CASEIN #108

Casein-Volume 1 #108 6/29/74

#108

VOLUME 1

GRAS MONOGRAPH SERIES

CASEIN

prepared for

THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION
AND WELFARE

JULY 29, 1974

prepared by

Tracor Jitco, Inc.

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This publication was prepared under Contract Number FDA 72-100
with the Public Health Service, Food and Drug Administration,
Department of Health, Education, and Welfare

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SUMMARY

Acute Toxicity

Purified casein is non-toxic at high levels. Oral MLD values in rats of 30,000-38,000 mg/kg BW have been found but death was due to aspiration asphyxia or gastric rupture (0180). The amount of purified casein that can be tolerated appears to be dependent on the mass that the stomach can accommodate (0180). The estimated oral LD_{50} is well over 1,000,000 mg/kg BW (0180). Sodium and calcium caseinates have oral MLDs of 75,000 mg/kg BW in rats (0180). The indicated oral LD_{50} value is in the order of 400,000 to 500,000 mg/kg BW (0180).

Short-term studies

Small amounts of casein administered to experimental animals over a long period of time cause amyloidosis, a disease characterized by deposits of a glycoprotein (amyloid) in spleen, kidneys, liver and other organs (0259). The disease occurs spontaneously also in man and laboratory animals. Immunologic phenomena, and toxic products of microbial contaminants, have been suggested as mechanisms (1401).

Glomerular and tubular kidney damage has been observed in animals fed high supplements of casein (0832).

Casein at a level of 18% in the diet was found to be very effective in building hemoglobin and protecting rats from anemia and granulocytopenia (0779).

Kittens on a semi-purified basal diet containing vitamin-free casein developed conjunctivitis, xerosis, keratitis, vascularization of the cornea, cataracts, and progressive destruction of the visual cells of the retina in spite of vitamin A supplements in amounts adequate for other test diets (1334). It has been suggested that the amino acid patrern may be unsuitable for cats (1334).

in vitro with digestive juices from the dog (1591). *In vivo* studies with Casein-I^{131} revealed maximum radioactivity in the blood stream in about 2 hours (1591). In the calf, casein digestion began soon after ingestion, and P^{32} was detected in the blood stream within 15 minutes although maximum levels were not reached until 13-23 hours later (0895). Liver, kidneys, rumen, omasum, and jejunum showed rapid rates of P^{32} exchange (0895).

Sodium caseinate infused rumenally or abosmally at a level of 900 mg/kg BW/day increased milk yield significantly, and the protein and nitrogen content and dry matter digestibility as well. The sequence of limiting amino acids for the lactating dairy cow was phenylalanine, methionine, lysine, threonine, leucine, isoleucine, histidine, valine, and arginine (0324).

Drug Interaction

Casein at a level of 30% in the diet reduced significantly the amount of kidney damage in rats due to therapy with 1% sulfadiazine (0780). Casein also protected rabbits from hyperglycemia in acetoacetate-induced diabetes (1067).

Consumer Exposure

Casein and caseinates are regarded as "complete" proteins with the proper amino acid composition for adequate nutrition and growth (1412). Large quantities are used in the preparation of infant formulas, children's foods, diets for the elderly, sick, and convalescent, diabetic foods, diets for the obese, and in calf starters and other animal feeds (1412).

Estimated daily intakes of casein from milk products range from 14 grams for children and adults to 33 grams for infants. Estimated maximum daily intakes are 54 and 140 grams, respectively (0420).

Calcium caseinate levels are 2 and 6 grams on the average and 7 and 17 grams maximum, for the above groups (0420).

Estimated average daily intakes of sodium caseinate from all food categories are 5 grams for infants and 7 grams for children and adults. Maximum intakes are estimated to be 13 and 14 grams, respectively, for the two groups (0421).

Foods in which sodium caseinate is used to the maximum level are milk products 9%, imitation dairy products and breakfast cereals 4.5%, and cheese 4% (0418). The total annual poundage reported to NAS and FEMA in 1972 was 13,231,488 pounds (0417).

In 1972, 105,401,000 pounds of casein were imported from abroad. Imports over the past ten years ranged from 87,878,000 pounds in 1963 to 135,288,000 pounds in 1970 (1474).

Casein is one of the substances that migrate to food by virtue of its wide use in the manufacture of synthetic textile fibers and paper coatings and glazes (1388).

Table 1

Amino Acid Composition of Casein (1094)

Name	Formula	%	Name	Formula	%
Alanine	$C_3H_7NO_2$	2.6	Lysine	$C_6H_{14}N_2O_2$	7.3
Arginine	$C_6H_{14}N_4O_2$	3.6	Methionine	$C_5H_{11}NO_2S$	2.5
Aspartic acid	$C_4H_7NO_4$	6.3	Phenylalanine	$C_9H_{11}NO_2$	4.4
Cystine	$C_6H_{12}N_2O_4S_2$	0.3	Proline	$C_5H_9NO_2$	10.1
Glutamic acid	$C_5H_9NO_4$	20.0	Serine	$C_3H_7NO_3$	5.6
Glycine	$C_2H_5NO_2$	2.4	Threonine	$C_4H_9NO_3$	4.3
Histidine	$C_6H_9N_3O_2$	2.7	Tryptophan	$C_{11}H_{12}N_2O_2$	1.1
Leucine	$C_6H_{13}NO_2$	8.2	Tyrosine	$C_9H_{11}NO_3$	5.6
Isoleucine	$C_6H_{13}NO_2$	5.4	Valine	$C_5H_{11}NO_2$	6.4

B. Calcium caseinate

Calcium 1.6%, amino acid components same as for casein above (1096).

C. Magnesium caseinate

No information.

D. Manganese caseinate

No information.

E. Potassium caseinate

No information.

F. Sodium caseinate

Sodium 1.3%; amino acid components same as for casein above (1095).

III. Structural formula

None

IV. Molecular Weight (Polymer weight)

A. Casein: 75,000 to 375,000. Varies according to purity (1412).

B. Calcium caseinate: No information.

C. Magnesium caseinate: No information.

D. Manganese caseinate: No information.

E. Potassium caseinate: No information.

F. Sodium caseinate: No information.

V. Specifications

A. Casein

Casein is manufactured in several grades of varying degrees of purity: certified, vitamin-free, high protein, purified, acid, rennet and so forth.

The United States Department of Agriculture (0050) has established the following U. S. Standards for grades of edible dry casein (acid):

U. S. Extra Grade Requirements

U. S. Extra Grade edible dry casein (acid) shall conform to the following requirements:

(a) Flavor and odor. Bland natural flavor and odor and free from offensive flavors and odors such as sour or cheesy.

(b) Physical appearance. Is white to cream colored; if pulverized, free from lumps that do not break up under slight pressure.

(c) Bacterial estimates. Standard plate count not more than 30,000 per gram. Coliform count negative per 0.1 gram.

(d) Protein content. Not less than 95 per cent, dry basis, (Nitrogen X 6.38).

(e) Moisture content. Not more than 10 per cent.

(f) Milk fat content. Not more than 1.5 per cent.

(g) Extraneous materials. Scorched particles not more 15 mg. and free from foreign materials in 25 grams.

(h) Free acid. Titrated to not more than 0.20 ml of 0.1N NaOH per gram.

U. S. Standard Grade Requirements

U. S. Standard Grade edible dry casein (acid) shall conform to the following requirements:

(a) Flavor and odor. Not more than slight unnatural flavors or odors and free from offensive flavors and odors such as sour or cheesy.

(b) Physical appearance. Is white to cream colored; if pulverized, free from lumps that do not break up under moderate pressure.

(c) Bacterial estimates. Standard plate count not more than 100,000 per gram. Coliform count not more than 2 per 0.1 gram.

(d) Protein content. Not less than 90 per cent, dry basis (Nitrogen X 6.38).

(e) Moisture content. Not more than 12 per cent.

(f) Milk fat content. Not more than 2 per cent.

(g) Extraneous material. Scorched particles not more 22.5 mg and free from foreign materials in 25 grams.

(h) Free acid. Titrated to not more than 0.27 ml of 0.1 N NaOH per gram

F. Sodium caseinate

SODIUM CASEINATE (1095)

TYPICAL ANALYSIS:

PROTEIN (N x 6.38) (dry basis)	92.5 - 94.5%
MOISTURE	5.0%
ASH	4.0%
FAT	1.5%
SODIUM	1.3%

AMINO ACID CONTENT: (calculated)	Per Cent
ALANINE	2.7
ARGININE	3.7
ASPARTIC ACID	6.4
CYSTINE	0.3
GLYCINE	2.4
GLUTAMIC ACID	20.2
HISTIDINE	2.8
ISOLEUCINE	5.5
LEUCINE	8.3
LYSINE	7.4
METHIONINE	2.5
PHENYLALANINE	4.5
PROLINE	10.2
SERINE	5.7
THREONINE	4.4
TRYPTOPHANE	1.1
TYROSINE	5.7
VALINE	6.5

VI. Description

A. Casein

Casein is a white or light cream-colored amorphous powder with a clean characteristic milk-like odor (1094, 1412).

It is very sparingly soluble in water and nonpolar organic solvents but dissolves readily in aqueous solutions of alkalies (1388).

Physical constants are (1412):

Specific Gravity: 1.25-1.31

Specific Rotation: D -61.7° to -92.2° (Levorotatory)

Refractive Index: 1.675

Casein may be stored safely up to 2 1/2 years if packed in clean, closely woven burlap bags or multiply paper bags in a clean, dry warehouse at a temperature below 80°F and a relative humidity of 65% or less. Higher temperatures and moisture favor microbial spoilage (1412).

The Warth incubation test is widely used to indicate probable storage safety of lots of casein:

> Fifteen grams of the sample is placed in 90 ml of sterile water in a cotton-stoppered flask and incubated at 37.5°C. A good product will stand up well for at least 48 hours and will show no sign of marked decomposition under 72 hours (1412).

Spoilage of casein under satisfactory storage conditions is usually due to poor quality or unsatisfactory processing conditions (1412).

Molds, moth larvae, and beetles are the most troublesome pests. Sulfur dioxide fumigation of warehouses is resorted to sometimes. Exteriors of bags are sprayed with sodium cresylate on occasion (1412).

B. Calcium caseinate

Calcium caseinate is an amorphous white powder.

C. Magnesium caseinate

No information.

D. Manganese caseinate

No information.

E. Potassium caseinate

No information.

F. Sodium caseinate

Sodium caseinate is an amorphous white powder with a clean bland flavor.

VII. Analytical Methods

The official AOAC (0084) quantitative analytical method for casein involves dissolving a representative sample in water, precipitating the casein with acetic acid and determining nitrogen on the washed precipitate by the Kjeldahl method. N X 6.38 gives the percentage of "casein" (total protein).

The major components of casein (α-, β-, and κ-casein) can be determined quantitatively by column chromatography of alkylated samples on DEAE-cellulose in the presence of urea by a procedure recently developed by Rose et al. (1256). The estimated accuracy of the method is 5% with reproducibility within 2 or 3%.

The authors point out that the procedure is applicable to a wide variety of casein preparations and that it is not affected by minor components of casein and varying amounts of carbyhydrate in κ-casein which influence the results with certain other methods.

VIII. Occurrence and levels

A. Plants

Casein and caseinates do not occur in the plant kingdom.

B. Animals

Casein is synthesized and secreted by the mammary gland of the lactating mammal. It exists in milk as a stable suspension of tiny calcium-protein-phosphorus particles, about 100mμ in diameter, referred to as micelles (0269). The actual amount in the milk of different species varies considerably (0975).

Human milk	- 0.4%
Cow's milk	- 2.8%
Goat milk	- 2.5%
Sheep milk	- 4.6%
Horse milk	- 1.3%
Llama milk	- 6.2%

Commercial casein is prepared from skim milk by precipitation with acid at pH 4.6 or by means of the enzyme rennin (1412).

Calcium, magnesium, manganese, potassium, and sodium caseinates are prepared by treating a good quality acid casein with the appropriate hydroxide ($Ca(OH)_2$, NaOH, etc.) to a pH of about 6.7. The solutions are then spray dried or roller dried and milled (0975, 1412).

C. Synthetics

Casein and caseinates are widely employed in the food industry as foods, emulsifiers, binders, stabilizers, and texturizers. At least 26 foods on the U. S. market contain one or more as nutrients or processing aids (0422, 1412).

Large quantities of casein are used also in the manufacture of plastics, synthetic textile fibers, paper coatings and glazes, adhesives, paints, and leather finishes (1388, 1412).

D. Inorganic sources

None.

BIOLOGICAL DATA

I. Acute Toxicity

Rats

Boyd et al. (0180) determined the oral (by intragastric canula) LD_{50} values of casein, vitamin-free casein, high-protein casein, sodium caseinate, calcium caseinate, in young male albino rats (Wistar strain), weighing 150-200 grams, that had been fasted overnight. Ten to 20 animals were used per group. The preparations were given in increasing amounts until death occurred. Distilled water was used as the vehicle excepting for casein (certified) which was dissolved in 0.12% ammonium hydroxide. Vehicle controls were included.

The animals were observed daily for clinical signs of toxicity. Body-weight, food consumption, water intake, colonic temperature, urinary volume, urinary glucose, protein output, and urinary pH were determined also each day.

Following death or sacrifice, gross pathologic changes were noted, organ weights determined, and histopathologic findings recorded. Mean differences from controls were analyzed by the t test for statistical significance. The results are summarized below and in Acute Toxicity Table 2. (See original article for clinical and histopathologic details).

The MLD of casein (certified), 30,000 mg/kg BW, killed 30% of the rats in 30 minutes to 14 hours. Death was caused by aspiration asphyxia or by gastric rupture!

The MLD of vitamin-free and high-protein casein was found to be 37,500 mg/kg BW. Death was caused by gastric rupture. Survivors exhibited weight loss, anorexia, diuresis, proteinuria, aciduria, and listlessness.

The authors estimated that casein has an LD_{50} of well over 1,000,000 mg/kg BW. They stated that the ability to survive large amounts of "natural" casein (certified, high-protein, or vitamin-free) appears to be limited mainly by the inability of the stomach to accomodate the large bulk.

The MLD of sodium caseinate was 75,000 mg/kg BW which killed 2 of 10 rats within 24 hours. Death was preceded by marked weight loss, anorexia, diuresis, proteinuria, alkaline urine, diarrhea, hypothermia, and listlessness.

Table 2
Acute Toxicity Table

(by substance or constituent thereof)

SUBSTANCE	ANIMAL (Species)	SEX & No. (M or F)	ROUTE (p.o., i.v., s.c., i.p., i.m., other)	DOSAGE mg/kg body wt.	MEASUREMENT (LD50, ED50 or other)	Ref. Bibliogr. No.
Casein (Certified)	Rats (Wistar)	20 males	p.o.	30,000	MLD	Boyd (0180)
Casein (High Protein)	Rats (Wistar)	20 males	p.o.	37,500	MLD	Boyd (0180)
Casein (Vitamin-free)	Rats (Wistar)	20 males	p.o.	37,500	MLD	Boyd (0180)
Sodium Caseinate	Rats (Wistar)	10 males	p.o.	75,000	MLD	Boyd (0180)
Calcium Caseinate	Rats (Wistar)	10 males	p.o.	75,000	MLD	Boyd (0180)

Twenty four hours after the last injection, the animals were sacrificed and studied grossly and histologically. The data are presented in Tables 3 and 4.

