent grade, Pfanstiehl Chemical Company.

Various fractionations of 1:20 liver extract were made and the fractions were fed to cotton rats which received rations 801 and 802. All the liver fractions were added at the expense of the entire ration. Data on the rate of growth are included for groups of animals from earlier dental caries studies in order to compare the growth results with the severity of tooth decay. The average gains per week for both the first 6 weeks and 14 weeks on experiment are presented in table 2. The incidence and extent of carious lesions obtained when the different carbohydrate diets were fed are shown in table 3.

RESULTS

When rations 801 and 802 were fed, the average rate of growth in the first 6 weeks was approximately the same, namely 6.7 and 6.9 gm. per week, respectively. The rate of growth was more uniform when ration 802 was fed; therefore, 24% casein was used in all rations subsequent to this observation. When whole liver substance, 1:20 liver extract or the alcohol extract of 1:20 liver extract were added to ration 801, the growth rate was increased to 10.3, 8.1, and 7.7 gm. per week, respectively. Similarly solubilized liver, 1:20 liver extract, or the alcohol extract of 1:20 liver extract increased the growth rate when added to ration 802, to 8.2, 9.0, and 7.8 gm. per week, respectively.

The growth rate and ability of the cotton rat to survive were markedly affected by the particle size of the complex carbohydrates. When fine dextrin, soluble starch or fine stock rations were ingested, inferior growth was obtained. This was largely attributed to the fact that the animals scattered these fine rations which resulted in low food consumption. It can be seen in table 2 that seven of the eight animals fed the fine dextrin ration, which contained the 18% casein, died within 6 weeks. In another series when 1:20 liver extract was added to the extent of 4% and the casein increased to 24%, all four cotton rats survived the 14-week experimental period but the growth was still poor. However, all nine animals died when the soluble starch ration, which contained 4% 1:20 liver extract and 24% casein, was fed.

In contrast to these results coarse dextrin diets afforded excellent growth, in fact better growth than with any of the other carbohydrates (table 2). Animals which received the maltose, sucrose, glucose, fructose or dextri-maltose rations grew at approximately the same rate, 8.6, 9.0, 8.4, 8.0, and 7.8 gm. per week, respectively, for the first 6 weeks on experiment and 5.6, 6.5, 6.0, 5.7 and 6.0 gm. per week for the entire 14-week period.

The incidence and extent of carious lesions were high when the soluble carbohydrates were ingested (table 3). These results are in direct contrast to the low incidence and extent observed when animals received coarse or fine dextrin rations. No appreciable difference was demonstrated between the coarse and fine dextrin rations. The replacement of 50% of the sucrose by fine dextrin did not reduce the severity of the tooth damage as compared to that observed when the sucrose basal

TABLE 2

Growth of cotton rats fed various diets.

(All results expressed as growth of males for reasons given in the text.)

RATION	NO. OF ANIMALS	RATE OF GROWTH PER WEEK	
		6 weeks	11 weeks
		gm.	*gm.*
801 [1]	13	6.7	4.9
801 + 4% 1:20 liver extract (L.E.)	7	8.1	6.1
801 + alcohol — ether extract ⅔ to 9% 1:20 L.E.	8	6.2	4.1
801 + alcohol extract ⅔ to 6% 1:20 L.E.	3	7.7	6.5
801 + 4% whole liver substance	2	10.3	
801 + additional vitamins ADEK	9	4.8	3.2
Sucrose replaced by coarse dextrin	9	6.4	3.1
Sucrose replaced by fine dextrin	8 [2]	2.3	
802	32	6.9	4.5
802 + 4% 1:20 L.E.	31	9.0	6.5
802 + 4% Sol. L.E.	7	8.2	5.8
802 + alcohol extract ⅔ to 6% 1:20 L.E.	8	7.8	4.7
802 + acetone extract ⅔ to 6% 1:20 L.E.	3	6.7	..
803 (soluble starch) + 4% 1:20 L.E.	9 [3]	..	
804 (glucose) + 4% 1:20 L.E.	7	8.4	6.0
805 (dextri-maltose) + 4% 1:20 L.E.	5	7.8	6.0
806 (½ of the sucrose replaced by lactose) + 4% 1:20 L.E.	4	6.1	4.2
807 (½ of the sucrose replaced by fine dextrin) + 4% 1:20 L.E.	4	7.8	6.1
808 (coarse dextrin) + 4% 1:20 L.E.	5	10.1	8.2
809 (fine dextrin) + 4% 1:20 L.E.	4	7.0	4.1
810 (maltose) + 4% 1:20 L.E.	5	8.6	5.6
811 (fructose) + 4% 1:20 L.E.	4	8.0	5.7
812 (¾ of the sucrose replaced by fine dextrin) + 4% 1:20 L.E.	4	7.8	4.0
Steenbock stock ration	6	8.3	5.0
Coarse stock ration	3	6.2	
Fine stock ration	3	5.3	
Dog Food stock ration	13	9.0	4.3

[1] Percentage composition of diet 801: sucrose 73, casein 18, salts IV 4, corn oil 5; adequate quantities of B vitamins were provided as supplements (McIntire, Schweigert and Elvehjem '44).

[2] Seven out of 8 animals died in 6 weeks.

[3] All animals died within 3 weeks after they were placed on experiment.

ration was fed. The replacement of 75% of the sucrose by fine dextrin afforded partial protection. Apparently one-sixth of the diet as sucrose is sufficient to cause a relatively high incidence of caries.

It can readily be seen that the number of lesions observed when the control diet (802 + 4% 1:20 liver extract) was fed varied somewhat between groups; the average incidence observed in this series of experiments ranged from 23.5 to 33.0 caries per rat. These values are

TABLE 3

The effect of different carbohydrates on the incidence and extent of carious lesions.

RATION	NUMBER OF ANIMALS	AVERAGE INCIDENCE OF CARIOUS LESIONS	AVERAGE EXTENT OF CARIOUS LESIONS
802 + 4% 1:20 liver extract (L.E.)	2	32.5	112 +
808 (coarse dextrin) + 4% 1:20 L.E.	2	5.5	10 +
809 (fine dextrin) + 4% 1:20 L.E.	4	10.3	16 +
802 + 4% 1:20 L.E.	3	25.3	74 +
807 (½ of the sucrose replaced by fine dextrin) + 4% 1:20 L.E.	4	27.7	96 +
802 + 4% 1:20 L.E.	3	33.0	113 +
812 (¾ of the sucrose replaced by fine dextrin) + 4% 1:20 L.E.	4	23.5	50 +
802 + 4% 1:20 L.E.	3	32.3	94 +
805 (dextri-maltose) + 4% 1:20 L.E.	3	32.7	100 +
805a (dextri-maltose + 2% NaCl) + 4% 1:20 L.E.	2	36.0	94 +
802 + 4% 1:20 L.E.	2	23.5	77 +
804 (glucose) + 4% 1:20 L.E.	7	27.6	95 +
806 (½ of sucrose replaced by lactose) + 4% 1:20 L.E.	4	22.5	64 +
802 + 4% 1:20 L.E.	3	26.3	77 +
810 (maltose) + 4% 1:20 L.E.	5	27.6	81 +
811 (fructose) + 4% 1:20 L.E.	4	29.8	92 +

of the same magnitude as observed in the earlier work. The average extent of the lesions varied from 74 + to 113 +. These differences are largely due to the variation in susceptibility to tooth decay of offspring from different parent stock. Therefore, it is important that each litter be equally represented in each group of any experiment. Records have been kept of the litters represented in each experimental series. An investigation of the susceptibility of different strains of cotton rats to caries formation is now in progress and the results from this investigation will be reported later.

DISCUSSION

The soluble carbohydrates appear to favor conditions for the formation of carious lesions while dextrin, a carbohydrate which is not readily fermented by lactic acid bacteria or other acid-producing organisms, does not favor such conditions. Unfortunately animals which received the soluble starch did not survive the experimental period, but this carbohydrate would not be expected to favor conditions for extensive tooth decay. The replacement of one-half of the sucrose by dextrin did not result in a decrease in the caries incidence. A similar result was obtained with dextri-maltose, which contains approximately 50% dextrin. Since the carbohydrate was the only dietary constituent varied in these experiments, its importance in the etiology of dental caries in the cotton rat must be emphasized.

Boyd ('42) concluded that carious lesions in children were arrested and prevented by the ingestion of diets whose quality was significantly superior to the diet of the average child. The ingestion of considerable quantities of sugar did not result in an extension of the caries or development of new carious lesions in a few cases where the effect of high quantities of sugar was specifically studied. In a later paper Boyd ('43) reported similar results from a study conducted over a 17-year period on the incidence and progression of carious lesions in children with diabetes mellitus. These experiments show that dental caries in humans can be controlled by the diet. Jay ('40) has suggested that the carbohydrate foods in the diet are largely responsible for decayed teeth.

The mechanism by which soluble carbohydrates favor conditions for a high caries incidence in the cotton rat may be much more complex than merely affecting conditions in the oral cavity. More extensive studies and many approaches to the problem will be necessary before the role of the dietary carbohydrates can be accurately evaluated.

SUMMARY

1. Data on the growth rate of cotton rats maintained on various carbohydrate diets and purified rations are presented. Additional growth responses could be demonstrated when either solubilized liver, 1:20 liver extract or whole liver substance was added to the sucrose rations.

2. Dextri-maltose, glucose, fructose, maltose and sucrose diets afforded approximately the same rate of growth. Fine dextrin, stock or soluble starch rations when fed to cotton rats produced inferior growth as compared to the sucrose control ration.

3. A very high incidence and extent of carious lesions were noted when glucose, dextri-maltose, fructose, maltose or lacto-sucrose diets were fed. However, fine dextrin and coarse dextrin diets did not favor conditions for severe tooth decay and a very low incidence and extent of the lesions were observed. There was no appreciable difference in the results obtained with coarse or fine dextrin.

4. The replacement of one-half of the sucrose with fine dextrin did not reduce the severity of tooth decay as compared to animals which received the sucrose diets. The replacement of three-quarters of the sucrose with fine dextrin reduced the incidence and extent to a small degree.

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LITERATURE CITED

Boyd, J. D. 1942 Nutrition as it affects tooth decay. J. Am. Dietet. Assn., vol. 18, p. 211.

———. 1943 Prevention of dental caries in late childhood and adolescence. J. Am. Dental Assn., vol. 30, p. 670.

Jay, P. 1940 The role of sugar in the etiology of dental caries. J. Am. Dental Assn., vol. 27, p. 393.

McIntire, J. M., B. S. Schweigert and C. A. Elvehjem 1944 The nutrition of the cotton rat (Sigmondon hispidus hispidus). J. Nutrition, vol. 27, p. 1.

Shaw, J. H., B. S. Schweigert, C. A. Elvehjem and P. H. Phillips 1944 Dental caries in the cotton rat. II. Production and description of the carious lesions. J. Dent. Res., vol. 23, p. 417.

Shaw, J. H., B. S. Schweigert, J. M. McIntire, C. A. Elvehjem and P. H. Phillips 1944 Dental caries in the cotton rat. I. Methods of study and preliminary nutritional experiments. J. Nutrition, vol. 28, p. 333.

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Influence of Mast Cells and Vasoconstrictors upon Various Acute Connective-Tissue Reactions

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Introduction

In previous publications, we have described several experimental models of acute or chronic connective-tissue reactions and their response to humoral agents (1–5). It is convenient to discuss the acute and chronic reaction forms separately, since they differ essentially in many respects.

Here we shall limit ourselves to seven prototypes of acute changes in connective tissue, the latter term being defined in its broadest sense (including cellular and intercellular elements as well as the microcirculation). Our first task will be to show which among these seven apparently quite dissimilar responses are independent of each other and which represent merely different manifestations of a single phenomenon.

We have selected mast-cell products and vasoconstrictors as agents likely to influence our experimental models, firstly because these compounds are especially powerful regulators of acute connective-tissue responses and, secondly, because being natural body constituents, their actions are of importance not only in pharmacology but also in physiology and pathology.

On the other hand, our seven prototypes have been chosen because they test very distinct properties of connective tissue and are easily reproducible in the same species, the rat, under strictly comparable standardized conditions.

The essential features of our models may be summarized as follows:

1. Anaphylactoid edema. This consists of a transient, purely serous inflammation affecting the 'acral' regions (paws, snout, ears, anogenital

region). It appears within less than one hour after systemic treatment with various mast-cell dischargers (e.g., ovomucoid, polymyxin, compound 48/80, dextran, dextrin) and disappears a few hours later, leaving no trace. It is generally ascribed to the discharge of mediators of inflammation (especially histamine and 5-HT) from the degranulating mast cells which, in the rat, are particularly numerous in the acral regions. The reaction is readily prevented by antihistamines and antiserotonins (1,6).

2. *Dye transudation.* Capillary permeability is increased in the course of many acute connective-tissue reactions, particularly inflammation. The standard procedure for testing this change is to inject certain water-soluble dyes (e.g., Evans blue) intravenously; wherever vascular permeability is increased, the connective tissue exhibits diffuse staining owing to localized dye leakage (7).

3. *Angiotaxis.* This test is often confused with the preceding one since it also manifests itself by selective staining of certain regions after intravenous administration of coloring matter. However, here, particulate pigments (e.g., colloidal carbon) are used and these do not stain connective tissue diffusely but are deposited in the form of fine precipitates in and around the vascular wall, mostly in phagocytes. Thus, in a sense, angiotaxis complements the function of the reticuloendothelial system in clearing the blood of particles through ingestion by sessile phagocytic elements (4).

4. *Thrombohemorrhagic anaphylactoid edema, or 'anaphylactoid purpura'.* This lesion is actually a combination of anaphylactoid edema and the thrombohemorrhagic phenomenon (THP). It represents a 'pluricausal disease' model in that it depends on the conjoint action of at least two factors: 1) a conditioner which induces THP-reactivity; 2) a challenger which makes the resulting disease proneness manifest and determines its localization. Morphologically, the THP is characterized by thrombosis and hemorrhage at sites where appropriate challengers (e.g., mast-cell dischargers, 5-HT, epinephrine, vasopressin) are applied immediately after intravenous conditioning with certain metals (e.g., rare-earth metals) or polysaccharides (e.g., agar, carrageenin). In addition to this local variant, there is a systemic form which occurs after similar conditioning when appropriate challengers (e.g., mast-cell dischargers, 5-HT, histamine or vasoconstrictors) are injected intravenously. Here, the anaphylactoid shock organs show not only edema but also thrombohemorrhagic lesions (1, 2).

5. *Calcifying anaphylactoid edema.* This phenomenon is closely related to the preceding one in that it also affects the anaphylactoid 'acral' shock organs selectively and is elicited by combined treatment with a conditioning and a challenging factor. However, here, the shock organs exhibit intense calcification, instead of thromboses and hemorrhages (4, 8).

6. *Tissue clearance.* Many of the agents that elicit anaphylactoid edema, or some of its just mentioned variants, can also delay the disappearance from the skin of intradermally injected, easily diffusible coloring matter (e.g., phenolsulfonphthalein). Normally, very diffusible dyes are so rapidly taken up into the blood and eliminated through the urine that there is not enough time for them to spread and stain a large area. However, when tissue clearance is delayed, enough of the dye remains *in situ* to act as a lasting reservoir from which continued spreading far into the surroundings can occur. Since here clearance is the limiting factor after treatment with drugs, the increase in the size of the stained surface reflects the degree of interference with its absorption from the injection site (9).

7. *Acute conditioned necrosis* (*ACN*). Following systemic conditioning with mast-cell dischargers (dextran, dextrin, polymyxin) or mast-cell products (histamine, 5-HT) acute topical necrosis of the skin occurs at sites challenged by otherwise well-tolerated amounts of non-specific tissue irritants (e.g., hypertonic saline, sugar or urea solutions, acetic acid) (10–12). Presumably, this predisposition for necrosis depends upon delayed tissue clearance which, by prolonging the contact of the exogenous irritants with the connective tissue, increases their topical damaging effect.

Materials and Methods

For the sake of uniformity all experiments were performed on female Sprague-Dawley rats with a mean body weight of 100 g (90–110 g). Altogether, 1530 animals were used for seven series of tests concerned with the acute connective-tissue reactions just described. Each of these series was further subdivided into seventeen groups, the first acting as a control, the others treated with various agents known to have a strong influence upon some forms of connective-tissue reactivity (Table I).

The procedures employed in the seven series (and the number of rats in each of their seventeen constituent groups) were:

I. *Anaphylactoid edema.* Thirty rats per group, the results being based on the mean of the combined readings in ten rats of each group in series II, III and VI.

The intensity of the resulting acral edema was appraised one hour after treatment in terms of an arbitrary scale of 0–3 as explained elsewhere (1).

II. *Dye transudation.* Twenty rats given 1% Evans blue (Fisher Scientific Co., Fair Lawn, N.J., USA) 1 ml i.v. Appraisal after one hour, based on the intensity of acral blueing in terms of an arbitrary scale of 0–3.

III. *Angiotaxis.* Twenty rats given 10% 'Pelikan' India ink (Pelikan-Werke Günther Wagner, Hannover, Germany) 1 ml i.v. Appraisal after twenty-four hours, judging by the intensity of acral blackening in terms of an arbitrary scale of 0–3. Histologic control of ink deposition in and around minute vessels in anaphylactoid shock organs.

IV. *Anaphylactoid purpura.* Ten rats given agar I (Anachemia Chemicals Ltd., Montreal, P.Q., Canada) 1 mg in 1 ml i.v. Appraisal two hours after treatment, based on the intensity of acral purpura, expressed in terms of an arbitrary scale of 0–3. Histologic control of hemorrhages and thromboses in microcirculation of acral shock organ.

V. *Calcifying anaphylactoid edema.* Ten rats given 16 mg of lead acetate [$Pb(C_2H_3O_2)_2 \cdot 3H_2O$, Fisher Scientific Co., Fair Lawn, N.J., USA] in 1 ml i.v. Appraisal after five days (when calcinosis reached its maximum) on the basis of acral calcification expressed in terms of an arbitrary scale of 0–3. Histologic control of calcification with the von Kóssa technique.

VI. *Tissue clearance.* Ten rats given 2.5 mg of phenolsulfonphthalein, 'PSP' (Matheson Coleman & Bell, Ohio, USA) in 0.05 ml, intradermally on the shaved back. Appraisal one hour after treatment, based on the size of the colored spot, the longest (D) and shortest (d) axes being measured (± 1 mm) and the area (S) calculated according to Madinaveitia (13), assumingthat the patches are regular ellipses ($S = \pi \cdot D \cdot d/4$).

VII. *Acute conditioned necrosis* (*ACN*). Twenty rats, given 20 mg of acetic acid (C.P. Reagent, C.I.L., Montreal, P.Q., Canada) in 2 ml water s.c. under the shaved skin of the back. The area of the necrotic region developing at the acetic acid injection site was appraised twenty-four hours after treatment and calculated as in series VI.

All i.v. injections were administered into the jugular vein under light ether anesthesia. The solvent for all agents used was distilled water.

Each of the seven series was subdivided into seventeen groups as follows:

1. *Controls*, given no treatment apart from that used in the seven series to determine connective-tissue reactivity under different test conditions.

2. *Egg-white* (fresh chicken egg-white diluted with distilled water and filtered through tissue paper).

3. *Dextran* (M.W. ≈ 75,000. A 6% solution stabilized by 5.7% sorbitol N.F., Abbott Laboratories, North Chicago, Ill., USA).

4. *Dextrin* (British gum, or starch gum, a polysaccharide [$(C_6H_{10}O_5)n \cdot xH_2O$, prepared by incomplete hydrolysis of starch, Testagar & Co. Inc., Detroit, Michigan, USA].

5. *Ferric dextran* (Imferon® containing 50 mg Fe/ml, Fisons Ltd., Toronto, Canada).

6. *Ferric dextrin* (Astrafer® a ferric dextrin chelate containing 20 mg Fe/ml, Astra, Worcester 6, Mass., USA).

7. *Thorium dextrin* (Thorotrast®, Testagar & Co. Inc., Detroit, Michigan, USA).

8. *Polymyxin* (Polymyxin-B sulfate, Pfizer Corporation, Montreal, P.Q., Canada).

9. *Compound 48/80* (a condensation product of p-methoxyphenethyl-methylamine with formaldehyde, Burroughs Wellcome and Co., Montreal, P.Q., Canada).

10. *Viomycin* (Pfizer, Montreal, P.Q., Canada).

11. *Compound L 1935* (Labaz Laboratories, Brussels, Belgium).

12. *Hexadimethrine* (Abbott Laboratories, North Chicago, Ill., USA).

13. *5-HT* (5-hydroxytryptamine, serotonin creatinine sulfate, Nutritional Biochemicals, Cleveland, Ohio, USA).

14. *Histamine* (Histamine phosphate, Abbott Laboratories, North Chicago, Ill., USA).

15. *Octapressin* ('PLV-2' a synthetic vasopressin derivative containing 100 μg (5 I.U.)/ml, Sandoz, Dorval, P.Q., Canada).

16. *Vasopressin* (Parke, Davis & Company, Detroit, Michigan, USA).

17. *Epinephrine* (Adrenaline bitartrate, Brickman Co., Montreal, P.Q., Canada).

All these compounds were given i.v. in 1 ml of distilled water under light ether anesthesia, simultaneously with the special treatments characteristic of each series. The individual doses are listed in Table I which also gives the mean readings $\pm$ standard error and the degree of significance. The significance was calculated by the application of Student's t-test. Only when one of two results to be compared was 'O' did we estimate the significance of the difference on the basis of the confidence limits.

Results

The prototypes of the reactions studied are illustrated in Figures 1–3. Under our experimental conditions, dye transudation resulted in blue (Evans blue), angiotaxis in black (colloidal carbon), anaphylactoid purpura in red (hemorrhages), and calcifying anaphylactoid edema in white (hydroxylapatite) discoloration of the acral shock organs. Hence, the reactive sites were very different upon naked-eye inspection, and of course even more so when examined histologically. However, since on black-and-white photographs the dark variants of anaphylactoid edema appeared to be essentially similar, only two examples are illustrated: anaphylactoid purpura, during the acute edematous stage, and angiotaxis, after the swelling has subsided.

Our results are summarized in Table I. It would be redundant to comment on each finding separately, but certain lawful correlations between the results obtained in the seven series deserve to be pointed out.

Dye transudation appears to be the most sensitive and specific test for anaphylactoid edema. It was significantly increased following treatment with every mast-cell discharger used (Groups 2–11) except hexadimethrine (Group 12), about which we shall have more to say later.

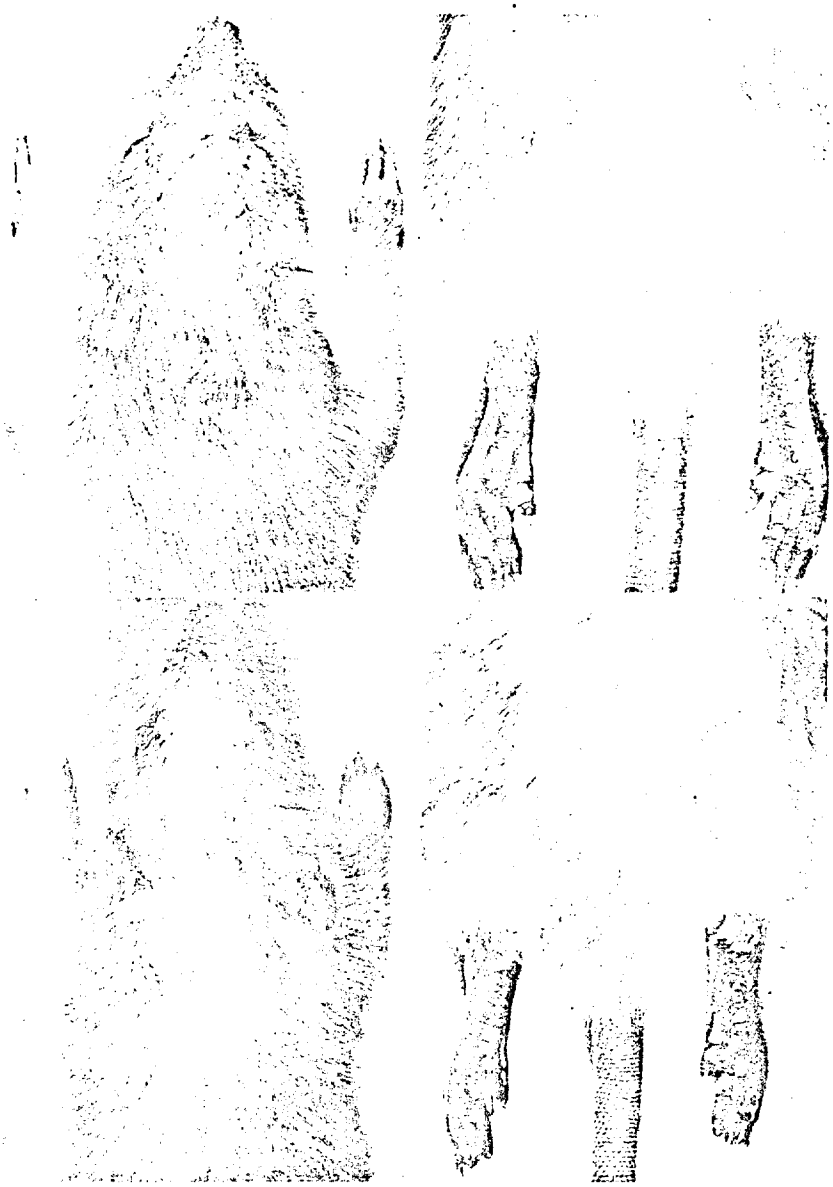


Fig. 1. *Angiotaxis.* – *Top:* Normal control. *Bottom:* Pronounced angiotaxis in anaphylactoid shock organs of a rat treated with 15 mg of dextrin – 1 ml of 20% Indian ink, both i.v. Photograph taken 24 h later, when edema has already disappeared but angiotactically fixed carbon is still present.

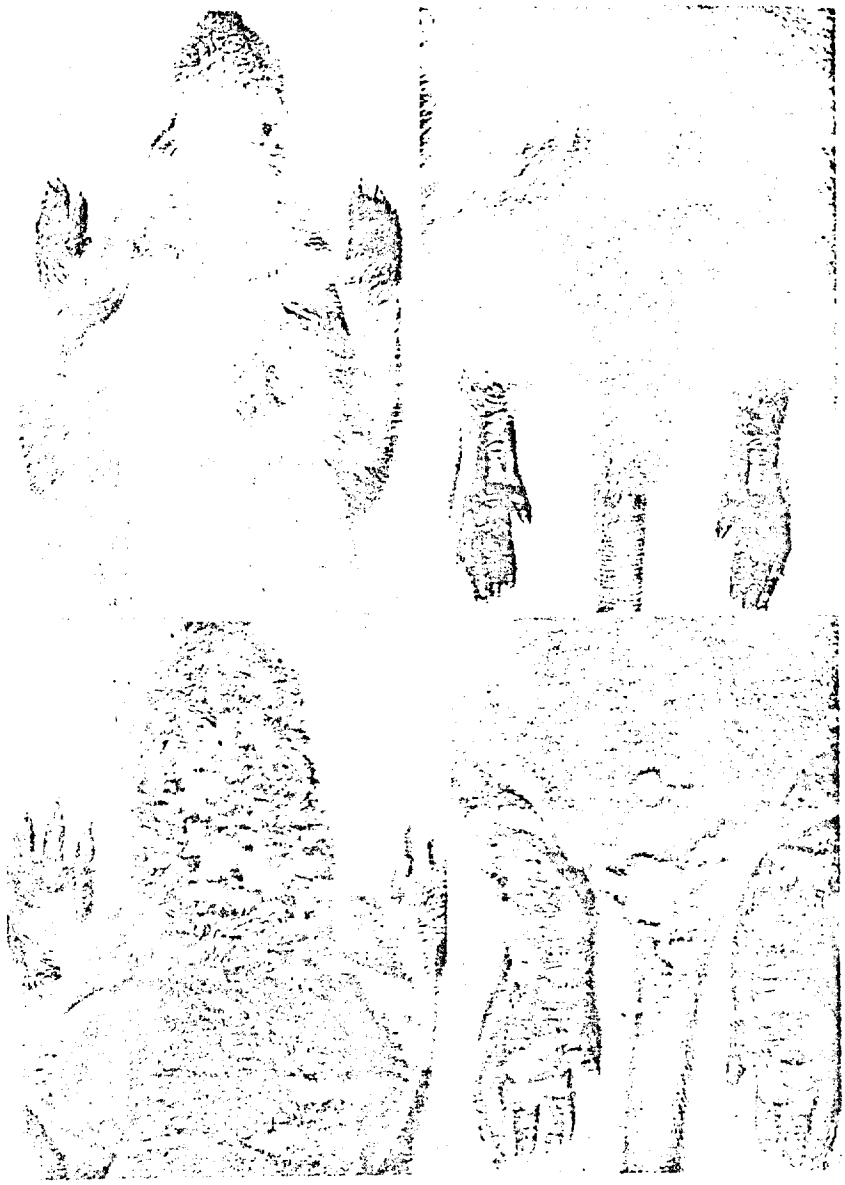
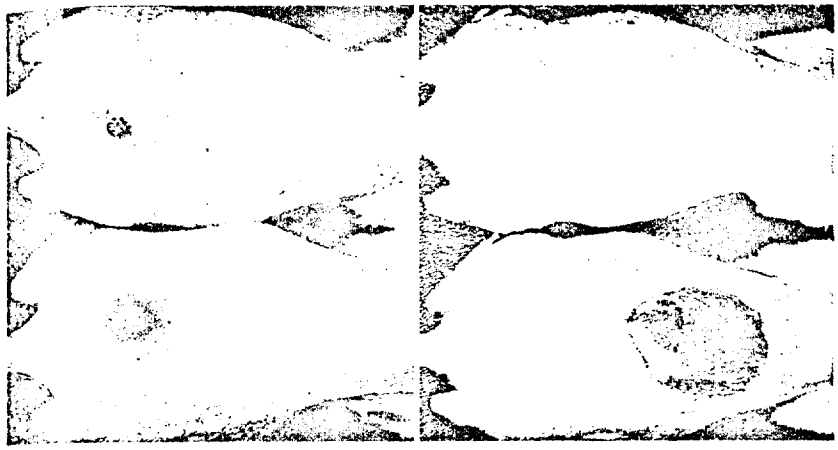


Fig. 2. Thrombohemorrhagic and calcifying variants of anaphylactoid edema. – Top: Anaphylactoid purpura in acral shock organs of a rat given 1 mg of agar + 1 ml of 50% egg-white, both i.v., two h. before this photograph was taken. *Bottom:* Calcifying anaphylactoid edema in a rat given 60 mg of dextran + 16 mg of lead acetate, both i.v. 4 days before this photograph was taken. Acral shock organs, which merely showed edema in acute stage, are now the principal sites of calcification; however, some calcinosis cutis is also seen in small patches between the shoulder blades and in the sacral region.



Delayed tissue clearance and acute conditioned necrosis. – *Left:* Both rats received 25 mg of PSP intradermally but only bottom animal was given 15 mg of dextran i.v. to delay tissue clearance of dye. *Right:* Both rats received 20 mg of acetic acid s.c. on the back, but only bottom animal was conditioned for acute necrosis by 15 mg of dextran i.v.

Conversely, it was not increased by any of the compounds tested that are not mast-cell dischargers (Groups 13–17).

Anaphylactoid purpura was also regularly elicited by all mast-cell dischargers – again with the notable exception of hexadimethrine – but in this test, mast-cell products (Groups 13, 14) and vasoconstrictors (Groups 15–17) likewise gave positive results.

The ability to delay the *tissue clearance* of PSP and to condition for the *ICN phenomenon* is even less specific since it is demonstrable with every compound tested, except epinephrine. On the other hand, in both these tests, even hexadimethrine gave highly significant positive results.

Clear evidence of *angiotaxis and ordinary or calcifying anaphylactoid edema* was not noted with all of the mast-cell dischargers. Furthermore, in these respects none of the compounds other than mast-cell dischargers showed significant activity.

The *singular behavior of hexadimethrine* deserves special consideration. This compound is a potent mast-cell discharger and it produces pronounced anaphylactoid edema in the acral shock organs when administered at higher dose levels subcutaneously to rats. Indeed, weight for weight, it is more potent as a local mast-cell discharger

TABLE I *Comparison of seven acute*

Group	Treatment	I Anaphylactoid edema (Scale 0–3)	Mort. (%)	II Dye transudation (Scale 0–3)	Mort. (%)	III Angiotens (Scale 0–3)
1	None	0	0	1.1 ± 0.07	0	0.2 ± 0.08
2	Egg-white, 50 %	0.9 ± 0.10[3]	10	2.1 ± 0.23[3]	0	1.1 ± 0.25[3]
3	Dextran, 15 mg	1.3 ± 0.14[3]	0	2.9 ± 0.10[3]	0	0.8 ± 0.25
4	Dextrin, 15 mg	0.9 ± 0.11[3]	0	2.8 ± 0.13[3]	0	1.7 ± 0.25
5	Ferric dextran = 10 mg Fe	1.3 ± 0.14[3]	0	2.8 ± 0.13[3]	0	1.8 ± 0.22
6	Ferric dextrin = 5 mg Fe	0.7 ± 0.11[3]	0	2.9 ± 0.10[3]	0	1.1 ± 0.15
7	Thorium dextrin = 15 mg dextrin	0.2 ± 0.07[1]	3	2.1 ± 0.23[3]	0	0.6 ± 0.22
8	Polymyxin, 0.1 mg	0	7	1.8 ± 0.13[3]	0	0.4 ± 0.16
9	Cpd. 48/80, 0.1 mg	0	23	2.0 ± 0.15[3]	0	0.6 ± 0.24
10	Viomycin, 10 mg	0.1 ± 0.05	0	2.1 ± 0.18[3]	0	0.5 ± 0.17
11	Cpd. L 1935, 0.3 mg	0	3	2.0 ± 0.00[3]	0	0.1 ± 0.11
12	Hexadimethrine, 2.5 mg	0.1 ± 0.05	7	0.5 ± 0.17[3]	0	1.3 ± 0.17[3]
13	5-HT, 0.3 mg	0	13	1.1 ± 0.12	20	0
14	Histamine, 20 mg	0.1 ± 0.06	0	1.2 ± 0.13	0	0.2 ± 0.13
15	Octapressin, 0.01 mg	0	0	1.3 ± 0.15	0	0
16	Vasopressin, 0.01 mg	0	10	1.0 ± 0.00	0	0
17	Epinephrine, 0.01 mg	0	7	1.0 ± 0.00	20	0

[1] = $0.01 < P < 0.05$
[2] = $0.001 < P < 0.01$ Indicates significance, compared to Group 1 of same series.
[3] = $P < 0.001$
[4] Because of toxicity, in this case 200 μg were used.

and produces more pronounced topical swelling when injected into the paw than any of the compounds tested up to now (1). However, because of its high toxicity when introduced directly into the blood, larger amounts could not be tested in this investigation where, for the sake of uniformity, all compounds had to be administered intravenously. Indeed, even the comparatively low dose of 2.5 mg used in the present work was uniformly fatal to rats conditioned for the calcifying form of anaphylactoid edema, although it caused little or no mortality when given conjointly with other conditioning factors. The most unexpected effect of hexadimethrine was that it not only failed to enhance the transudation of Evans blue into the

...ced by various agents

TABLE I

II	V Calcifying anaphylactoid edema (Scale 0–3)	Mort. (%)	VI Tissue clearance mm²	Mort. (%)	VII Acute conditioned necrosis (ACN) mm²	Mort. (%)
[illegible]	0	10	23±2	0	3±2	0
[illegible]	1.9±0.23[3]	30	96±13[3]	0	962±116[3]	0
[illegible]	2.3±0.16[3]	20	59±10[2]	0	413±96[3]	0
[illegible]	1.6±0.37[2]	0	72±13[2]	0	1031±85[3]	0
[illegible]	0	0	50±8[2]	0	587±115[3]	0
[illegible]	—	100	71±13[2]	0	1030±104[3]	10
[illegible]	2.0±0.00[3]	70	122±20[3]	0	1004±57[3]	0
[illegible]	1.0±0.37[1]	50	130±18[3]	0	1232±66[3]	0
[illegible]	1.5±1.50	80	225±55[3]	20	1177±52[3]	0
[illegible]	1.3±0.33[1]	50	88±7[3]	0	956±121[3]	10
[illegible]	0[4]	50	94±16[3]	0	1121±63[3]	30
[illegible]	—	100	63±10[3]	10	178±64[3]	10
[illegible]	0.5±0.22	60	46±8[1]	0	758±125[3]	0
[illegible]	0	30	51±6[3]	0	359±66[3]	0
[illegible]	0.1±0.12	20	118±17[3]	0	517±135[3]	0
[illegible]	0	30	88±24[2]	30	784±109[3]	10
[illegible]	0	20	27±2	0	421±101[3]	0

acral shock organs, but actually inhibited this particularly constant and characteristic indicator of anaphylactoid edema.

A number of additional tests was performed, therefore, with hexadimethrine to determine the mechanism of its singular effect upon dye distribution. It was found that in rats killed one hour after the treatment given to Group 12, the liver became deeply colored whereas the acral organs, the hepatic lymph nodes and the renal papilla remained virtually colorless; the reverse was true in controls given only Evans blue. Presumably hexadimethrine actively retains Evans blue in internal organs, thus deviating it from the usual sites of deposition.

Discussion

The primary object of these experiments was to compare the effect of various mast-cell dischargers, mast-cell products and vasoconstrictors upon seven acute reaction forms of connective tissue. The latter were selected because they put connective tissue through its paces. Although they differ essentially from each other in their manifestations, these reactions appear to be related in some way since, under appropriate conditions, all of them can be elicited by compounds capable of producing an anaphylactoid edema of the acral shock organs in the rat. Our comparative studies revealed numerous similarities and differences between mast-cell dischargers, mast-cell products and vasoconstrictors. Depending upon controllable conditions, these compounds can participate in the production of such diverse changes as simple edema, transudation of water-soluble dyes into connective tissue, angiotaxis of colloidal particles, purpura or calcification in the acral shock organs, the tissue clearance of intradermally injected materials and the provocation of necroses by irritants normally well tolerated by connective tissue.

The principal generalizations that can be made regarding the effect of the agents tested upon these seven reaction forms are the following:

1. Compounds which delay tissue clearance are also active in conditioning for anaphylactoid purpura and acute conditioned necrosis (ACN). We found only two exceptions to this rule: firstly, hexadimethrine fails to condition for anaphylactoid purpura, although it moderately delays tissue clearance and predisposes for the ACN; secondly, epinephrine fails to delay tissue clearance under our experimental conditions, although it does moderately predispose for anaphylactoid purpura and the ACN. It must be remembered, however, that hexadimethrine differs from other mast-cell dischargers in many respects. Furthermore, in these experiments all agents had to be administered intravenously to create comparable conditions; hence, we could give only small amounts of epinephrine. It is known, however, that larger doses, given subcutaneously, can cause a delay in tissue clearance (1). Therefore, these apparent exceptions may not be fundamental.

2. Some mast-cell dischargers and mast-cell products, which do not produce anaphylactoid edema under our experimental conditions, can nevertheless elicit an ACN, delayed tissue clearance and anaphylac-

toid purpura. Apparently, these latter reaction forms are not strictly dependent upon the development of anaphylactoid edema.

3. Such potent vasoconstrictors as octapressin, vasopressin and epinephrine fail to produce ordinary or calcifying anaphylactoid edema; in fact, earlier work has shown that they actively inhibit anaphylactoid edema (1). Yet, all three vasoconstrictors delay tissue clearance (with the aforementioned exception of epinephrine given i.v. at low dose levels) and predispose for the production of both anaphylactoid purpura and the ACN.

4. Only mast-cell dischargers that cause angiotaxis can also elicit anaphylactoid edema and vice versa (the few apparent exceptions are statistically doubtful).

Curiously, hexadimethrine which, under our experimental circumstances produced no anaphylactoid edema, nevertheless induced a statistically highly significant angiotaxis. This finding further illustrates the exceptional properties of this compound. As we have said before, there is no doubt that hexadimethrine is a potent mast-cell discharger and that, upon subcutaneous injection of large doses, it does produce a typical acral anaphylactoid edema. Like other mast-cell dischargers it also delays tissue clearance and conditions for the ACN phenomenon as well as for angiotaxis. Still, unlike other mast-cell dischargers, it inhibits, instead of promoting, dye transudation into the acral shock organs and does not condition for anaphylactoid purpura.

5. All mast-cell dischargers which produce an anaphylactoid edema and angiotaxis also enhance dye transudation (with the repeatedly mentioned exception of hexadimethrine). However, not all mast-cell dischargers that enhance dye transudation cause visible anaphylactoid edema or angiotaxis in the acral shock organs. Apparently, dye transudation is a more sensitive indicator for compounds of this group than demonstrable edema or angiotaxis.

6. All agents which delay tissue clearance also enhance the ACN. This fact suggests that the ability of normally well tolerated irritants to cause such necrosis depends upon the prolongation of their contact with the surrounding tissues.

7. Vasoconstrictors not normally endowed with the capacity to produce an anaphylactoid edema (and indeed known to be potent inhibitors of this response) can elicit an anaphylactoid purpura in the acral shock organs, which is essentially a thrombohemorrhagic variant of anaphylactoid edema. There appears to exist a close

parallelism between agents capable of delaying tissue clearance, producing anaphylactoid purpura and conditioning for the ACN phenomenon. This similarity of action suggests the possibility of a common link in the mechanism underlying these seemingly distinct reaction forms. The nature of this relationship remains to be established.

Since so many agents – different in their chemical structure and pharmacologic activity – share the property of eliciting a delay in tissue clearance as well as the ACN and anaphylactoid purpura, it was thought that these particular phenomena may be due to nonspecific stress. However, this possibility had to be rejected, because a variety of typical stressors proved to be ineffective in delaying tissue clearance (9), predisposing to the ACN (10) or eliciting anaphylactoid purpura (2).

Of course, these conclusions do not necessarily hold for experiments performed under different conditions of dosage or route of administration. Yet, our findings do show that, using standardized techniques, it is possible to detect close correlations between the ability of certain compounds to elicit some, but not all, of the acute connective-tissue reactions examined.

Summary

Seven essentially different acute connective-tissue reactions have been studied under comparable standardized conditions in the rat, namely: anaphylactoid edema, the transudation of intravenously injected dyes, angiotaxis, anaphylactoid purpura, calcifying anaphylactoid edema, delay in tissue clearance, and acute conditioned necrosis. Certain mast-cell dischargers can induce all these reaction forms; others elicit only some of them selectively.

When given intravenously, 5-HT, histamine, octapressin, vasopressin and epinephrine do not produce typical anaphylactoid edema, increased transudation of an intravenously injected dye, angiotaxis or calcifying anaphylactoid edema; yet, they do produce anaphylactoid purpura, delay in tissue clearance, and the ACN phenomenon under appropriate conditions. Special emphasis is placed upon the unique properties of hexadimethrine, which differ essentially from those of all other mast-cell dischargers tested.

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References

1. SELYE, H.: Anaphylactoid edema (Warren H. Green, Inc., St. Louis 1968).
2. SELYE, H.: Thrombohemorrhagic phenomena (Charles C. Thomas, Springfield 1966).
3. SELYE, H.: Stress (Acta Inc., Med. Publ., Montreal 1950).
4. SELYE, H.: The mast cells (Butterworth, Washington 1965).
5. SELYE, H.: Calciphylaxis (The University of Chicago Press, Chicago 1962).
6. SELYE, H.: Studies on adaptation. Endocrinology *21:* 169 (1937).
7. SELYE, H.; SOMOGYI, A. and VÉGH, P.: Inflammation, topical stress and the concept of pluricausal diseases. 1st Symp. of the Int. Inflammation Club, Augusta, Mich., May 31–June 4, 1967. Biochem. Pharmacol. (in press).
8. SERAFIMOV, N.; TUCHWEBER, B.; GABBIANI, G.; JEAN, P. and SELYE, H.: The action of histamine liberators on calcification produced by various metals. Arch. int. Pharmacodyn. *154:* 249 (1965).
9. SELYE, H.: Mast cells, vasoconstrictors and absorption from connective tissue. Prof. J. Charvat's Jubilee Vol., Endocr. exp. *1:* 131 (1967).
10. SELYE, H.: Mast cells and necrosis. Science *152:* 1371 (1966).
11. SELYE, H.; ROHAN, P. and PAHK, U. S.: Humoral conditioning for necrosis. Part I: The conditioning factors. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmak. *255:* 133 (1966).
12. SELYE, H. and ROHAN, P.: Humoral conditioning for necrosis. Part II: The challengers. Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmak. *255:* 142 (1966).
13. MADINAVEITIA, J.: Studies on diffusing factors. Active preparations from mammalian testicle and their biological assay. Biochem. J. *32:* 1806 (1938).

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248

Significance of the 'Protein-Sparing' Effect of Dextrin[1]

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ABSTRACT

SPIVEY, H. E., M. C. KATAYAMA, M. YOSHIDA AND A. E. HARPER. *Significance of the 'protein-sparing' effect of dextrin.* Am. J. Physiol. 193(3): 479–482. Among the hypotheses proposed to explain the greater food intake and rate of gain of young rats fed ad libitum on low protein diets in which dextrin or corn starch has been substituted for sucrose are: *a*) increased synthesis of essential amino acids by intestinal microorganisms; *b*) improved digestion and absorption of protein owing to slower passage of diets containing dextrin through the small intestine; *c*) reduced destruction of amino acids in the animal body owing to more prolonged absorption of glucose from dextrin. The results of nitrogen balance and feeding experiments indicate that improvement in the efficiency of protein utilization when dextrin is substituted for sucrose is too small for any of the above hypotheses to account for the apparent 'protein-sparing' effect of dextrin.

IT WAS RECOGNIZED some time ago that the more rapid growth of rats fed on low protein diets in which corn starch or dextrin had been substituted for glucose or sucrose as the dietary carbohydrate was associated with increased food intake (1). It has generally been assumed that the increased food intake in itself was not primarily responsible for the more rapid growth but that both the greater rate of growth and the larger food intake were reflections of the nutritional superiority of diets containing complex carbohydrates. The background of this subject has recently been reviewed and several of the explanations that have been suggested for the apparent protein-sparing effect of complex carbohydrates have been discussed (2). Among the hypotheses put forward to explain the apparent nutritional superiority of dextrin are: *a*) synthesis of essential amino acids by the intestinal microflora of rats consuming the less soluble carbohydrates; *b*) improved digestion and absorption of protein owing to the slower passage of complex carbohydrates through the gastrointestinal tract; *c*) reduced destruction of amino acids in the animal body owing to more prolonged absorption of the glucose from the complex carbohydrates.

The experiments reported in this paper and those in the accompanying paper were performed in order to test these various hypotheses and in order to determine whether the apparent protein-sparing action might not be a direct result of increased food intake due to the physical properties of complex carbohydrates rather than to an indirect nutritional or metabolic effect.

MATERIALS AND METHODS

Male weanling rats of either the Sprague-Dawley or Wistar strain weighing from 40 to 50 gm were used throughout these studies. They were maintained in individual cages with raised screen bottoms and unless otherwise noted they were fed ad libitum.

The ration used in the experiment with sulfasuxidine-treated and cecectomized rats was identical with that previously reported (1). In the experiments with amino acids substituted for casein, a mixture of essential

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TABLE 1. EFFECT OF CECECTOMY OR SULFA DRUG ON RATE OF GROWTH OF RATS FED 9% CASEIN WITH EITHER SUCROSE OR CORNSTARCH

Group	Carbohydrate	No. of Rats	Average 1-Wk. Gain, gm
1	Cornstarch (sham-operated)	6	23±1.8*
2	Sucrose (sham-operated)	6	11±1.2
3	Cornstarch (cecectomized)	5	20±2.3
4	Sucrose (cecectomized)	6	11±1.2
5	Cornstarch + sulfasuxidine, 1%	6	24±1.7

* Standard error of the mean.

amino acids (mixture XXVI) (3) which was sufficient only for maintenance was used. To this was added 4.67% of ammonium citrate to provide a source of nitrogen other than amino acids which would permit slow growth and would provide some additional nitrogen for microorganisms and for the synthesis of the dispensible amino acids (3).

The composition of the basal diet for the rest of the experiments reported was as follows: sucrose, 81.6; casein, 9.0; DL-methionine, 0.3; corn oil, 5.0; salts 4 (4), 4.0; and choline chloride, 0.15%. Vitamins were included to provide, in milligrams per 100 gm of ration, thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 2.5; calcium pantothenate, 2.0; pyridoxine, 0.25; biotin, 0.01; folic acid, 0.02; vitamin B_{12}, 0.002; and inositol, 10.0. Two drops of halibut liver oil, fortified to provide vitamin A, 1,000 IU; vitamin D, 10 IU; 2-methyl, 1-4 naphthoquinone, 0.04 mg; and α-tocopherol, 0.8 mg; were administered weekly.

The carbohydrate component of each diet is listed in the tables of experimental results.

The casein was commercial casein extracted three times with boiling alcohol. The term 'dextrin' used throughout this paper refers to the product obtained by heating moist cornstarch in an autoclave at 15 lb. pressure for 3 hours.

The rats that received their entire daily ration during a single 1.5-hour period were trained in the manner described by Finlayson and Baumann (5).

Nitrogen was determined by the Kjeldahl method using mercuric oxide as the catalyst.

The cecectomies were performed as described by Schweigert *et al.* (6).

RESULTS

The results presented in table 1 show that neither cecectomy nor the feeding of sulfasuxidine, procedures which tend to suppress the growth of microorganisms, depressed the growth rate of rats receiving the 9% casein diet containing cornstarch. The difference between the rate of gain of groups receiving sucrose and that of groups receiving cornstarch was comparable to previously reported differences (1).

The results presented in table 2 were obtained in a nitrogen balance experiment in which rats receiving either sucrose or dextrin as the dietary carbohydrate were allowed to consume, ad libitum, diets containing just above maintenance levels of a mixture of essential amino acids supplemented with ammonium citrate. It was assumed that if intestinal synthesis of amino acids were of significance, a substantial improvement in nitrogen utilization should be observed under these conditions. The rats receiving cornstarch gained slightly, but not significantly, more than those receiving sucrose; they consumed somewhat more food and retained somewhat more nitrogen. Also the value for percentage of ingested nitrogen retained was slightly greater for the group receiving cornstarch than for the group receiving sucrose.

In an attempt to determine whether slower disappearance of cornstarch or dextrin from the intestinal tract (7) was responsible for the improved growth rate of rats receiving these carbohydrates, a paired-feeding technique was used with rats trained to consume their entire daily ration in a single 1.5-hour period. The first group (table 3) was fed the complete sucrose diet during the regular feeding period while the second received the same diet but with only 25% of the sucrose during the regular feeding period. This group received the re-

TABLE 2. EFFECT OF SUCROSE OR CORNSTARCH ON GROWTH, FOOD INTAKE AND NITROGEN RETENTION OF RATS FED AMINO ACID MIXTURE*

	Av. Food Intake	Wt. Gain	Av. Nitrogen Intake	Av. Nitrogen Retention	Nitrogen Retention
	gm/day	*gm/4 wk.*	*mg/day*	*mg/day*	%
Sucrose	4.6	16.5±1.5†	39.5	21.0±1.2†	53.3±
Cornstarch	5.2	20.0±3.0	47.3	27.2±1.6	57.4±

* For amino acid mixture, see text.

† Mean ± standard error of the mean for 6 rats.

TABLE 3. EFFECT OF PROLONGED INGESTION OF SUCROSE ON GROWTH OF RATS RECEIVING A LOW-PROTEIN (9% CASEIN) DIET*

Group	Method of Feeding Sucrose	Rate of Gain,† gm/wk.
1	Sucrose in diet	15.3±2.3‡
2	25% of the sucrose in diet; 75% of the sucrose given by stomach tube over a 6-hr. interval	16.8±1.8

* Rats fed for a single 1.5-hr. interval daily.
† Experiment was of 1 week duration.
‡ Mean ± standard error of the mean for 8 rats.

maining 75% of the sucrose by stomach tube, one-third every 2 hours, during the succeeding 6 hours. The stomach tube was passed down the esophagus of each control rat (*group 1*) the same number of times in order to eliminate any bias from the effects of trauma. It is evident that the prolonged ingestion of sucrose was ineffective in stimulating growth.

Since Womack and associates (8, 9) had shown that adult rats fed on diets containing low levels of amino acids and dextrin utilized nitrogen more efficiently than similar animals receiving comparable diets containing sucrose, it seemed important to determine whether this also the case with young growing rats. In table 4 are presented the results of nitrogen balance experiment in which young rats were fed ad libitum on diets containing 9% of casein with either sucrose or dextrin as the dietary carbohydrate. Each series represents a 3-day collection period, collections being made over the last 3 days of each week of the 3-week experimental period.

The growth rates of 12 gm/wk. for rats receiving sucrose and 21 gm/wk. for those receiving dextrin were comparable to rates usually obtained with these groups. The food intake, and therefore the nitrogen intake, of the group receiving sucrose was only 65–70% as great as that of the group receiving dextrin. Fecal nitrogen excretion, as Mitchell (10) has emphasized, paralleled food intake and was considerably greater for the dextrin group. Total nitrogen retention, as would be expected, paralleled growth, but in each series the percentage of the dietary nitrogen retained was somewhat greater for rats receiving dextrin than it was for those receiving sucrose. However, since the values for the percentage of ingested nitrogen retained varied by as much as 5% between series and since there was considerable variation within the sucrose series and differences observed are of doubtful significance.

DISCUSSION

It is evident that neither microorganisms eliminated by feeding sulfasuxidine nor the intact cecum are in any way responsible for the beneficial effect of dextrin in stimulating the growth of rats fed on low protein diets. The results of the nitrogen balance experiment with rats fed only maintenance levels of essential amino acids together with ammonium citrate indicate that such synthesis of essential amino acids as may occur when sucrose is replaced by dextrin under these conditions, is insufficient to stimulate growth appreciably. Steele (11) has shown that none of the carbon of uniformly C^{14}-labeled sucrose fed to rats can be detected in the essential amino acids of the carcass. Rose and associates (12, 13) have demonstrated that the ingestion of sulfasuxidine had little effect on the growth of rats fed inadequate quantities of amino acids with a mixed carbohydrate and that the nitrogen

TABLE 4. EFFECT OF SUCROSE OR DEXTRIN ON GROWTH, FOOD INTAKE AND NITROGEN RETENTION OF WEANLING RATS FED A 9% CASEIN DIET

Carbohydrate	Nitrogen Intake	Nitrogen in Feces	Nitrogen in Urine	Total Nitrogen Retained	Nitrogen Retained*	Av. Wt. of Rats†
	mg	*mg*	*mg*	*mg*	*%*	*gm*
			Series 1			
Dextrin	313 ±12‡	26.6 ±4.5	49.8 ±4.5	236.6 ±9.4	75.6 ±0.6	71.5
Sucrose	218 ±12	13.6 ±2.5	51.0 ±9.5	155.3 ±20.6	70.4 ±6.7	60.0
			Series 2			
Dextrin	395 ±34	56.2 ±1.1	49.3 ±3.5	289.0 ±23.6	73.2 ±1.0	94.0
Sucrose	260 ±17	25.6 ±5.1	64.1 ±6.2	170.3 ±19.7	65.5 ±4.3	73.5
			Series 3			
Dextrin	535 ±29	72.3 ±5.2	53.3 ±1.6	409.4 ±29.4	76.5 ±0.8	111.8
Sucrose	340 ±17	22.5 ±2.4	79.6 ±1.3	237.9 ±18.1	69.9 ±1.6	85.2

Each series represents a 3-day collection period.
* % of ingested nitrogen retained.
† Represents average weight of rats at end of 3-day collection period.
‡ Mean ± standard error of the mean for 5 rats.

of N^{15}-labeled urea appeared primarily in the dispensible amino acids and to a small extent in histidine into which it could enter through metabolic reactions. These observations, together with the realization that essential amino acids are required in milligram quantities/day to stimulate growth, in contrast to the requirement for only microgram quantities of the vitamins known to be synthesized by the intestinal flora, make it unlikely that intestinal synthesis of amino acids is sufficiently great to stimulate growth.