Table 3

Hepatic and Splenic Amyloidosis* (0676)

Material injected §	Amount injected (mgm)	Day killed	Weight gain (gm)	Organ weights † (% body weight) Liver	Spleen	Incidence of amyloidosis Liver	Spleen
No injection (5)	—	62	8.0 ± 1.4	5.1 ± 0.31	0.31 ± 0.017	—	—
Subcutaneous injections							
$NaHCO_3$ (5)	—	14	1.8 ± 0.37	5.7 ± 0.48	0.37 ± 0.025	—	—
" (7)	—	21-23	1.5 ± 0.83	5.2 ± 0.33	0.13 ± 0.063	—	—
" (4)	—	28	3.4 ± 0.26	5.5 ± 0.2	0.31 ± 0.055	—	—
" (9)	—	35-41	3.5 ± 0.7	5.3 ± 0.16	0.30 ± 0.06	—	—
" (4)	—	51	3.1 ± 0.62	5.5 ± 0.22	0.30 ± 0.017	—	—
" (8)	—	70	4.2 ± 0.85	5.7 ± 0.64	0.34 ± 0.024	—	—
Casein (5)	238	14	1.1 ± 0.66	5.9 ± 0.34	0.37 ± 0.077	—	—
" (14)	337-388	21-23	1.8 ± 0.7	5.5 ± 0.3	0.42 ± 0.08	—	—
" (6)	460	28	2.3 ± 0.59	6.2 ± 0.27	0.39 ± 0.06	—	4/6
" (8)	520	35	—	—	—	6/8	8/8
" (14)	600-675	35-41	2.7 ± 1.34	6.0 ± 0.23	0.54 ± 0.077	13/14	14/14
" (6)	862	51	2.7 ± 1.34	7.3 ± 0.94	0.64 ± 0.052	6/6	6/6
" (6)	1151	79	1.6 ± 0.87	8.0 ± 0.46	0.71 ± 0.077	6/6	6/6
Casein Hydrolysate (5)	600	35	2.5 ± 0.5	5.6 ± 0.05	0.35 ± 0.083	—	—
" (5)	720	41	4.9 ± 1.58	5.3 ± 0.09	0.44 ± 0.087	—	—
" (5)	1320	62	6.6 ± 1.75	5.3 ± 0.31	0.35 ± 0.028	—	—
Gelatin (5)	600	35	3.1 ± 0.78	5.4 ± 0.27	0.30 ± 0.054	—	—
" (5)	720	41	4.3 ± 1.26	5.6 ± 0.17	0.37 ± 0.035	—	—
" (5)	1320	62	4.7 ± 0.91	5.5 ± 0.22	0.41 ± 0.041	—	—
" (4)	1600	35	5.9 ± 0.81	5.6 ± 0.42	0.45 ± 0.067	—	—
Intraperitoneal injections							
$NaHCO_3$ (4)	—	35-41	3.2 ± 0.36	5.2 ± 0.2	0.32 ± 0.04	—	—
" (4)	—	62	4.4 ± 1.7	5.6 ± 0.51	0.41 ± 0.052	—	—
Casein (5)	600	35	1.1 ± 1.08	6.1 ± 0.44	0.4 ± 0.1	4/5	5/5
" (4)	720	41	2.9 ± 0.63	5.8 ± 0.4	0.44 ± 0.07	4/4	4/4
Casein Hydrolysate (5)	1320	62	5.2 ± 2.5	5.5 ± 0.24	0.36 ± 0.023	—	—
Gelatin (5)	1320	62	5.3 ± 1.34	5.4 ± 0.22	0.33 ± 0.017	—	—

* Mice given daily injections (casein, casein hydrolysate and gelatin) 5 days a week for varying periods of time. All mice killed 24 hours after last injection.

† All weight values represent means with standard deviations.

§ Number of animals in parenthesis.

Table 4

Incidence of Hepatic and Splenic Amyloidosis* (0676)

Number of mice	Amount injected (mgm)	Day killed	Incidence of amyloidosis Liver	Spleen
5	500	22	4/5	5/5
5	648	29	5/5	5/5

* Mice given daily injections of casein 7 consecutive days a week for 3 and 4 weeks, and killed 24 hours after the last injection.

Although gelatin and casein hydrolysate failed to cause amyloidosis in contrast to casein, the author pointed out that involvement of an immune mechanism in the disease was by no means clear. In addition to the occurrence of spontaneous amyloidosis in several species, its induction by such diverse substances as phenol, turpentine, cholesterol, vitamin C deficiency, and goitrogens, further complicates the subject of mechanisms.

3. Ranlov et al. (1207) transferred isotopically-labelled (H^3) spleen cells from mice hyperimmunized with casein i.v. to normal syngenic mice subsequently treated with nitrogen mustard in an effort to determine the route of migration of the donor cells and their possible relation to amyloid deposits in the resultant amyloidosis.

A spleen cell suspension containing 10^8 cells/0.5 ml, from pooled spleens of 12 inbred C3H mice which had been immunized with 17 daily s.c. injections of 0.5 ml of a 5% sodium caseinate solution and two injections of 0.5 microcurie of H^3-thymidine, was prepared. Twenty-six normal syngenic mice were then given 10^8 labelled nucleated spleen cells i.v. Six animals that died within 15 minutes of the injection constituted the Group 0. The remaining 20 mice were given an injection of 0.05 mg of nitrogen mustard (Erasol R). Five mice were then sacrificed after 1, 2, 3, and 4 days and studied histologically and radiographically.

All recipient mice developed splenic amyloidosis one to four days after transfer of labelled spleen cells from the casein-immunized donor mice. Autoradiographic determinations indicated that the major part of the hyperimmunized donor spleen cells apparently migrated to the perifollicular regions of the recipient spleen within 2-3 days. The appearance of donor spleen cells coincided with amyloid deposits in the same region. Amyloid deposition was found only in the spleen.

4. Stora et al. (1401) investigated the effect of Bacillus cereus on the production of experimental anyloidosis in the mouse by casein and sodium caseinate. The study was prompted by the discovery of the organism in several lots of commercial purified casein (Hammersten).

Swiss mice, 5 weeks of age, 26 to 50 per group, were injected s.c. with 0.3 ml of sterilized or non-sterilized 10% casein or sodium caseinate five times each week. A total of 12 casein and 40 sodium caseinate injections were given. Equal numbers of non-treated control animals were also included

in each experiment. Terramycin (50 mg/kg BW) was administered to the control and test mice to prevent bacterial infections.

None of the animals injected with sterile sodium caseinate or azo-casein showed any evidence of amyloidosis. Non-sterile sodium caseinate, however, caused diffuse splenic amyloidosis in 22 of 26 animals injected, and 15 of these showed hepatic amyloidosis also. Only 5 of 20 animals survived the non-sterile azo-casein injection; all had typical diffuse amyloidosis. The authors discussed the probable mechanism of the Bacillus cereus effect.

B. Rats

Louhija et al. (0900) injected s.c. 17 male Sprague-Dawley rats weighing 175 to 265 g every third day for 5 months with 3 ml of a 3-3.5% casein solution. Twelve control animals were injected in like manner with 0.9% sodium chloride solution. Blood samples were collected prior to injections, and after 1, 2 1/2, and 5 months. All animals were sacrificed at the end of the 5th month and the heart, spleen, and kidneys studied histologically. Sialic acid and electrophoretic distribution of protein-bound carbohydrates were determined on the blood specimens.

All organs were found to be negative for amyloidosis, thus confirming previous evidence that the rat is insusceptible to this disorder. Casein treatment caused a significant increase in the serum sialic acid concentration up to one month, followed by a gradual decline to control values by the fifth month in spite of continuous administration of casein. An elevation of glycoprotein in the α_1-, α_2-, and particularly in the β-globulin, fractions was found also. The relationship between amyloidosis and hyperproteinemia was discussed.

C. Hamsters

Irwin et al. (0656) studied the influence of low and normal casein diets on amyloidosis induced by Leishmania donovani infection in hamsters. The authors pointed out that several chronic diseases like leprosy, tuberculosis, and rheumatoid arthritis, predispose patients to amyloidosis. Moreover, since the disease is much more prevalent in American leprosy patients than in Mexican sufferers, a dietary factor has been postulated.

Sixty-two male Golden hamsters, 4 months of age, were inoculated i.v. with a dose of Leishmania donovani containing approximately 5 parasites. Thirty-five inoculated animals were then placed on a low casein diet (8%) and

the remaining 27 on a normal casein diet (27%). Seven uninfected animals were included as controls on each diet.

Several hamsters from each group, selected at random, were sacrificed at 8, 20, and 28 weeks post-inoculation. The remainder were allowed to die of the infection. All animals were autopsied and spleen, liver, kidneys, adrenals, heart, and gastro-intestinal tract examined grossly and microscopically for evidence of infection and amyloid deposits.

Seventy-five per cent of the animals on the normal casein diet developed amyloidosis with extensive deposits in liver, spleen, adrenals, and kidneys. Hamsters on the low casein diet, on the other hand, showed a lower incidence of amyloidosis ($P < 0.001$) with delay in appearance of amyloid deposits. There was little correlation, however, between the concentration of parasites in the spleen and the presence of amyloid.

D. Rabbits

Cohen et al. (0259) determined the incidence and histopathology of casein-induced amyloidosis in the rabbit. Thirty-four New Zealand white female rabbits were injected s.c. with 5 ml portions of sodium caseinate (10% aqueous) twice weekly over a period of 12 months. A group of 8 untreated animals were included as controls. The diet consisted of Purina Rabbit Chow (15% protein) with water ad libitum.

Animals were sacrificed at monthly intervals and examined for amyloid development by several standard procedures to determine the sequence of tissue alterations and degree of organ involvement (See original article for details). The incidence and extent of the disease in the casein-treated animals is presented in Table 5. No evidence of the condition was detected in any of the untreated control animals.

The earliest site of amyloid deposition was the spleen. All animals given casein injections for 4 months or more showed deposits. Although the extent of involvement was variable, the spleen of some animals showed over 50% replacement with amyloid. Kidney involvement was invariable after 5 months with deposits primarily glomerular in location. The degree of amyloid involvement here, too, varied from animal to animal. Liver involvement was not marked; replacement never exceeded 50% of the organ.

The authors pointed out that the overall distribution in casein-induced anyloidosis in the rabbit was parenchymal, and thus resembled the so-called "secondary" amyloidosis in man.

Table 5

Incidence and Extent of Casein-Induced Amyloidosis in 42 Rabbits Examined at Monthly Intervals* (0259)

Rabbit no.	Months of casein†	Amount of amyloid‡		
		Spleen	Kidney	Liver
164	0	0	0	0
165	0	0	0	0
182	0	0	0	0
184	0	0	0	0
186	0	0	0	0
188	0	0	0	0
191	0	0	0	0
196	0	0	0	0
120	1	0	0	0
127	1	0	0	0
156	1	0	0	0
171	1	0	0	0
175	1	0	0	0
178	1	0	0	0
98	2	2+	0	0
100	2	2+	0	0
111	2	0	0	0
121	2	0	0	0
123	2	0	0	0
136	2	0	0	0
148	2	1+	0	0
150	2	0	0	0
113	3	4+	4+	2+
116	3	3+	1+	0
132	3	3+	0	0
170	3	2+	0	0
172	3	0	0	0
174	3	0	0	0
67	4	4+	0	1+
115	4	4+	2+	2+
97	5	4+	1+	0
99	5	3+	2+	1+
47	6	2+	2+	0
56	6	4+	2+	0
58	6	3+	4+	0
87	6	4+	4+	0
89	6	3+	3+	0
94	6	4+	2+	1+
92	7	4+	2+	1+
59	9	3+	3+	0
14	10	2+	3+	0
8	12	4+	1+	0

* The first 8 rabbits are controls.

† All time intervals adjusted to the nearest month.

‡ Graded as follows:

0 = no amyloid
1+ = 1 to 25 per cent replacement of the organ
2+ = 26 to 50 per cent replacement
3+ = 51 to 75 per cent replacement
4+ = 76 to 100 per cent replacement

Kidney Damage

High concentrations of dietary casein have caused glomerular and tubular renal damage in a relatively short period of time in experimental animals, in addition to amyloidosis where relatively long exposures are usually required.

A. Mice

Nayak et al. (1068) studied the effect of low, intermediate, and high protein (casein) diets on the liver and kidneys in mice. Mice of three different strains were used in the overall study but all of the pertinent results were obtained with the C3H line. Males, or mixed males and females, approximately 8 weeks old, in groups of 6 or 10, were placed on either a high protein diet (20% vitamin-free casein), an intermediate diet (12% vitamin-free casein), or a low protein diet (4% vitamin-free casein), for a period of 100 days. Control mice were fed Purina Laboratory Chow. At the conclusion of the experimental period, the animals were sacrificed and the vital organs studied grossly and microscopically.

A moderate degree of renal tubular degeneration and necrosis was found in C3H mice on the high casein (20%) diet but not in the Hauschka strain. Amyloidosis was observed in CFW and Hauschka lines. Periportal fatty changes and mild cytological alterations were detected in the liver, but there was no evidence of fibrosis or cirrhosis.

B. Rats

1. Lalich et al. (0832) investigated the effect of dietary protein overload with casein, peanut meal, or soybean meal, on renal glomerulosclerosis in partially-nephrectomized rats.

Male Sprague-Dawley rats weighing 100-110 g were partially nephrectomized (left kidney, and in some instances, part of the right organ) and placed on a commercial diet (25%) supplemented with 200 g/kg of crude casein, soybean meal, or peanut meal for a period of 151-254 days. Non-nephrectomized animals and basal diet controls were included also. The animals were observed for toxic signs and weight changes throughout the feeding period. At the end of the treatment, all animals were sacrificed and the kidneys removed, weighed, and subjected to a detailed histopathologic study.

The authors pointed out that their findings supported previous observations that excess protein in the diet induced degeneration of the glomeruli and tubules in the rat kidney. The severity of the protein nephropathy seemed

to be related to the nature of the protein overload; soybean meal caused the greatest amount of damage.

2. Hay et al. (0564) investigated the effect of diet on nephrosclerosis in rats induced by lyophilized anterior pituitary extract (L.A.P.)

Groups of ten castrated, partially-nephrectomized, male piebald rats, weighing 40 to 60 g, were placed on test diets 1 or 2 (See Table 6) and injected daily with 25 mg of L.A.P. for 21 days. Food consumption and water intake were determined during the experimental period. At autopsy, the fixed adrenals and kidneys were weighed and the incidence and severity of nephrosclerosis diagnosed histopathologically.

A similar experiment was conducted also to determine the effect of substituting various proteins (gelatin, lactalbumin, zein, egg albumin, and gluten) for casein in the test diet.