The failure to obtain any increase in the rate of gain by administering sucrose over a prolonged period of time (table 3) indicates that if the prolonged absorption of carbohydrate reduces the amount of protein used for energy, the effect is insufficient under these conditions to cause a growth response.

The results of the nitrogen balance experiments indicate, as was found by Womack and associates (8, 9) for adult rats, that there is a small increase in the percentage of ingested nitrogen retained when sucrose in a low protein diet is replaced by dextrin however the values for percentage retention, were quite variable, as can be seen from the standard errors (table 4). Since in a previous study (14) no growth difference was observed when rats fed on low protein diets containing dextrin were pair-fed with those receiving similar diets containing sucrose and since reduced energy intake or food intake may reduce the efficiency of protein utilization (15–17) it seems more plausible that the trend toward higher percentage nitrogen retention of rats receiving dextrin is a result of their greater food intake and rate of gain rather than a cause of it.

The small differences in the efficiency of nitrogen utilization observed in these experiments make it unlikely that any of the hypotheses previously proposed (1, 8, 9, 14) [illegible] account for the beneficial effect of dex[illegible] stimulating food intake and growth rate [illegible] rats consuming low protein diets. It [illegible] more likely, from results presented in [illegible] accompanying paper (18), that these effe[illegible] depend directly upon the physical pro[illegible] of the complex carbohydrate.

Some of the crystalline vitamins were kind[illegible] vided by Merck Sharp & Dohme, Rahway, [illegible] Jersey.

REFERENCES

1. Harper, A. E. and M. C. Katayama. *J. Nu[illegible] tion* 49: 261, 1953.
2. Harper, A. E. and C. A. Elvehjem. *J. A[illegible] Food Chem.* 5: 754, 1957.
3. Rose, W. C., L. C. Smith, M. Womock and [illegible] Shane. *J. Biol. Chem.* 151: 307, 1949.
4. Hegsted, D. M., R. C. Mills, C. A. Elvehjem and E. B. Hart. *J. Biol. Chem.* 138: 459, 1941.
5. Finlayson, J. S. and C. A. Baumann. *J. Nu[illegible] tion* 59: 211, 1956.
6. Schweigert, B. S., J. M. McIntire, L. M. Ha[illegible] derson and C. A. Elvehjem. *Arch. Biochem.* [illegible] 403, 1945.
7. Dreisbach, L. and E. S. Nasset. *J. Nutrition* [illegible] 523, 1954.
8. Womack, M., M. W. Marshall and A. B. Pa[illegible] *J. Nutrition* 51: 117, 1953.
9. Marshall, M. W. and M. Womack. *J. Nu[illegible] tion* 52: 51, 1954.
10. Mitchell, H. H. In: *Proteins and Amino Acids [illegible] Nutrition*, edited by M. Sayhun. New Yo[illegible] Reinhold, 1948, p. 46–81.
11. Steele, R. *J. Biol. Chem.* 198: 237, 1952.
12. Rose, W. C. and L. C. Smith. *J. Biol. Chem.* 187: 687, 1950.
13. Rose, W. C. and E. E. Dekker. *J. Biol. Chem.* 223: 107, 1956.
14. Harper, A. E., W. J. Monson, D. A. Arata, D. A. Benton and C. A. Elvehjem. *J. Nutrition* [illegible] 523, 1953.
15. Geiger, E. *Fed. Proc.* 10: 670, 1951.
16. Forbes, R. M. and M. Yohe. *J. Nutrition* [illegible] 409, 1955.
17. Munro, H. N. *Physiol. Rev.* 31: 449, 1951.
18. Harper, A. E. and H. E. Spivey. *Am. J. Physiol.* 193: 483, 1958.

Am. J. Physiol., 148, 1947

258

THE INTESTINAL SYNTHESIS OF NIACIN AND FOLIC ACID IN THE RAT[1]

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That vitamins are synthesized by intestinal microorganisms has been proved beyond any reasonable doubt. In some cases, significant amounts of these vitamins are absorbed from the intestines and utilized in the animal body. The evidence is particularly strong in the case of folic acid. Intestinal microorganisms have been shown to synthesize folic acid *in vitro* (1, 2) and the vitamin has been found in a simple water extract of the cecal contents as well as in the washed intestinal walls (3). Rats on certain highly purified diets containing very small amounts of folic acid grow well, but when sulfa drugs are added, poor growth and granulocytopenia are observed (4, 5), concurrent with marked changes in the intestinal flora (6, 7). When folic acid concentrates or pure folic acid compounds are administered, growth is resumed and the blood picture improves (8, 9, 10). The addition of sulfa drugs to synthetic diets definitely reduces the vitamin B_c potency of rat livers; when sources of vitamin B_c are added, the vitamin B_c potency of the livers increases several fold (11, 12).

Similar evidence does not exist in the case of niacin, though it is clear that this vitamin is synthesized somewhere in the rat's body (13, 14). Dann (14) concluded that intestinal synthesis is not an important source of niacin for the rat because its addition to purified diets containing 1 per cent sulfaguanidine did not produce increased growth. However, a response would not be expected because folic acid was not supplied in his ration. Furthermore, it is well known that sulfa drugs may change the species comprising the intestinal flora without changing the total number of organisms, and in the light of present knowledge, the amount and type of drug fed in the experiments of Dann would not be expected to produce a maximum effect.

Thus the question of whether or not sufficient synthesis of niacin for the rat's needs takes place in its tissues remained unanswered and the present studies were designed to throw some light on the matter. Data on the intestinal synthesis of niacin on various rations were needed as a basis for the study of the pellagra-preventing action of such foods as milk which is low in niacin and the pellagra-promoting action of corn. Folic acid determinations were made as an adjunct to the main study.

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We are indebted to Merck and Company, Rahway, New Jersey, for some of the crystalline B vitamins and to Abbott Laboratories, North Chicago, Illinois for haliver oil.

The choice of a method for studying intestinal synthesis is dictated by available facilities and the type of information desired. The use of sterile animals has been suggested as an ideal approach but this involves a great deal of expense and effort, and while it provides valuable information, it does not offer final proof of what happens under normal conditions. The excretion of nutrients may be measured and compared with the intake (16). In addition the average amount of the vitamin found in the entire animal at the beginning and end of the experiment may be determined (14, 17). Additional techniques include the use of drugs, cecectomy, dietary modification and careful studies of the intestinal flora. None of these methods obviates the possibility of synthesis in the tissues and subsequent excretion into the intestinal tract.

Under most conditions the cecum contains the greatest part of the intestinal contents and a number of studies point to this structure as an important seat of vitamin synthesis (18, 19). Taylor, Pennington, and Thacker (20) found that cecectomy of rats on a poor diet had a deleterious effect on growth while cecectomy on a good diet had no effect. Wakim, Krider and Day (20) have reported that cecectomy produces more consistent hypoprothrombinemia in rats on purified rations containing sulfa drugs. Kornberg, Daft and Sebrell (21) tested the cecal contents of rats on diets with and without sulfa drugs in the diet and found that the latter had greater activity in counteracting prothrombin deficiency in rats.

In the present studies the needed information was obtained through the determination of niacin and folic acid in the cecal contents of rats on various diets after they had been on experiment for 1 and 2 weeks. In addition, a highly purified diet complete in all known factors for the rat except niacin was fed to rats together with sulfa drugs in an attempt to produce a niacin deficiency.

EXPERIMENTAL. Twenty-one day old male weanling Sprague-Dawley rats were placed on the different rations and at the end of 1 and 2 weeks the cecal contents of 3 rats in each group were analyzed for niacin and folic acid. The basal diet had the following composition:

Carbohydrate	78 grams
Casein	15 grams
Corn oil	3 grams
Salts IV[2]	4 grams
Cystine	0.15 gram
Thiamine	0.2 mgm.
Riboflavin	0.3 mgm.
Pyridoxine	0.25 mgm.
Ca-pantothenate	2.0 mgm.
Biotin	0.005 mgm.
2-methyl-1,4-naphthoquinone	0.1 mgm.
Choline	100 mgm.
Inositol	10 mgm.
Folic acid preparation $\cong$ 1 per cent solubilized liver extract powder containing 11.5 ug. B_c (*S. faecalis* assay) per 100 grams ration.	
α-tocopherol	1 mgm. per week
3 drops Haliver Oil diluted 1–6 with corn oil per week.	

[2] Phillips, P. H. and E. B. Hart. J. Biol. Chem. 109: 657, 1935.

The folic acid preparation contained only 1.6 ug. of niacin per 11.5 ug. B_c (22). The basal diet without the folic acid preparations contained less than 0.01 mgm. niacin per 100 grams.

Phthalylsulfathiazole was added at the expense of the entire ration. The lactose and skim milk powder were added to the synthetic rations at the expense of sucrose. Mineralized skim milk powder was prepared so that each 100 grams of ration contained the following amounts of added minerals: 15 mgm. iron, 15 mgm. copper and 1.5 mgm. manganese. Vitamins A, D and E were supplemented by dropper as on the basal ration.

"Synthetic milk" had the following composition:

Carbohydrate (lactose unless specified)	40
Casein	28
Fat	26
Salts IV	6
Vitamins as in basal diet above	

In all cases the casein (Labco) was extracted three times with hot 95 per cent ethanol.

The animals were killed by decapitation and the cecal contents were suspended in 0.1 N KOH and autoclaved at 121°C. for 1 hour. The suspensions were neutralized with HCl and stored in the cold under chloroform and toluene.

Niacin was determined with *Lactobacillus arabinosus* according to the method of Krehl, Elvehjem and Strong (23). The folic acid potency was measured by the method of Teply and Elvehjem (24) using *Streptococcus faecalis* with vitamin B_c as the standard. In preliminary studies the vitamin B_c potency of cecal material was not materially increased by various treatments with enzymes, acid or alkali. However, since autoclaving for 1 hour with 0.1 N KOH did not destroy the activity of vitamin B_c for either *S. faecalis* or *Lactobacillus casei* this treatment was used as standard procedure. P-aminobenzoic acid was added to the tubes to counteract any sulfa drug present in the cecal contents.

In all cases the cecal contents represented the greatest part of the intestinal contents. On lactose diets and on some of the diets containing sulfa drugs the ceca were greatly enlarged. There was a variation in color and consistency ranging from watery white material on the lactose diets to black pithy material on the dextrin diet.

The figures given in table 1 are averages of three analyses. In most cases there was remarkably close agreement within each group.

The largest amount of niacin and folic acid per gram of cecal contents was found in the rats on the dextrin ration. On a high lactose diet there was little synthesis of either vitamin during the second week. This was probably due to an excessive amount of unabsorbed lactose in the cecum. The addition of sulfa drug to the lactose ration actually caused an increased amount of niacin in the cecum. This effect also occurred in the case of some of the milk powder diets.

In general the inclusion of 2 per cent phthalylsulfathiazole caused a marked decrease in cecal niacin on a per gram basis, but the ceca increased in size so

that there was practically no change in the total amount present. In contrast, the folic acid content was greatly decreased even on a "total amount" basis.

TABLE 1

Niacin and folic acid in rat cecal contents (averages of values from three different rats)

DIET	NIACIN		WT. OF CECAL CONTENT		FOLIC ACID**	
	1st week	2nd week	1st week	2nd week	1st week	2nd week
	ug. per gm.	*ug. per gm.*	*gm.*	*gm.*	*ug. per gm.*	*ug. per gm.*
Sucrose basal	19.3	24.7	0.28	0.40	0.53	0.85
Sucrose basal + 2% phthalylsulfathiazole	2.8	3.5	1.9	3.9	0.02	0.03
Lactose basal	42.6	0.9	0.33	3.5	1.8	0.03
Lactose basal + 2% phthalylsulfathiazole	4.3	8.8	2.3	5.5	0.03	0.01
Glucose basal	18.6	13.3	0.49	0.67	0.6	0.3
Glucose basal + 2% phthalylsulfathiazole	3.0	4.8	2.3	2.7	0.02	0.02
Dextrin basal	31.1	48.7	0.66	1.0	1.9	1.0
Dextrin basal + 2% phthalylsulfathiazole	2.6	3.3	2.1	5.3	0.01	0.006
Sucrose basal + "1% folic acid"*	30.1	31.5	0.56	0.82	1.3	0.4
Sucrose basal + "1% folic acid"* + 2% phthalylsulfathiazole	2.3	5.3	3.5	3.8	0.2	0.04
Sucrose basal + 0.5 mgm.% niacin	33.9	24.0	0.40	0.69	1.0	
Sucrose basal + 0.5 mgm.% niacin + 2% phthalylsulfathiazole	3.2	3.6	1.7	3.1	0.07	
Sucrose basal + 15% skim milk powder	42.1	35.6	0.83	1.2	1.7	0.3
Sucrose basal + 15% skim milk powder + 2% phthalylsulfathiazole	3.9	9.9	1.4	2.3	0.01	
Sucrose basal + 7½% lactose	31.9	30.9	0.41	0.55	0.9	
Sucrose basal + 7½% lactose + 2% phthalylsulfathiazole	2.9	4.9	2.2	3.4	0.03	
Mineralized skim milk powder + 30% corn oil	11.9	4.1	2.1	3.9	0.03	
Mineralized skim milk powder + 30% corn oil + 2% phthalylsulfathiazole	5.4	15.9	2.2	4.0	0.02	
Mineralized skim milk powder + 30% butterfat	6.9	15.8	1.5	3.8	0.1	
Mineralized skim milk powder + 30% butterfat + 2% phthalylsulfathiazole	15.6	16.4	1.7	3.4	0.04	
"Synthetic milk" (corn oil)	12.5	17.0	0.85	1.9	0.1	
"Synthetic milk" (butterfat)	20.8	36.6	0.82	1.1	0.4	
"Synthetic milk" (butterfat-sucrose)	15.3	16.1	0.46	0.60	1.0	

* Special "folic acid" concentrate = 1% solubilized liver extract or 11.5 ug. B_c per 100 grams of ration.

** Potency compared with vitamin B_c using *S. faecalis*.

This may explain the relative difficulty of producing niacin deficiency with sulfa drugs particularly when adequate amounts of folic acid are present.

On the "synthetic milk" ration only moderate amounts of folic acid were found. Replacement of lactose with sucrose increased the folic acid activity of the cecal

contents and decreased the niacin content. Substitution of butterfat for corn oil allowed an increased synthesis of both vitamins.

On mineralized milk powder +30 per cent corn oil or butter fat only moderate amounts of folic acid were found in the cecum. Phthalysulfathiazole diminished the amount slightly. The amount of niacin in the cecum of rats fed the corn oil ration was low the first week and increased during the second week. When butterfat was used instead of corn oil the niacin content in the cecum was high

TABLE 2

Growth depression with phthalylsulfathiazole and its counteraction with niacin (weekly average gains of three rats in each group)

DIET USED	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK	AVERAGE
	gm.	*gm.*	*gm.*	*gm.*	*gm.*
Basal diet	32	32	30	31	31
Basal diet + 2% phthalylsulfathiazole	19	15	8	5	12
Basal diet + 2% phthalylsulfathiazole + "1% folic acid"*	14	15	17	17	16
Basal diet + 2% phthalylsulfathiazole + 0.5 mgm.% niacin	19	9	4	7	13
Basal diet + 2% phthalylsulfathiazole + "1% folic acid" + 0.5 mgm.% niacin	23	27	29	31	28

* As in table 1.

TABLE 3

Growth depression with high levels of phthalysulfathiazole and its partial counteraction with niacin (average weekly gains of three rats in each group)

DIET USED	1ST WEEK	2ND WEEK	3RD WEEK	4TH WEEK
	gm.	*gm.*	*gm.*	*gm.*
Basal diet	24	36	32	37
Basal + 4% phthalylsulfathiazole	12	11	9	1
Basal + 4% phthalysulfathiazole + "1% folic acid*	10	16	27	22
Basal + 4% phthalysulfathiazole + "1% folic acid" + 1 mgm. % niacin	18	21	26	20

* As in table 1.

the first week and remained at a high level. It is interesting that only in the case of milk powder diets was there no change in the size of the cecum on the addition of phthalysulfathiazole.

The addition of 7½ per cent lactose or 15 per cent skim milk powder to the sucrose basal ration caused a marked increase in niacin and folic acid in the ceca. The addition of phthalylsulfathiazole reduced the amounts to those obtained with the basal diet plus phthalylsulfathiazole. Although milk may provide "bound folic acid" which can be used by the rat (25) it probably contributes to the synthesis of this vitamin because of its effects on the intestinal flora.

Addition of excess niacin increased the synthesis of folic acid and excess folic acid increased niacin synthesis. These effects were eliminated by feeding phthalylsulfathiazole.

Although the present studies are primarily concerned with synthesis of vitamins, the concurrent destruction of niacin probably plays an important rôle in determining the final niacin concentration (26).

Attempts to produce niacin deficiency. The basal diet described above was employed. In preliminary experiments succinylsulfathiazole at a 1 per cent or 2 per cent level produced severe diarrhea. Phthalylsulfathiazole caused much less diarrhea and was used exclusively in the experiments described.

Table 2 shows that with a diet containing 20 per cent casein and 73 per cent sucrose the addition of 2 per cent phthalylsulfathiazole produced a growth depression that could not be counteracted by an amount of folic acid concentrate $\cong$ 1 per cent solubilized extract powder. Supplementation with both folic acid and 0.5 mgm. per cent niacin almost completely counteracted the effect of phthalylsulfathiazole.

The results in table 3 were obtained in an experiment where the level of the drug was raised to 4 per cent. After the first two weeks niacin did not potentiate the action of folic acid. At the 4 per cent level of phthalylsulfathiazole niacin and folic acid together could not counteract the effect of the drug. Whether the limitation is related to one or both of these two vitamins or due to some other effect is not known. Neither substituting glucose for sucrose nor reducing the casein content of the diet to 15 per cent has made the niacin deficiency more marked.

The increased effectiveness of folic acid after the second week on experiment can probably be attributed to the increased intestinal synthesis of niacin as a result of cecal growth. Taylor et al. (20) state "At the time of weaning, the rat's cecum is only about one-fourth as large relative to the digestive tract as it will be in the adult. This may account in part for the well-recognized fact that rats at this stage are less resistant to some vitamin deficiencies than they are a week or so later."

As far as the authors are aware these experiments are the first to indicate that a niacin deficiency may be produced in the rat through the use of sulfa drugs. It has been shown that the ration must be highly purified and high levels of a suitable sulfa drug must be used if positive results are to be obtained. It is hoped that the availability of pure folic acid and new drugs will facilitate further studies.

SUMMARY

1. The ceca of rats that had been on various diets with and without 2 per cent phthalylsulfathiazole for 1 and 2 weeks were analyzed for niacin and folic acid.

2. Dextrin caused the greatest synthesis of both niacin and folic acid.

3. In general, 2 per cent phthalylsulfathiazole produced a marked decrease in the concentration of niacin in the cecal contents, but the ceca increased in size so that the total amount remained about the same. The folic acid content was greatly decreased even on a total basis.

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4. Excess niacin increased the synthesis of folic acid and excess folic acid increased the synthesis of niacin. These effects were prevented by feeding 2 per cent phthalylsulfathiazole.

5. Addition of lactose or milk powder to the basal ration increased the synthesis of both niacin and folic acid. When phthalylsulfathiazole was fed this increase did not occur.

6. On "synthetic milk" diets butterfat caused more vitamin synthesis than did corn oil.

7. Through the use of a highly purified diet growth depression was produced with phthalylsulfathiazole which could be counteracted only by feeding niacin along with folic acid.

REFERENCES

(1) Hutchings, B. L., N. Bohonos and W. H. Peterson. J. Biol. Chem. **141**: 521, 1941.

(2) Thompson, R. C. Univ. Tex. Pub. no. 4237, p. 87, 1942.

(3) Mitchell, H. K. and E. R. Isbell. Univ. Tex. Pub. no. 4237, p. 125, 1942.

(4) Black, S., J. M. McKibbin and C. A. Elvehjem. Proc. Soc. Exper. Biol. and Med. **47**: 308, 1941.

(5) Kornberg, A., F. S. Daft and W. H. Sebrell. Science **98**: 20, 1943.

(6) Gant, O. K., B. Ransone, E. McCoy and C. A. Elvehjem. Proc. Soc. Exper. Biol. and Med. **52**: 276, 1943.

(7) Miller, A. K. J. Nutrition **29**: 143, 1945.

(8) Nielsen, E. and C. A. Elvehjem. J. Biol. Chem. **145**: 713, 1945.

(9) Axelrod, A. E., P. Gross, M. D. Bosse and K. F. Swingle. J. Biol. Chem. **148**: 721, 1943.

(10) Kornberg, A., F. S. Daft and W. H. Sebrell. Proc. Soc. Exper. Biol. and Med. **58**: 46, 1945.

(11) Schweigert, B. S., I. Tatman-Greenhut, L. J. Teply and C. A. Elvehjem. This Journal **144**: 74, 1945.

(12) Wright, L. D., H. R. Skeggs and A. D. Welch. Arch. Biochem. **6**: 15, 1945.

(13) Shourie, K. L. and M. Swaminathan. Ind. J. Med. Res. **27**: 697, 1940.

(14) Dann, W. J. J. Biol. Chem. **141**: 803, 1941.

(15) Schweigert, B. S., J. M. McIntire, L. M. Henderson and C. A. Elvehjem. Arch. Biochem. **6**: 403, 1945.

(16) Briggs, G. M., T. D. Luckey, L. J. Teply, C. A. Elvehjem and E. B. Hart. J. Biol. Chem. **148**: 517, 1943.

(17) Guerrant, N. B., R. A. Dutcher and R. A. Brown. J. Nutr. **13**: 305, 1937.

(18) Griffith, W. H. J. Nutrition **10**: 667, 1935.

(19) Nielsen, E., G. M. Shull and W. H. Peterson. J. Nutrition **24**: 523, 1942.

(20) Taylor, A., D. Pennington and J. Thacker. Univ. Texas. Pub. no. 4237, p. 135, 1942.

(21) Kornberg, A., F. S. Daft and W. H. Sebrell J. Biol. Chem. **155**: 193, 1944.

(22) Krehl, W. A., P. S. Sarma, L. J. Teply and C. A. Elvehjem J. Nutr. **31**: 85, 1946.

(23) Krehl, W. A., F. M. Strong and C. A. Elvehjem. Ind. Eng. Chem. Anal. Ed. **15**: 471, 1943.

(24) Teply, L. J. and C. A. Elvehjem. J. Biol. Chem. **157**: 303, 1945.

(25) Wright, L. D., H. R. Skeggs, A. D. Welch, K. L. Sprague and P. A. Mattis. J. Nutrition **29**: 287, 1945.

(26) Benesch, R. Lancet **1**: 718, 1945.

277

Brit. J. Pharmacol. (1963), **21**, 235–237.

ANAPHYLACTOID OEDEMA PRODUCED IN RATS BY CERTAIN DEXTRINS

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The author has previously shown the conditioning role of the anaphylactoid reaction in the calcification of the snout and paws of the rat after the injection of certain metallic compounds. The present experiments show that it is the dextrin fraction of these compounds which causes these anaphylactoid phenomena before the calcifying response. This fact deserves special attention since dextrin is frequently used in the preparation of certain drugs.

After administration of dihydrotachysterol the rat responds to the injection of certain metallic compounds by a typical syndrome characterized by heavy calcium deposits in the snout, paws and oesophagus (Selye, Gentile & Veilleux, 1961). These lesions develop over 2 or 3 days and they are preceded by an evanescent anaphylactoid reaction which immediately follows the injection of the compound.

After we had demonstrated the essential conditioning role of this initial anaphylactoid reaction in the later deposition of calcium (Veilleux, 1963), our attention was attracted by the fact that the metallic compounds used contained dextrin. Our first attempt to produce an anaphylactoid reaction with one particular dextrin was unsuccessful. We therefore resorted to dextrins of different origins which, in the present experiment, are compared as to their aptitude to induce an anaphylactoid reaction.

METHODS

We used four different dextrins which are identified by the letters A, B, C and D (Table 1).

Dextrin A is Bacto-Dextrin (Difco, Detroit). According to the manufacturer, it is a highly purified product obtained from potato starch. It contains low molecular weight dextrins and is substantially free from dextrose and maltose as well as the high molecular weight soluble starches (Cowles, personal communication).

We have been unable to obtain precise information concerning Dextrin B (No. 1297, Eastman Kodak, Rochester, N.Y.) but we think it is similar to Bacto-Dextrin since it behaves in a similar manner, at least with regard to the anaphylactoid response.

Dextrin C (Astra, A.B., Södertälje, Sweden) corresponds exactly to the substance used by this company in the preparation of its iron-dextrin compound Ferrigen. It is obtained from potato starch and its average molecular weight, measured by gel-filtration through "sephadex," was approximately 6,000 (Sjögren, personal communication).

Dextrin D (Testagar, Detroit) is the one used by this firm in manufacturing its thorium dioxide preparation known as Thorotrast. It is a tapioca extract which is lyophilized and

pyrogen-free. It contains approximately 2.5% of dextrose. Its molecular weight is not known, but is believed to be relatively low because of the low viscosity and high grade of conversion of Dextrin D (Carrigan, personal communication).

Eighty rats with a mean initial body weight of 100.4 g (range 98 to 104 g) were divided into eight equal groups and treated as indicated in Table 1.

The different dextrins, in 25% solutions, were injected intravenously (Groups 1 to 4) and intraperitoneally (Groups 5 to 8) in doses of 0.3 and 0.5 ml. respectively. The anaphylactoid oedema of the snout and paws was evaluated 0.5 and 1 hr after the injection in all groups and registered in terms of an arbitrary scale of 0 to 3 (0=no reaction, 1=minimal reaction, 2=moderate reaction and 3=maximal reaction).

RESULTS

The results clearly show (Table 1) that Dextrins A and B are completely devoid of any anaphylactoid properties by either route of administration (Groups 1, 2, 5 and 6; Fig. 1, 1). On the other hand, Dextrins C and D both elicit an anaphyl-

TABLE 1

ANAPHYLACTOID OEDEMA OF THE SNOUT AND PAWS OF THE RAT AS OBSERVED 0·5 AND 1 HR AFTER THE INJECTION OF FOUR DIFFERENT DEXTRINS (25% SOLUTION) BY EITHER THE INTRAVENOUS OR THE INTRAPERITONEAL ROUTE

Groups 1 to 4 received 0·3 ml. intravenously, and Groups 5 to 8 0·5 ml. intraperitoneally. See text for method of assessing anaphylactoid reaction

		Anaphylactoid reaction			
		0·5 hr		1 hr	
Group	Dextrin given	Snout	Paws	Snout	Paws
1	A	0	0	0	0
2	B	0	0	0	0
3	C	1·8	2·0	1·8	1·6
4	D	1·6	1·3	1·3	1·2
5	A	0	0	0	0
6	B	0	0	0	0
7	C	2·8	3·0	2·7	3·0
8	D	2·7	3·0	2·7	3·0

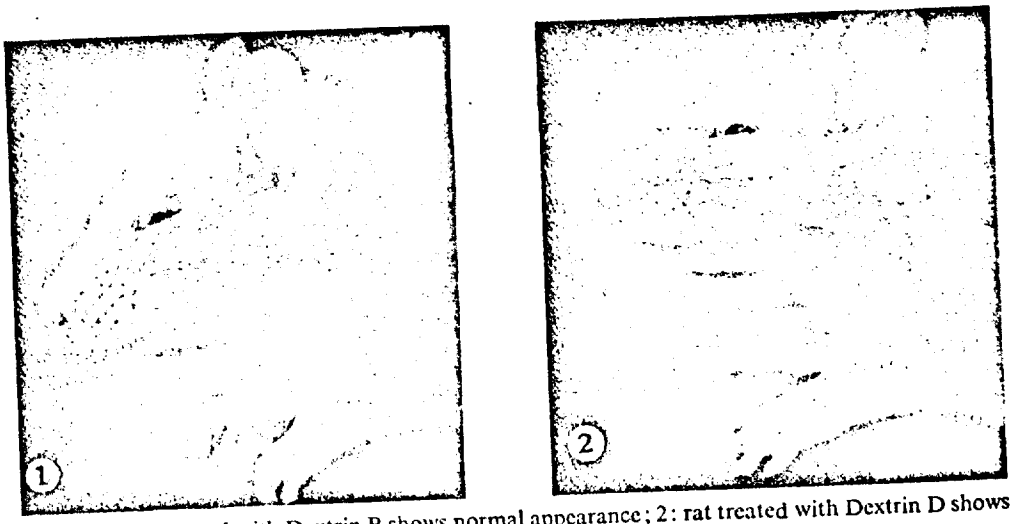

Fig. 1. 1: Rat treated with Dextrin B shows normal appearance; 2: rat treated with Dextrin D shows swelling of paws, lips and snout.

actoid oedema (Fig. 1, 2), the reaction being more marked when the substance is injected intraperitoneally (Groups 7 and 8).

DISCUSSION

Dextran has been well known for its anaphylactoid properties since Pulaski's first observation (Morrison, Bloom & Richardson, 1951), and similar clinical side-effects of iron-dextran in man have been mentioned occasionally (Callender & Smith, 1954; Koszewski & Walsh, 1958; Shafer & Marlow, 1959; Samuels, 1962). About 40 years ago, Hanzlik & Karsner (1920), in a survey of "anaphylactoid phenomena" in the guinea-pig, reported the occurrence of systemic anaphylactoid shock in response to the injection of dextrin, but this substance has not yet been found to elicit the characteristic anaphylactoid oedema of the snout and paws that we have just described. It is interesting to note that only certain dextrins of relatively low molecular weight are able to produce the reaction.

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REFERENCES

CALLENDER, S. T. & SMITH, M. D. (1954). Intramuscular iron. *Brit. med. J.*, ii, 1487.

HANZLIK, P. J. & KARSNER, H. T. (1920). Anaphylactoid phenomena from the intravenous administration of various colloids, arsenicals and other agents. *J. Pharmacol. exp. Ther.*, **14**, 379–423.

KOSZEWSKI, B. J. & WALSH, J. R. (1958). Intramuscular iron therapy with iron dextran. *Amer. J. med. Sci.*, **235**, 523–531.

MORRISON, J. L., BLOOM, W. L. & RICHARDSON, A. P. (1951). Effect of dextran on the rat. *J. Pharmacol. exp. Ther.*, **101**, 27–28.

SAMUELS, L. D. (1962). Leukemoid reaction to parenteral iron-dextran complex. *J. Amer. med. Ass.*, **182**, 1334–1335.

SELYE, H., GENTILE, G. & VEILLEUX, R. (1961). Production of calciphylactic facial, oesophageal and mediastinal lesions by combined treatment with dihydrotachysterol and thorium dioxide. *Brit. med. J.*, ii, 1194–1196.

SHAFER, A. W. & MARLOW, A. A. (1959). Toxic reaction to intramuscular injection of iron. *New Engl. J. Med.*, **260**, 180.

VEILLEUX, R. (1963). Rôle de la réaction anaphylactoïde dans un syndrome calcifiant expérimental. *Rev. canad. Biol.*, **22**, 15–18.

286

J. Nutr., 3, 1931

THE METABOLIC RATE AND RESPIRATORY QUOTIENTS OF RATS FOLLOWING THE INGESTION OF DEXTRIN AND DURING FASTING*

By

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BECAUSE of the increasing use of the white rat in metabolism studies, there has been considerable interest during the past few years in the metabolic rate and respiratory quotients of the rat immediately after feeding, during the basal condition, and during fasting. The length of time required to reach the basal condition is still a matter of uncertainty, as are also the actual basal values. This uncertainty is partly due to the fact that sufficient effort has not been made to carry out these determinations under what might be called standard and uniform conditions. In the work described in the present paper, an attempt has been made to control and standardize the conditions under which the determinations were carried out, and to allow the rat to reach under this environment what is believed to be a more nearly basal condition.

Temperature. It is well known that the metabolic rate of the rat increases as one changes the environmental temperature below or above 28°C. On the basis of this, Goto (1) and Benedict and MacLeod (2) make the statement that the basal rate of the rat should be determined only at 28°. A number of investigators (3, 4, 5, 6,) have selected 27° or 28° as the temperature at which their determinations upon rats were made.

While the metabolic rate changes quickly with variations in the surrounding temperature, a further response is obtained when the subject remains for some time under the influence of the new temperature conditions. A number of investigators (7, 8, 9, 10, 11, 12, 13, 14) have shown that in the tropics or even in the semi-tropics the basal rate of men and women averaged lower and in some cases even 20 per cent less than in temperate climates. von Hoesslin (15) believes that the rate of metabolism of the

* This paper was read in outline form before the American Physiological Society at the Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, March 26-29, 1930.

cells slowly lessens in the hot climate, and that body surface is not the only deciding factor in determining the metabolic rate at a given temperature. Sundstroem (16) gave his rats ample time to adapt themselves to climate environment, but has given no data on the results of his experiments. Hill (17) believed that the metabolism of body cells is raised by an open air life and exposure to cold. Cori and Cori (6) kept their rats at approximately 27° for 24 hours prior to their metabolic rate determinations. Benedict and MacLeod (2) found a higher metabolic rate in rats kept at 21° than in those kept at 28° for 24 hours prior to the determinations which were made at 28°. The seasonal variation in rats found by Benedict and MacLeod (2) may be partially due to differences in the previous environmental temperature.

In the following series of determinations, we have maintained the rats with an environmental temperature of 28° for 3 or more days prior to the beginning of the test period.

Atmospheric humidity and air velocity. These factors are closely allied to that of temperature, as they modify the rate of surface cooling at a given dry bulb temperature. A number of investigators (18, 19, 20) have well shown that the effective temperature and air velocity and not the dry bulb temperature are controlling factors in the rate of metabolism. Their measurements were made on humans, however, in a naked or nearly naked condition where the exposure of the skin would be expected to produce the maximum effect. Clothing, as would be expected, greatly reduces the effect of humidity and air velocity on metabolism. Sundstroem (16) has applied these factors to basal determinations on rats, but has given no figures to show the magnitude of the observed effects when applied to animals well protected by fur. As Richet (21) showed in 1885 by shaving the fur from rabbits, the metabolic rate at a given temperature was enormously increased in the shaved rabbits, while if protected by a cloth covering, these rabbits gave quite normal results. Cluzet (22), working with guinea pigs, found that at 28° there was a slight decrease in the CO_2 output in moisture-saturated air as contrasted with dry air. The effect of normal variations of atmospheric humidity and slight air currents on the metabolic rate of rats at 28° is apparently of secondary importance. We have not attempted to modify the humidity nor to determine the velocity of the mild air currents in the metabolism room used in the following series of determinations.

Barometric pressure. Variations of atmospheric pressure within extremely wide limits are known to have no appreciable effect on the respiratory gas exchange (23 to 40).

Light conditions. Although the rat is a nocturnal animal, in the continuous absence of light and consequently of diurnal variations of light, its activity, as shown by kymographic records, is no greater than under normal conditions. In the following series of measurements we have maintained rats in the practically complete absence of light for 3 or more days prior to the determinations, which were made under the same conditions. This factor has been neglected in previous biocalorimetric work.

TABLE I

SURFACE AREA OF NORMAL RATS AND DEXTRIN REQUIREMENTS PER 24 HOURS

Rat	Surface		Dextrin		Rat	Surface		Dextrin	
Wt.	Area	Increment per gm.	Per 24 hrs.	Increment per gm.	Wt.	Area	Increment per gm.	Per 24 hrs.	Increment per gm.
gm.	cm²	cm²	gm.	gm.	gm.	cm²	cm²	gm.	gm.
50	131	1.5	2.55	0.030	230	328	0.9	6.39	0.016
60	146	1.4	2.85	0.028	240	337	0.9	6.55	0.016
70	160	1.4	3.13	0.026	250	345	0.8	6.71	0.016
80	174	1.4	3.39	0.025	260	353	0.8	6.87	0.016
90	187	1.3	3.63	0.024	270	361	0.8	7.03	0.015
100	199	1.2	3.87	0.023	280	369	0.8	7.18	0.015
110	211	1.2	4.10	0.022	290	377	0.8	7.33	0.015
120	222	1.1	4.32	0.021	300	385	0.8	7.48	0.015
130	233	1.1	4.53	0.021	310	393	0.8	7.63	0.015
140	244	1.1	4.74	0.020	320	401	0.8	7.78	0.015
150	254	1.0	4.94	0.019	330	408	0.7	7.93	0.014
160	264	1.0	5.13	0.019	340	415	0.7	8.07	0.014
170	274	1.0	5.32	0.019	350	422	0.7	8.21	0.014
180	283	0.9	5.51	0.018	360	429	0.7	8.35	0.014
190	292	0.9	5.69	0.018	370	436	0.7	8.49	0.014
200	301	0.9	5.87	0.017	380	443	0.7	8.63	0.013
210	310	0.9	6.04	0.017	390	450	0.7	8.76	0.013
220	319	0.9	6.21	0.017	400	457	0.7	8.89	0.013

Period of fasting. The usual method of determining the time of the beginning of fasting has been to remove the food cups from the animals without a knowledge of when the animals last ate. Cori and Cori (6), however, in a study of the rate of formation of glycogen, fed glucose by stomach tube and determined the metabolic rate and respiratory quotients for 3 hours after feeding. Árvay (41) fed meat powder and lard and determined the gas exchange for 30 minutes. In the following work we have given the rat a definite amount of dextrin to eat. This was eaten immediately, for the most part, and if a residue remained it was fed to the rat, so that the duration of

the meal was not more than 30 minutes. The periods were then timed from the beginning of this test meal.

Amount and kind of food available for metabolism. Aside from the investigations mentioned in the preceding paragraph, no attempt has been made to control the amount of metabolites available to the animals at the beginning of the fasting period. In this work this was done by a preliminary fast of 18 to 24 hours, followed by a test meal consisting of an amount of dextrin that, based upon an average metabolic rate, would furnish sufficient calories for 12 hours. By the preliminary fasting, available metabolites were reduced to body fat and protein and a minimum of glycogen. At the end of the test meal the rat has an amount of food and food stores that we believe is as definite in amount and kind as can be obtained in a normal animal.

The weight of dextrin for the test meal was calculated on the basis of previous determinations by others of the metabolic rate of the male rat. An average value of 800 Cals. per square meter of body surface per 24 hours, or 0.04 Cal. per square centimeter per 12 hours was assumed. As 1 gm. dextrin by its complete combustion to CO_2 and liquid H_2O furnishes 4.11 Cals. (42), 0.04 Cal. would require 0.009734 gm. dextrin. The weight of dextrin in grams required for the test meal can be found by multiplying by 0.009734 the surface area in square centimeters as calculated from Lee's formula (43 and p. 513). Table I shows the surface area in sq. cm. and the weight of dextrin required for the calories per 24 hours for normal male rats of various body weights on the assumption that all of the 800 Cals. per square meter of skin area are obtained from the dextrin.

Method

In our series the metabolic rate and respiratory quotients were determined for periods up to 44 hours from the beginning of the test meal. These determinations were made at 28° and in the dark in 2 distinct types of apparatus, as follows: 1.—A bell jar in which the rat was placed, and from which the expired air was collected at intervals for analysis in a Henderson-Haldane gas analysis apparatus. 2.—A closed circuit apparatus previously described (44), but now improved in convenience and accuracy. The 20 rats of this series were run repeatedly as follows. Rats A and B were kept in a dark, constant temperature room for 3 or 4 days prior to the fasting and feeding. They were then run at 1-hour periods for the intervals 1 to 10 hours, and 22 to 35 hours, A in the bell jar and B in the closed circuit apparatus. They were then restored to light and the stock diet, and several weeks later were placed in the dark room again and run for the in-

terval 11 to 21 hours, and 34 to 44 hours, in the same apparatus as before. Later the same procedure was repeated, placing B now in the bell jar and A in the closed circuit apparatus. By a similar rotation of the 20 rats it was possible to avoid the effect of the individual variations of the rats on the average of the approximately 500 determinations made.

The rats were male animals, 120 to 220 grams in weight, and maintained, as recommended by Osborne and Mendel (45), on a stock diet of special dog biscuits[1] with greens and milk on alternate days. These determinations were made in September, October and November.

Apparatus

Constant temperature metabolism room (46). A well-insulated inside room of 600 cu. ft. capacity is kept at an approximate temperature of 28 ± 0.5°C. Total darkness is obtained at all times except when a person is in the room. At these times the room is illuminated by an incandescent light bulb operated by a door switch. Individual cages in which the rats are kept are supported on an insect-proof rack in this room.

Bell jar apparatus for metabolic rate and respiratory quotient determinations. This apparatus is situated in the constant temperature metabolism room. A bell jar (47) of about 12.5 liters capacity is provided with an inlet tube which reaches to the bottom and an outlet tube which extends from the top, both tubes extending through the rubber stopper of the bell jar and being provided with stopcocks. The bell jar is placed on a glass plate and the ground-glass flange at the bottom is made air-tight by means of petrolatum. Tests for leaks are made before each period by means of a manometer. A small motor manufactured for use in a malted milk mixer was fitted with a short shaft and a fan, and suspended near the top of the inside of the bell jar. The electrical connections for the motor pass through the stopper. The inlet tube to the bell jar is connected to a small, oil-sealed, rotary pump that may be used for pumping air from the outside of the building in and out of the bell jar. A 4-mesh screen in the bell jar ½″ from the glass plate bottom prevents the droppings from being accessible to the rat. The air of the bell jar is sampled by means of gas sampling bulbs and subsequently analyzed in the Henderson-Haldane gas analysis apparatus, with the usual check by duplicate analyses.

To use the apparatus, the animal is placed in the bell jar, and a current of outside air passed through the jar from ½ hour to 1 hour. The stopcocks on the outlet and inlet tubes are then closed, and the expired air is allowed to accumulate for a period which is judged from the size of the rat to be

[1] Manufactured by Potter-Wrightington Co., Inc., Boston.

long enough to give a CO_2 content of 1.5 to 2.5 per cent CO_2. The fan motor is then run for 5 seconds to stir the air, after which an air sample is withdrawn and analyzed in the adjacent laboratory. Blank runs on the apparatus with outside air furnish the CO_2 and O_2 values to be used as a basis for the calculation.

Closed circuit apparatus for metabolic rate and respiratory quotient determinations. This apparatus is also located in the constant temperature

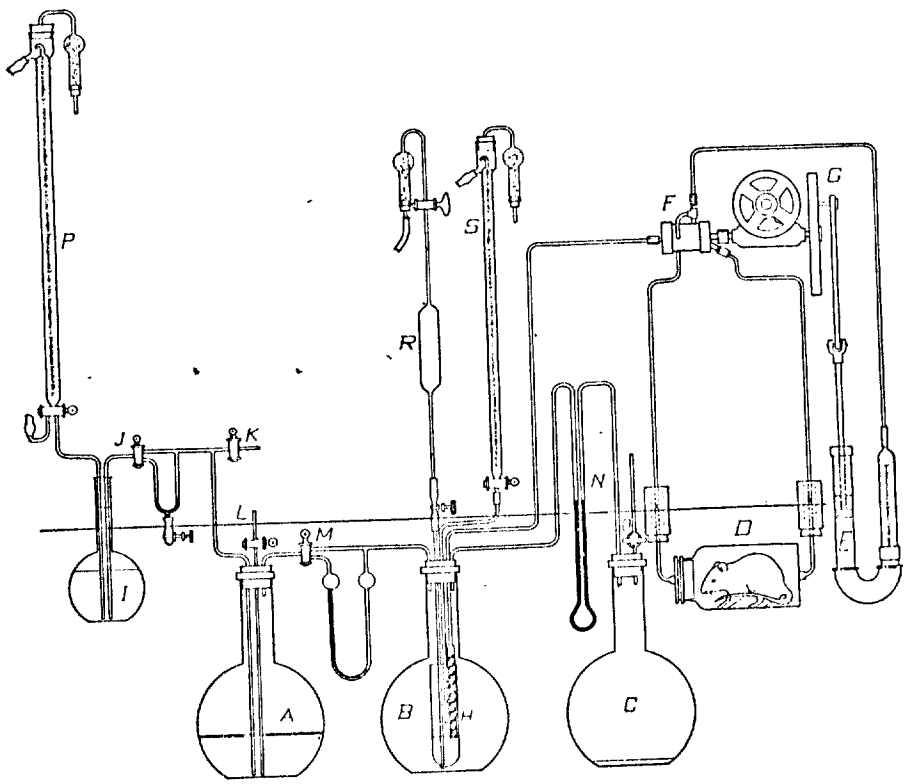

FIGURE 1.

metabolism room. The air in this apparatus is circulated by means of a single-acting, piston pump (E, Figure 1) run by a motor at a speed of about 12 cycles per minute. Liquid petrolatum which fills the cylinder both above and below the piston as far as the U bend of the pump extends, insures that any leak past the piston can be only liquid petrolatum, the meniscus of which may be measured at any time by means of graduations on the glass part of the pump (E). Each down stroke of the pump propels about 80 cc. of air through the 1-liter chamber (D) that contains the ani-

mal, and into a 6-liter flask (B, air storage flask), while each up stroke of the pump sucks about 80 cc. of air from this flask directly into the barrel of the pump without its passing through the animal chamber. The direction that the air takes is determined by the rotary valve (F) attached to the shaft of the crank wheel (G) of the pump.

The CO_2 breathed out by the animal is removed from the air which comes from the animal chamber or jar by means of a spiral scrubber (H) moistened with standardized NaOH solution (approximately half normal). This solution is contained in a test tube, 48 mm. in diameter, supported near the bottom of the flask. The spiral scrubber (H) through which air both enters and leaves the flask is kept moistened with NaOH solution in the following way. When air is drawn from the flask by each up stroke of the pump, a few cubic centimeters of liquid are at first sucked up with it to the top of the scrubber (H) because the end of the scrubber dips a few millimeters below the surface of the solution. When on the down stroke of the pump air is forced into the flask, it passes through the same tube and scrubber by which it left the flask, and thus over a large, freshly moistened surface. In this manner satisfactory absorption of CO_2 is obtained. The amount of CO_2 absorbed in a given period of time is determined at the end of that period by withdrawing exactly 50 cc. out of approximately 55 cc. of the alkali and titrating with standardized HCl in the presence of an excess of $BaCl_2$ solution and phenolphthalein.

A simple form of pump (48) has been successfully adapted to this apparatus and used by several members of the staff of this laboratory. Its pump action depends on the alternate raising and lowering of a mercury reservoir, the direction of the air current being determined by mercury valves. The air, after passing the animal chamber (D), is bubbled into the NaOH solution and passes thence from the top of the air storage flask (B), back to the mercury valve and pump. Since mercury vapor may be harmful in some metabolism work, this form of pump is not recommended in connection with a respiratory calorimeter for general use.

As oxygen is used by the animal, the gas pressure in the system diminishes, as is indicated by a water manometer (N) on the air storage flask. A compensatory flask (C) is connected to the other arm of this manometer. This flask is provided with a stopcock (O) which opens to the air of the room and which is closed at the beginning of the run. The compensatory flask is thus entirely shut off throughout the duration of a run. The temperature and barometric pressure at this time are recorded and are used in the calculations. Moreover, as the compensatory flask contains a small amount of the NaOH solution, such as is used in the air storage flask, the

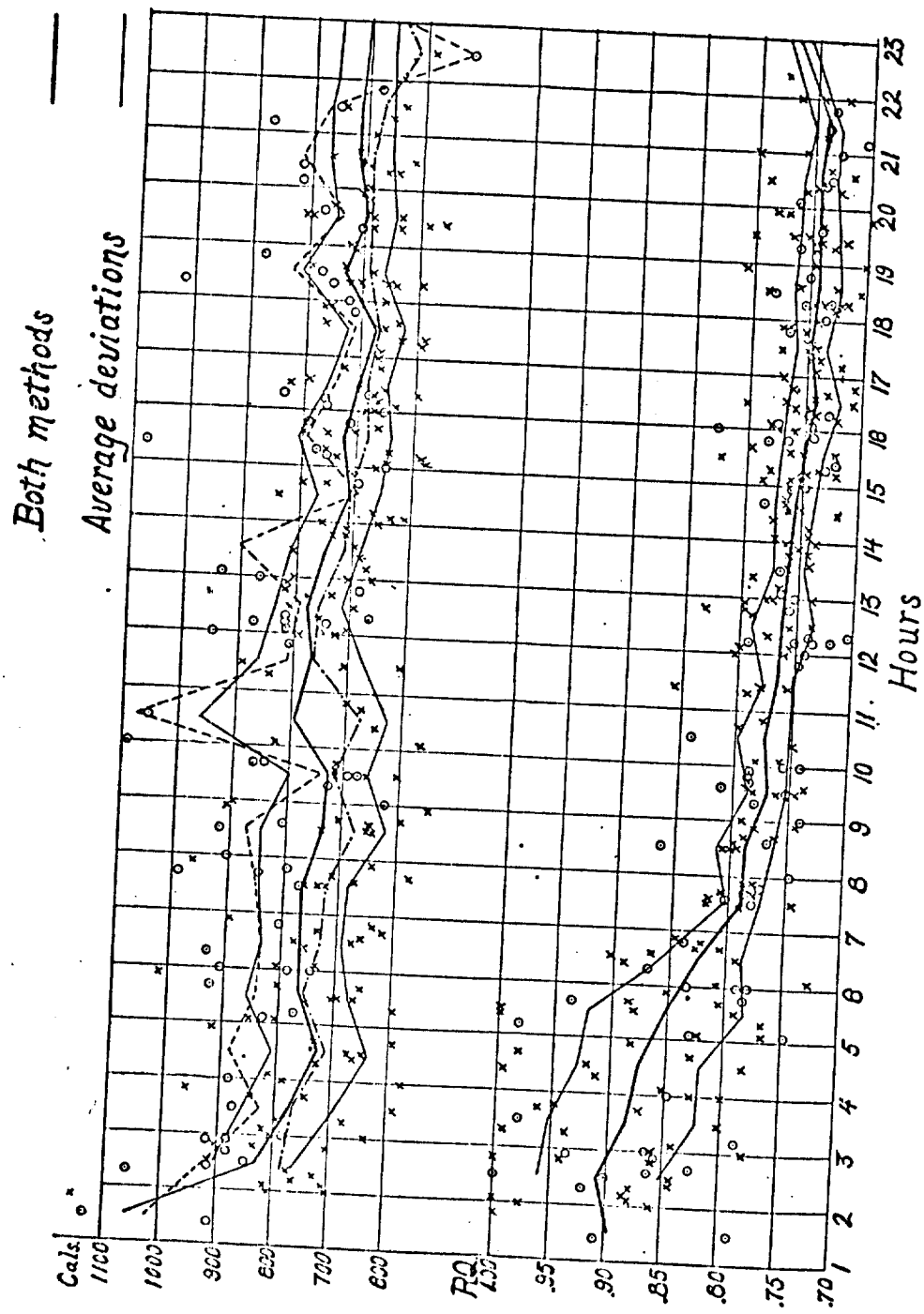
Both methods
Average deviations
Cals.
1100
1000
900
800
700
600
RQ
1.00
.95
.90
.85
.80
.75
.70
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Hours
Averages by hours.
Bell jar apparatus
Closed circuit apparatus
Cals.
Bell jar apparatus
Closed circuit apparatus

FIGURE 2.

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289

Lipid Metabolism of Puppies as Affected by Kind and Amount of Fat and of Dietary Carbohydrate [1]

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ABSTRACT Feeding 37 young growing puppies diets similar in composition to those fed infants demonstrated that sucrose, Dextri-Maltose, and corn syrup are equally suitable as types of dietary carbohydrate. No significant differences were observed in rate of growth or response of blood sugar levels or serum levels of protein and total fatty acids between groups of animals fed these sugars as the major source of carbohydrate. Cholesterol levels were lowest when sucrose was fed in diets containing corn or hydrogenated coconut oil. Lactose was not acceptable as the sole source of carbohydrate for the young puppy. This may be attributed to a deficiency of the enzyme lactase in the intestinal mucosa of the puppy. Rate of growth and serum levels of unsaturated fatty acids in these animals differed from those fed sucrose, Dextri-Maltose, or corn syrup when the diet was low in linoleic acid. Differences in serum unsaturated fatty acids between puppies fed lactose and those fed sucrose, Dextri-Maltose, or corn syrup were very slight when corn oil diets were fed. It is concluded that during the period of rapid growth dietary fat affects the level and composition of serum lipids to a greater extent than does dietary carbohydrate.

Intense interest in the possible role of diet in development of aortic atheromatous lesions in young subjects has prompted considerable study of effects of various dietary components on the lipid fractions in blood and tissues. Studies with experimental animals (1–5) and with adult human subjects [2] have suggested the possibility of a relationship between type of dietary carbohydrate and lipid metabolism. Carbohydrate studies with young human subjects have been concerned primarily with the absorption of different types of carbohydrate in relation to blood sugar levels and urinary excretion after the administration of test doses of carbohydrate (6, 7). Scant attention has been given to the effect of type of carbohydrate on the metabolism of other nutrients in the infant's diet. Since linoleic acid has been demonstrated to be an essential component of fat for young growing infants (8, 9) as well as for experimental animals, the present study with puppies was undertaken to determine the physiologic response of 4 types of carbohydrate on fat metabolism during growth (a) when the diet is deficient in fat, (b) when the dietary fat is deficient in linoleic acid, and (c) when the dietary fat is high in linoleic acid content.

The young puppy was chosen as the experimental animal because it has been found to react to diets deficient in linoleic acid in a manner similar to young infants fed diets deficient in this nutrient. Changes in composition of blood serum lipids as well as gross and histologic alterations in skin occur in babies and puppies under these dietary conditions (9–12).

MATERIALS AND METHODS

Experimental diets. Twelve experimental diets were prepared to simulate infant formulas. There were 4 low fat basal diets which were considered fully adequate in calories, protein, vitamins, and minerals. Each of these 4 diets contained a different type of carbohydrate commonly used in infant feeding, i.e., β-lactose,[3] sucrose,

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[1] This work was supported in part by a U.S. Department of Agriculture Contract sponsored by the Human Research Division, Agriculture Research Service and was inaugurated under the direction of Dr. Arild E. Hansen (deceased).

[2] Wells, W. W., and S. C. Andersen 1962 The effect of dietary lactose on the serum cholesterol level of human subjects. Federation Proc., *21:* 100 (abstract).

[3] Beta-Lactose, The Borden Company, New York.

Dextri-Maltose[4] and corn syrup.[5] Four diets similar to those low in fat were prepared by isocaloric substitution of 30% of the carbohydrate calories by a hydrogenated coconut oil product[6] which was low in linoleic acid. Four additional diets, similar to those low in fat, were prepared by isocaloric substitutions of 30% of the carbohydrate calories with corn oil.[7] The caloric distribution of the diets low in fat and those moderate in fat content are shown in table 1.

All diets were fed at normal caloric levels, 150 kcal/kg/day from weaning to 4 months of age; 125 kcal/kg/day from 4 to 6 months of age and 90 to 100 kcal/kg/day thereafter.

The fat was weighed or measured daily and mixed with a weighed amount of dry basal diet and the vitamin mixture just before the addition of sufficient water to make a palatable mixture. The animals were fed once daily and notations made when all the food was not consumed.

Experimental animals. To eliminate possible variations in response to diet due to differences in breed, only Beagles were used in the study. All animals were started on the study at the time of weaning (6 to 9 weeks of age) and were housed in separate metal cages in an air conditioned room maintained at 23.5°. Care was taken to see that they were free from intestinal parasites and immunized for distemper and canine hepatitis. The animals were weighed weekly before feeding.

Each of the 12 experimental diets was fed to 5 or more puppies. Most of the animals were maintained with more than one diet. The length of period that each diet was fed varied depending on the acceptability of the diet and the physical condition of the animal. The shortest period was one week for each of 2 dogs that were fed the low fat-lactose diet. Each animal was observed with respect to acceptance of the diet, rate of growth, gross and histologic condition of the skin. Metabolic response to the diets was determined by analysis of whole blood for reducing sugars and true glucose, blood serum for total protein and lipid components and urine for reducing sugars.