The results obtained indicated that under the experimental conditions of the study, nephrosclerosis developed only in those rats consuming diets containing 30% or more of casein, or 15% casein plus 15% of one of the other proteins tested. Casein, egg albumin, and wheat gluten, appeared to have a greater effect than lactalbumin, zein, or gelatin. A normal supply of vitamins had no protective effect against the high casein diet. No lesions were observed in any of the rats on diets containing only 15% casein.

Blood Changes

The influence of casein on blood components and on the blood-forming tissues has been the subject of numerous studies both in man and experimental animals.

A. Rats

1. Kornberg et al. (0779) studied the effect of casein and L. casei factor (L.C.F.) alone, and in combination, on anemia and granulocytopenia in rats.

Weanling Osborne-Mendel albino rats (other specifications not given), eight per group, were placed on a protein-free diet with and without L.C.F., in parallel with an 18% casein diet (See Table 7). Total white blood cell counts, polymorphonuclear granulocyte counts, and hematocrit determinations were made over a 28-day period. The results are presented in Table 7.

In a second experiment, rats with experimental granulocytopenia and anemia were treated with casein and L.C.F., alone, and combined, in an effort to correct the condition. These results are shown in Table 8.

Table 6

Composition of Two Basic Synthetic Diets (0564)

	Diet No. 1	Diet No. 2
Composition, %		
Cornstarch	54	69
Casein*	30	15
Fat**	1	1
Cod liver oil ***	1	1
Bulk†	1	1
Sodium chloride ††	4	4
Mineral mixture‡	4	4
Water †††	5	5
Supplements, mgm. per 100 gm. of diets		
Thiamine hydrochloride‡‡	0.4	
Riboflavin‡‡	0.4	
Pyridoxin‡‡	0.4	
Calcium pantothenate‡‡	2.0	
Nicotinic acid‡‡	1.0	
α-Tocopherol acetate‡‡	About 10 mgm. per rat once weekly introduced by dropper into mouth	

* *Not vitamin-free. It was considered that this would supply the choline requirement.*

** *Crisco, Domestic Shortening, or Primex.*

*** ***Ingram and Bell.***

† *Agar agar, acacia, or "Cellu-flour". When agar became unavailable we used acacia until Cellu-flour could be obtained. No difference was seen between them.*

†† *This was added to the diet to sensitize the rats to the nephrosclerotic activity of L.A.P. instead of giving it in the drinking water.*

††† *The starch and casein in the first lot of diets was found to contain an amount of water corresponding to 5% of the diet. On the dry weight basis these diets contain 31.6 and 15.8% protein, respectively. Subsequently the water content of the starch and casein was determined and the amount weighed out calculated to give 54% or 69% starch and 30% or 15% casein by dry weight.*

‡ *Slightly modified mixture recommended by Steenbock and Nelson (52).*

$NaCl$	23.4 gm.
$MgSO_4 . 7H_2O$	24.6 gm.
Na_2HPO_4	14.2 gm.
K_2HPO_4	69.6 gm.
$CaHPO_4 . 2H_2O$	69.8 gm.
Ca-lactate . $5H_2O$	15.4 gm.
Fe-citrate	1.2 gm.
KI	0.10 gm.

‡‡ ***Kindly supplied by Hoffman-LaRoche.***

Table 7

Granulocytopenia and Anemia in Rats Fed Protein-Free Diets and Prevention with Casein (0779)

Group*	Diet	No. of rats	No. of rats with granulocytopenia‡	No. of rats with anemia‡
A	0 per cent casein	8	8	6§
B	0 per cent casein — 20 γ L.C.F. daily†	8	8	7§
C	18 per cent casein — pair-fed with Group A	8	2	0
D	18 per cent casein	8	0	0

* The 4 groups were equal with respect to sex, litter, and weight distribution. Food intake was ad libitum in groups A, B, and D.

† This crystalline fermentation product (2) was administered by pipette to each rat from the outset of the experiment.

‡ Noted within 28 days.

§ The rats which failed to develop anemia died after 19 to 25 days.

Table 8

Treatment of Granulocytopenia (0779)

Treatment	No. of rats	No. of rats with positive response	Poly. granulocytes per cu. mm. (average)	
			Before treatment	After treatment
L.C.F.*—100 γ	8	2	350	750
L.C.F.†—200 γ subcutaneously	2	0	300	750
L.C.F.†—100 γ + L.C.F. conc.‡ —50 γ	3	0	33	417
Casein diets—18 per cent or 30 per cent	9	0	217	39
L.C.F.†—100 γ + L.C.F. conc.‡ —50 γ + casein diet—18 per cent	3	3	83	4,833
L.C.F.*—100 γ + casein diet—18 per cent	5	5	350	3,090
L.C.F.‖—100 γ + amino acid mixture	6	6	317	2,433

* Crystalline fermentation L.C.F. (2) or synthetic L.C.F. (Stokstad). No differences were observed between the activity of the two substances in these experiments.

† Crystalline fermentation L.C.F. (2).

‡ Contained in 0.058 grams of liver concentrate.

‖ Synthetic L.C.F. (Stokstad).

Severe granulocytopenia and anemia developed in rats on a protein-free diet. Casein at a level of 18% in the diet was very effective in preventing these dyscrasies but was incapable of effecting a cure after the condition had become established (Tables 7 and 8). L. casei factor was ineffective as a preventative or therapeutic agent. A combination of L.C.F. (50γ) and 18% casein, however, was highly effective in correcting both the anemia and granulocytopenia (Table 8).

2. Pearson et al. (1140) investigated the effect of casein and eight other proteins on the rate of hemoglobin regeneration in nutritional anemia in the rat.

Young rats (specifications not given) which had been made severely anemic (hemoglobin 3-4 g/100 ml blood) by an exclusive milk diet were then placed on test diets containing various proteins at a level of 17.5% approximately (See Table 9) and the effect on body weight and hemoglobin formation determined. Seven to ten rats were employed per group. The results obtained are presented in Tables 10 and 11.

Casein, liver, egg albumin, soybean oil meal, and rat blood were equally effective in building hemoglobin. Anemic rats on diets containing these proteins showed normal hemoglobin values within eight days (Tables 10 and 11).

Gelatin and gliadin were the least effective proteins tested in inducing hemoglobin regeneration. Corn gluten meal and wheat gluten were intermediate between the other two groups in effect on growth as well as on hemoglobin formation.

Table 9

Percentage Composition of Diets (1140)

Diet No.	100-P	101-P	110-P	111-P	112-P	117-P	118-P	119-P	120-P	121-P	122-P	123-P
Dextrin	76 0	89 0	86 6	67 0	94 0	76 0	76 0	52 0	76 0	53 0	70 3	76 0* 91 2
Blood												
Casein	18 0	5 0								41 0		
Corn gluten meal							18 0					
Egg albumin									18 0			
Gelatin						18 0						
Gliadin												
Liver			7 4	27 0				42 0				
Soy bean oil meal											23 7	
Wheat gluten												
Salts*	4 0	4 0	4 0	4 0	4 0	4 0	4 0	4 0	4 0	4 0	4 0	4 0
Cod liver oil	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
Wheat germ oil	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0	1 0
Protein	17 35	4 82	4 76	17 43		17 07	15 87	17 87	17 64	18 22	18 86	15 74

* Phillips and Hart (9).

Table 10

Effect of Various Proteins on Hemoglobin Formation and Body Weight (1140)

Protein	Diet No.	No. of animals	Hb, gm. per 100 ml. blood					Weight, gm.				
			Initial	4th day	8th day	12th day	16th day	Initial	4th day	8th day	12th day	16th day
Casein	100-P	10	3.19	7.38	11.15	11.96	12.10	48.8	65.1	74.2	84.4	96.4
Low casein	101-P	7	3.43	6.50	9.68	11.48	11.83	49.3	52.0	54.1	55.7	55.7
Liver	111-P	10	3.30	7.65	11.87	12.31	12.50	53.8	64.0	77.5	90.0	102.1
Low liver	110-P	7	3.31	5.86	8.68	10.12	11.05	55.4	56.0	56.7	58.0	56.1
Egg albumin	118-P	10	3.66	7.25	11.29	11.66	12.01	56.0	70.7	83.7	94.6	105.7
Soy bean oil meal	119-P	10	3.46	7.72	10.84	11.54	11.91	53.5	68.9	78.5	87.2	95.8
Blood	123-P	8	3.52	7.99	11.60	12.57	12.13	48.5	54.6	59.4	66.4	72.2
Corn gluten meal	121-P	10	3.69	6.69	10.05	10.81	11.25	50.2	53.4	55.3	58.8	60.6
Wheat gluten	122-P	10	3.60	6.40	9.37	11.23	11.71	47.7	51.0	52.9	53.2	54.0
Gliadin	117-P	7	3.24	6.51	8.15	10.27	10.38	51.6	50.6	49.7	49.3	47.7
Gelatin	120-P	7	3.48	5.95	7.99	9.50	10.40	52.6	51.3	46.1	45.0	42.3
No protein	112-P	7	3.41	6.05	8.05	9.13	9.56	59.7	54.4	49.1	46.7	43.8

Table 11

Average Gain in Hemoglobin Values and Change in Weight over 8 Day Period (1140)

Protein	Diet No.	Hb per 100 ml. blood		Weight
		Mean ± σ*	Range	
		gm.	gm.	gm.
Casein	100-P	7.96 ± 0.24	6.83 - 9.54	25.4
Low casein	101-P	6.25 ± 0.46	4.51 - 7.82	4.8
Liver	111-P	8.57 ± 0.41	6.02 - 10.01	23.7
Low liver	110-P	5.36 ± 0.24	4.52 - 6.15	1.3
Egg albumin	118-P	7.61 ± 0.38	5.93 - 10.19	27.7
Soy bean oil meal	119-P	7.38 ± 0.25	6.41 - 9.15	25.0
Blood	123-P	8.08 ± 0.44	6.73 - 10.12	10.9
Corn gluten meal	121-P	6.36 ± 0.23	5.23 - 7.53	5.1
Wheat gluten	122-P	5.77 ± 0.17	5.01 - 6.61	5.2
Gliadin	117-P	4.91 ± 0.22	4.05 - 5.75	−1.9
Gelatin	120-P	4.52 ± 0.49	3.23 - 6.27	−6.5
No protein	112-P	4.64 ± 0.38	3.64 - 6.26	−10.6

* σ represents the standard error of the mean.

3. Damodaran et al. (0283) also determined the relative efficiency of casein, egg albumin, and wheat gluten on hemoglobin formation in rats suffering from experimental anemia.

Three groups of young rats, 90-95 g BW, were placed on a basal diet of 75% starch, 8% butter fat, 8% sugar, 4% salt mixture (Steenbock's #40 with a small quantity of copper sulfate) and 5% protein (casein, egg albumin, or wheat gluten). Yeast and shark-liver oil were given twice weekly to supply the necessary vitamins. The animals were then made anemic by injection of phenylhydrazine. The condition was followed by red blood cell counts and hemoglobin determinations made every four days over a period of 3 to 4 weeks. The data are presented in Table 12.

Casein and egg albumin were very efficient in blood regeneration bringing about restoration of normal red blood cell counts and hemoglobin concentrations within 15 days. Wheat gluten was inferior in this respect, 25 days being required for restoration of normal values.

Table 12

Effect of Casein, Egg Albumin and Wheat Gluten on Hemoglobin Formation (0283)

	Gr. 1 Casein			Gr. 2 Wheat gluten			Gr. 3 Egg albumin		
	Days		% increase	Days		% increase	Days		% increase
	4	12		4	12		4	12	
R.B.C. in millions per c.mm.	3·40	5·51	62·05	3·48	4·75	36·87	3·35	5·77	72·39
	Days		% increase	Days		% increase	Days		% increase
	5	13		5	13		5	13	
Haemoglobin gm. per 100 c.c.	9·14	13·72	50·12	9·14	12·41	35·78	9·04	14·29	58·08

		R.B.C.	Haemoglobin
Standard error of difference between Grs. 1 and 2	..	3·17	2·79
do. do. do. do. Grs. 2 and 3	..	2·38	1·10

4. McGlory et al. (0973) isolated the pressor material "pepsitensin" in highly purified form following pepsin digestion of vitamin-free casein and studied its chemical nature and hypertensive properties in rats. The substance was found to be a polypeptide or mixture of polypeptides and produced a graded pressor response comparable to dosage increase. Pepsitensin caused the maximal blood pressure possible without exhibiting tachyphylaxis.

B. Humans

1. Natelson et al. (1066) studied the effect of non-casein protein to casein ratios of feeding formulas on the levels of certain blood constituents in infants during the first month of life.

Three groups of healthy infants (equally matched as to sex and weight), fifteen per group, were placed on the following diets: (A) Non-casein protein to casein, 3:2, (B) Breast-fed, and (C) Non-casein protein to casein, 1:8. (The protein content of the formulas was 2.1 as compared with 0.8-1.31% for the breast milk). The following clinical determinations were made at intervals over a period of 30 days: hematocrit, pH, plasma sodium, potassium, chloride, carbon dioxide, sugar, urea, protein, calcium, and phosphorus.

In this study, the non-casein proteins of cow's milk were more efficiently utilized in building plasma protein than casein. The differences in hematocrit values were not great, however, and there was no significant difference in weight gain among the three groups.

2. Vartiainen et al. (1493) determined the effect of casein on circulating eosinophils, blood sugar, and blood pressure in healthy human subjects.

One group of volunteers (males), 20-23 years of age, 63-82 kg BW, were given vitamin-free casein (Merck) at a level of 0.6 g protein/kg BW; a second group of like number and specifications were given gelatin (Burr bacteriologic grade) at the same level; both groups had been fasted overnight. The subjects were then confined to bed, and blood pressure, blood sugar, and the number of circulating eosinophils determined at 2-hour intervals.

Casein at the level used caused a marked decrease in the number of circulating eosinophils in comparison with the gelatin control preparation. Blood pressure and blood sugar were unaffected by either preparation.

3. Bazzano et al. (0115) studied the influence of casein, lactalbumin, beef protein, and casein fortified with cystine, on the serum lipids and

lipoproteins of human volunteers.

Six healthy normolipemic male university students, 20-25 years of age, were placed on one or more of the various test or control diets used (See original article for detailed diet tables). The experimental period of 10 weeks was divided into a control phase, followed by one or more experimental phases, and a return to the control phase again at the end of the study. Serum lipids (cholesterol, phospholipids, triglycerides) were measured twice weekly, and serum lipoprotein (β-lipoprotein) once each week, throughout the investigation. The results are presented in Tables 13 and 14.

The authors pointed out that although the results obtained definitely indicated the hypocholesterolemic effect of casein when used as the sole source of protein, previous dietary studies indicated a much greater effect in this respect. They reported that in several experiments where casein was used in place of beef protein, the drop in serum cholesterol was of the order of 40 mg%.