Chemical analyses. At the end of each dietary regimen, fasted blood specimens, 24-hour urine specimens and skin biopsies were obtained from each animal. Heparinized arterial blood was used for determination of total reducing sugars by the Somogyi method (13) and true glucose with glucose-oxidase (14). The total protein content of blood serum was determined with the Biuret reagent according to a modification of the Kingsley method (15). Blood serum lipid analyses included total cholesterol (16), total fatty acids (17) and silicic acid separation of the glyceride, cholesterol ester and phospholipid fractions (18). Fatty acids from each of the above fractions were methylated at 70° with 2% sulfuric acid in methanol. The methyl esters were chromatographed in a Beckman GC-2A instrument using 183-cm diethylene glycol succinate columns at 220° and helium as the carrier gas. Peaks were identified by comparison with retention times of known methyl esters.[8] The 5, 8, 11-eicosatrienoic acid was identified by its conformity to the theoretical retention time of an eicosatrienoic acid which appeared in serum from linoleic acid deficient animals and subsequently disappeared from serum of the animals after feeding linoleic acid. This retention time also conformed to that of 5, 8, 11-eicosatrienoic acid reported by Ackman (19). Peak areas were quantitated by measurement of peak heights and relative retention times. Correction factors were applied to compensate for differences in detector response to individual fatty acid components. Detector response was linear within a given instrument sensitivity setting and injected sample sizes were chosen to allow use of a single sensitivity setting. Urine specimens were examined for total reducing sugars by the Somogyi method. Determination of true glucose in urine was not possible because toluol or thymol were used as preservatives during collection of the urine. Identification of individual sugars in urine was made by an adaptation[9] of the paper chromatographic method of White and Hess (20). Histologic sections of skin biopsies taken from the dorsal surface of the thigh were stained with hematoxylin and eosin.

Statistical methods. Equality of variance for serum cholesterol and fatty acids (mg/100 ml) within each group of animals fed the low fat, hydrogenated coconut oil or corn oil diets was determined by Bartlett's test (21). Equality of means for serum cholesterol and fatty acids (mg/100 ml) within each of the above groups was determined by Student's t test (21).

[4] Mead Johnson and Company, Evansville, Indiana.
[5] Karo (light), Corn Products Company, New York.
[6] Cobee 92, The Drew Company, Bolton, New Jersey.
[7] Mazola, Corn Products Company, Argo, Illinois.

TABLE 1

Caloric distribution for experimental diets

Diets low in fat	% calories	Diets with fat	% calories
Protein		Protein	
Casein	10	Casein	10
Skim milk powder	10	Skim milk powder	10
Carbohydrate		Carbohydrate	
Skim milk powder	14	Skim milk powder	14
Experimental	65	Experimental	36
Fat		Fat	
Skim milk powder	1	Skim milk powder	1
		Experimental	29
Vitamins [1]		Vitamins [1]	
Salt-bone ash		Salt-bone-ash	
Cellulose [2]		Cellulose [2]	

[1] Vi-Penta no. 3 (Roche Laboratories, Nutley, New Jersey)/supplied daily: vitamin A, 2,000 IU; vitamin D, 160 IU, 0.4 mg each of thiamine, riboflavin, pyridoxine, niacin and Ca pantothenate; vitamin C, 20 mg; biotin, 12 μg. Vitamin E, 25 mg weekly as d-α-tocopheryl acetate.
[2] Cellu Flour, Chicago Dietetic Supply House, Chicago.

RESULTS

Data are presented for 37 dogs from the time of weaning to approximately 9 months of age.

Acceptability of the diets and rate of growth. It became evident early in the study that the 4 carbohydrates used in the diets were not equally acceptable to weanling puppies. Lactose as the sole source of carbohydrate was not tolerated by the weanling puppy. This may be attributed to a deficiency of the enzyme lactase in the intestinal mucosa. Diarrhea and usually anorexia occurred in all young animals fed the lactose diets. Death occurred during the first 12 weeks of life in 6 of the 9 weanling puppies fed lactose diets deficient in linoleic acid, compared with one death each among those fed the sucrose, Dextri-Maltose, or corn syrup diets deficient in linoleic acid. Growth was poor for young puppies fed lactose diets but for dogs over 8 months of age, weight loss was not a consistent feature. However, diarrhea was a common symptom at all ages when lactose was the dietary carbohydrate irrespective of the amount or type of fat in the diet.

Comparison of groups of 5 or more animals fed the same diet demonstrated that sucrose, Dextri-Maltose and corn syrup were equally acceptable as a source of carbohydrate in the diet of young growing puppies. With diets having the same amount and kind of fat, there were no consistent trends in rate of growth which could be attributed to sucrose, Dextri-Maltose or corn syrup as the source of carbohydrate.

Under our experimental conditions, improvement in growth rate was noticeable for only 2 dogs when corn oil was substituted for 30% of the carbohydrate calories in the low fat diet or 30% of the calories as hydrogenated coconut oil.

Gross and histologic changes in skin. Gross evidence of linoleic acid deficiency occurred only after prolonged feeding of

[8] Applied Science Laboratories, Inc., State College, Pennsylvania.
[9] Modifications introduced were (a) removal of substances insoluble in 66% methanol; (b) desalting with an electric desalter (Res. Specialty Model 1930, Berkeley, California) before concentration of urine; (c) use of the mixture 2:2:1 butanol-pyridine-water as solvent in a one dimensional descending chromatograph.

either a diet low in fat or one containing hydrogenated coconut oil. An unkempt appearance with desquamation and slight infection of the ears were characteristic of 3 growing puppies fed the low fat diet for 18 weeks. Desquamation was generalized but less marked for 3 dogs fed hydrogenated coconut oil for a similar length of time. There were no signs of involvement of the paws. Effects were similar whether the carbohydrate was sucrose, Dextri-Maltose, or corn syrup. Because of the generally debilitating effects of lactose, it was not possible to feed young puppies the lactose diets deficient in linoleic acid for long enough periods to note development of deficiency signs. Furthermore, as demonstrated by Wiese et al. (22), the poor growth exhibited by these animals would tend to prevent the development of gross and histologic signs of linoleic acid deficiency.

In general, histologic changes paralleled the gross appearance of the skin. The degree of histologic alterations in the epidermis and dermis were related to the linoleic acid intake, the length of time the diet was fed and to the composition of the previous diet when more than one diet was fed to an animal. Increased density of the keratin layer, which is one of the early signs of linoleic acid deficiency, was evident when puppies were fed either a diet low in fat or one containing hydrogenated coconut oil from the time of weaning. Severe histologic changes in the stratum corneum with peg formation and extensive parakeratosis were not observed in skin sections from animals that had been fed diets deficient in linoleic acid. Histologically, skin from animals fed diets containing hydrogenated coconut oil appeared to differ from those fed the diets low in fat in the rate of development of the sebaceous glands. In the former animals these glands often were more numerous and moderately large even when there was no evidence of increased density of the keratin or thickening of the epidermis.

Skin from animals fed diets containing corn oil was essentially normal except in instances of incomplete recovery from a previous linoleic-deficient diet. Definite histologic changes which occur during recovery from the deficient state were evident for 5 animals.

Among the puppies that were growing normally, there were no histologic differences which could be attributed to the type of carbohydrate fed.

Total protein in blood serum. Total protein in blood serum was determined as an indication of normal protein metabolism in the animals. Table 2 shows the mean values for groups of 5 or more animals fed the same diet. The mean protein levels for puppies fed the fat-deficient lactose diet was lower than for any other group. All other levels for total protein in blood serum can be considered satisfactory. There was an insignificant trend for mean serum protein levels to be higher in animals fed sucrose than for those fed Dextri-Maltose, or corn syrup. Serum protein levels of the animals that received these 3 carbohydrates with corn oil were slightly higher than of the animals fed the low fat or hydrogenated coconut oil diets. Because the dogs fed the lactose diets had diarrhea and some anorexia, they were always maintained with these diets for a shorter time than with the sucrose, Dextri-Maltose, or corn syrup diets.

TABLE 2

Mean serum protein and reducing sugars in blood and urine in relation to dietary fat and dietary carbohydrate

No. dogs	Dietary CHO	Dietary Fat	Feeding period	Age	Protein	Blood Reducing sugar	Blood Glucose	Urinary reducing sugar
			weeks	*weeks*	*g/100 ml*	*mg/100 ml*		*g/24 hr*
6	Lactose	no	3.3	25.2	4.49	88	81	2.6
7	Sucrose	no	7.3	30.5	5.43	92	86	0.9
8	Dextri-Maltose [1]	no	8.4	29.7	5.25	90	84	0.4
6	Corn syrup	no	11.2	31.1	5.25	82	70	0.3
6	Lactose	HCO [2]	3.3	29.6	5.28	85	82	7.4
8	Sucrose	HCO	7.3	31.5	5.16	82	82	0.3
6	Dextri-Maltose	HCO	7.7	31.6	5.12	82	74	0.3
5	Corn syrup	HCO	10.4	26.5	4.95	93	91	0.4
6	Lactose	corn oil	6.9	23.0	5.16	96	85	2.6
12	Sucrose	corn oil	17.1	36.0	5.80	91	87	0.4
11	Dextri-Maltose	corn oil	14.5	38.9	5.50	88	83	0.2
7	Corn syrup	corn oil	15.3	31.7	5.51	90	83	0.2

[1] Mead Johnson and Company, Evansville, Indiana.
[2] Hydrogenated coconut oil product, Cobee 92, The Drew Company, Bolton, New Jersey.

Reducing sugars in whole blood. Total reducing sugars and true glucose were determined on arterial blood after an overnight fast. Table 2 shows the mean values for groups of 5 or more animals fed the 12 experimental diets. Mean values for blood sugar were considered normal for all animals. Trends with type of carbohydrate or dietary fat were not evident. Differences between total reducing sugars and true glucose often are considered to be due to galactose. However, Haworth and Ford (6) did not identify galactose by paper chromatography in the blood of infants fed lactose diets. They concluded that galactose was not present in amounts greater than 10 to 20 mg/100 ml of blood. With 2 exceptions differences between total reducing sugars and glucose were less than 10 mg/100 ml of blood for all groups of puppies fed the 4 types of carbohydrate. No attempt was made to identify sugars other than glucose in the blood.

Young dogs fed the lactose diets showed normal fasting blood sugar levels even though absorption of lactose appeared to be poor as evidenced by diarrhea. With adult dogs, Bennett and Coon[10] have demonstrated normal fasting blood sugar levels but lower glucose absorption curves following the ingestion of lactose than when sucrose, Dextri-Maltose, or corn syrup was the source of dietary carbohydrate. Similar absorption data have been reported for infants with an intolerance to lactose (23).

Sugars in urine. Table 2 shows a summary of mean values for total reducing sugars in urine as determined by the Somogyi method. Interpretation of the high values observed for animals that received diets containing lactose is not possible because of diarrheal contamination. Mean values for reducing sugars in urine of dogs receiving sucrose, Dextri-Maltose, and corn syrup were low, although sugars were present in all urines.

Individual sugars in urine were identified by means of paper chromatography. Because of persistent diarrhea in all animals receiving a lactose diet, no estimate could be made of the excretion of sugars by these animals. The sensitivity of the chromatographic method used for detection of urinary sugars for the dogs fed diets containing sucrose, Dextri-Maltose, or corn syrup was of the order of 2.5 mg/100 ml original urine for lactose, galactose and glucose and 10 mg/100 ml original urine for maltose, sucrose and fructose. When the major dietary carbohydrate was sucrose, 4 sugars were identified in most of the urine samples, i.e., lactose, sucrose, galactose and glucose. Fructose appeared in approximately 35% of these samples. When Dextri-Maltose was fed, lactose appeared in nearly all urines and galactose in 45% of the specimens. When corn syrup was fed, lactose and sucrose were identified in most of the urine samples, galactose in 50% and fructose in approximately 25% of the samples. The identification of lactose and galactose in most urine specimens regardless of the type of carbohydrate fed, may be attributed to the ingestion of 14% of the calories as lactose derived from the skim milk in all diets.

Maltose was not identified in any urine.

The chromatographic identification of lactose, sucrose and glucose in urine of dogs fed our experimental diets is in agreement with the observations of Haworth (7) with infants and children who were fed test doses of lactose, sucrose and maltose. The latter disaccharide was not noted in blood or urine from children fed this sugar.

Blood serum lipids. Figure 1 illustrates the mean serum levels for total cholesterol, total fatty acids and fatty acids in the glyceride, cholesteryl ester, and phospholipid fractions for groups of 5 or more dogs fed the same diet.

[10] Unpublished data.

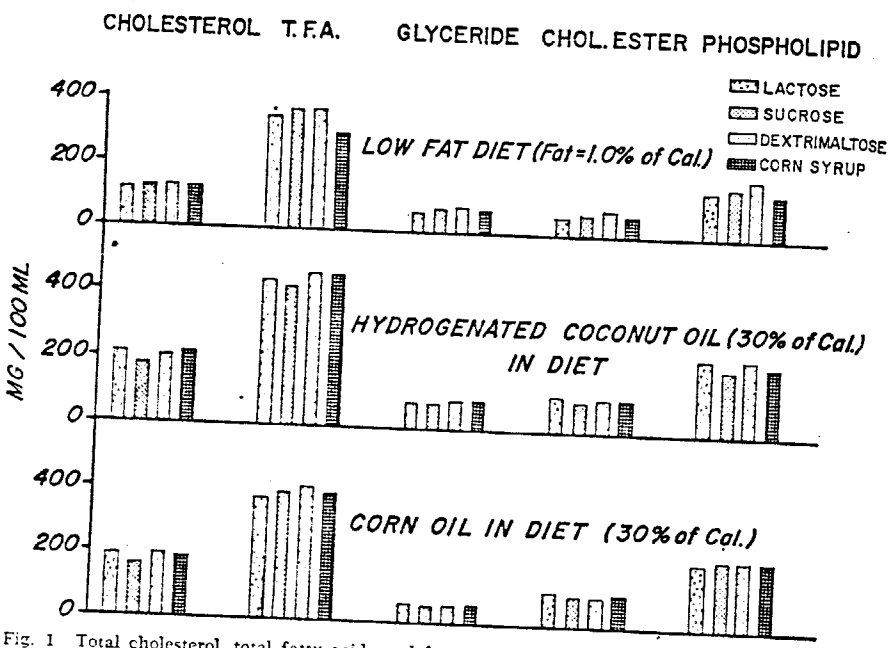

Fig. 1 Total cholesterol, total fatty acids and fatty acids in the glyceride, cholesteryl ester and phospholipid fractions of serum.

Differences in serum levels for these lipid components in relation to dietary fat were as follows:

1. Total cholesterol and total fatty acids were lowest for all dietary sugars when the low fat diets were consumed.
2. Glycerides were lowest when corn oil was fed.
3. Cholesteryl esters were lowest when low fat diets were consumed.
4. Phospholipids constituted the major lipid fraction in dog serum and were lowest when low fat diets were fed.

Except for diets containing sucrose, there were no consistent trends in serum levels for lipid components which were related to the type of dietary carbohydrate. For diets containing corn or hydrogenated coconut oil, serum cholesterol levels were lowest for animals fed the sucrose diets.

Statistical comparisons were made for mean serum levels of cholesterol, total fatty acids and fatty acids from the glyceride, cholesteryl ester and phospholipid fractions among the groups fed diets low in fat, those fed hydrogenated coconut oil and those fed corn oil irrespective of the type of carbohydrate. Using Student's *t* test as a measure of significance, the following differences between groups were noted:

Total cholesterol:	
low fat < hydrogenated coconut oil	$P < 0.01$
low fat < corn oil	$P < 0.01$
corn oil < hydrogenated coconut oil	$P > 0.05$
Total fatty acids:	
low fat < hydrogenated coconut oil	$P < 0.01$
low fat < corn oil	$P < 0.01$
corn oil < hydrogenated coconut oil	$P > 0.05$
Glyceride fatty acids:	
low fat < hydrogenated coconut oil	$P < 0.01$
corn oil < low fat	$P < 0.01$
corn oil < hydrogenated coconut oil	$P < 0.01$
Cholesteryl ester fatty acids:	
low fat < hydrogenated coconut oil	$P < 0.01$
low fat < corn oil	$P < 0.01$
hydrogenated coconut oil < corn oil	$P > 0.05$
Phospholipid fatty acids:	
low fat < hydrogenated coconut oil	$P < 0.01$
low fat < corn oil	$P < 0.01$
corn oil < hydrogenated coconut oil	$P > 0.05$

Mean levels for saturated and unsaturated fatty acids in serum for each of the

12 experimental diets are illustrated in figures 2, 3 and 4.

When the diet was low in fat (fig. 2) there were essentially no differences in mean serum levels for saturated fatty acids among the 4 types of carbohydrate in the total; glyceride; cholesteryl ester; or phospholipid fractions. However, phospholipids had the highest and cholesteryl esters the lowest percentage of saturated fatty acids. Total fatty acids were made up of approximately equal amounts of palmitic and stearic acids; glycerides and cholesteryl esters had predominantly palmitic acid and phospholipids had stearic acid as the major saturated fatty acid.

In all fractions of serum (total, glycerides, cholesteryl esters and phospholipids) similar trends with the 4 carbohydrates were noted in the unsaturated fatty acid serum levels. Monoene and the trienoic acid characteristic of fat deficiency tended to be lowest in the animals fed lactose and highest in the animals fed Dextri-Maltose and corn syrup. Linoleic and arachidonic acid levels were higher in the puppies fed the lactose diet than those fed the sucrose, Dextri-Maltose or corn syrup diets. In view of the effects of caloric intake on growth and unsaturated fatty acid levels in young dogs (22) it appears reasonable to assume the differences in serum unsaturated fatty acids between the dogs fed lactose and the other 3 carbohydrates were related to differences in degree of absorption and utilization of the carbohydrate calories which in turn were reflected in the growth rate of the young puppies.

Figure 3 illustrates mean serum levels for the saturated and unsaturated fatty acids when the diet contained hydrogenated coconut oil. Trends with type of carbohydrate were similar to those observed for diets low in fat.

Figure 4 illustrates mean levels for saturated and unsaturated fatty acids when the diet contained corn oil. Linoleic and arachidonic acids showed higher serum levels than when the diet was deficient in linoleic acid. No 5, 8, 11-eicosatrienoic

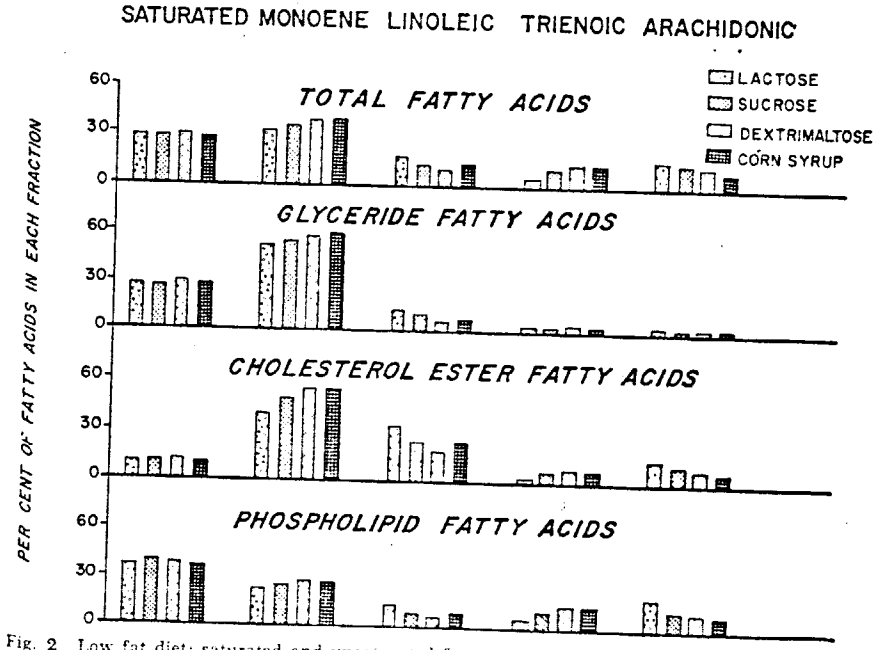

Fig. 2 Low fat diet; saturated and unsaturated fatty acids in the total fatty acids, glyceride, cholesteryl ester and phospholipid fatty acids in serum.

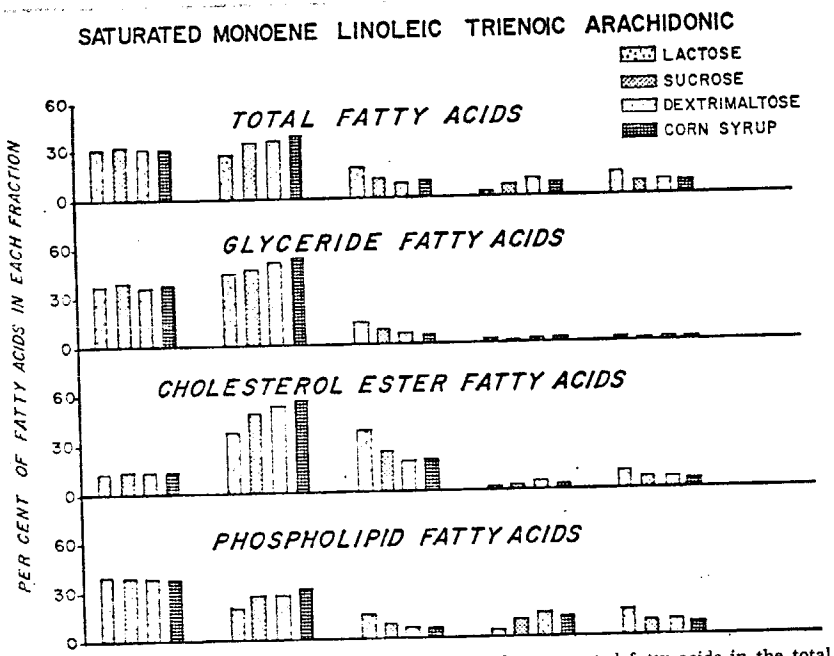

Fig. 3 Hydrogenated coconut oil diet; saturated and unsaturated fatty acids in the total fatty acids glyceride, cholesteryl ester and phospholipid fatty acids in serum.

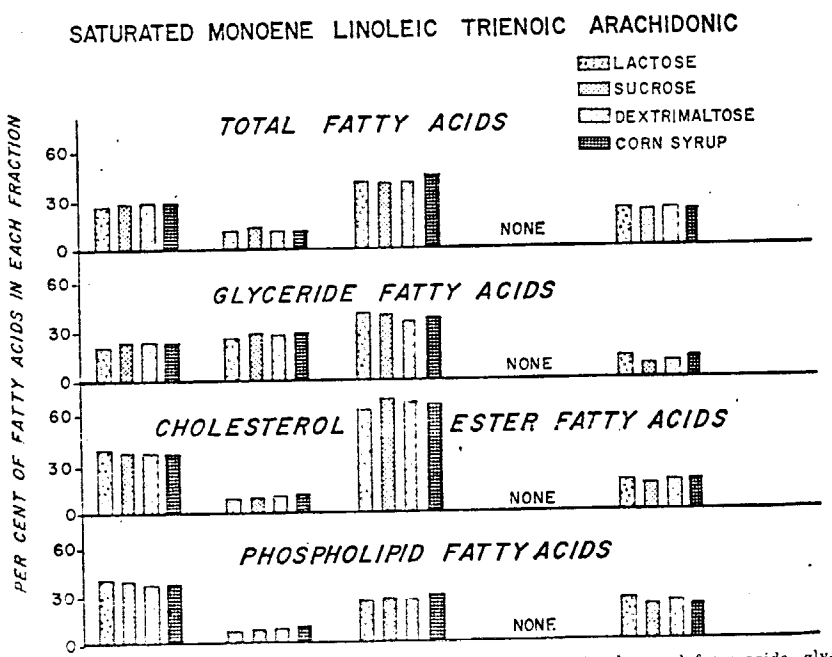

Fig. 4 Corn oil diet; saturated and unsaturated fatty acids in the total fatty acids, gly-

acid was observed in serum when corn oil was fed. Trends with the type of carbohydrate were much less evident than when the diets were low in linoleic acid content.

DISCUSSION

Effects of dietary carbohydrate on the serum lipids of young puppies during the period of rapid growth were less dramatic than the effects of dietary fat. With the exception of total cholesterol, there were no consistent trends in the level of lipid components of blood serum which were characteristic for the kind of carbohydrate fed. Cholesterol levels were lower when sucrose was fed with corn or hydrogenated coconut oil than when the dietary carbohydrate was lactose, Dextri-Maltose or corn syrup. Similar effects have been reported by Wells and Andersen[11] for young human adults whose serum cholesterol levels were lower after sucrose than after lactose feeding. Macdonald and Braithwaite (24) observed lower serum levels for glycerides for adult subjects after a raw starch diet than after a sucrose diet. However, when receiving the same amount and kind of fat there were no significant differences in serum glycerides among the 4 types of carbohydrate fed to our young dogs.

When the diet was deficient in linoleic acid, retardation in growth and persistent diarrhea were prominent features for young puppies fed the lactose diets. Failure to thrive was reflected in the serum levels for linoleic, arachidonic and trienoic acids. These metabolic effects in young puppies fed lactose as the sole source of carbohydrate are attributed to poor absorption of lactose as a source of calories for growth. It is well known that the human infant is normally provided with the necessary enzyme system for absorption and utilization of the high lactose content of human milk. Hence metabolic effects of dietary lactose observed for the young puppy would not be applicable to the young infant unless he has hereditary lactase deficiency only recently appreciated clinically (23, 25–27).

It has been demonstrated by Carroll (28, 29) that responses to dietary carbohydrate by enzyme systems in livers of rats are modified by the type of dietary fat. Effects of dietary glucose, sucrose and rice starch on the cholesterol and fat content in the livers appeared to be a function of the type of fat fed. In our young puppies metabolic response to sucrose, Dextri-Maltose, or corn syrup did not show consistent trends when the intake of fat was the same and only differed when lactose was fed. However, irrespective of the type of carbohydrate, lipid components of blood serum differed significantly among the groups fed the low fat, the hydrogenated coconut oil and the corn oil diets. It is concluded from these data that in the young growing puppy, dietary fat affects the level and composition of serum lipids to a greater extent than does the dietary carbohydrate.

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LITERATURE CITED

1. Portman, O. W., E. Y. Lawry and D. Bruno 1956 Effect of dietary carbohydrate on experimentally induced hypercholesteremia and hyperbetalipoproteinemia in rats. Proc. Soc. Exp. Biol. Med., *91*: 321.
2. Grant, W. C., and N. J. Fahrenbach 1959 Effect of dietary sucrose and glucose on plasma cholesterol in chicks and rabbits. Proc. Soc. Exp. Biol. Med., *100*: 250.
3. Tomarelli, R. M., R. Hartz and F. W. Bernhart 1960 The effect of lactose feeding on the body fat of the rat. J. Nutrition, *71*: 221.
4. Macdonald, I. 1962 Some influences of dietary carbohydrate on livers and depot lipids. J. Physiol., *162*: 334.
5. Wells, W. W., and C. R. Cook 1962 Lactose diets and cholesterol metabolism. III. Inhibition of cholesterol biosynthesis from acetate 1-C^{14} and mevalonate-2-C^{14} by lactose or succinylsulfathiazole-feeding in the rat. J. Nutrition, *76*: 48.
6. Haworth, J. C., and J. D. Ford 1960 Blood sugar in infants after lactose feeding. Lancet, *2*: 794.
7. Haworth, J. C. 1960 Sugars in the blood and urine of children following the ingestion of disaccharides. Arch. Dis. Child., *35*: 552.
8. Hansen, A. E., M. E. Haggard, A. N. Boelsche, D. J. D. Adam and H. F. Wiese 1958 Essential fatty acids in infant nutrition. III. Clinical manifestations of linoleic acid deficiency. J. Nutrition, *66*: 564.

[11] See footnote 2.