Table 13

Serum Lipids During Various Dietary Regimens (0115)

Subject: J. C.	FCD	Casec Formula	Casec Formula c̄ Cystine	Milk-Beef Formula	FCD
Average time (days)	21	21	18	17	14
Cholesterol (mg %)	152[1] ± 9.1	146 ± 7.7	137 ± 9.9	141 ± 3.8	145 ± 4.7
Phospholipids (mg %)	186 ± 5.2	179 ± 12.6	170 ± 10.3	162 ± 7.4	153 ± 1.8
Triglycerides (mg %)	71 ± 9.3	63 ± 7.6	67 ± 2.5	83 ± 14.7	64 ± 8.1
β-Lipoproteins					
S_f 0-12 (mg %)	231 ± 36.2	200 ± 16.5	213 ± 22.9	204 ± 17.2	233 ± 25.5
S_f 12-20 (mg %)	7 ± 3.6	13 ± 7.6	16 ± 7.5	14 ± 2.0	13 ± 1.2
S_f 20-100 (mg %)	29 ± 15.6	41 ± 7.5	45 ± 16.4	44 ± 11.0	41 ± 11.4
S_f 100-400 (mg %)	9 ± 4.2	9 ± 6.4	5 ± 1.2	8 ± 2.0	10 ± 1.5
Total 12-400 (mg %)	44 ± 22.0	63 ± 5.7	67 ± 21.5	66 ± 9.5	64 ± 15.4
Total 0-400 (mg %)	275 ± 47.5	259 ± 15.5	279 ± 27.2	270 ± 19.3	365 ± 93.3

[1] Average ± standard deviation (S.D.).

Table 14

Serum Lipids During Various Dietary Regimens (0115)

Subject: J. I.	FCD	Casec Formula	Casec c̄ Cystine	Egg Albumin	FCD
Average time (days)	33	23	21	21	21
Cholesterol (mg %)	180[1] ± 9.1	155 ± 20.1	138 ± 5.2	146 ± 5.8	174 ± 7.5
Phospholipids (mg %)	218 ± 11.0	192 ± 15.8	180 ± 9.5	177 ± 10.2	211 ± 32.3
Triglycerides (mg %)	77 ± 11.3	101 ± 15.5	83 ± 21.5	84 ± 15.0	87 ± 6.8
β-Lipoproteins					
S_f 0-12 (mg %)	246 ± 21.2	245 ± 58.4	181 ± 2.1	205 ± 44.3	251 ± 30.9
S_f 12-20 (mg %)	9 ± 3.6	17 ± 7.3	9 ± 4.2	12 ± 7.2	22 ± 9.2
S_f 20-100 (mg %)	39 ± 8.6	75 ± 13.5	42 ± 3.5	59 ± 2.0	62 ± 19.9
S_f 100-400 (mg %)	9 ± 4.4	8 ± 2.1	12 ± 5.7	12 ± 5.3	8 ± 2.4
Total 12-400 (mg %)	57 ± 12.9	101 ± 20.8	63 ± 13.4	83 ± 7.2	92 ± 30.8
Total 0-400 (mg %)	303 ± 27.4	346 ± 61.8	245 ± 15.6	288 ± 51.1	338 ± 67.5

[1] Average ± standard deviation (S.D.).

Eye Damage

Several investigators have studied the effect of caseins of varying degrees of purity on cataract formation and other forms of eye damage in experimental animals.

A. Rats

1. Kamath et al. (0704) placed vitamin A-depleted rats (young, weanling Wistar males) on test diets containing 10, 20, or 40% protein as casein supplemented with either carotene or retinyl acetate (See Table 15). Eight to ten animals were used per group over an experimental period of 28 days. Weight changes and retinol content of liver and spleen were determined. The results are presented in Table 15.

A significant increase in hepatic retinol each week in parallel with increase in protein (casein) from 10 to 20% was found. Increasing the protein dietary content to 40% also increased liver retinol but not as rapidly as the change from 10 to 20% casein. Utilization of carotene was found to be linearly dependent on dietary protein. Protein deficiency reduced utilization of the higher levels of retinyl acetate but had no effect on the lower levels.

2. Day et al. (0299) demonstrated the need for rigorous purification of casein preparations to obtain valid results in nutritional studies.

Rats, 21 days of age (other specifications not given) were placed on a basic experimental riboflavin-deficient (cataract-producing) diet #625 (casein, 18%; cod liver oil, 2%; butterfat, 8%; Osborne & Mendel salt mixture, 4%; cornstarch, 68%). Seven different casein preparations were employed in the study.

The following criteria were used in assessing the purity of the caseins used: (1) Incidence of cataracts; (2) average time of appearance of cataracts; (3) average maximum growth; (4) average time required to reach maximum weight; (5) average period of survival.

The results presented in Table 16 and Fig. 1 clearly indicate marked differences in response of the animals according to several of the criteria which are related to riboflavin impurities.

B. Kittens

Scott et al. (1334) placed fifteen kittens (specifications not mentioned) on a semi-purified diet containing vitamin-free casein with, and without, vitamin A supplements and determined the effects on eyes and retinas (See Table 17).

Table 15

Mean Values of Body Weight Gains, Hepatic and Renal Retinol Deposits in Young, Vitamin A-Depleted Rats Fed Vitamin A-Supplemented Diets for 28 Days (0704)

Supplement	Dose: Level	Dose: Retinol equiv./day	Casein protein in diet	Number of rats	Body weight gain	Retinol deposits per organ: Hepatic	Retinol deposits per organ: Renal	Retinol deposits per organ: Hepatic plus renal	Efficiency of utilization [1]
		μg	%		g	μg	μg	μg	%
Carotene	Adequate	20	10	10	64	36 ± 9 [2]	36 ± 3	72 ± 8	13 ± 2
			20	10	85	91 ± 12	26 ± 3	116 ± 10	21 ± 2
			40	10	94	170 ± 10	20 ± 4	190 ± 10	34 ± 2
	Moderately high	64	10	9	66	215 ± 31	23 ± 2	238 ± 29	13 ± 2
			20	10	83	510 ± 38	17 ± 3	528 ± 36 [a,3]	29 ± 2
			40	9	97	557 ± 56	12 ± 2	569 ± 53 [a]	32 ± 3
	High	182	10	8	65	645 ± 51	28 ± 3	673 ± 48	13 ± 1
			20	9	85	1068 ± 60	16 ± 2	1085 ± 55	21 ± 1
			40	9	103	1373 ± 83	11 ± 2	1384 ± 78	26 ± 2
Retinyl acetate	Adequate	25	10	9	66	196 ± 20	25 ± 5	221 ± 18 [b]	32 ± 3
			20	9	87	181 ± 9	20 ± 2	201 ± 10 [b]	29 ± 2
			40	10	93	218 ± 11	17 ± 2	235 ± 11 [b]	34 ± 2
	Moderately high	74	10	9	68	740 ± 96	17 ± 3	758 ± 90	37 ± 1
			20	10	91	1054 ± 54	13 ± 4	1067 ± 51 [c]	51 ± 3
			40	10	96	1100 ± 40	12 ± 2	1112 ± 38 [c]	53 ± 2
	High	214	10	10	70	2834 ± 174	25 ± 4	2859 ± 164	48 ± 3
			20	10	89	3784 ± 85	17 ± 3	3801 ± 86 [d]	63 ± 2
			40	10	95	3796 ± 118	14 ± 2	3808 ± 117 [d]	64 ± 2

F values

Source of variation		
A. Level of dietary protein (2) [4]	187.9 ** [5]	78.4 ** [6]
B. Level of vitamin A dose (2)	1.9	1708.2 **
C. Source of supplement (1)	1.3	881.4 **
AB. Interaction (4)	0.4	9.1 **
BC. Interaction (2)	0.4	208.1 **
AC. Interaction (2)	3.6 *	4.1 *

[1] Efficiency of utilization = $\frac{\text{Hepatic + renal retinol deposits in } \mu g \text{ retinol equivalent}}{\text{Total dose fed for 28 days in } \mu g \text{ retinol equivalent}} \times 100.$

[2] ± SEM

[3] Means with same superscript for a given vitamin A intake are not significantly different ($P > 0.05$, t test)

[4] Degrees of freedom in parentheses.

[5] ** $P < 0.01$, * $P < 0.05$

[6] Performed on means of square root of data for hepatic plus renal retinol.

Eyes were examined regularly with an ophthalmascope in addition to other observations for toxic effects. Vitamin A estimations on diets, liver, kidneys, and plasma were carried out. Gross and microscopic examinations were made on eyes, retinas, and other organs at autopsy.

The authors observed that animals on the casein diet, after normal growth for several months, developed classical signs of vitamin A deficiency in 6 to 20 months in spite of receiving supplements of vitamin A (5000 IU Vitamin A palmitate 3 times weekly) which were more than adequate on other test diets. Conjunctivitis, xerosis with keratitis, vascularization of the cornea,

Table 16

Summary of data regarding growth, survival and incidence and time of appearance of cataract in groups of rats receiving riboflavin-deficient diets containing different casein preparations. (0299)

	No. of rats	No. showing cataract	Av. wt. at start g.	Av. maximum gain g.	Av. no. days to reach max. wt.	Av. no. days for appearance of cataract	Av. no. days survival
I. Alcohol-extracted	15	13	41·1	17	37	83	119
II. Acid-extracted	9	8	39·9	20	28	56	81
III. Labco	17	12	39·6	16	33	50	90
IV. Harris	9	8	37·6	16	23	52	83
V. Glaxo AB	10	7	38·5	42	65	113	133
VI. Glaxo ABE	14	6	38·1	40	62	86	117
VII. Glaxo ABE extracted	5	2	35·8	22	43	75	101

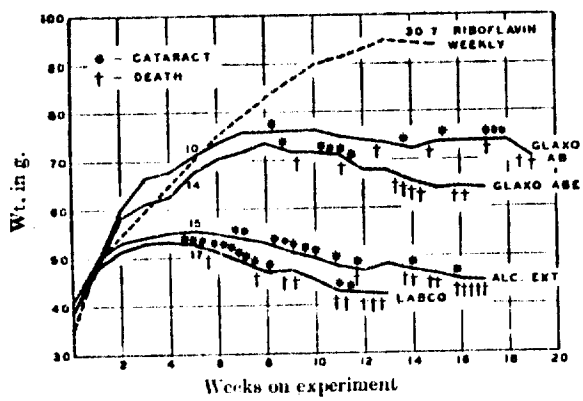


Fig. 1 - Composite weight curves of groups of rats which received riboflavin-deficient diets identical in all respects except for the caseins that they contained. The numbers on the curve indicate the number of rats in each group. The asterisks mark the time of appearance of cataract in individual animals, and the daggers mark the death of individual animals. Each curve was terminated at the point of average survival for that group; consequently, animals showing cataract and those dying after that time are not indicated on the chart. The dotted line indicates the average growth of a group of rats receiving the Labco casein diet supplemented with 30γ riboflavin weekly; these animals were killed at the end of 15 weeks. (0299)

Table 17

Semi-Purified Diet Fed to Kittens (1334)

Ingredients	g/100 g dry diet
Casein (vitamin free)	35
Sucrose *or* dextrin	36·7
Lard	10
Arachis oil, Violmul K *or* coconut oil	12
Sugar beet residue	3
Salt mixture	2
Nutricon (vitamin supplement)	1
Choline chloride	0·3
Inositol	0·2

progressive destruction of the visual cells of the retina, and formation of cataracts were the major toxic visual effects noted. A marked reduction in the vitamin A content and a decrease in the liver reserve were found also.

Supplementation of the test diet with other vitamins and variation of constituents other than casein, did not prevent the visual damage. The authors suggested that the amino acid pattern of casein may be unsuitable for cats.

Allergy

One of the hazards of casein to the consumer is its potential as an allergen. Certain individuals develop a hypersensitivity to dietary casein absorbed from the gastrointestinal tract. In one survey, 60% of milk allergic infants and children reacted to casein (0975). Commercial casein preparations as well as the natural form in milk, are capable of inducing hypersensitive states. Fortunately, casein is not the main allergen in milk (1216).

Milk allergy usually develops during the first few months of life and creates a serious problem in infant nutrition (0975). It vanishes early, however, and only 2% of the patients remain allergic beyond the age of six (0975). Hypersensitivity to casein may take any of the major forms indicated in Table 18. Studies have indicated that the caseins of various species are quite similar antigenically and patients allergic to one type are often allergic to others as well.

Table 18

Types of Allergy to Casein (0975)

Form	Prevalence
Gastrointestinal	25-40%
Respiratory	30-45%
Atopic Dermatitis	42%
Urticaria	8%
Anaphylaxis	5%

In addition to the usual allergies, hypersensitivity to casein may be involved in more serious diseases such as steatorrhea, coeliac disease, and the sudden infant death syndrome, according to the results of several investigations.

A. Guinea pigs

1. Ratner et al. (1216) determined the relative allergenicities of the three major milk proteins, α-casein, β-lactoglobulin, and α-lactalbumin by means of in vivo anaphylaxis tests in guinea pigs.

Three groups of ten animals each (200-250 g BW) were sensitized by injection of a single 1 mg dose of the protein s.c.; three weeks later, the animals were challenged with 0.1 ml of pasteurized skim milk i.v.

The results presented in Table 19 clearly show that β-lactoglobulin is a stronger allergen than either α-casein or α-lactalbumin. More rigorous sensitization, however, as indicated in Table 20, indicates that all three major milk proteins are capable of causing allergic reactions if exposure is sufficient.

2. Anderson et al. (0039) demonstrated the close antigenic relationship between cow, goat, and human casein as early as 1932 by active and passive sensitization tests in guinea pigs.

In one series of experiments, groups of 3 to 46 guinea pigs were sensitized by the i.p. route to cow, goat, or human casein and later challenged i.v. with one of the heterologous preparations. Positive and negative controls were included in each experiment. The data are given in Tables 21 and 22.

Table 19

Allergenic Superiority of Beta-Lactoglobulin as Compared with Alpha-Lactalbumin and Alpha-casein (1216)

Number of Animals	Single Sensitizing Injection, 1.0 mg Sc	Challenge 3 Weeks Later, 0.1 ml PSM, Iv Result	
10	Alpha-lactalbumin	All negative	
10	Alpha-casein	All negative	
10	Beta-lactoglobulin	7	+ + + +
		1	+ +
		1	+
		1	0

Table 20

Enhancement of Allergenicity of Alpha-lactalbumin and Alpha-casein by Sensitization with Multiple Doses (1216)

Number of Animals	Multiple Sensitizing Injections; 1 mg Sc + 1 mg Ip; 2 Days Later 1 mg Sc	Challenge 3 Weeks Later 0.1 ml PSM, Iv Result	
10	Alpha-lactalbumin	4	+ + + +
		1	+ + +
		2	+ +
		1	+
		2	0
10	Alpha-casein	3	+ + + +
		2	+ +
		4	+
		1	0
10	Beta-lactoglobulin	8	+ + + +
		1	+
		1	0

* Abbreviations in Table:

Sc = subcutaneously; Iv = intravenously; Ip = intraperitoneally.
PSM = pasteurized skimmed milk.
+ + + + = Anaphylactic death - dyspnea, convulsions, collapse, apnea and death.
+ + + = Severe anaphylaxis - dyspnea, convulsions, collapse and recovery.
+ + = Moderate anaphylaxis - dyspnea, convulsive movements, moderate collapse, recovery.
+ = Mild anaphylaxis - dyspnea and scratching.
0 = No reaction.