9. Hansen, A. E., H. F. Wiese, A. N. Boelsche, M. E. Haggard, D. J. D. Adam and H. Davis 1963 Role of linoleic acid in infant nutrition. Pediatrics, *31:* suppl. 1, part 2.
10. Wiese, H. F., A. E. Hansen and D. J. D. Adam 1958 Essential fatty acids in infant nutrition. I. Linoleic acid requirement in terms of serum di-, tri- and tetraenoic acid levels. J. Nutrition, *66:* 345.
11. Hansen, A. E., J. G. Sinclair and H. F. Wiese 1954 Sequence of histologic changes in skin of dogs in relation to dietary fat. J. Nutrition, *52:* 541.
12. Wiese, H. F., A. E. Hansen and M. A. Baughan 1957 Effect of fat in diet on unsaturated fatty acids in phospholipid, cholesterol ester and glyceride fractions in serum of dogs. J. Nutrition, *63:* 523.
13. Somogyi, M. 1945 A new reagent for the determination of sugars. J. Biol. Chem., *160:* 61.
14. Saifer, A., and S. Gerstenfeld 1958 The photometric microdetermination of blood glucose with glucose oxidase. J. Lab. Clin. Med., *51:* 448.
15. Kingsley, G. R. 1942 Direct biuret method for determination of serum proteins as applied to photoelectric and visual colorimetry. J. Lab. Clin. Med., *27:* 840.
16. Sperry, W. M., and F. C. Brand 1943 The colorimetric determination of cholesterol. J. Biol. Chem., *150:* 315.
17. Wiese, H. F., and A. E. Hansen 1953 Semimicromethod for unsaturated fatty acids of blood serum. J. Biol. Chem., *202:* 417.
18. Lis, E. W., J. Tinoco and R. Okey 1961 A micromethod for fractionation of lipids by silicic acid chromatography. Ann. Biochem., *2:* 100.
19. Ackman, R. G. 1963 Influence of column temperature in gas-liquid chromatographic separation of methyl esters of fatty acids on polyester substrates. J. Gas Chromat., *1:* 11.
20. White, A. A., and W. C. Hess 1956 Paper chromatographic detection of sugars in normal and dystrophic human urines. Arch. Biochem. Biophysics, *64:* 57.
21. Dixon, W. J., and F. J. Massey, Jr. 1957 Introduction to Statistical Analysis. McGraw-Hill Book Company, New York, p. 121, 179.
22. Wiese, H. F., A. E. Hansen and E. Coon 1962 Influence of high and low caloric levels on fat deficiency of dogs. J. Nutrition, *76:* 73.
23. Sunshine, P., and N. Kretchner 1964 Studies of small intestine during development. III. Infantile diarrhea associated with intolerance to dissacharides. Pediatrics, *34:* 38.
24. Macdonald, I., and D. A. Braithwaite 1964 The influence of dietary carbohydrates on the lipid pattern in serum and in adipose tissue. Clin. Sci., *27:* 23.
25. Durand, P. 1958 Lattosuria idiopathica paziente con diarrea cronica ed acidosi. Minerva Pediat., *10:* 706.
26. Holzel, A., V. Schwarz and K. W. Sutcliffe 1959 Defective lactose absorption causing malnutrition in infancy. Lancet, *1:* 1126.
27. Weijers, H. A., and J. H. van de Kamer 1962 Diarrhoea caused by deficiency of sugar splitting enzymes. II. Acta Paediat., *51:* 371.
28. Carroll, C. 1963 Influences of dietary carbohydrate-fat combinations on various functions associated with glycolysis and lipogenesis in rats. I. Effects of substituting sucrose for rice starch with unsaturated and with saturated fat. J. Nutrition, *79:* 93.
29. Carroll, C. 1964 Influences of dietary carbohydrate-fat combinations on various functions associated with glycolysis and lipogenesis in rats. II. Glucose vs. sucrose with corn oil and two hydrogenated oils. J. Nutrition, *82:* 163.

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INFLUENCE OF CARBOHYDRATE, NITROGEN SOURCE AND PRIOR STATE OF NUTRITION ON NITROGEN BALANCE AND LIVER COMPOSITION IN THE ADULT RAT

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Previous studies showed that adult rats fed low levels of amino acids (0.1%) were in negative nitrogen balance when the dietary carbohydrate was sucrose, whereas nitrogen equilibrium was attained when the carbohydrate was corn dextrin or when the essential amino acid intake was increased. Diammonium citrate had no significant effect on the nitrogen balances (Womack, Marshall and Parks, '53). A preliminary investigation of the fat content of the livers of the animals indicated that rats receiving sucrose and 0.1% of the amino acids had more fat in the liver than those fed corresponding diets with corn dextrin. The present study was designed to investigate further the effect of a different source of nitrogen, glutamic acid, upon nitrogen balances when fed as a supplement to diets containing low levels of amino acids, and to determine the effects of the two carbohydrates, sucrose and corn dextrin, on the amount of fat in the liver.

EXPERIMENTAL

Determination of nitrogen balances

The compositions of diets and amino acid mixtures have been previously described (Womack et al., '53). The basal nitrogen-free diet consisted of a source of carbohydrate (su-

(6)

crose or dextrin), 80.65%; salt mixture (Jones and Foster, '42), 4%; corn oil, 3%; lard, 12%; vitamin A and D concentrate,[1] 0.05%; inositol, 0.1%; and choline chloride, 0.2%. The following vitamins were added per kilogram of ration: thiamine HCl, 5 mg; pyridoxine HCl, 5 mg; nicotinic acid, 5 mg; riboflavin, 10 mg; Ca pantothenate(*d*), 25 mg; *p*-aminobenzoic acid, 300 mg; α-tocopherol acetate, 25 mg; 2-methyl-1,4-naphthoquinone, 2 mg; biotin, 100 μg; folic acid, 2 mg; and vitamin B_{12}, 30 μg.

The physiologically available forms of the essential amino acids were fed at levels of either 0.1 or 0.2%. Non-essential amino acids were included at levels of 0.1%. Glutamic acid, when fed as a source of additional nitrogen, was added at levels of either 1.65 or 6.45% of the rations. All substitutions were made at the expense of the carbohydrate. All animals were offered 12 gm food each day, except those fed nitrogen-free rations, which were fed ad libitum. In calculating the essential amino acid intake, no allowance was made for the small amounts of amino acids present in the corn dextrin.

The animals used for these investigations were male rats, housed individually when 21 to 23 days old and given the commercial stock ration and water ad libitum until they attained the desired age and weight. This ration contained approximately 25% protein and 5% fat. Animals to be used in the undepleted series were removed from the stock diet when 70 to 76 days of age and given diets 2 to 5, containing either sucrose (series A) or corn dextrin (series B), for three weeks. Animals to be protein-depleted were removed from the stock ration when 90 to 100 days old, fed the nitrogen-free rations containing either sucrose or corn dextrin for 18 days, and then given diets 2 to 5, containing either sucrose (series C) or corn dextrin (series D), for three weeks. This experimental design was varied for both the undepleted and the protein-depleted animals fed diet 2 and sucrose. At the end of the three-week period, one-half of each group of ani-

[1] Squibb's Navitol, containing 65,000 U.S.P. units of vitamin A and 13,000 units of vitamin D per gram.

mals was transferred to diet 2, containing corn dextrin, for three additional weeks, while the other half continued to receive diet 2 containing sucrose for a total of 6 weeks (series A-1 and C-1). All animals were divided into groups according to litter and body weight before being placed on the experimental diets.

The first week during which amino acids were fed was an adjustment period and no collections of urine and feces were made. Nitrogen balances were determined for each animal during the next two 7-day periods. For most of the animals of series C and D, nitrogen balances were also determined during the last 7 days of the 18-day protein-depletion period.

Weekly weighings were made of the animals and of their uneaten food. When food was scattered during a collection period, the data for the animal for that period were discarded.

The methods of collecting the urine and feces and of the nitrogen analyses have been previously described (Womack et al., '53).

Liver analyses

On the 22nd day of amino acid feeding, all of the animals were anesthetized with 1% sodium amytal except those of series A-1 and C-1, which were killed on the 43rd day. The livers were removed, blotted free of excess blood, placed in dishes and weighed; they were dried under infra-red lamps at a distance of 13 inches, ground, and duplicate samples analyzed for total lipids (ether-extractable material), nitrogen and moisture.[2] When an excessive amount of fat was present in the dried sample, grinding was impossible without loss of fat; therefore, the whole sample was extracted. All extractions were made with ethyl ether on the Bailey-Walker apparatus.

RESULTS AND DISCUSSION

Nitrogen balances

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TABLE 1

Average weights and daily nitrogen balance data for undepleted (series A and B) and protein-depleted (series C and D) adult male rats receiving diets containing low levels of amino acids with and without supplementation with glutamic acid and with either sucrose or corn dextrin as the source of carbohydrate

SERIES NO.	DIET NO.	DESCRIPTION OF DIET	NO. ANIMALS/ GROUP	INITIAL WEIGHT	WT. AT END OF ADJUSTMENT PERIOD	FINAL WEIGHT	FOOD INTAKE	CALORIE INTAKE	NITROGEN INTAKE		NITROGEN EXCRETION		NITROGEN BALANCE AND STANDARD ERROR [2]
									Total	EAAN [1]	Feces	Urine	
				gm	*gm*	*gm*	*gm*		*mg*	*mg*	*mg*	*mg*	*mg*
A (undepleted, sucrose)	2	0.1% EAA, 0.1% NEAA [3]	6	299	280	272	12.0	54	37.1	14.8	13.0	48.0	— 23.8 ± 0.9
	3	0.1% EAA, 0.1% NEAA + 1.65% GA [4]	6	298	282	276	12.0	54	55.0	14.7	12.2	59.9	— 17.1 ± 1.0
	4	0.1% EAA, 0.1% NEAA + 6.45% GA	6	299	281	276	12.0	54	108.0	14.8	13.0	116.7	— 21.7 ± 1.7
	5	0.2% EAA, 0.1% NEAA	6	301	285	290	12.0	54	55.2	29.5	11.6	41.6	+ 2.0 ± 1.0
B (undepleted, corn dextrin) [5]	2	0.1% EAA, 0.1% NEAA	6	297	278	276	11.8	54	40.1	14.5	15.6	40.1	— 15.5 ± 1.8
	3	0.1% EAA, 0.1% NEAA + 1.65% GA	6	295	275	276	12.0	55	58.6	14.7	15.3	56.9	— 13.5 ± 1.1
	4	0.1% EAA, 0.1% NEAA + 6.45% GA	6	297	275	271	11.9	54	110.7	14.6	16.2	106.1	— 11.6 ± 1.3
	5	0.2% EAA, 0.1% NEAA	6	293	275	276	12.0	55	58.7	29.4	17.7	40.8	+ 0.1 ± 0.9
C (protein-depleted, sucrose)	1	Nitrogen-free	15	328	...	275	9.4	42	1.9	...	10.2	49.5	— 57.9 ± 1.4
	2	0.1% EAA, 0.1% NEAA	15	275	277	273	11.1	50	34.9	13.6	10.5	37.1	— 12.7 ± 0.7
	3	0.1% EAA, 0.1% NEAA + 1.65% GA	5	269	270	267	10.4	47	49.0	12.8	9.6	47.5	— 8.1 ± 1.3
	4	0.1% EAA, 0.1% NEAA + 6.45% GA	5	267	265	265	11.2	50	102.7	13.7	10.8	98.9	— 7.0 ± 1.0
	5	0.2% EAA, 0.1% NEAA	6	267	266	269	10.8	48	50.8	26.6	9.8	33.6	+ 7.5 ± 0.6
D (protein-depleted, corn dextrin)	1	Nitrogen-free	15	322	...	285	12.9	58	7.7	...	16.6	44.1	— 53.0 ± 1.2
	2	0.1% EAA, 0.1% NEAA	6	277	280	270	11.6	52	40.1	14.2	14.7	29.4	— 4.0 ± 0.9
	3	0.1% EAA, 0.1% NEAA + 1.65% GA	6	277	281	277	11.5	52	57.5	14.1	15.4	46.6	— 4.4 ± 1.4
	4	0.1% EAA, 0.1% NEAA + 6.45% GA	5	280	286	283	11.7	53	110.2	14.4	16.4	97.6	— 3.8 ± 1.9
	5	0.2% EAA, 0.1% NEAA	6	278	288	290	11.7	53	57.4	28.8	15.7	34.9	+ 6.7 ± 0.6

[1] Essential amino acid nitrogen.

[2] $S_x = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n(n-1)}}$.

[3] EAA, essential amino acids; NEAA, non essential amino acids.

the nitrogen balances was determined by the "t" test.[3] The value of "t" required for significance at the 1% level of probability was 2.92.

In series A (undepleted rats fed sucrose-containing diets), increasing the total nitrogen intake by the addition of 1.65% glutamic acid (diet 3) caused a statistically significant improvement in the balances when compared with those of control animals receiving diet 2 ($t = 5.06$), but when 6.45% of glutamic acid was fed (diet 4), the balances were not significantly different from those of animals fed the diet containing only 0.1% of the amino acids. No explanation is readily apparent for the finding that the addition of 17.9 mg of nitrogen from glutamic acid (diet 3) improves the balances, while 70.9 mg nitrogen per day (diet 4) does not. In series B, when corn dextrin was used instead of sucrose in the diets, neither level of glutamic acid improved the balances significantly. When a comparison was made between the balances obtained in series A and B on each diet, the differences were significant for diets 2, 3 and 4, but not for diet 5 (diet 2, $t = 4.17$; diet 3, $t = 2.43$, significant at the 5% level only; and diet 4, $t = 9.48$). The smaller difference obtained with diet 3 (3.6 mg per day) in comparison with the differences obtained on diets 2 and 4 (8.3 and 10.1 mg) suggests that the improvement in the balances brought about by glutamic acid and by corn dextrin are not additive. The animals did not achieve nitrogen equilibrium, as did those of an earlier study when corn dextrin was substituted for sucrose following a 4-week regimen of sucrose-containing diets during which large negative balances were maintained (Womack et al., '53).

In series C, using sucrose-containing diets and protein-depleted animals, both levels of glutamic acid brought about improvement; the nitrogen balances of animals fed diets 3

[3] The t value of the statistically significant difference between the averages of two sets of values was determined by the formula:

$$t = \frac{M_1 - M_2}{\sqrt{(S_{x_1})^2 + (S_{x_2})^2}}.$$

and 4 were significantly less negative than those of animals fed diet 2 ($t = 3.09$ and 4.76). In series D, using corn dextrin-containing diets and protein-depleted rats, supplementation with glutamic acid brought about no significant changes in nitrogen balances (table 1, diets 2, 3 or 4). Comparison of the nitrogen balances of series C and D indicates that there was a significant difference for the two groups of animals fed diet 2 ($t = 8.06$) but no significant difference between the balances of animals fed either level of glutamic acid (diets 3 and 4). However, this evaluation is complicated by small differences in weight, food and essential amino acid intakes of the groups of animals. For the depleted animals, it appears that if an improvement is brought about by the addition of glutamic acid to the sucrose rations, no further significant change is brought about by corn dextrin.

In all series (A to D), doubling the intake of essential amino acids (diet 5), irrespective of carbohydrate source, caused a significant improvement in the balances and the animals were in nitrogen equilibrium or in positive balance. Protein-depleted animals were able to store nitrogen at levels of essential amino acid intake at which undepleted animals were in equilibrium. Differences between the groups receiving the carbohydrates were not significant. Therefore, when the essential amino acid intake was adequate, the substitution of corn dextrin for sucrose was of no benefit. These findings are in accord with results recently reported by Harper and Katayama ('53), that when growing rats received sucrose diets containing 9% casein supplemented with essential amino acids which were limiting in casein, the difference in growth produced by feeding cornstarch instead of sucrose was almost eliminated.

Animals protein-depleted by being fed the nitrogen-free ration (diet 1) with sucrose had nitrogen balances significantly different only at the 5% level ($t = 2.58$) from those of animals depleted by being fed diet 1 with corn dextrin. Nitrogen intake was 5.8 mg per day greater for animals receiving corn dextrin. The total nitrogen excretion of the

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299

Studies on the Utilization of Various Starches and Sugars in the Rat

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ABSTRACT Various types of dietary carbohydrates were fed to growing rats for 28 days with either casein or wheat gluten as the source of dietary protein. Unmodified potato starch and potato amylose resulted in increases in body weight gains and enlarged ceca when casein supplied a dietary protein level of 6%. The same effect was shown when wheat gluten was the dietary protein at either a 9 or 15% protein level. These 2 sources of carbohydrate gave relatively poor weight gains and protein efficiency values when the casein level of the diet was increased to 15% protein. Unmodified corn, wheat, rice, tapioca, and amioca starches, as well as maltose, corn amylopectin, and potato amylopectin gave normal body weight gain values, protein efficiency ratios and ceca weights at both the high and low dietary protein levels. Lactose, unmodified arrowroot starch, fructose, and dextrose gave inferior body weight gains and protein efficiency values. The prevalance of diarrhea and an enlargement of the ceca gave evidence that commercial samples of corn dextrin and pregelatinized amioca starch were not as well utilized as unmodified corn starch or unmodified amioca starch.

Recently there has been an increased interest in the biological utilization of carbohydrates because of the development of new starch products for the food industry. During the past several years, amylose has been produced in large quantities from both corn starch and potato starch. Many new pregelatinized starch products have been developed, also, with special functional properties for food use.

It has been claimed that starches from various sources and modified starches produce varying growth responses in the rat. Booher et al. (1) carried out an extensive study in which it was shown that unmodified potato, arrowroot, and sago starches were not completely available to the growing rat, whereas the starches from wheat, corn, waxy maize, rice, and cassava were well-utilized for rat growth. It was also shown that commercial samples of hydrochloric acid-modified and hypochlorite-oxidized wheat starch performed as well as unmodified wheat starch. Commercially modified corn starch products were shown by Whistler and Belfort (2) to give body weight gains in rats which were similar to those obtained from feeding unmodified corn starch. However, these workers also showed that a very highly oxidized, laboratory-prepared starch gave poor body weight gains.

In vitro studies with amylase enzymes have indicated that whole starches of various types, and starch fractions are digested at different rates. Waxy maize starch was hydrolyzed more slowly than unfractionated corn starch, and much more slowly than the linear fraction from corn starch according to Caldwell and Adams (3); Sandstedt et al. (4) found that the resistance of high amylose corn starch products to digestibility by α-amylase was not directly associated with the amylose content of the starches, and Borchers (5) reported that high amylose corn starch gave digestibility values of 66 to 77% in the rat, which were much lower than the value of 95% given by normal corn starch.

Various dietary carbohydrates have been reported to affect protein metabolism. Thus, Harper et al. (6) showed that at low dietary protein levels, dextrin gives better rat growth than sucrose and fructose, and glucose gives a growth rate between that of dextrin and sucrose. When sucrose was the source of dietary carbohydrate in low protein diets, from 2 to 3% more protein was required to support rat growth equivalent to that obtained from

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a similar diet in which corn starch was the source of dietary carbohydrate (7). Chang (8) found that raw potato starch, cooked potato starch, dextrin, sucrose, corn starch, and glucose had different effects on the availability of lysine in wheat gluten diets.

The purpose of this study was to compare the protein efficiency values, and rat growth data obtained from feeding a series of unmodified starches, pregelatinized starches, disaccharides, and monosaccharides, as well as several amylose and amylopectin products.

EXPERIMENTAL

Groups of 10 rats each were fed semipurified diets containing various sources of dietary carbohydrate. The animals were Wistar strain males weighing from 50 to 55 g at the beginning of the assays. The animals were housed in individual raised-bottom wire cages in an air conditioned room kept at 75° ± 2°F. The diets contained 4% USP salts XIV, 10% corn oil, either casein[2] or wheat gluten[3] to supply protein, ample vitamins and 2% cellulose. No adjustments were made to equalize the moisture contents of the diets because previous experiments had shown that the addition of water to diets containing high levels of some sugars resulted in a poor diet texture. The maximal moisture content of the diets used in these studies was 12%.

The 15% protein diets contained 66% of each carbohydrate. Casein was also fed at 6% protein, and wheat gluten at 9% protein. These latter diets contained 77 and 73%, respectively, of the carbohydrates being evaluated. Reagent grade sugars were used as sources of fructose, maltose, dextrose, sucrose and galactose. The pregelatinized starches were obtained from various manufacturers, and therefore were subjected to different processing conditions. The amylose and amylopectin samples were also from commercial sources and probably contained small amounts of the respective branched-chain and linear fractions.

Three different rat assays were carried out to study the nutritional effects of dietary carbohydrate. At the termination of the first rat study, it was noted that the ceca of some of the animals were greatly enlarged and engorged with food. This condition would give erroneous body weight gain values in some of the test groups. Therefore, at the termination of the assays the ceca were removed and weighed so that corrections might be made for the large amounts of incompletely digested food that remained in this portion of the gastrointestinal tract. In the first assay, ceca were weighed from at least 6 animals in most test groups. In the other 2 assays all the ceca were weighed except where the ceca burst due to extreme enlargement. The cecal weights were not deducted from the body weight gains given in the tables since they were not available from all the animals in each assay.

The body weight gains, with and without the cecal weights, and the protein efficiency values were analyzed statistically by analysis of variance technique as described by Federer (9). The variation of animals within each diet was calculated. No differences in these variances could be detected among diets within an assay. Hence a weighted average variance was used for each assay as the measure of animal to animal variation. Such an average variance is more precise than the variance of a single diet because the average variance is based on more observations. Also, because it is based on more observations, the average variance permits more sensitive tests of differences among diets.

The 95% confidence limits in the tables show ± 2 standard deviations of the average, based on the averaged variance of each assay.

RESULTS AND DISCUSSION

First study with casein as dietary protein. A large selection of starches and sugars was included in the first study to determine the effects of dietary carbohydrate on the utilization of protein. As indicated in table 1 there was considerable variation in the protein efficiency values obtained from feeding different carbohydrates to weanling rats. Of the unmodified

[2] A.N.R.C. Casein, Sheffield Chemical Company Norwich, New York.
[3] Pro 80 Wheat Gluten, General Mills, Minneapolis Minnesota.

TABLE 1

Summary of data from first 28-day study with casein as the dietary protein

Carbohydrate source	6% Dietary protein level				15% Dietary protein level			
	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]	Body wt gain[1]	Protein efficiency[1]	Dry food	Cecum wt/100 g

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	g		*g*	*g*	*g*		*g*	*g*
Unmodified starches								
Corn	46.3 ± 7.1[3]	2.43 ± 0.36[3]	290	1.3(9)	143 ± 15.0[3]	2.42 ± 0.20[3]	361	1.4(10)
Potato	64.5 ± 7.5[4]	2.54 ± 0.37	375	9.5(7)	101 ± 15.0[4]	1.65 ± 0.20[4]	362	6.4(9)
Wheat	42.9 ± 7.1	2.27 ± 0.36	287	1.3(6)	142 ± 15.0	2.47 ± 0.20	350	1.0(7)
Arrowroot	33.1 ± 7.5[4]	1.56 ± 0.37[4]	312	5.3(10)	111 ± 15.0[4]	2.09 ± 0.20[4]	315	4.3(7)
Rice	41.9 ± 7.1	2.22 ± 0.36	285	1.3(7)	132 ± 15.0	2.27 ± 0.20	352	1.0(7)
Tapioca	37.0 ± 7.1	2.21 ± 0.36	251	1.5(7)	146 ± 15.0	2.33 ± 0.20	376	0.9(7)
Pregelatinized starches								
Potato	40.8 ± 7.5	2.62 ± 0.37	249	1.3(8)	131 ± 15.0	2.30 ± 0.20	359	1.2(10)
Arrowroot	35.1 ± 7.5[4]	2.17 ± 0.37	259	1.6(9)	118 ± 15.0[4]	2.30 ± 0.20	329	1.2(8)
Tapioca	42.2 ± 7.1	2.15 ± 0.36	315	1.2(7)	126 ± 15.0	2.21 ± 0.20	363	1.1(7)
Amioca	39.4 ± 7.1	2.33 ± 0.36	259	4.4(9)	115 ± 15.0[4]	2.03 ± 0.20[4]	348	2.8(8)
Starch derivatives								
Corn dextrin	42.6 ± 7.1	2.15 ± 0.36	314	2.8(7)	126 ± 15.0	1.97 ± 0.20[4]	401	2.2(7)
Potato amylose	62.7 ± 7.1[4]	2.49 ± 0.36	377	4.8(9)	124 ± 15.0	2.04 ± 0.20[4]	367	4.3(8)
Potato amylopectin	49.4 ± 7.1	2.65 ± 0.36	282	2.1(10)	120 ± 15.0[4]	2.37 ± 0.20	308	2.8(9)
Disaccharides								
Sucrose	27.2 ± 7.1[4]	1.57 ± 0.36[4]	285	1.2(7)	131 ± 15.0	2.42 ± 0.20	353	1.1(7)
Maltose	38.8 ± 7.1	2.16 ± 0.36	294	1.1(6)	149 ± 15.0	2.57 ± 0.20	376	1.0(7)
Lactose	1.2 ± 10.1[4]	0.13 ± 0.50[4]	157	14.4(1)	48.2 ± 21.2[4]	1.10 ± 0.28[4]	287	— (0)
Lactose-dextrose (50:50)	13.3 ± 7.1[4]	0.88 ± 0.36[4]	241	6.5(6)	102 ± 15.0[4]	2.06 ± 0.20[4]	316	4.1(7)
Monosaccharides								
Fructose	24.8 ± 7.5[4]	1.16 ± 0.37[4]	352	1.4(6)	110 ± 15.0[4]	2.00 ± 0.20[4]	357	1.5(7)
Galactose-dextrose	47.8 ± 7.1	2.29 ± 0.36	335	1.6(6)	122 ± 15.0	1.93 ± 0.20[4]	405	1.3(7)
Dextrose	37.4 ± 7.1	1.62 ± 0.36[4]	368	1.8(7)	115 ± 15.0[4]	1.95 ± 0.20[4]	364	1.3(7)

[1] Mean values based on 10 animals/group where no mortality occurred. Five animals died when fed the lactose diets at both protein levels and one death occurred with the unmodified potato starch, pregelatinized potato starch, unmodified arrowroot starch and fructose, all at the 6% protein level.

[2] Mean value with number of animals in parentheses. These include both the cecum and its contents.

[3] Mean and 95% confidence limit on the mean.

[4] Means are significantly different at the 5% significance level when compared to those obtained with unmodified corn starch.

starches, potato and arrowroot gave enlarged ceca at both the 6 and 15% protein levels and poor body weight gains at the higher protein level when casein was the dietary protein. At the lower protein level, feeding of unmodified potato starch resulted in significantly better body weight gains than those produced by the other dietary carbohydrates, and a high protein efficiency value was obtained. This superiority in weight gain from the diet containing potato starch was also evident when the cecal weights were deducted from the terminal body weights. The animals that were fed the unmodified potato starch diet consumed much more food than the other groups in this study. On the other hand, arrowroot gave a very poor protein efficiency value at the 6% protein level, and unmodified potato starch gave an unusually poor protein efficiency value at the 15% protein level. The unmodified corn, wheat, rice, and tapioca starches all gave good growth and protein efficiency values at both the high and low dietary protein levels. No evidence of enlarged ceca were noted from these starches.

Throughout these studies unmodified corn starch was used for comparisons because this carbohydrate has been used frequently as a "normal" control in the literature. In the series of pregelatinized starches which were tested at both protein levels, the arrowroot product gave growth values which were significantly lower than those obtained from unmodified corn starch. Feeding of pregelatinized amioca starch in a diet containing 15% protein resulted in significantly poorer growth and protein efficiency values when compared to corn starch in the 15% protein diets. This pregelatinized starch is a commercial product made from waxy maize corn starch. The diet containing pregelatinized amioca starch gave a diarrhea throughout the test and resulted in ceca which were enlarged as compared to the cecal weights obtained from the animals fed the other pregelatinized starches. Also, the corn dextrin diets caused a slight diarrhea. This processed starch gave ceca which were about twice the weight of those obtained with unmodified corn starch. The protein efficiency value obtained from the corn dextrin diet was also significantly poorer than the value obtained from unmodified corn starch when the dietary level of protein was 15% for both diets.

A commercial sample of potato amylose gave significantly better body weight gains and protein efficiency values at the lower casein levels when compared with the responses given by either corn dextrin or corn starch. However, as shown with the unmodified potato starch, this superiority by the amylose product was not evident when the protein level was increased to 15%. The potato amylose also resulted in enlarged ceca at both levels of dietary protein. Potato amylopectin supported normal body weight gains at the low protein level and slightly poorer body weight gains than corn starch at the higher protein level. Very good protein efficiency values were evident at both protein levels with the amylopectin. The amylopectin diets gave ceca which were slightly enlarged, but smaller than those observed in the animals fed the amylose. These data indicate that the amylopectin fraction of potato starch was better utilized than the amylose fraction at both dietary protein levels.

Of the lower molecular weight sugars, lactose produced poor weight gains, even when diluted with an equal part of dextrose. When lactose was fed as the sole source of carbohydrate there was a 50% mortality at both dietary protein levels. This was to be expected since it is known that lactose is poorly utilized by the rat at high dietary levels. Maltose and sucrose gave good growth and protein efficiency values at the 15% dietary protein level, whereas the sucrose diet gave significantly poorer body weight gains and protein efficiency when compared with unmodified corn starch at the 6% dietary protein level. Fructose or dextrose as the only source of carbohydrate gave relatively poor body weight gain and protein efficiency ratings. These values for fructose at the 6% dietary protein level were the lowest of any of the carbohydrates evaluated, with the exception of lactose.

The fructose diets resulted in somewhat larger livers, on a unit body weight basis, than the other diets. The sucrose diets also produced slightly larger livers than

the other carbohydrates (table 2). The data in the same table do not indicate that the larger livers were related to a high fat content. However, at the low casein level the sucrose diet resulted in slightly greater liver fat values than most of the diets. Most of the liver fat values were in the normal range for these levels of dietary protein with the possible exceptions of the low liver fat values obtained from feeding lactose at both protein levels, and unmodified arrowroot starch at the 6% dietary protein level. The 14.1% liver fat value obtained from the maltose diet with the higher casein level was higher than expected. Usually diets producing good protein efficiency values have liver fat values in the range of 9 to 12% on a dry basis. The animals fed sucrose, fructose, dextrose or lactose had a very "unthrifty" appearance which was especially apparent in those fed dextrose at the 15% dietary protein level.

During the last 2 weeks of the 28-day study the apparent gross digestibility of some diets was determined by a quantitative collection of the feces from 6 animals at each protein level. The average digestibility value obtained from the potato amylose was 91%, and the unmodified potato starch gave a value of 82%. The potato amylopectin, pregelatinized potato starch, pregelatinized amioca starch, and unmodified potato starch gave values of 97 to 98%. This was a further indication that the amylose, and unmodified potato starch were not completely digested.

Second study with casein as dietary protein. A second rat study was carried out to obtain data on the utilization of the amylose and amylopectin fractions of corn starch. A high amylose corn starch sample containing 55% amylose[4] and a potato amylose product from a different commercial source than the one evaluated in the first study were also included.

The data in table 3 show that the second potato amylose sample also resulted in a significantly greater body weight gain and a higher protein efficiency value at the low dietary protein level when compared with the unmodified corn starch. At both protein levels the corn amylose resulted in smaller ceca than the potato

[4] Amylon, National Starch and Chemical Corporation, New York City.

TABLE 2

Summary of liver weights and liver fat contents from first casein series[1]

Dietary carbohydrate	Liver weight/ 100 g body wt		Liver fat content on dry basis[2]	
	6% Protein level	15% Protein level	6% Protein level	15% Protein level
	g	*g*	%	%
Unmodified potato starch	3.7	4.2	9.4	9.8
Unmodified arrowroot starch	3.5	3.8	6.2	9.5
Unmodified tapioca starch	3.8	4.0	9.4	10.7
Unmodified wheat starch	3.8	3.6	8.2	9.4
Unmodified rice starch	3.7	3.9	8.0	9.4
Unmodified corn starch	3.8	3.9	8.2	11.1
Pregelatinized amioca starch	3.8	4.3	8.1	8.4
Pregelatinized potato starch	4.0	3.9	10.4	8.7
Pregelatinized tapioca starch	4.1	4.3	12.0	8.7
Pregelatinized arrowroot starch	4.1	3.9	9.3	8.1
Corn dextrin	4.0	3.6	9.7	7.8
Potato amylose	3.6	3.8	9.0	9.2
Potato amylopectin	3.9	4.3	11.8	10.6
Sucrose	5.0	4.7	12.3	9.5
Maltose	4.1	3.9	10.0	14.1
Lactose	3.8	4.2	6.5	4.0
Lactose-dextrose (50:50)	3.9	4.2	6.7	11.4
Fructose	5.7	5.2	10.3	7.6
Galactose-dextrose	4.3	4.5	10.0	14.1
Dextrose	3.9	3.8	10.0	11.9

[1] Average liver weights determined from all the animals surviving in each group.
[2] Average liver fat data calculated from the livers which were pooled and dried before extraction with ethyl ether.

TABLE 3

Summary of data from second 28-day rat study with casein as the dietary protein

Carbohydrate source	6% Dietary protein level				15% Dietary protein level			
	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]
	g		*g*	*g*	*g*		*g*	*g*
Unmodified corn starch	51.3 ± 6.2[3]	2.95 ± 0.20[3]	260	1.7(10)	162 ± 20.2[3]	2.74 ± 0.32[3]	355	1.1(8)
Unmodified amioca starch	46.4 ± 6.2	2.58 ± 0.20	269	1.8(9)	150 ± 18.1	2.67 ± 0.28	336	1.2(10)
Pregelatinized amioca starch	42.9 ± 6.2[4]	2.90 ± 0.20	250	4.0(10)	119 ± 18.1[4]	2.60 ± 0.28	291	2.4(10)
High amylose corn starch	49.4 ± 6.2	2.55 ± 0.20	286	6.5(4)	124 ± 19.0[4]	2.38 ± 0.30[4]	350	4.5(9)
Corn amylopectin	48.6 ± 6.2	3.15 ± 0.20	258	1.6(9)	128 ± 18.1[4]	2.71 ± 0.28	301	1.1(10)
Corn amylose	52.7 ± 6.2	3.09 ± 0.20	262	4.1(10)	151 ± 18.1	2.60 ± 0.28	354	3.4(7)
Potato amylose no. 2	73.2 ± 6.2[4]	3.25 ± 0.20	332	5.6(10)	142 ± 18.1	2.46 ± 0.28	342	4.1(10)

[1] Mean values based on an average of 10 animals/group except 8 animals in the corn starch group and 9 in the high amylose corn starch group when the protein level was 15% and 8 animals in the high amylose corn starch group when the protein level was 6%.

[2] Mean value with the number of animals in parentheses. These include both the cecum and its contents.

[3] Mean and 95% confidence limit on the mean.

[4] Means are significantly different at the 5% significance level when compared to those obtained with unmodified corn starch.

amylose. The corn amylose did not result in the large weight gains and food intake at the lower dietary protein level as shown by both the unmodified potato starch and the potato amylose in the 2 studies where casein was the protein source. This suggests that the corn amylose may have been better utilized than the potato amylose product at the low dietary protein level. The high amylose corn starch gave the largest ceca in this series of diets, however, the enlarged ceca were not accompanied by increases in body weight gains at the lower level of protein intake. When the 15% protein diets were compared, the high amylose corn starch produced significantly poorer body weight gains and protein efficiency than the unmodified corn starch.

The corn amylopectin gave relatively poorer body weight gains at the higher dietary protein level. This appeared to be due to a depression in food intake since the protein efficiency values and ceca sizes were normal at both levels of dietary protein.

In this second study the pregelatinized amioca starch gave larger ceca and significantly poorer body weight gains than either the unmodified amioca starch or the unmodified corn starch in both the high and low protein diets. This pregelatinized starch product resulted in a diarrhea which was not observed in the animals fed the unmodified amioca starch. These data indicate that the processing conditions used in manufacturing the pregelatinized amioca starch could have been responsible for the different responses obtained. Moinuddin and Lee (10) reported that the addition of cellobiose, a poorly digested carbohydrate, to a high protein casein diet also resulted in diarrhea, poor body weight gains, and enlarged ceca in the rat.

Wheat gluten as the dietary protein. A third rat assay included a series of carbohydrates with wheat gluten as the protein source at 9 and 15% dietary protein levels. The 9% protein level was fed in this series since wheat gluten is a protein of relatively poor quality and it was doubtful whether the animals would grow on a level of 6% protein.

The data in table 4 show that unmodified potato and arrowroot starches gave

TABLE 4

Summary of 28-day data with wheat gluten as the dietary protein

	9% Dietary protein level				15% Dietary protein level			
			Dry	Cecum			Dry	Cecum

id not result food intakes vel as shown o starch and tudies where e. This sug- e may have potato amyl- tary protein n starch gave ies of diets; were not ac- body weight rotein intake. s were com- n starch pro- body weight than the un-

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that unmodi- starches gave

TABLE 4

Summary of 28-day data with wheat gluten as the dietary protein

Carbohydrate source	9% Dietary protein level				15% Dietary protein level			
	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]
	g		*g*	*g*	*g*		*g*	*g*
Unmodified starches								
Potato	15.1±3.2[3,4]	0.41±0.12[3]	358	7.2(9)	48.1±5.1[3,4]	0.66±0.11[3]	431	6.8(9)
Arrowroot	7.4±3.0	0.19±0.12[4]	384	4.9(10)	23.9±5.1	0.38±0.11[4]	383	5.3(9)
Corn	10.4±3.0	0.36±0.12	286	1.4(10)	29.1±4.8	0.57±0.10	331	0.9(10)
Modified starches and starch derivatives								
Pregelatinized potato starch	14.1±3.0	0.54±0.12[4]	284	1.2(10)	32.3±5.1	0.70±0.11	290	1.0(10)
Pregelatinized amioca starch	10.5±3.0	0.40±0.12	277	3.7(10)	26.9±4.8	0.65±0.10	260	3.1(10)
Pregelatinized arrowroot starch	11.3±3.0	0.49±0.12	249	1.8(10)	26.5±4.8	0.51±0.10	310	1.3(10)
Potato amylose	17.8±3.2[4]	0.71±0.12[4]	253	5.6(10)	50.4±4.8[4]	0.90±0.10[4]	344	5.0(10)
Potato amylopectin	13.1±3.0	0.50±0.12	260	1.8(10)	37.1±4.8[4]	0.80±0.10[4]	283	1.6(10)
Corn dextrin	10.8±3.0	0.38±0.12	331	2.5(10)	36.1±4.8	0.71±0.10	323	2.4(10)
High dextrose dextrin	11.1±3.0	0.42±0.12	293	1.4(10)	32.5±4.8	0.72±0.10	291	1.1(10)
Di- and monosaccharides								
Maltose	8.6±3.2	0.39±0.12	238	1.6(9)	30.0±4.8	0.68±0.10	298	1.0(10)
Sucrose	5.2±3.0	0.26±0.12	211	1.6(10)	27.5±4.8	0.71±0.10	256	1.1(10)
Dextrose-fructose (50:50)	9.6±3.4	0.35±0.13	281	1.4(10)	31.2±5.1	0.59±0.11	357	1.0(9)
Dextrose	13.0±3.0	0.45±0.12	315	1.4(10)	30.5±4.8	0.57±0.10	347	1.0(10)

[1] Mean values based on 10 animals/group with the exception of 9 animals in the groups fed unmodified potato starch, pregelatinized potato starch, unmodified arrowroot starch and dextrose-fructose in the 15% protein diets; also 9 animals in the group fed amylose, unmodified potato starch, and maltose and 8 animals from the diet containing dextrose-fructose in the 6% protein diets.

[2] Mean value with number of animals in parentheses. These include both the cecum and its contents.

[3] Mean and 95% confidence limit on the mean.

[4] Values are significantly different at the 5% significance level when compared to those obtained with unmodified corn starch.

enlarged ceca and greater food intakes than the other carbohydrates included in the study. The sucrose diet resulted in poor food consumption which was below that obtained from feeding a mixture of fructose and dextrose at the levels equivalent to their levels in sucrose. At the 15% dietary protein level, potato amylose and unmodified potato starch gave significantly better body weight gains than the unmodified corn starch, even when the cecal weights were deducted from the terminal body weights. This is similar to the data obtained at the 6% dietary protein level with casein as the source of protein. At both wheat gluten protein levels, the amylose gave significantly better protein efficiency values than unmodified corn starch.

The unmodified arrowroot starch did not result in large body weight gains in spite of the presence of greatly enlarged ceca. In fact, at both protein levels the protein efficiency values obtained from the unmodified arrowroot starch were significantly lower than the corresponding values obtained from unmodified corn starch. Another similarity to the first study was the cecal enlargement noted in the rats fed either the pregelatinized amioca starch or corn dextrin. The dextrin product containing the higher dextrose level, which was equivalent to 29% reducing sugars as dextrose, gave normal ceca. This again gives an indication that the process used in making the corn dextrin with the lower dextrose content resulted in a product which was more resistant to digestion. The low dextrose corn dextrin used in these studies had been prepared from corn starch which was heated in the presence of "mineral acid." In a recent publication, Leach and Schoch (11) have shown that an 80-fluidity corn starch which had been treated with acid was more resistant to solubilization by dimethyl sulfoxide than unmodified corn starch. These authors noted that poorly soluble starches were resistant to digestion by α-amylose.

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LITERATURE CITED

1. Booher, L. E., I. Behan and E. McMeans 1951 Biological utilization of unmodified starches. J. Nutrition, *45:* 75.
2. Whistler, R. L., and A. M. Belfort 1961 Nutritional value of chemically modified starches. Science, *133:* 1599.
3. Caldwell, M. L., and M. Adams 1950 Action of certain alpha amylases. Adv. Carbohyd. Chem., *5:* 229.
4. Sandstedt, R. M., D. Strahan, S. Ueda and R. C. Abbott 1962 The digestibility of high amylose corn starch compared to that of other starches. The apparent effect of the Ae gene on susceptibility to amylase action. Cereal Chem., *39:* 123.
5. Borchers, R. 1962 A note on the digestibility of the starch of high amylose corn in rats. Ibid., *39:* 145.
6. Harper, A. E., W. J. Monson, D. A. Arata and D. A. Benton 1953 Influence of various carbohydrates on the utilization of low protein rations by the white rat. J. Nutrition, *51:* 523.
7. Harper, A. E., and C. A. Elvehjem 1957 A review of the effects of different carbohydrates on vitamin and amino acid requirements. J. Agr. Food Chem., *5:* 754.
8. Chang, Yet-Oy 1962 Effect of carbohydrates on utilization of protein and lysine by rats. J. Nutrition, *78:* 21.
9. Federer, W. T. 1955 Experimental Design, Theory and Application. Macmillan Company, New York, pp. 18–21.
10. Moinuddin, J. Fischer and H. Wing-Tsit Lee 1958 Effects of feeding diets containing sucrose, cellobiose or glucose on the dry weights of cleaned gastrointestinal organs in the rat. Am. J. Physiol., *192:* 417.
11. Leach, H. W., and T. J. Schoch 1962 Structure of the starch granule. III. Solubilities of granular starches in dimethyl sulfoxide. Cereal Chem., *39:* 318.

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SOME FACTORS AFFECTING NITROGEN BALANCE IN THE ADULT RAT

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The experiments described in this paper were undertaken to determine the effect of different levels and sources of dietary nitrogen and the nature of the dietary carbohydrate on nitrogen balance in the adult rat at low levels of amino acid feeding. For this purpose two levels of essential amino acids were fed and the nitrogen intake was varied by incorporating in the rations two levels of diammonium citrate. Preliminary experiments had indicated a difference in nitrogen balances of animals depending upon the nature of the carbohydrate moiety of the ration. Therefore, after the nitrogen output had been determined on a diet containing sucrose, the animals were transferred to similar rations containing corn or potato dextrin.

Three series of experiments were conducted. In the first two, the response of undepleted or protein-depleted adult rats to variations in level and source of nitrogen and in type of carbohydrate was investigated. In the third series, variations in nitrogen balances due to type of carbohydrate in relation to the length of time of amino acid feeding were studied.

EXPERIMENTAL

The compositions of the amino acid mixtures and of the diets employed are given in tables 1 and 2. The non-essential amino acids were incorporated in the rations at a level of 0.1%. The physiologically effective forms of the essential amino ac-

ids were included in the rations at levels of either 0.1 (mixture 1) or 0.2% (mixture 2). Diammonium citrate was added to diets 3 and 4 at levels of 0.73 and 2.50%. When the animals ingested 12 gm of the sucrose-containing rations, diet 2 furnished 37 mg total nitrogen, diet 3 48 mg, diet 4 72 mg, and diet 5 48 mg. Diets 2, 3 and 4 supplied the same amounts of essential amino acids with three levels of total nitrogen; diets 3 and 5 furnished equal amounts of total nitrogen with two levels of essential amino acid nitrogen. In order to keep the nitrogen intake on diet 5 equal to that of diet 3 without decreasing the caloric intake, 10.8 gm of diet 5 and 1.2 gm per day of the nitrogen-free ration (diet 1) were fed, and thereby the daily allotment of each of the non-essential amino acids was decreased to 10.8 mg, as compared with 12.0 mg received by the animals ingesting diets 2, 3 and 4. This small decrease is believed to be without bearing on the results of the study. Animals ingesting 12 gm of the rations containing dextrin received about 5 mg more nitrogen per day than the amounts furnished in the sucrose rations. Since information was lacking as to the character of this nitrogen, it was counted only as total nitrogen.

Male rats from the laboratory stock colony[1] were housed individually and fed a commercial pelleted diet from weaning until the start of the experiments. The laboratory was air-conditioned, with the temperature maintained at 80 ± 2°F. In series 1, 36 animals 70 to 79 days old, weighing 265 to 357 (ave. 302) gm, were placed directly upon experimental diets 2 to 5, without prior depletion of their nitrogen stores. From 8 to 10 animals were placed on each diet, the several groups being equalized as closely as possible according to litter and body weight. They were fed ad libitum for 4 days to allow for adjustment to the experimental rations, after which weighed amounts of the rations were offered daily for 8 weeks and urine and fecal collections were made for each 7-day period.

[1] A purebred strain resulting from the crossing of albino and black and white hooded strains of *mus norvegicus*. The litters may include white, black, and black and white rats.

During the first 4 weeks sucrose was the carbohydrate in the rations, and in the final 4 weeks corn dextrin was substituted for sucrose. The first period in which the animals received corn dextrin was considered to be an adjustment period and is not included in any evaluation of the data.

TABLE 1

Composition of amino acid mixtures

CONSTITUENTS	MIXTURE 1		MIXTURE 2	
	Physiologically active	As used	Physiologically active	As used
	gm	*gm*	*gm*	*gm*
DL-alanine	0.1	0.1	0.1	0.1
L-arginine	0.1		0.1	
L-arginine HCl		0.12		0.12
L-aspartic acid	0.1	0.1	0.1	0.1
L-cystine	0.1	0.1	0.1	0.1
L-glutamic acid	0.1	0.1	0.1	0.1
Glycine	0.1	0.1	0.1	0.1
L-histidine	0.1		0.2	
L-histidine·HCl·H_2O		0.135		0.27
L-hydroxyproline	0.1	0.1	0.1	0.1
DL-isoleucine	0.1	0.2	0.2	0.4
L-leucine	0.1	0.1	0.2	0.2
L-lysine	0.1		0.2	
L lysine HCl		0.125		0.25
DL-methionine	0.1	0.1	0.2	0.2
DL phenylalanine	0.1	0.1	0.2	0.2
L-proline	0.1	0.1	0.1	0.1
DL-serine	0.1	0.1	0.1	0.1
DL-threonine	0.1	0.2	0.2	0.4
DL-tryptophan	0.1	0.1	0.2	0.2
L-tyrosine	0.1	0.1	0.1	0.1
DL-valine	0.1	0.2	0.2	0.4
$NaHCO_3$		0.160		0.27
	1.9	2.440	2.8	3.81

In series 2, 48 rats 91 to 106 days old, weighing 307 to 388 (ave. 341) gm, were depleted of their protein stores by feeding them ad libitum for 18 days a nitrogen-free ration (diet 1) containing sucrose. At the end of this time, when their body weights varied from 270 to 328 (ave. 292) gm, the ani-

mals, distributed into groups of 12 according to litter and body weight, were placed on diets 2 to 5 containing sucrose as the carbohydrate. After three weeks the source of the carbohydrate was changed and the experiments continued for

TABLE 2

Composition of diets[1]

CONSTITUENTS	DIET 1	2	3	4	5
	gm	*gm*	*gm*	*gm*	*gm*
Amino acid mixture 1		2.44	2.44	2.44	
Amino acid mixture 2					3.81
Diammonium citrate			0.73	2.50	
Carbohydrate[2]	80.65	78.21	77.48	75.71	76.84
Salt mixture[3]	4.00	4.00	4.00	4.00	4.00
Corn oil[4]	3.00	3.00	3.00	3.00	3.00
Lard	12.00	12.00	12.00	12.00	12.00
Vitamin A and D concentrate[5]	0.05	0.05	0.05	0.05	0.05
Inositol	0.10	0.10	0.10	0.10	0.10
Choline chloride	0.20	0.20	0.20	0.20	0.20
	100.00	100.00	100.00	100.00	100.00

[1] The following vitamins were added per kilogram ration:

Thiamine HCl, 5 mg; pyridoxine HCl, 5 mg; nicotinic acid, 5 mg; riboflavin, 10 mg; Ca pantothenate(d), 25 mg; *p*-aminobenzoic acid, 300 mg; α-tocopherol acetate, 25 mg; 2-methyl-1,4-naphthoquinone, 2 mg; biotin, 100 μg; folic acid, 2 mg; vitamin B_{12}, 30 μg.

The caloric contents of the diets were calculated to be as follows:

4.4 cal. per gram: diet 4, sucrose.

4.5 cal. per gram: diet 1, sucrose; diet 2, sucrose; diet 3, sucrose; diet 4, dextrin; and diet 5, sucrose.

4.6 cal. per gram: diet 2, dextrin; diet 3, dextrin; diet 5, dextrin.

[2] Sucrose, diets 1 to 5; corn dextrin, diets 1 to 5; potato dextrin, diets 2 and 5.

[3] Jones and Foster ('42).

[4] Mazola.

[5] Squibb's Navitol containing 65,000 U.S.P. units of vitamin A and 13,000 units of vitamin D per gram.

three additional weeks. One-half of the animals previously fed diets 2 and 5 containing sucrose received corn dextrin as the source of carbohydrate, while the remainder received potato dextrin. Six of the animals of diet 3 were given corn dextrin and the others received corn dextrin plus 1% sulfa-

suxidine. One-half of the animals receiving diet 4 were changed from sucrose to corn dextrin and the others were used in another study, the results of which are not reported here. The first week of each three-week period was used as an adjustment period before collection of urine and feces.

In series 3, 21 male rats 90 to 99 days old, weighing 288 to 362 (ave. 324) gm, were depleted by feeding the nitrogen-free diet 1 containing sucrose (groups A and B) or corn dextrin (group C) ad libitum for 18 days, at which time their body weights varied from 251 to 306 (ave. 275) gm. The 7 animals comprising group A received diet 2 containing amino acid mixture 1 and sucrose for 6 weeks. The 8 animals in group B were given diet 2 containing sucrose for three weeks and then placed on diet 2 containing corn dextrin for three weeks. The 6 animals of group C received diet 2 containing corn dextrin for three weeks from the end of the nitrogen-free period. No collections of urine or feces were made during the first week after the nitrogen-free period.

Except where otherwise indicated, all animals were offered 12 gm of diet each day. The rats and the uneaten food were weighed weekly. If food was scattered, or if the essential amino acid nitrogen intake fell below 13.0 mg per day on diets 2, 3 and 4, or below 23.0 mg on diet 5, in series 1 and 2, the data from those periods were discarded. In series 3, the food intakes of the animals were in general lower than those in the first two experiments and all periods except those in which scattering occurred are included in the averages.

During the periods when nitrogen balances were determined, the animals were placed in wire metabolism cages with one-half-inch mesh bottoms supported above 250-mm glass funnels. Under the funnels were glass bottles containing toluene and sufficient sulfuric acid to acidify the urine samples. Fine mesh screens over the tops of the funnels retained the feces, which were collected daily and placed in concentrated sulfuric acid. Two or three times during each period the feces in sulfuric acid were partially digested to prevent the foaming which occurred if the entire sample was digested at once.

One per cent ferric oxide was added to the food at the beginning of each 7-day period to serve as a feces marker. Several times during each period the screens and funnels were washed down with a fine stream of hot 2% boric acid solution, this procedure again being employed at the end of the period when the animals were transferred to clean cages. The used cages were washed in a similar manner, and all washings added to the weekly urine collections. The further treatment of the urine and feces has already been described (Womack, Harlin and Lin, '53). Analysis of variance was used to aid in evaluation of the results.

RESULTS AND DISCUSSION

Increasing the average daily total nitrogen intake of the undepleted animals from 35.8 to 46.4 or 72.6 mg by the addition of diammonium citrate without change in essential amino acid nitrogen caused small but not statistically significant changes in the negative nitrogen balances, from −23.2 to −19.4 to −17.2 mg (table 3, series 1). When the total nitrogen intake was raised to 47.7 mg by increasing the essential amino acid nitrogen intake to 25.1 mg per day, the average nitrogen balance of −7.2 was significantly better than that of −19.4 for the animals receiving 46.4 mg of total nitrogen but only 14.5 mg of essential amino acid nitrogen (diet 3). It is apparent that 12 gm per day of the sucrose ration containing amino acid mixture 1 do not meet the essential amino acid requirement of adult rats. The nitrogen of the diammonium citrate appears to have little influence on the nitrogen balances of undepleted animals when added to rations containing these levels of amino acids. These results were confirmed in a group of animals partially depleted of protein (table 3, series 2).

Comparison of the nitrogen balances of the animals receiving diets 2, 3 and 4 containing sucrose revealed that there were greater nitrogen losses in the undepleted (series 1) than in the depleted animals (series 2) at comparable levels of total and essential amino acid nitrogen intake. This is to be

TABLE 3

Average daily nitrogen balance data for undepleted (series 1)[1] or protein-depleted (series 2) adult male rats receiving rations containing low levels of amino acids with and without supplementation with diammonium citrate and with either sucrose, corn dextrin or potato dextrin

SERIES NO.	CARBOHYDRATE OF RATION	DIET NO.	INITIAL WEIGHT	FINAL WEIGHT	NO. OF 7-DAY PERIODS	INTAKE				NITROGEN EXCRETION		NITROGEN BALANCE
						Food	Calories	Total nitrogen	E.A.A.N.[2]	Feces	Urine	
			gm	*gm*		*gm*		*mg*	*mg*	*mg*	*mg*	*mg*
1	Sucrose	2	292[3]	278	30	11.9	54	35.8	14.7	12.8	46.2	— 23.2
		3	284[3]	271	28	11.8	53	46.4	14.5	12.0	53.8	— 19.4
		4	281[3]	271	34	11.8	52	72.6	14.5	12.3	77.5	— 17.2
		5	294[3]	281	31	11.9	54	47.7	23.1	12.4	42.5	— 7.2
	Corn dextrin[4]	2	282	287	13	11.8	54	41.4	14.5	14.4	30.6	— 3.6
		3	271	281	21	11.6	53	50.3	14.3	13.5	35.1	+ 1.6
		4	272	276	12	11.4	51	74.2	14.0	13.7	61.9	— 1.3
		5	283	297	21	11.9	55	52.7	24.9	14.8	34.7	+ 3.2
2	Sucrose	1	341	292	47	9.8	44	2.0		10.5	52.0	— 60.6
		2	291	296	23	11.9	53	36.2	14.6	11.9	37.0	— 12.7
		3	293	294	21	11.7	52	45.7	14.4	11.4	45.7	— 11.3
		4	294	299	18	11.8	52	71.3	14.5	12.9	70.9	— 12.5
		5	292	298	18	11.8	53	45.0	24.7	11.3	36.7	— 3.0
	Corn dextrin[4]	2	302	302	11	11.7	53	41.1	14.4	14.5	30.2	— 3.6
		3	301	306	9	11.5	52	49.5	14.1	14.9	36.6	— 2.1
		4	297	297	12	11.7	52	75.8	14.3	14.2	60.9	+ 0.7
		5	305	307	12	11.8	54	52.0	24.7	14.4	35.2	+ 2.4
	Potato dextrin[5]	2	293	292	10	11.9	54	41.8	14.7	23.3	28.0	— 9.5
		5	286	290	12	12.0	54	51.4	25.0	21.6	31.9	— 2.1
	Corn dextrin with 1% sulfasuxidine	3	288	296	10	11.6	52	64.0	14.3	26.9	32.4	+ 4.7

[1] Two animals from each group were killed at the end of the period of sucrose feeding.
[2] Essential amino acid nitrogen.
[3] Weight after 4-day adjustment period. Losses during that period: diet 2, 16 gm; diet 3, 17 gm; diet 4, 18 gm; diet 5, 8 gm.
[4] Amidex, from Corn Products Refining Co.
[5] From Morningstar, Nicol, Inc.

expected, since undepleted animals apparently have large stores of labile protein to call upon when their amino acid requirements are not met by intake. It might be expected that over a period of a month on low nitrogen rations the protein reserves would be gradually reduced and the nitrogen balances of the undepleted would approach those of the depleted group. This is not the case, however, as is shown by average nitrogen balances of −25.2, −23.8, −22.7 and −20.9 mg per day for 4 successive 7-day periods for the animals of series 1, diet 2, on which the largest decrease was observed. Apparently the amino acid intake met a large enough proportion of the requirement so that the body stores were not approaching depletion even at the end of the 4th week on the ration.