Table 21

Active Sensitization Studies with Cow, Goat, and Human Casein in Guinea pigs (0039)

Sensitizing casein	Number of Animals	Challenge casein	Number of allergic animals
Cow	46	Human	32
Human	37	Cow	16
Goat	12	Human	9
Human	4	Goat	4
Cow	8	Goat	5
Goat	3	Cow	3
Cow	–	Cow	Violent anaphylaxis (100%)
Human	–	Human	"
Goat	–	Goat	"
None	–	Cow	No reaction
None	–	Human	"
None	–	Goat	"

Table 22

Passive Sensitization Studies With Cow and Human Casein in the Guinea pig (0039)

Sensitizing antibody	Number of Animals	Challenge casein	Number of allergic animals
Cow	27	Human	15
Human	20	Cow	9
Cow	6	Cow	6
Human	4	Human	4
None	5	Human	0
None	4	Cow	0

In the passive sensitization experiments, guinea pigs were sensitized by the i.p. injection of anti-cow casein rabbit serum or anti-human casein rabbit serum and later challenged i.v. with the heterologous casein. Positive and negative controls were included also.

3. Ratner at al. (1216) studied the response of guinea pigs to heat-denatured milks and milk proteins in an effort to determine the effect of commercial processing methods on the allergenicity of milk proteins and milk formulas. The heat-denatured milks used were the same as commercially-available preparations which are heated before evaporation (see original article for brands and details).

In one series of experiments, guinea pigs were sensitized with pasteurized, or other heat-treated skim milk, and challenged with purified α-casein, β-lactalbumin, or β-lactoglobulin. In the second series of experiments, the sensitizing and challenging preparations were reversed.

The data presented in Tables 23 and 24 clearly show that heat-denatured milks still retain the allergenicity of the α-casein fraction while having lost all of that of the α-lactalbumin component, and much of that of the β-lactoglobulin constituent (main allergenic milk protein).

4. The same authors (1216) later investigated the effect of heat treatment on milk allergenicity further by using the oral route for sensitization and challenge of guinea pigs. The data are presented in Tables 25 and 26.

Two of ten animals sensitized with α-casein showed allergic effects when challenged 21 days later with heat-denatured milk. When challenged 52 days after sensitization, however, one of 6 animals died from anaphylactic shock and two others showed allergic symptoms.

None of the animals sensitized with α-lactalbumin or β-lactoglobulin showed allergic reactions on oral challenge with heat-denatured milk. Pasteurized skim milk orally, on the other hand, sensitized about 2/3 of the animals used to later oral challenge with the same preparation.

5. The same authors (1216) in another study investigated the allergenic properties of raw, evaporated, and boiled milk, dried milk formulas, acidified milk, and purified milk proteins, both orally and parenterally in guinea pigs.

Parenteral hypersensitivity tests were conducted by sensitizing the animals by i.p. injection and giving challenge doses by the i.v. route. In oral tests, both sensitizing and shock doses were by feeding.

Table 23

Effect of Heat Denaturation of Milk on the Sensitizing Qualities of Alpha-lactalbumin, Beta-lactoglobulin and Alpha-casein (1216)

	Animal	Sensitization, 5 ml Ip	Challenge, 0.3 mg Iv	Result	Second Challenge, Iv	Result
		Powdered HDM	Alpha-lactalbumin		Alpha-casein 0.3 mg	
A	1			0		+
	2			0		++++
	3			0		+
	4			0		++++
	5			0		++++
	6			0		++++
	7			0		++++
	8			0		0
	9			0		++++
	10			0		0
		Powdered HDM	Beta-lactoglobulin			
B	11			0		
	12			++++		
	13			++++		
	14			++++		
	15			++++		
	16			++++		
	17			++++		
	18			++++		
	19			++++		
	20			++++		
		Liquid HDM	Alpha-lactalbumin		Beta-lactoglobulin, 0.1 mg	
C	21			0		++
	22			0		++++
	23			0		++++
	24			0		+
	25			0		++++
	26			0		++++
	27			0		++++
	28			0		0
	29			0		++++
	30			0		++++

* In this and subsequent tables:
Ip = intraperitoneal; Iv = intravenous; Sc = subcutaneous.
HDM = heat denatured milk; PSM = pasteurized skimmed milk.
++++ = Anaphylactic death—dyspnea, convulsions, collapse, apnea, death.
+++ = Severe anaphylaxis—dyspnea, convulsions, collapse and recovery.
++ = Moderate anaphylaxis—dyspnea, convulsive movements, moderate collapse and recovery.
+ = Mild anaphylaxis—dyspnea and scratching.
0 = No reaction.

Table 24

Effect of Heat Denaturation of Milk on its Allergenicity, as Tested by Challenge of Animals Sensitized to Protein Fractions from Milk (1216)

	Animal	Sensitizing Injection, 1 mg Sc+1 mg Ip; 9 Days Later, 1 mg Sc	Parenteral Challenge 3 Weeks Later, 0.1 ml Iv		
			Result of First Injection of HDM	Second 1 hr Later	Result
		Alpha-lactalbumin		PSM	
A	1		0		++++
	2		0		+++
	3		0		++
	4		0		++++
	5		0		++
	6		0		++++
	7		0		+
	8		0		0
	9		0		++++
	10		0		0
		Beta-lactoglobulin		PSM	
B	11		0		0
	12		0		+
	13		0		++++
	14		0		++++
	15		1		++++
	16		0		++++
	17		0		++++
	18		0		++++
	19		1		++++
	20		1		++++
		Alpha casein		PSM	
C	21		0		0
	22		++++		
	23		0		1
	24		0		+
	25		++++		
	26		++++		
	27		1		+
	28		1		+
	29		1		++
	30		1		++

Table 25

Allergenic Superiority of Beta-lactoglobulin as Compared with Alpha-lactalbumin and Alpha-casein (1216)

Number of Animals	Single Sensitizing Injection, 1.0 mg Sc	Challenge 3 Weeks Later, 0.1 ml PSM, Iv	
		Result	
10	Alpha-lactalbumin	All negative	
10	Alpha-casein	All negative	
10	Beta-lactoglobulin	7	++++
		1	++
		1	+
		1	0

Table 26

Enhancement of Allergenicity of Alpha-lactalbumin and Alpha-casein by Sensitization with Multiple Doses (1216)

Number of Animals	*Multiple Sensitizing Injections; 1 mg Sc + 1 mg Ip; 2 Days Later 1 mg Sc*	*Challenge 3 Weeks Later, 0.1 ml PSM, Iv*	
			Result
10	Alpha-lactalbumin	4	++++
		1	+++
		2	++
		1	+
		2	0
10	Alpha-casein	3	++++
		2	++
		4	+
		1	0
10	Beta-lactoglobulin	8	++++
		1	+
		1	0

* Abbreviations in Table:

Sc = subcutaneously; Iv = intravenously; Ip = intraperitoneally.

PSM = pasteurized skimmed milk.

++++ = Anaphylactic death—dyspnea, convulsions, collapse, apnea and death.

+++ = Severe anaphylaxis—dyspnea, convulsions, collapse and recovery.

++ = Moderate anaphylaxis—dyspnea, convulsive movements, moderate collapse, recovery.

+ = Mild anaphylaxis—dyspnea and scratching.

0 = No reaction.

The results obtained indicated that, parenterally, casein, lactalbumin, lactoglobulin, and bovine serum globulin were highly anaphylactogenic with respect to themselves and raw milk. Evaporation and boiling destroyed most of the parenteral sensitizing properties of bovine serum globulin and greatly diminished that of the lactalbumin fraction. These treatments were without appreciable effect, however, on the sensitizing properties of casein.

Oral allergy studies demonstrated that the oral sensitizing properties of evaporated, and boiled milk (100°C for 4 hours), were greatly diminished in contrast to dried milk formulas. The authors pointed out that the marked difference between parenteral and oral sensitizing properties of the milks tested was probably due to their coagulation in the stomach and subsequent

digestion which prevented absorption into the circulation in the unaltered state.

6. Saperstein et al. (1284) tested seven commercial infant formulas for antigenicity against purified casein (purified by ten re-precipitations), and other milk proteins, by the precipitin ring test and the passive cutaneous anaphylaxis test in guinea pigs.

All of the preparations tested gave antigenic reactions with purified casein and several other milk proteins (See Tables 27 and 28). The authors suggested that the term "heat-denatured milks" be abandoned as implying non-antigenicity of the proteins in the preparations.

7. Cole et al. (0262) studied the allergenic properties of skim milk, evaporated milk, a commercial milk formula, as well as purified casein, α-lactalbumin, β-lactoglobulin, and bovine serum albumin in guinea pigs. The casein preparation was obtained by acid precipitation from pasteurized skim milk and purified by re-precipitation five times.

Twenty-one days after a sensitizing injection had been given to the guinea pigs (male and female, English strain, 200-300 g BW), the animals were challenged with 0.1 mg of milk or purified protein in buffered saline, injected into the juglar vein. The authors reported that the severity and number of reactions was greatest in the animals sensitized with casein and the milk formula. Four of nine animals sensitized with casein suffered fatal anaphylactic shocks when challenged, and the other five had mild or severe reactions.

8. Parish et al. (1129) challenged milk-sensitized guinea pigs (specifications not given) with reconstituted National Dried Milk Powder (the sensitizing preparation), stomach contents from "cot death", purified casein, α-lactalbumin, or β-lactoglobulin via the respiratory tract in a study prompted by the possible role of milk protein allergy in the "sudden death symdrome of infancy" or "cot death" syndrome. The challenge via the glottis was designed to parallel as closely as possible, accidental inhalation of regurgitated stomach contents by a sleeping infant.

Four of five sensitized guinea pigs challenged with 0.25 ml of 1% casein died rapidly and without struggling. Fifteen of 23 sensitized (milk) animals challenged with stomach contents from cases of "cot death" succumbed in like manner. All of the fatalities showed histopathologic changes in the lungs which resembled those found in "cot death".

Table 27

Antigenicity of bovine serum albumin, alpha-lactalbumin, beta-lactoglobulin, and casein of reconstituted milk formulas as determined by passive cutaneous anaphylaxis in guinea pigs* (1284)

Products**	Anti BSA (0.13 μg AbN/ml.)	Anti alpha-lactalbumin (0.046 μg AbN/ml.)	Anti-beta-lactoglobulin (0.023 μg AbN/ml.)	Anti-casein (0.008 μg AbN/ml.)
Liquid				
Bremil	-	+	+	+
SMA	-	+	+	+
Enfamil	-	+	+	+
Modilac	-	+	+	+
Lactum	-	+	+	+
Similac	-	+	+	+
Bakers	-	+	+	+
Powdered				
Bremil	-	+	+	+
SMA	+	+	+	+
Enfamil	+	+	+	+
Lactum	+	+	+	+
Similac	+	+	+	+
Bakers	+	+	+	+

*Skin sites sensitized with 0.1 ml. of diluted specific antiserum (rabbit). Antibody N content is an approximate value.

**All reactions were identical after terminal heating of reconstituted formulas at 99°C. (210°F.) for 30 minutes, except SMA powdered, Lactum powdered, and Bakers powdered, which became BSA negative.

Table 28

Protein antigenicity of milk products measured in vivo by passive cutaneous anaphylaxis in the guinea pig (1284)

Milk Product	Antigenicity of proteins				
	Casein	Alpha-lactalbumin	Beta-lactoglobulin	BSA	BGG
Evaporated milk	+	+	+	-	-
Dried instant skim milk (Starlac)	+	+	+	+	+
Pasteurized fluid cow's milk	+	+	+	+	+
Prepared infant formulas					
Liquid Similac	+	+	+	-	-
Liquid Bakers	+	+	+	-	-
Liquid Bremil	+	+	+	-	-
Liquid SMA	+	+	+	-	-
Liquid Enfamil	+	+	+	-	-
Liquid Modilac	+	+	+	-	-
Liquid Lactum	+	+	+	-	-
Powdered Similac	+	+	+	+	-
Powdered Bremil	+	+	+	-	-
Powdered SMA	+	+	+	+	-
Powdered Enfamil	+	+	+	+	-
Powdered Lactum	+	+	+	+	-
Powdered Bakers	+	+	+	+	-

9. Spies et al. (1381) made an important contribution to a better understanding of the problem of milk allergy with the discovery of new antigens resulting from brief pepsin digestion of bovine casein and other milk proteins.

Purified bovine serum albumin, casein, α-lactalbumin, and β-lactoglobulin were digested with swine pepsin (100 mg) at pH 2 and 37°C for 8 minutes. The mixture was then immediately poured onto ice cubes to stop the reaction. After adjusting the pH to 7.5 with NaOH, the hydrolysate was recovered by lyophilization. The preparations were then dialyzed against several changes of distilled water with resultant separation of each digestion mixture into a dialysate (D) and endo fraction (E). Care was taken to eliminate the possibility of either fraction becoming contaminated with undigested protein.

Hypersensitivity tests were performed by the Schultz-Dale technique using uterine strips from virgin female guinea pigs (225 g) which had been sensitized with the preparation emulsified in Freund's complete adjuvant. (The uterine strips employed were non-sensitive to pepsin).

The Ouchterlony double-diffusion method, as well as ring and tube precipitin tests, were employed for determination of other antigenic relationships. The major results are presented in Tables 29 and 30.

The data obtained clearly showed that each of the milk proteins yielded a new allergen on digestion with pepsin. The authors pointed out that this phenomenon might explain the diagnostic puzzle presented by milk allergy patients who give negative skin tests with milk or milk proteins.

B. Humans

1. Wright et al. (1574) found that most newborns have circulating antibodies to purified casein at birth. Ninety-one percent of 100 newly-delivered infants had antibodies in the cord blood and 88% of the mothers were positive also. Seventy-six percent of the infants had antibodies to α-lactalbumin and 66% were positive for β-lactoglobulin.

2. Goldman et al. (0490) tested 45 milk allergy patients with purified casein and other milk proteins in individual oral challenge studies. The median age of the group was six months with a range between two weeks and six years. The casein employed had been purified by repeated solubilization and precipitation until it contained 2% or less of whey proteins.

Table 29

Response of the dialysates of the pepsin hydrolyzates of bovine serum albumin, casein, α-lactalbumin and β-lactoglobulin in Schultz-Dale tests (1381)

Protein	Sensitizing antigen*	Challenge dose of sensitizing antigen (μg of total nitrogen†)	Results		
			Animals tested (No.)	Positive for new antigen (No.)	Doubtful (No.)
BSA	BSAPD	10	5	2	0
	BSAPD	300	5	4	1
	None	300	4	0	0
Casein	CPD	10	8	0	2
	CPD	300	10	2	2
	None	300	3	0	1
α-Lactalbumin	LaPD	10	5	5	0
	LaPD	300	5	5	0
	None	300	4	0	0
β-Lactoglobulin	LgPD	10	5	5	0
	LgPD	300	5	5	0
	None	300	4	0	0

*The dialysate of the pepsin hydrolyzate of respective proteins.

†The ovarian halves of the 2 uterine horns from each animal were used separately, one for the 10 μg and one for the 300 μg challenge.