When the animals of series 1 and 2 were changed from rations containing sucrose to those containing corn dextrin, some unexpected results were obtained. Nitrogen losses were lower in all groups receiving amino acid mixture 1, and the balances of the animals receiving diets containing either mixture 1 or mixture 2 were not significantly different from zero. The effect on nitrogen balance of a change from sucrose to corn dextrin, therefore, was approximately equivalent to an increase in essential amino acid intake. In this study there were no statistically significant differences among the balances for the animals on any of the rations containing corn dextrin, nor between the depleted and undepleted animals on those rations. There were, however, statistically significant differences between the values for each pair of rations containing sucrose or corn dextrin. When 1% sulfasuxidine replaced an equal amount of corn dextrin in diet 3, the average daily nitrogen balance of +4.7 mg was the highest obtained on any ration, and was significantly higher than the value of −2.1 obtained for litter mates receiving diet 3 and corn dextrin but no sulfasuxidine. The difference between the balances for the animals receiving sucrose or potato dextrin is significant, but is overshadowed by the much greater improvement brought about by the change to corn dextrin (diet 2,

series 2). The difference between the balances of the animals receiving sucrose or potato dextrin and the higher levels of essential amino acids (diet 5, series 2) is not significant, while the change to corn dextrin results in a significant improvement in the balances.

The largest increases in nitrogen intake resulting from the change to corn dextrin were from 35.8 to 41.4 mg per day for the animals of series 1, diet 2, which was associated with a significant change in nitrogen balance from — 23.2 to — 3.6; and from 45.0 to 52.0 mg per day for the animals of series 2, diet 5, which was associated with a significant change in nitrogen balance from — 3.0 to + 2.4. In the latter instance it is possible that the change in nitrogen balance of 5.4 mg can be accounted for by the increase in intake of 7.0 mg. In the former case, however, it seems unlikely that an increase in intake of 5.6 mg would improve the nitrogen balance by 19.6 mg. Moreover, increases in the nitrogen intake of many times that amount by the addition of diammonium citrate brought about no such changes in nitrogen balances on the sucrose-containing rations. Also, in the undepleted animals of series 1, diet 4, there was an increase in average nitrogen intake of only 1.6 mg and yet the nitrogen balances rose from — 17.2 to — 1.3 mg per day.

Initial and final weights of the animals of series 1 and 2 are included in table 3. Except for the losses during the periods when nitrogen-free rations were fed, the only appreciable variations in weight were shown by the undepleted animals of series 1 during the periods of sucrose feeding.

The nitrogen balances of the protein-depleted animals of series 3 are presented in table 4. Although the effects on nitrogen balance of small differences among the groups in the food, calorie, total nitrogen and essential amino acid nitrogen intake cannot be evaluated, it seems clear that the improved nitrogen balances are due to the change of the carbohydrate to corn dextrin and not to a gradual change in the balances when the animals remain on the rations containing sucrose for relatively long periods of time.

TABLE 4

Average daily nitrogen balance data for protein-depleted adult male rats (series 3)[1] receiving rations containing low levels of amino acids (diet 2) and with either sucrose or corn dextrin

GROUP NO.[1]	CARBOHYDRATE OF RATION	WEEK OF AMINO ACID FEEDING	INTAKE				NITROGEN EXCRETION		NITROGEN BALANCE
			Food	Calories	Total nitrogen	E.A.A.N.[2]	Feces	Urine	
			gm		*mg*	*mg*	*mg*	*mg*	*mg*
A	Sucrose	2	11.0	49	34.6	13.5	11.0	37.6	— 14.1
B	Sucrose	2	11.0	49	34.9	13.6	10.4	37.7	— 13.3
C	Corn dextrin	2	11.3	51	39.5	13.9	14.8	28.3	— 3.7
A	Sucrose	3	11.3	50	35.3	13.8	10.2	37.6	— 12.4
B	Sucrose	3	11.1	50	34.9	13.7	10.6	36.5	— 12.2
C	Corn dextrin	3	11.8	53	40.7	14.5	14.6	30.5	— 4.3
A	Sucrose	4	10.6	47	32.6	13.0	9.7	33.1	— 10.1
B	Corn dextrin[3]	4	11.0	50	37.3	13.5	12.5	30.3	— 5.6
A	Sucrose	5	10.6	48	32.9	13.1	9.3	33.8	— 10.2
B	Corn dextrin[3]	5	11.2	51	38.2	13.8	13.6	29.3	— 4.6
A	Sucrose	6	10.2	46	31.6	12.5	8.8	33.2	— 10.8
B	Corn dextrin[3]	6	11.3	52	39.6	13.9	14.2	28.8	— 3.5

[1] Average starting weights after protein-depletion: group A, 276 gm; group B, 273 gm; group C, 277 gm. Average final weights after amino acid feeding: group A, 270 gm; group B, 276 gm; group C, 270 gm.

[2] Essential amino acid nitrogen.

[3] These animals were fed sucrose for the first three weeks of amino acid feeding.

There are several possible explanations for the effect of dextrin in the ration on the utilization by the adult rat of low levels of amino acids. The dextrin may provide small amounts of either additional essential or non-essential amino acids; the small increase in caloric content of the dextrin diet may be beneficial to the animal; the possibility of the existence of a different type of bacterial flora when the dextrin is present could conceivably affect the availability of dietary amino acids to the host organism; some difference between the physical states of sucrose and dextrin may produce a delay in the elimination of the intestinal contents, thereby making the amino acids available for absorption for a longer time; or a delay in absorption of the dextrin or its digestive products may furnish energy for intestinal synthesis of additional essential or non-essential amino acids. Whether or not such synthesized amino acids, if formed, would be available to the animal is a much-debated question. Although the animals were housed in cages with large mesh bottoms, undoubtedly some feces were consumed, so that amino acids formed might be available in this fashion even if not absorbed directly at the site of synthesis.

From the results of the present study it would seem that the change from soluble carbohydrate to modified starch, as represented by the change from sucrose to potato dextrin, is not the predominant factor in the improvement in nitrogen balances. The results obtained when 1% sulfasuxidine was incorporated in the rations are in agreement with the belief that the bacteria of the intestinal tract are in competition with the host organism for certain nutrients, and if they are changed in kind or amount the host may benefit.

Attention should be directed to the increases in fecal nitrogen excretion of the animals ingesting dextrin-containing rations. The animals of series 2 receiving corn dextrin excreted an average of 1.4 to 3.5 mg per day more fecal nitrogen than the same animals receiving the sucrose-containing rations. This was somewhat lower than the increase in nitrogen intake due to the change to the rations containing corn

dextrin. For the animals receiving potato dextrin, however, the increase in fecal nitrogen excretion was considerably higher than the increase in nitrogen intakes.

Booher, Behan and McMeans ('51) found that unmodified potato starch was relatively poorly digested when fed to rats, and stated that the apparent digestibility coefficients for protein varied directly with the digestibility of the starch component. Although no *in vivo* digestion trials were conducted in the present studies, it was apparent that the feces of the animals receiving the rations containing potato dextrin were bulkier and more numerous than those of the animals receiving either sucrose or corn dextrin. Harper, Katayama and Jelinek ('52) found that unmodified potato starch, autoclaved potato starch or potato dextrin incorporated in casein-containing rations fed to young rats induced greater excretion of certain amino acids in the feces than either sucrose or glucose. Further investigation is needed before speculating on the source of the extra fecal nitrogen observed when potato dextrin was fed in rations containing amino acids, in the present studies.

Dietrich, Monson and Elvehjem ('52) have shown that chicks and hyperthyroid rats fed purified rations gain at a greater rate if the rations contain corn dextrin than if sucrose is the carbohydrate. Moreover, if a 70% ethanol extract of dextrin is added to the sucrose-containing rations, the rates of gain approach those of the animals receiving dextrin. Further experiments will be required to determine whether the effect on nitrogen balance of a change in the carbohydrate from sucrose to corn dextrin is related to the unknown growth factor found in ethanol extracts of corn dextrin by these workers.

The present studies indicate that at these low levels of nitrogen intake, amino acid requirements for maintenance of nitrogen balance in the adult rat are significantly influenced by the carbohydrate moiety of the ration.

Since the preparation of this manuscript, a paper has appeared by Harper and Katayama ('53) demonstrating that

young rats fed rations containing 9% casein and cornstarch grew better than those receiving the same rations and sucrose. Substituting dextrin made from either cornstarch or potato starch supported growth equivalent to that obtained with cornstarch. The results of the present study, while differing at certain points, support the suggestion of Harper and Katayama that the type of carbohydrate in the ration may modify the requirement of the rat for amino acids, and extend the observations to adult rats and to rations containing free amino acids as well as intact proteins.

SUMMARY

The negative nitrogen balances of adult protein-depleted or undepleted rats fed rations containing low levels of amino acids (14.4 to 14.7 mg essential amino acid nitrogen per day) with sucrose as the carbohydrate were not significantly improved when the total nitrogen intake was increased by the addition of two levels of diammonium citrate. The negative nitrogen balances were significantly improved when the essential amino acid nitrogen intake was increased to 24.7 and 25.1 mg per day. Nitrogen losses of protein-depleted animals were less than those of undepleted animals at the same levels of total and essential amino acid nitrogen intake. When corn dextrin was substituted for sucrose in the rations, the animals achieved nitrogen equilibrium at levels of essential amino acid intake which resulted in large negative balances with the rations containing sucrose. The beneficial effects of feeding corn dextrin appeared to be due to the change in the carbohydrate and not to a gradual lowering of the negative nitrogen balances when animals received rations containing low levels of amino acids and sucrose for several weeks. Feeding 1% sulfasuxidine with corn dextrin brought about an additional improvement in the balances. When potato dextrin was substituted for sucrose there was a small but significant improvement in the nitrogen balances at the lower levels of amino acid intake but no difference at the higher levels.

LITERATURE CITED

BOOHER, L. E., I. BEHAN AND E. MCMEANS 1951 Biological utilizations of unmodified and modified food starches. J. Nutrition, *45:* 75–100.

DIETRICH, L. S., W. J. MONSON AND C. A. ELVEHJEM 1952 Studies on an unidentified growth factor for chicks and hyperthyroid rats fed purified rations. Arch. Biochem. Biophys., *38:* 91–96.

HARPER, A. E., AND M. C. KATAYAMA 1953 The influence of various carbohydrates on the utilization of low protein rations by the white rat. J. Nutrition, *49:* 261–275.

HARPER, A. E., M. C. KATAYAMA AND B. JELINEK 1952 The influence of dietary carbohydrate on levels of amino acids in the feces of the white rat. Canadian J. Med. Sci., *30:* 578–584.

JONES, J. H., AND C. FOSTER 1942 A salt mixture for use with basal diets either low or high in phosphorus. J. Nutrition, *24:* 245–256.

WOMACK, M., H. A. HARLIN AND P.-H. LIN 1953 The influence of the nonessential amino acids on the requirement for isoleucine, methionine and threonine. Ibid., *49:* 513–526.

It results in a reduction in the size of the molecules and a decrease in the viscosity as well as an increase in reducing value.

2. Transglucosidation—This involves breaking glucosidic linkages and reforming them at another point to yield more highly branched molecules. This reaction is favored by heat and apparently does not predominate until the moisture has been removed. It does not alter the molecular weight or reducing value, but improves the stability of the dextrin solution by reducing the amount of linear component.
3. Repolymerization—Glucose is capable of polymerizing at high temperatures in the presence of catalytic amounts of acid. During this process the reducing value decreases.

In addition, levoglucosan formation undoubtedly occurs at high temperatures.

Depending upon which of these reactions or combinations of reactions are involved, three major types of products may be obtained—white dextrins, yellow dextrins or British gums. In white dextrin conversions, hydrolysis is the dominant reaction. Moisture, high acidity, and relatively low converting temperatures are used in making them. They cover a wide range of viscosities and cold water solubility depending upon the degree of conversion. The most highly converted types have an average degree of polymerization approaching 20, and are quite soluble. For a given level of conversion (as measured by viscosity) they tend to have light color, relatively low cold water solubility, and their dispersions tend to have limited stability.

Yellow dextrins are made with moderately high amounts of acid, low moisture and fairly high converting temperatures. During the initial stage of the conversion hydrolysis predominates, but in the later stages transglucosidation and repolymerization reactions are dominant. All yellow dextrins are low in viscosity and are essentially completely soluble in cold water. Their degree of polymerization runs in the neighborhood of 20–50. They are more soluble, darker in color and more stable than a white dextrin of comparable viscosity.

Little or no acid, in some cases buffers, and high temperatures characterize British gum conversions. While hydrolysis initially occurs, transglucosidation is the predominant reaction. Since hydrolysis is minimized, British gums are not as low in viscosity as yellow dextrins or the thinnest white dextrins. For the same viscosity they are substantially darker in color, more soluble in cold water and lower in reducing value than white dextrins. In addition, their solutions are much more stable than those of white dextrins.

Modification of Starch by Cross-Linking

The characteristics of a starch dispersion change markedly during the cooking process. The unswollen starch behaves as a suspension in water. However, when the granules begin to swell the dispersion becomes a short, salve-like paste. This quickly changes to an elastic, and in some cases, rubbery texture when the swollen granules rupture forming dispersions of molecules and molecular aggregates.

The short, salve-like consistency of the highly swollen granules is usually associated wth the stage at which the starch reaches its maximum viscosity and thickening power. It also provides textural qualities which are palatable, and particularly suited for use in pie fillings, sauces, puddings, etc. However, in raw starches this condition is very tenuous being readily destroyed by overcook-

and arginine nitrogen. Protein precipitated from the alkali extract of pecan flour (8) showed notably higher arginine nitrogen and lower histidine nitrogen than was found in work done directly on the ground pecan kernel without previous extraction to remove the oil (57). The specific rotation, $[\alpha]_D^{20}$, of nut proteins was found to be as follows: almond, −56.44°; Brazil nut, −42.94°; butternut, −45.40°; Persian walnut, −45.21°; and eastern black walnut, −44.43° (58). The closest resemblance between proteins from unrelated seeds of plants is presented by corylin from filbert and juglansin from Persian walnut, which are distinguished only by a slight difference in the nitrogen content. With the use of a special ultracentrifuge, the peanut globulin arachin has been separated into five groups with molecular weights of 600,000, 400,000, 140,000, 30,000, and 20,000 (17).

Oil. Most nuts are characterized by a high oil or fat content—usually about 60%, but as high as 76% in some varieties of pecans. For the most part the oil is contained in the kernel or embryo of the seed, but it occurs mainly in the endosperm of coconut, palm, and pine nuts, and in the flesh of the ginkgo fruit. Some fatty acid values for a few of the nuts are reported in Table 5. But considerable variations in percent of fatty acids have been reported in both pecan and peanut oils (Table 6). For main physical characteristics and composition of nut oils, see Drying oils; Fats and fatty oils.

Carbohydrates. The carbohydrates of pecan kernel (21), coconut meal (ref. 96, p. 385), English walnut (43), European chestnuts (ref. 96, p. 299), and chufa and the ivory nut (ref. 96, p. 388) were found to contain some of the following: reducing sugars, sucrose, raffinose, mannitol, dextrin, pentosans, amyloids, starch, cellulose, tannins, gums, waxes, resins, and some undetermined material. Several investigators studied the carbohydrates of developing coconuts and found mannitol to be the main sugar derivative in milk of young coconuts, while the principal sugar in the milk of the mature nut was sucrose with no mannitol present. The starch in peanuts (33) is reduced by roasting, as seen from the figures in Table 7.

Table 7. Starch Contents of Peanuts, % (33)

Lot	Raw runners	Raw Spanish	Lightly roasted runners	Lightly roasted Spanish	Fully roasted runners	Fully roasted Spanish
0	3.81		2.71		1.15	
1		3.02		2.39		1.50
2	4.62		2.88		1.32	
3			2.99		1.15	

Ash Constituents (Minerals). Nuts are considered a good source of minerals essential in nutrition, supplying in particular the elements copper, manganese, iron, and sulfur. The mineral constituents of a number of nuts shown in Table 2 are averages of available analytical data. Other values for the percentages of minerals in the peanut kernel (26) and percentages of ash constituents in the macadamia kernel (25) have been reported. Chufa was found to have a high silicon content.

Some nut trees have been found to be accumulators of certain mineral elements. Hickory is notable as an accumulator of aluminum (65), the ash of its leaves containing up to 37.5% Al_2O_3. Walnut, unlike hickory, does not accumulate aluminum; the ash of autumnal leaves of Persian walnut was found to contain only 0.032% Al. Hickory was found to be an accumulator of rare earth elements, greatly exceeding all other

alcohol and pantothenates were almost identical. At 60° and pH 4 pantothenyl alcohol was dec more slowly (about 10% loss in 4 weeks) than pantothenic acid (about 65% loss in 4 weeks). *See* Rubin, *J. Am. Pharm. Assoc., Sci. Ed.* **37**, 502 (1948). Aq solns can be stabilized with pantolactone: U.S. pat. 2,898,373 (1959).

MED USE: To increase peristalsis and intestinal tone. *Dose:* im 250 or 500 mg.

VET USE: Nutritional factor for all species. Horses and ruminants normally need no dietary sources.

Dextran. Macrose; Macrodex; Intradex; Polyglucin; Dextraven; Onkotin; Expandex; Gentran; Plavolex. A term applied to polysaccharides produced by bacteria growing on a sucrose substrate, contg a backbone of D-glucose units linked predominantly α-D(1 → 6). Evans, Hibbert in *Advances in Carbohydrate Chemistry*, vol. 2, p 209 (1946); W. Brock Neely, *ibid.* vol. 15, p 341 (1960). Empirical formula $(C_6H_{10}O_5)_n$. Mol wt about 75,000. Dextran is produced by the action of certain strains of chain-forming microorganisms on sucrose: Hucker, Pederson, *N. Y. Agr. Expt. Sta., Tech. Bull.* **167**, 3 (1930). These microorganisms are classified as: family *Lactobacteriaceae*, tribe *Streptococceae*, genus *Leuconostoc*, species *L. mesenteroides* and *L. dextranicum*. Tarr, Hibbert, *Can. J. Res.* **5**, 414 (1931), have described a method for the prepn of dextran. The nutrient soln (pH 8.0) consists of 10% sucrose, 0.1% peptone, 0.1% potassium chloride, and 0.2% disodium hydrogen phosphate. The yield may be increased by the addition of tomato juice, yeast extract, maple syrup and molasses. *See also* Novak, Stoycos, U.S. pat. 2,841,578 (1958 to Commonwealth Engineering Co.). Enzymic synthesis: Sugg, Hehre, *J. Immunol.* **43**, 119 (1942). Behrens, Ringpfeil, U.S. pat. 3,044,940 (1962 to Serum Werk Bernburg). The crude dextran may be isolated from the culture by precipitation with methanol. Continuous dialysis process: Shurter, U.S. pat. 2,717,853 (1955 to Commercial Solvents). Elimination of pyrogens: Levi, Lozinski, U.S. pat. 2,762,727 (1956 to Charles E. Frosst & Co.). Structure: Fowler *et al.*, *Can. J. Res.* **B15**, 486 (1937). Their results indicate that dextran is made up entirely of D-glucose units; there is a cross linkage for every repeating unit of five glucose molecules, and 1,6 linkages within each repeating unit. Fairhead *et al.*, *Can. J. Res.* **B16**, 151 (1938). Peat *et al.*, *J. Chem. Soc.* **1939**, 581, apparently encounter different dextrans, but all authors agree that dextran is a linear glucose polymer in which the terminal units of one chain are in chemical union with members of adjacent chains to form a network structure. Structural studies of dextrans: Goldstein, Whelan, *ibid.* **1962**, 170, 176. Method of producing clinical dextran: Novak, Witt, U.S. pat. 2,972,567 (1961).

USE: In soft center confections, as a partial substitute for barley malt. Mixed ethers and esters of dextran can be used in lacquers.

MED USE: Plasma expander for emergency treatment of shock; not a substitute for whole blood.

VET USE: Blood substitute in animals requiring increased blood vol (vascular shock). *Dose:* 2–20 ml/lb body weight iv (6% soln in saline soln). *Caution:* Epinephrine should be on hand to control possible reactions.

Dextran Iron Complex. Iron-dextran complex; Imferon; Fenate. Prepn: London, Twigg, U.S. pat. 2,820,740 (1958 to Benger Labs. Ltd.); U.S. reissue 24,642 (1959); Herb, U.S. pat. 2,885,393 (1959 to Laros Co.). Marketed as a soln contg 2–5% elemental iron. LD_{50} i.v. in mice: 1 g Fe/kg.

MED USE: In iron deficiency anemia when parenteral (im) administration is indicated.

VET USE: For iron deficiency anemia, particularly in baby pig anemia.

Dextran Sulfate Sodium. Dextrarine; dexulate; dextran sulfuric acid ester sodium salt. Mol wt about 7300. Contains about 17% S. Has up to three sulfate groups per glucose unit. Prepd by boiling dextran with sulfuric acid to reduce the mol wt and esterifying the degraded dextran with chlorosulfonic acid in pyridine: Ricketts, Walton, U.S. pat. 2,715,091 (1955 to Nat. Res. Dev. Corp.).

White powder from alcohol + ether. Freely sol in water. Activity about 17 international heparin units/mg. Aq solns must be buffered (*e.g.*, with sodium bicarbonate) to prevent dex during autoclaving.

MED USE: Has been used as anticoagulant.

Dextri-Maltose®. Maltose and dextrins obtained by enzymic action of barley malt on corn flour.

Light, amorphous powder. Readily sol in water or milk. One leveled tablespoonful (8 grams) supplies 27 calories.

USE: As carbohydrate modifier for use with milk and milk products in infants' formulas.

Dextrin. British gum; starch gum; gommelin; Leiocom; Fortodex. $(C_6H_{10}O_5)_n.xH_2O$. Produced by the incomplete hydrolysis of starch with dil acids, or by heating dry starch.

White or yellow, amorphous powder. The white is practically odorless; the yellow has a characteristic odor. It is dextrorotatory. $[\alpha]_D$ generally above 200°. Soluble in 3 parts boiling water to a gummy soln; less sol in cold water; insol in alcohol and ether. Does not reduce Fehling's soln; gives a reddish-brown color with iodine.

USE: Excipient for dry extracts and pills; for preparing emulsions and dry bandages; for thickening dye pastes and mordants used in printing fabrics in fast colors; sizing paper and fabrics; printing tapestries; preparing felt; manuf printer's inks, glues and mucilage; polishing cereals; in matches, fireworks, and explosives.

Dextroamphetamine Sulfate. *d*-Amphetamine sulfate; *d-α-methylphenethylamine sulfate;* *d*-1-phenyl-2-aminopropane sulfate; *d*-β-phenylisopropylamine sulfate; Dexedrine Sulfate; Afatin; Dexamphetamine; *d*-Amfetasul; Domafate; Obesedrin; Dexten; Maxiton; Sympamin; Simpamina-D; Albemap; Dadex; Ardex; Dexalone; Amsustain; Betafedrina; *d*-Betaphedrine; Diocurb. $[C_6H_5CH_2CH(NH_2)CH_3]_2.H_2SO_4$; mol wt 368.49. $(C_9H_{13}N)_2.H_2SO_4$; C 58.67%, H 7.66%, N 7.60%, S 8.70%, O 17.37%. *See* Amphetamine for structural formula. Prepn by resolution of amphetamine with *d*-tartaric acid followed by treatment with 10% H_2SO_4: Temmler, Brit. pat. 508,757 (1939); Nabenhauer, U.S. pat. 2,276,508 (1942 to Smith, Kline & French); Magidson, Garkusha, *J. Gen. Chem.* (*U.S.S.R.*) **11**, 339 (1941).

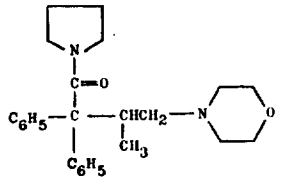
Plates, rods [different from amphetamine sulfate crystals, *see* Keenan, *J. Am. Pharm. Assoc.* **37**, 519 (1948)]. Slightly bitter taste followed by a sensation of numbness. $[\alpha]_D^{20}$ +21.8° (c = 2). mp above 300°. One part dissolves in approx 10 parts of water, in 500 parts of 95% alcohol. A soln of one gram in 10 ml water has a pH between 5.0 and 6.0. LD_{50} s.c. in rats: 200 mg/kg.

A compd formed from dextroamphetamine and carboxymethylcellulose is marketed as ***Carboxyphen,*** mol wt 568,000. About 40 mg of Carboxyphen equals 15 mg of dextroamphetamine sulfate.

MED USE: Sympathomimetic, CNS stimulant. *Dose:* Oral 5 mg. *Side Effects: See* Amphetamine Sulfate.

VET USE: As analeptic in horses, cattle, dogs; as stimulant and for weight reduction in dogs, cats.

Dextromoramide. ***1-(3-Methyl-4-morpholino-2,2-diphenylbutyryl)pyrrolidine;*** 4-[2-methyl-4-oxo-3,3-diphenyl-4-(1-pyrrolidinyl)butyl]morpholine; *d*-morpholylmethyldiphenylbutyrylpyrrolidine; (+)-1-(3-methyl-4-morpholino-2,2-diphenylbutyryl)pyrrolidine; *d*-2,2-diphenyl-3-methyl-4-morpholinobutyrylpyrrolidine; *d*-3-methyl-2,2-diphenyl-4-morpholinobutyrylpyrrolidine; *D*-Moramide; R-875; Palfium; Palphium; Jetrium; Dimorlin; SKF 5137; pyrrolamidol. $C_{25}H_{32}N_2O_2$; mol wt 392.52. C 76.49%, H 8.22%, N 7.14%, O 8.15%. Synthesis: Janssen, *J. Am. Chem. Soc.* **78**, 3862 (1956); Brit. pat. 822,055 (1959 to Lab. Pharmaceutica Dr. C. Janssen N.V.).



Crystals, mp 180–184°. $[\alpha]_D^{20}$ +25.5° (c = 5 in benzene). Absorption max in 0.01*N* isopropanol-HCl: 254, 260, 264 mμ. Practically insol in water. Solubility in 0.1*N* HCl = 1:25 (w/v). Solubility in ethanol 50 g/100 ml; in methanol 40 g/100 ml; in acetone 50 g/100 ml; in ethyl acetate 40 g/100 ml; in benzene 5 g/100 ml; in chloroform 5 g/100 ml. Soluble in ether.