Table 30

Response of the endo fractions of the pepsin hydrolyzates of bovine serum albumin, casein, α-lactalbumin, and β-lactoglobulin in Schultz-Dale tests (1381)

Protein	Sensitizing antigen*	Challenge dose of sensitizing antigen (μg of total nitrogen†)	Results		
			Animals tested (No.)	Positive for new antigen (No.)	Doubtful (No.)
BSA	BSAPE	10	4	4	0
	BSAPE	300	4	4	0
	None	300	4	0	0
Casein	CPE	10	4	0	0
	CPE	300	4	0	0
α-Lactalbumin	LaPE	10	5	1	1
	LaPE	300	5	1	1
β-Lactoglobulin	LgPE	10	4	0	0
	LgPE	300	5	0	0

*The endo fraction of the pepsin hydrolyzate of respective proteins.

†See footnote †, Table II.

Fifty-seven percent of the patients were allergic to casein, 51% to bovine serum albumin, 66% to β-lactoglobulin, and 54% to α-lactalbumin. The allergic symptoms resulting from the reaction to casein and the other proteins were similar to those to skim milk: allergic rhinitis, bronchial asthma, atopic dermatitis, urticaria, anaphylaxis, vomiting, diarrhea, abdominal pain, and central nervous system effects.

3. The same authors (0490) demonstrated the unreliability of skin tests for diagnosing allergy to purified milk proteins. In the study reported, the ratios of positive skin reactions to tests conducted were: casein 16/180, α-lactalbumin 15/180, β-lactoglobulin 13/180, and serum albumin 14/180.

4. Hill (0587) skin tested 44 eczematous infants who were hypersensitive to cow lactalbumin, with cow and goat casein and goat lactalbumin, in an investigation of possible hypersensitivity inter-relationships of these milk proteins in infantile eczema. The results obtained are summarized in Table 31.

Table 31

Hypersensitivity Interrelationships Between Cow and Goat Lactalbumin in Infantile Eczema (0587)

Skin Test Antigen	Result of Test	Positive/Total Number
Cow Lactalbumin	Sensitivity	44/44
Goat Lactalbumin	Sensitivity	25/44
Goat Lactalbumin	Questionable Sensitivity	9/44
Goat Lactalbumin	No sensitivity	10/44

Ten of the 25 patients who gave positive tests with goat lactalbumin were negative to both goat and cow casein. (Previous studies demonstrated that cow and goat casein are almost immunologically identical). Results of this study indicated that immunologic cross reactivity between cow and goat lactalbumin further limits the safety of goat's milk for infants allergic to cow's milk.

5. The same author (0588) skin tested a larger group of eczematous infants with specifically purified casein and lactalbumin preparations in view of the fact that earlier reports with these substances in the scientific literature may be invalid because of contamination of the casein preparations

with lactalbumin. The casein skin test antigen was diluted 1:2000 on the basis of previous findings that this dilution would eliminate the possibility of non-specific irritation in the eczematous infant.

Thirty-five (46%) of the infants tested were hypersensitive to both casein and lactalbumin. Thirty-five (46%) were positive to lactalbumin alone. Five (6.7%) reacted to casein alone.

The author concluded, on the basis of a series of tests in guinea pigs, that the casein skin test antigen employed was free from lactalbumin in amounts that would give cross reactions. One of the discussants disagreed with some of the author's conclusions.

6. Buckley et al. (0194) compared nutritional responses and allergic reactions of 488 babies to a commercial high whey: casein formula - SMA-526 (247 infants) and a standard evaporated cow's milk formula (241 infants). The results are presented below and in Table 32. Both preparations were generally satisfactory dietetically and gave comparable growth rates.

Positive skin tests for milk hypersensitivity were found in 5% of the babies. Forty-two percent of these patients were positive to β-lactoglobulin and bovine serum albumin, and 25% were positive to casein and lactalbumin.

The authors point out that their allergenicity data are in agreement with those of previous investigators in this area.

Table 32

Antigenic Effects of Two Bovine Milk Preparations in Infants (0194)

Over-all incidence of positive tests to cow's milk	
Skin tests	5%
Tanned cell hemagglutinins	76%
Precipitins	2.2%

7. Taylor et al. (1434) tested the sera of 24 children with coeliac disease and 60 adults with steatorrhea against casein and the other major proteins of milk along with gluten fraction III of wheat, in an effort to shed light on the etiology of these diseases. Geldiffusion, complement-fixation, and hemagglutination techniques were employed. Fifty healthy children and 64 normal adults were included in the study as controls. The results are presented in Figures 2 and 3.

The data showed clearly that patients with coeliac disease as well as those with idiopathic steatorrhea had significantly higher antibody titers to a proteolyzed fraction of wheat gluten and to purified cow's milk, casein, α-lactalbumin, and β-lactoglobulin, than those of the healthy controls. The authors discussed these findings in relation to the etiology and treatment of the diseases.

8. According to a review by Heiner et al. (0570), milk allergy may be involved in hypochromic microcytic anemia in children. All of the symptoms of this disease disappear when milk is removed from the diet and recur when it is introduced again.

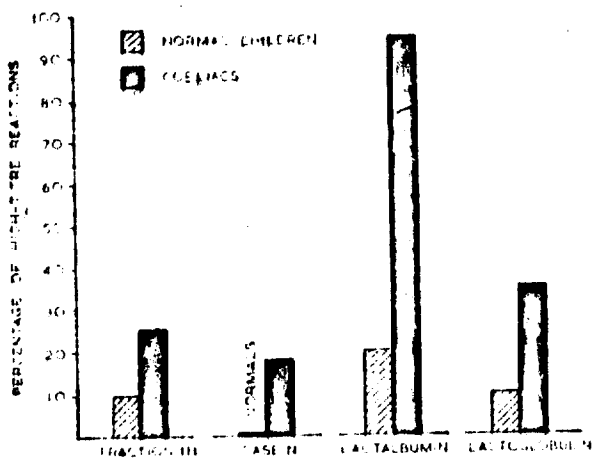


Figure 2

Serological reactions in coeliac disease (coated tanned-red-cell test). (1434)

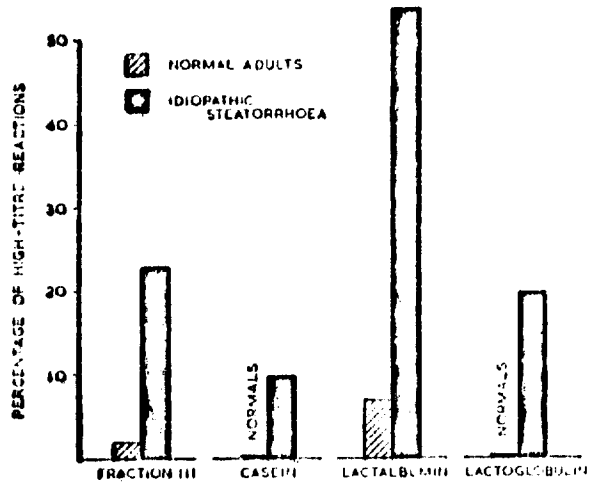


Figure 3

Serological reactions in idiopathic steatorrhoea in adults (coated tanned-red-cell test). (1434)

III. Special Studies

Effects on Carcinogenesis

Numerous studies on the effect of high dietary concentrations of casein on spontaneous and experimentally-induced cancer have been reported. In some instances, protein, amino acid, and/or vitamin controls were included. In others, however, specific effects are difficult or impossible to assess. The following studies are representative of the nature of these investigations and results obtained.

A. Mice

1. Walters *et al.* (1516) studied the effect of high (25%) and low (15%) dietary casein on the incidence of lung tumors in weanling BALB/c mice injected with 30 μg of *p*-dimethylaminobenzanthracene 24 hours after birth. Control groups (untreated and those injected with the gelatin vehicle) were included also. Thirty-five to forty animals were used per group. The mice were observed daily and those exhibiting sudden weight losses or showing definite signs of illness were killed and autopsied. Survivors were sacrificed during the 40th week and studied for gross and microscopic evidence of neoplasms. The data are presented in Table 33.

The authors concluded that the incidence of lung tumors was significantly greater in animals on the high casein diet than at the lower level ($P < 0.01$).

Table 33

Results of Experiment (1516)

Group	Diet	Other treatment	Number of mice weaned	Number survivors at 40 wks.	Number (per cent) of survivors bearing lung tumours*	Average number of lung tumours per survivor	Mice with other tumours including malignant lymphoma
1	High casein	30 μg. DMBA/3% aqueous gelatine	36	26	26 (100)	30·8	3-malignant lymphoma 1-hepatoma
2		3% aqueous gelatine	39	30	7 (28)	0·21	0
3		None	35	34	5 (14)	0·14	0
4	Low casein	30 μg. DMBA/3% aqueous gelatine.	41	36	36 (100)	20·5	3-hepatoma 1-granulosa cell tumour of ovary
5		3% aqueous gelatine	37	37	6 (16)	0·18	0
6		None	46	45	14 (24)	0·31	0

* i.e. Pulmonary adenomas and adenocarcinomas visible on surfaces on lobes.

B. Rats

1. Gyorgy et al. (0534) investigated the effect of casein and other supplements on necrosis, cirrhosis, and cancer of the liver in rats fed a diet containing dimethylaminoazobenzene. In one experiment, the incidence of cancer due to butter yellow was reduced from 80-100 to 40% by adding 18% casein to the basal diet of rice, carrots, and vitamins.

In a second experiment, 20 rats on a diet of 18% casein, 68% cane sugar, 8% butterfat, 4% salt mixture, and cod liver oil supplemented with other vitamins, were protected from the carcinogenic effect of p-dimethylaminoazobenzene (0.6g/kg in diet) over a period of 175 days. Two rats showed mild patchy necrosis and slight cirrhosis only. When the dietary level of casein was reduced to 6%, the protective effect was not evident. Addition of 50 mg of cystine and 20 mg of choline, however, protected 75% of the animals from cancer and other forms of liver damage. The authors attributed the protective effect of casein for the liver to its lipotrophic activity.

2. Miller et al. (0998) fed Sprague-Dawley rats, both sexes, 8 to 9 weeks of age (other specifications not given), synthetic diets containing 0.06% of p-dimethylaminoazobenzene and varying concentrations of casein and pyridoxine (the rats received one drop of halibut liver oil monthly also). Prior to the experiment, half of the rats had been reared on a low pyridoxine diet (1.5 mg/kg) and the other half on a high pyridoxine diet (6 mg/kg of diet). Food intake values were determined at 3-week intervals.

After 120 days on the carcinogenic diets, the livers were examined by laparotomy and the animals were continued on the same diets as before, but minus the carcinogen, for an additional 60 days. The rats were then sacrificed and the livers examined for tumors. The data are presented in Table 34.

Casein at a level of 48% in the low pyridoxine diet (0.2 mg/kg) decreased the incidence of liver tumors from 50 to 7% in comparison with the basal diet concentration of casein of 12%. An adequate amount of pyridoxine (2.5 mg/kg), however, nullified the protective effect of casein.

The authors pointed out that the nutritional history of the animals also influenced tumor incidence. Rats raised on the low pyridoxine diet had fewer hepatomas than rats reared on the high pyridoxine diet, when they were later placed on the carcinogen: low pyridoxine diet.

Table 34

The Incidence of Hepatic Tumors in Rats Raised and Maintained on Synthetic Diets High and Low in Pyridoxine and Casein (0998)

Group no.	Growth diet Pyridoxine per kgm., mgm.	Maintenance diet * Pyridoxine per kgm., mgm.	Maintenance diet * Casein, per cent	Average weight increment at 4 mo., gm.	Average daily food intake,† gm. per rat	Survival ‡ at 4 mo.	Incidence § of tumors 4 mo.	Incidence § of tumors 6 mo.	Negative survivors at 6 mo.	Cirrhosis
1	6.0	2.5	12	36	10.1	15/15	7/15	15/15	0	Moderate
2	6.0	0.2	12	−19	8.3	16/17	3/16	13/16	3	Mild
3	1.5	2.5	12	20	9.7	15/15	9/15	13/15	1	Moderate
4	1.5	0.2	12	−29	7.7	16/17	3/16	8/16	8	Mild
5	1.5	2.5	48	41	11.3	12/15	9/12	11/12	1	Mild
6	1.5	0.2	48	−13	7.6	11/17	0/14	1/14	10	Mild

* Contained 0.06 per cent *p*-dimethylaminoazobenzene for first 4 months.
† The average of 5 determinations at 3 week intervals of the food consumed over a 3 day period.
‡ Survival = number alive at end of 4 months over number at start.
§ Incidence = number with tumors over number of survivors at 4 months.

3. Kensler et al. (0724) demonstrated that "vitamin-free", alcohol-extracted casein contained one of the two factors that protected rats against the carcinogenic effect of p-dimethylaminoazobenzene on a susceptibility diet; riboflavin was the other factor.

Twenty-six rats (specifications not given) placed on a diet of unpolished rice and carrot, containing 20 ml of 3% butter yellow per 1000 g, supplemented with 2 g of casein and 200 µg of riboflavin daily, had a cancer incidence of only 3% as compared with 70-80% for the supplements alone, and 97% for the basal control diet, over an experimental period of 110-150 days (See Fig. 4).

Protective Effect of Various Dietary Supplements against the Carcinogenic Effect of Butter Yellow

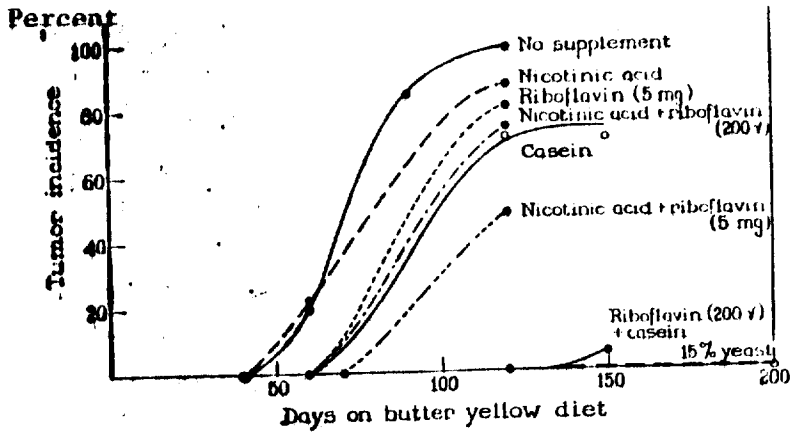


Figure 4 (0724)

4. Griffin et al. (0518) fed young male rats, diets containing either 12 or 24% casein together with the carcinogen p-dimethylaminoazobenzene (or a derivative) for periods of 14 to 20 weeks, and determined the influence of these components on the incidence of liver tumors. In a second series of experiments, the relative effects of methionine, and high and low levels of casein on the liver riboflavin of rats fed azo dyes was determined. The results are presented in Tables 35 and 36.

Rats fed casein at a level of 24% developed fewer hepatic tumors than those on the same basal diet with a casein level of only 12%. Supplementation of the 12% casein diet with methionine, however, also reduced the tumor incidence. Both casein and methionine promoted liver retention of riboflavin in rats fed carcinogenic azo dyes.

5. Harris et al. (0553) determined the effect of casein (20%) and varying concentrations of liver extract, riboflavin, cystine, cysteine, and several other substances, on the incidence of hepatic tumors induced by p-dimethylaminoazobenzene.

Wistar strain rats, 100-110 g BW, (other specifications not given), in groups of 25 to 90, were placed on one of the various diets containing 0.6 or 0.9 mg of carcinogen/g (See Tables 37 and 38). The animals were weighed semi-weekly or weekly and, after the third month, the abdomen was palpated once each week. In most instances, the animals were killed soon after tumors were detected. The livers of all animals included

Table 35

The Effect of Protein or Methionine on the Development of Liver Tumors in Rats Fed Certain Azo Dyes (0518)

Group*	% Carcinogen†	Diet	Time dye was fed (wk.)	Average initial weight (gm.)	Average weight at end of dye-feeding (gm.)	Average food intake (gm/rat/day)	Survival at end of dye-feeding	Final number of tumors	Negative survivors	Per cent tumors
1	0.06% m'Me.DAB	12% casein	18	166	199	11.8	14/14	14	0	100
2	0.06% m'Me.DAB	24% casein	18	165	225	10.0	13/14	6	7	47
3	0.06% m'Me.DAB	24% casein—90 mg. riboflavin/kg	18	168	226	11.1	14/14	1	13	7
4	0.06% m'Me.DAB	12% casein	14	179	182	10.0	15/15	13	2	87
5	0.06% m'Me.DAB	12% casein+3.6 gm. methionine/kg	14	173	203	10.3	15/15	10	5	67
6	0.06% m'Me.DAB	24% casein	14	172	210	10.3	15/15	10	5	67
7	0.096% o'Me.DAB	12% casein	10	178	184	8.0	14/15	11	3	79
8	0.096% o'Me.DAB	12% casein+3.6 gm. methionine/kg	10	179	234	10.7	13/15	4	9	31
9	0.096% o'Me.DAB	24% casein	10	174	224	11.0	14/15	7	7	50
10	0.06% DAB	12% casein	10	180	192	10.1	15/15	3	11	20
11	0.06% DAB	12% casein+3.6 gm. methionine/kg	10	182	221	10.5	15/15	3	12	20
12	0.06% DAB	24% casein	10	180	230	11.0	15/15	4	10	26
13	0.06% DAB	12% casein	20	207	254	13.9	12/15	4	7	33
14	0.06% DAB	12% casein+7.2 gm. methionine/kg	20	201	257	14.1	14/15	2	11	14
15	0.06% DAB	24% casein	20	203	285	15.6	12/15	2	10	17
16	0.06% DAB	36% casein	20	208	307	17.7	14/15	7	7	50

* Groups 1–6 and 4–12 received 2 mg. of riboflavin per kilogram of diet; groups 13–16 received 1 mg. of riboflavin per kilogram.

† DAB = p-dimethylaminoazobenzene.
m'Me.DAB = m'methyl-p-dimethylaminoazobenzene.
o'Me.DAB = o'methyl-p-dimethylaminoazobenzene.

Table 36

Relative Effect of Methionine and Protein on Liver Riboflavin in Rats Fed Azo Dyes (0518)

Group	Diet* and dye fed	No. animals	Initial weight	Growth increment (gm/rat/wk)	Daily food intake (gm/rat/day)	Liver riboflavin: μgm/gm	Liver riboflavin: Range	Liver riboflavin: Total μgm
83	12% casein—control	3	246	10	15.0	19.3	16.3–20.3	182
84	12% casein+0.06% DAB†	6	180	−2	11.5	15.6	12.6–17.5	114
85	12% casein+0.06% DAB+4 gm. methionine/kg	6	181	2	11.5	19.4	16.7–20.5	145
86	12% casein+0.064% m'Me.DAB	3	240	−10	10.0	15.5	14.8–16.2	100
87	12% casein+0.064% m'Me.DAB+4 gm. methionine/kg	3	215	2	11.5	15.9	15.0–17.6	108
88	12% casein, restricted control	3	240	2	11.0	20.8	19.0–21.5	162
89	18% casein+0.06% DAB	3	258	−2	12.0	17.2	17.1–17.3	180
90	18% casein+4 gm. methionine/kg+0.06% DAB	3	250	0	14.0	19.6	15.7–22.0	184
91	24% casein+0.06% DAB	3	270	0	14.0	20.8	19.9–22.3	220
92	18% casein+0.04% m'Me.DAB	3	270	−12	10.0	14.9	13.4–16.2	109
93	18% casein+4 gm. methionine/kg+0.04% m'Me.DAB	3	255	−13	8.1	15.6	14.7–16.5	145
94	24% casein+0.04% m'Me.DAB	3	270	−2	14.0	16.8	13.8–19.2	157
95	18% casein+0.096% o'Me.DAB	3	250	−1	12.5	17.0	16.0–17.9	176
96	18% casein+0.096% o'Me.DAB+4 gm. methionine/kg	3	270	−4	13.0	19.3	16.7–23.3	200

* Diets 83–88 contained 2 mg. riboflavin/kg; diets 89–96 contained 1 mg./kg. Feeding period for all groups was 3 weeks.
† DAB = p-dimethylaminoazobenzene.

Table 37

Composition of Test Diets (0553)

	Basal Diets No. 1	No. 2	No. 3	No. 4	No. 5
Casein, vitamin-free, Labco	200.00	0.0	200.00	200.00	100.00
Crisco or Primex	50.00	50.00	50.00	50.00	50.00
McCollum's Salt Mixture No. 185* (modified)	40.00	40.00	40.00	40.00	40.00
Agar†	20.00	20.00	20.00	20.00	20.00
Carotene	0.01	0.01	0.01	0.01	0.01
Vitamin D Concentrate in cottonseed oil (400,000 U. per gm.)	0.005	0.005	0.005	0.005	0.005
Thiamin	0.005	0.005	0.005	0.005	0.0025
Riboflavin	0.005	0.001	0.0015	0.008	0.0015
Vitamin B_6	0.003	0.003	0.003	0.003	0.002
Nicotinic acid	0.01	0.005	0.01	0.01	0.01
α-Tocopherol‡	0.01	0.01	0.01	0.01	0.01
Calcium pantothenate	0.0056	0.0056	0.0056	0.0056	0.0056
Choline chloride	1.0	0.0	1.0	1.0	0.0
Cerelose, refined§	689.0	0.0	689.0	689.0	790.0
Ground polished rice	0.0	890.0	0.0	0.0	0.0

* This was modified by the addition of 0.4 gm. of KI, 0.72 gm. of $CuSO_4$, and 1.0 gm. of $MnCl_2$ to 5916.6 gm. of salts mixed according to McCollum's formula.

† After February, 1942, agar had to be omitted from the diets, and pieces of filter paper were fed to the rats. Some of the animals of diets 1, 10, 17, 19, 20, 24, 25, and 26 were subjected to this change. Animals receiving diets 27 to 42 never had agar. An additional 2% of carbohydrate was substituted for agar.

‡ In the later experiments this was supplied as distilled natural tocopherols.

§ In February, 1943, it became necessary to replace cerelose by starch. Some of the rats on diets 30 to 35, inclusive, were subjected to this change.

Table 38

The Composition of the Diets Made from the Various Basal Diets (0553)

Diets

1.	4850	gm.	1st Basal
	150	"	Carcinogen solution
2.	4704.5	gm.	1st Basal
	150	"	Carcinogen solution
	145.5	"	Liver Extract, Lilly
3.	4845	gm.	1st Basal
	150	"	Carcinogen solution
	24.5	cc.	Liver Extract Solution, Purified, Lilly*
5.	4845	gm.	1st Basal
	150	"	Carcinogen solution
	4.85	"	*p*-Aminobenzoic acid
8.	4850	gm.	1st Basal
	150	"	Carcinogen solution
	165	mgm.	Pantothenic acid
10.	4800	gm.	1st Basal
	150	"	Carcinogen solution
	50	"	*l*-Cysteine hydrochloride
17.	4850	gm.	1st Basal
	150	"	Carcinogen solution
	125	mgm.	Riboflavin
19.	4842.5	gm.	1st Basal
	150	"	Carcinogen solution
	7.5	"	Choline hydrochloride
20.	4845	gm.	1st Basal
	150	"	Carcinogen solution
	5	"	Inositol
9.	4850	gm.	Ground polished rice
	150	"	Carcinogen solution
15.	4850	gm.	2d Basal
	150	"	Carcinogen solution
16.	4704.5	gm.	2d Basal
	150	"	Carcinogen solution
	145.5	"	Liver Extract, Lilly
27.	4850	gm.	2d Basal
	100	"	Carcinogen solution
	50	"	*l*-Cysteine hydrochloride
28.	4895	gm.	2d Basal
	100	"	Carcinogen solution
	5	"	*l*-Cysteine hydrochloride
29.	4850	gm.	2d Basal
	100	"	Carcinogen solution
	50	"	*l*-Cystine
30.	4100	gm.	2d Basal
	150	"	Carcinogen solution
	750	"	Liver Extract, Lilly
21.	4850	gm.	3d Basal
	150	"	Carcinogen solution
22.	4800	gm.	3d Basal
	150	"	Carcinogen solution
	50	"	*l*-Cysteine hydrochloride
35.	4600	gm.	3d Basal
	150	"	Carcinogen solution
	250	"	Lipoic acid
23.	4850	gm.	4th Basal
	150	"	Carcinogen solution
24.	4800	gm.	4th Basal
	150	"	Carcinogen solution
	50	"	*l*-Cysteine hydrochloride
34.	4825	gm.	4th Basal
	150	"	Carcinogen solution
	25	"	Succinic acid
41.	4800	gm.	4th Basal
	150	"	Carcinogen solution (2% Dye)
	50	"	*l*-Cystine
42.	4800	gm.	4th Basal
	150	"	Carcinogen solution (2% Dye)
	50	"	*l*-Cysteine hydrochloride
25.	4900	gm.	5th Basal
	100	"	Carcinogen solution
26.	4754	gm.	5th Basal
	100	"	Carcinogen solution
	146	"	Liver Extract, Lilly

* Equivalent to twice the amount of fresh liver represented by the crude liver extract used in diet No. 2.

in the tumor-incidence calculations were examined microscopically. The results are presented in Table 39.

Table 39

Microscopic Liver Examinations
and Tumor Incidences (0553)

Diet No.	Date begun 1948	Total used	Effective Total	Tumors	Minimal latent period, days	50% Tumor incidence, days	In Tumor Rats: Cirrhosis: Much %	Slight %	Focal %	Fibrosis %
1	Jan. 14	90	62	55	104	212	0	6	31	72
1*	July 29	40	31	28						
2	Jan. 14	30	26	24	112	184	0	0	8	52
3	Jan. 15	30	28	26	139	205	4	0	24	48
5	"	30	23	22	132	183	5	5	14	77
8	Mar. 15	30	17	17	124	174	0	12	41	76
9	Apr. 25	44	19	15	90	163	73	27	0	47
10	Jan. 15	30	28	22	119	296	0	6	21	48
10*	Sept. 11	30	19	12						
15	May 19	55	34	30	87	139	83	13	3	43
16	"	40	28	22	143	401	0	9	18	73
17	Aug. 21	30	27	23	96	173	4	0	31	65
19	Sept. 16	30	23	21	134	250	10	33	33	50
20	"	30	17	15	126	235	20	40	20	73
21	Sept. 11	30	20	15	72	153	100	0	0	33
22	Sept. 22	30	26	25	113	152	52	36	8	48
23	Sept. 12	30	21	18	131	227	6	33	39	45
24	Sept. 29	30	18	9	198	408	11	11	22	44
25	Dec. 16	50	32	29	120	162	86	14	0	31
26	"	25	23	13	162	455	0	31	15	54
	1942									
27	Mar. 16	25	23	22	114	176	90	5	5	72
28	"	25	20	19	121	142	100	0	0	50
29	"	25	22	21	127	169	81	19	0	43
30	Apr. 21	25	20	17	100	266	24	18	30	41
34	Apr. 28	25	20	19	176†	232	10	25	35	10
35	May 8	25	23	20	155†	213	0	40	35	50
	1944									
41	July 24	25	22	11	148	561	0	25	25	34
42	"	34	20	9	222	never	0	9	36	9

* Repeat.
† Latent period too long, unknown circumstances having prevented palpation sooner than five months.

Casein (20%) without supplements in the basal diet had little or no effect on the incidence of hepatic tumors in this study. When coupled with liver extract (3%), riboflavin (5-8 mg/kg diet), cystine (1%), or cysteine (1%), however, there was a marked protective effect on tumor induction by the carcinogen. Choline, inositol, p-aminobenzoic acid, pantothenic acid, lipoaic, and succinic acids, were without appreciable effect on carcinogenesis. The protective effects of exceptionally high concentrations of casein (48-60%) reported by other workers, may be due to its high content of cystine (0553).

6. Sugiura and Rhoads (1406) investigated the effect of casein on the induction of liver cancer in rats by butter yellow (p-dimethylamino-azobenzene).

Twenty animals (Wistar and Sherman strains), both sexes, weighing 65-185 g, were fed a diet consisting of 82% unpolished rice and 18% casein, 0.6 g/kg of butter yellow plus approximately 1 g of fresh carrot per rat daily, over a period of 284 days and determined weight changes and incidence of liver tumors. The data are presented in Table 40.

The results of this study clearly indicated that 18% casein in a diet of unpolished rice and carrot did not protect the animals against the carcinogenic action of p-dimethylaminoazobenzene. The effects of yeast and rice bran were investigated also in parallel with the study on casein.

Table 40

Effect of Feeding Rice-Bran Extract and Unpolished Rice on the Production of Liver Cancer by Butter-Yellow (1406)

Rat no.	Sex	No. of days fed	Body weight (gm.) Initial	Maximum	Final	Liver findings*
1	♂	100	134	157	157	—
2	♂	100	121	136	120	—
3	♀	100	125	145	142	—
4	♂	106	150	155	154	—
5	♂	110	147	177	170	—
6	♂	110	150	151	142	±
7	♂	112	115	122	122	±
8	♀	114	117	122	100	—
9	♂	116	119	136	133	—
10	♀	120	142	142	127	—
11	♀	120	132	132	118	—
12	♂	120	137	137	70	±
13	♀	125	124	151	149	—
14	♀	128	121	121	100	—
15	♂	129	130	155	155	—
16	♂	130	126	126	108	—
17	♀	130	129	130	144	±
18	♂	138	122	144	144	±
19	♂	139	120	151	151	—
20	♂	139	132	132	122	—
21	♂	139	116	127	120	±
22	♂	149	148	148	130	—
23	♀	150	127	160	147	—
24	♀	150	117	128	120	±
25	♂	150	122	122	117	±
26	♀	153	145	145	109	—
27	♀	161	115	133	100	—
28	♂	161	159	159	120	—
29	♂	161	150	150	124	—
30	♂	161	167	167	165	—
31	♀	161	135	138	117	±
32	♂	161	151	151	110	+
33	♂	161	179	179	168	+
34	♂	170	158	165	161	—
35	♀	172	113	118	118	—
36	♂	185	140	140	120	±
37	♀	200	121	135	130	±
38	♀	200	122	129	130	+
39	♂	218	174	160	100	—
40	♂	218	133	133	95	±
41	♂	218	162	173	144	±
42	♂	218	134	150	138	++
43	♂	228	108	128	112	+
44	♂	250	140	140	135	—
45	♂	250	148	148	133	±
46	♀	250	135	149	139	±
47	♂	250	135	135	111	+
48	♀	250	141	173	162	++

* — indicates smooth, practically normal liver; ± indicates nodular cirrhosis with adenomatous hyperplasia; + indicates distinct areas of cholangioma or hepatoma, or both; ++ indicates extensive liver cancer with or without metastasis. In all cases the diagnoses were confirmed by microscopic examination.

7. Shay et al. (1356) investigated the effect of high and low casein, lactalbumin, and ovalbumin diets on the incidence of 3-methylcholanthrene-induced mammary adenomas in rats.

Female Wistar rats, 4 to 5 weeks old, weighing 60-80 g, in groups of 22 to 66, were given daily oral doses containing graded amounts of 3-methylcholanthrene and placed on one of the test diets outlined in Table 41, or on the Rockland Complete Rat/Mouse control diet, and observed for the development of tumors. Growths were excised when they reached the 1-2 cm diameter size and the animals continued in the experiment. All tumors were examined microscopically for verification of mammary adenocarcinoma diagnosis. The observation period was 52-56 weeks. Major findings are presented in Tables 42 and 43.

The data showed that rats on the high casein diet (64%), with or without 3-methychloanthrene, definitely had a higher incidence of mammary adenocarcinomas than rats on the control diet. Results with ovalbumin at the 64% level were inconclusive because it was not as acceptable dietarily as the casein or control diets.

Table 41

The Special Diets were Prepared by the Nutritional Biochemical Company, Cleveland, Ohio, and their compositions were as follows: (1356)

Diet No. 1:
64% Vitamin-free casein
22% Sucrose
8% Vegetable oil
2% Brewer's yeast, USP
4% Salt mixture, USP, No. 2, plus Vitamine-diet fortification mixture.

Diet No. 2:
27% Vitamin-free casein
59% Sucrose
Oil, yeast, salt, and vitamin mixture as in diet 1.

Diet No. 3:
64% Purified lactalbumin
22% Sucrose
Oil, yeast, salt, and vitamin mixture as in diet 1.

Diet No. 4:
64% Ovalbumin
22% Sucrose
Oil, yeast, salt, and vitamin mixture as in diet 1.

Diet No. 5:
13.5% Vitamin-free casein
13.5% Ovalbumin
59% Sucrose
Oil, yeast, salt, and vitamin mixture as in diet 1.

Diet No. 6:
7% Vitamin-free casein
20% Ovalbumin
59% Sucrose
Oil, yeast, salt, and vitamin mixture as in diet 1.

Table 42

Mammary Carcinoma in Female Wistar Rats Maintained on 3 Diets-No Methylcholanthrene Given (expt. 1) (1356)

Group and diet	Weeks:	0	4	8	12	16	20	24	28	32	36	40	44	48	52	Breast tumors Number	Breast tumors Percent	Additional breast tumors Number	Additional breast tumors Tumors/rat	Other tumors
1 Rockland	Rats alive	54	54	54	54	54	54	54	52	52	52	52	51	51	30					
	Rats with breast cancer														1	1	2	1	0.03	0
	Mean body weights	75	153	186	199	210	215	222	230	233	237	241	242	254						
2 64% casein	Rats alive	66	66	66	66	64	62	62	61	61	61	60	60	59	0					
	Rats with breast cancer								2	2	1			1		6	10	4	0.15	1F*
	Mean body weights	72	144	181	198	210	223	228	241	246	257	265	272	284						
3 27% casein	Rats alive	24	24	24	24	24	23	23	23	23	23	23	22	22	6					
	Rats with breast cancer									1			1			2	10	4	0.25	1F*
	Mean body weights	71	152	174	190	204	213	223	236	246	250	254	266	275						

F = fibroadenoma of breast.

Table 43

Mammary Carcinoma in Female Wistar Rats Receiving 5 mg 3-Methylcholanthrene for 10 Days (total dose 50 mg) and Maintained on Rockland Rat Diet and Casein Diets (1356)

Group and diet	Weeks:	0	4	8	12	16	20	24	28	32	36	40	44	48	52	Breast tumors Number	Breast tumors Percent	Second tumors	Other tumors
6 Rockland	Rats alive	46	46	46	46	46	46	44	44	44	43	41	41	39	6				
	Rats with breast cancer				2		1		1			1	1		1	7	15	2	1F* 1L†
	Mean body weights	70	139	168	185	202	207	215	221	227	230	235	242	249					
7 24% Casein	Rats alive	42	42	41	41	40	39	38	38	37	35	35	35	35	8				
	Rats with breast cancer				2	2	3	2	1	1	1	1		2		15	36	6	2F*
	Mean body weights	70	141	173	194	204	206	213	221	226	231	241	246	245					
8 27% Casein, high weight	Rats alive	22	22	22	22	22	22	22	21	21	21	20	18	18	5				
	Rats with breast cancer				1	3		2	2		2			2		12	55	4	1L†
	Mean body weights	70	156	192	212	227	232	241	254	258	271	289	301	321	317				
9 27% Casein, low weight	Rats alive	21	21	20	18	18	18	17	17	17	16	15	15	15	6				
	Rats with breast cancer				2				1	1	2	1		1	1	9	43	3	1F*
	Mean body weights	73	142	167	182	193	199	211	217	224	226	233	237	250					

adenoma of breast.
phoma.

The incidence of tumors in rats on a 64% lactalbumin diet was 24% in comparison with 15% for the control diet. Casein at the 64% level ad libitum was not included in this experiment. For comparative purposes, however, in another experiment (See Table 43), when the tumor incidence was 15% on the control diet, 36% of rats on the high casein diet developed tumors.

The authors concluded that casein contains some factors that accelerate carcinogenesis induced in the breast by 3-methylcholanthrene.

8. Dunning et al. (0363) placed pedigreed Fischer line 344 female rats on a high casein (40-45%) diet containing varying amounts of yeast or purified DNA (See Table 44) and determined the effect of the combination on 2-acetylaminofluorene-induced cancer over a period of more than 400 days. The most striking observation in this study as indicated in Table 45 was the high incidence of mammary carcinoma (25%) in rats on the 45% casein diet as compared with an incidence of 0-6% in animals on the other diets.

Table 44

Composition of Diets (0363)

Material	Diet 6	Diet 13	Diet 14	Diet 15	Diet 16	Diet 17
	%					
Casein	45.0	40.0	44.0	43.0	40.0	40.0
Brewers yeast	0	0	0	0	5.0	0
High nucleic acid yeast*	0	5.0	0	0	0	0
Desoxyribonucleic acid	0	0	1.0	2.0	0	5.0
Salt mixture	4.0	4.0	4.0	4.0	4.0	4.0
Cello flour	2.0	2.0	2.0	2.0	2.0	2.0
Dextrin	34.0	34.0	34.0	34.0	34.0	34.0
Crisco	15.0	15.0	15.0	15.0	15.0	15.0
Halibut liver oil	.4	.4	.4	.4	.4	.4
2-Acetylaminofluorene	.06	.06	.06	.06	.06	.06

Vitamin supplement per kilo of diet†

	mg		mg		mg
Thiamin	4	Niacin	4	Choline HCL	2,000
Riboflavin	8	Calcium pantothenate	20	α tocopherol	150
Pyridoxine HCL	4				

* Supplied through the courtesy of Dr. Joel B. Peterson, Standard Brands, Inc., New York City.

† The crystalline B vitamins were dissolved in distilled water and added as a daily supplement to each food cup.

Table 45

Average Survival in Days ± P.E. and % of Rats with Induced Cancer (0363)

Group %	No. rats	Survival, days ± P.E.	Liver cancer %	Liver cancer % met.	Ear cancer %	Ear cancer Avg days	Mammary cancer %	Mammary cancer Avg days
45 casein	16	349 ±11.	75	67	44	323	25	327
5 high nucleic acid yeast	18	409 ± 9.4	83	47	33	338	6	406
1 nucleic acid	12	324 ±11.	67	62	67	290	0	
2 " "	12	322 ± 6.1	92	54	50	280	0	
5 " "	12	315 ± 6.1	83	30	75	286	0	
5 Brewer's yeast	11	317 ± 6.5	82	62	64	319	0	

9. Engel et al. (0389) fed weanling female rats (Alabama Experiment Station Strain), 20-22 days old, weighing 40-60 grams, a semi-synthetic diet containing varying concentrations of casein and 300 mg/kg of 2-acetylaminofluorene. The animals were weighed and examined for visible tumors every 7 days over an experimental period of 40 weeks. Results are presented in Table 46.

The data obtained indicated that increasing the concentration of casein in the diet from 9-27% to 40-60% reduced mammary tumor incidence from 86% to 12% (0389). Casein appeared to be partially protective against induction of ear duct and liver tumors also.

Addition of desoxyribonucleic acid or yeast nucleic acid had no effect on the protective action of casein (0389). Supplementation of the 60% casein diet with vitamin B_{12}, however, almost completely eliminated the beneficial effect of the high protein intake on survival. The authors pointed out that a decreased concentration of sucrose in the high casein diet should be considered in interpreting the results obtained.

Table 46

Influence of Dietary Casein Level on Tumor Induction by 2-Acetylaminofluorene (AAF) (0389)

Exp. no.	Group no.	Dietary casein level (per cent)	Av. body weight Initial (gm.)	Av. body weight 46 wks. (gm.)	Av. daily intake per rat Feed (gm.)	Av. daily intake per rat AAF (gm.)	No. rats	No. rats with tumors Mammary	No. rats with tumors Ear duct	No. rats with tumors Liver	No. rats with tumors None	Av. survival (wks.)
1	1	9	52	173	6.6	1.98	17	14(22)*	12(25)*	10	0	27
	2	20	51	197	7.0	2.10	8	7(21)	5(25)	4	0	28
	3	27	49	210	7.0	2.10	4	3(21)	2(24)	3	0	30
	4	40	49	196	7.0	2.10	8	2(25)	3(28)	4	0	32
	5	60	53	168	6.5	1.95	9	0	2(37)	4	3	40†
2	6	12	48	198	7.2	2.16	18	16(10)	12(24)	8	0	27
	7	12‡	56	216	7.6	2.28	6	6(20)	3(25)	2	0	28
	8	12§	57	220	7.6	2.28	6	6(21)	4(24)	3	0	27
3	9	60	47	222	7.7	2.31	6	5(29)	4(31)	4	0	42
	10	60#	46	216	8.0	2.40	12	10(18)	7(23)	4	0	32
4	11	9	47	162	6.2	1.86	7	5(21)	4(24)	5	0	28
	12‖	60	48	184	6.4	1.92	8	1(31)	3(31)	2	3	32**
	13	60	46	214	7.2	2.16	8	5(25)	6(29)	4	0	32**

* The numbers in parentheses are the average tumor induction periods in weeks.

† All these animals were still alive after the 40-week experimental period. They were sacrificed at that time to determine the liver tumor incidence.

‡ The diet was supplemented with 1 per cent of desoxyribonucleic acid.

§ The diet was supplemented with 3 per cent of yeast nucleic acid.

The diet was supplemented with 40 μg. of vitamin B_{12} and 2 mg. of folacin per kilogram.

‖ Rats of this group were pair-fed with those of group 11.

** All these animals were still alive after the 32-week experimental period. They were sacrificed at that time to determine the incidence of liver tumors.

10. Korpassy et al. (0783) investigated the effect of high and low dietary casein on the incidence of liver cirrhosis and tumors in rats treated with tannic acid. Three groups of young white rats, 17 males and 23 females per group, were treated as follows: Group I was fed a high casein/low fat diet (25% casein, 5% sunflower oil); Groups II and III were placed on a low casein/high fat diet (3% casein, 20% sunflower oil), and 1.25 mg of pyridoxine ("Benaden") each day.

Groups I and II were injected subcutaneously every 5th day with at first 150 mg, then 200 mg, and finally 250 mg of tannic acid per kilogram bodyweight in the form of a 1.5-2.5% aqueous solution. Group III served as the carcinogen-free controls.

The animals were observed throughout for toxic effects, and prior to the 180th day, all survivors were subjected to laparotomy. Animals on the low casein-high fat diet (Group II) had a much higher incidence of tumors (67.5%) than those on the high casein-low fat diet (29.7%). None of the carcinogen-free control animals (Group III) showed any gross or microscopic evidence of hepatic tumors. The authors noted however, that high casein did not protect against the cirrhogenic action of tannic acid; actually, cirrhosis seemed to occur more commonly on a high casein diet.

Protection from Liver Damage

Low to moderate dietary supplements of casein have been found to have a protective effect on certain types of experimentally-induced liver damage.

Rats

1. Litwack et al. (0892) investigated the effect of casein, gelatin, and various amino acid mixtures on liver fat deposition in the rat. Three-week-old Sprague-Dawley rats, 6 to 9 per group, were placed on a basal ration supplemented with gelatin, casein, or an amino acid mixture (See Table 47). Fat soluble vitamins in the form of 2 drops of halibut liver oil were given each week in addition to the supplement; the latter were at the expense of carbohydrate. The animals were weighed regularly and the average weekly growth circulated over the five-week feeding period. At the end of the 5th week, the rats were sacrificed and the fat content of the livers determined by the standard A.O.A.C. method. The major results are presented in Table 48.

The data obtained indicated that both casein and gelatin, at the levels employed, were very effective in preventing abnormal liver fat deposition in rats. The various amino acids tested, alone, and in combination, as supplements in the basal ration, were ineffective in this respect.

Table 47

Basal Ration for Fat Deposition Studies (0892)

Component	Level in diet (per 100 g ration)
Casein	9 g
Lecythine	.2
Sucrose or dextrin	81.9
Corn oil	5
Salt - 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

Table 48

Effect of Increasing Protein and Single Amino Acids Supplemented to Sucrose Basal Ration Without Cystine (0892)

Supplement, %	No. of animals	% fat of dry liver		Avg growth rate (g/wk)	
		Avg	Range	Avg	Range
0	6	20	(13.0-35.2)	7.6	(5.8-11.6)
3 Casein	6	11.5	(6.0-17)	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	13.0	(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)

2. Schwarz (1325), in 1952, discovered that crude and alcohol-extracted casein, at a level of 3% in the diet, contained a factor (Factor 3) which protected rats against acute fatal dietary liver necrosis produced by a vitamin E-free diet. The protective factor occurred in certain yeasts also. An amino acid mixture similar to that of casein was without effect. Factor 3 was found to be different from vitamin E or cystine, two substances already known to protect rats against the condition.

3. Naftalin (1044) in a study of acute dietary liver necrosis in rats discovered that the condition was markedly affected by the type of casein used and the weaning age of the animals. Male rats (hooded Lister strain), 8 to 10 per group, weaned at different ages, were placed on high and low protein diets as indicated in Table 49. The median survival time and incidence of liver necrosis were determined. The data are presented in Table 50.

Rats weaned at 17-19 days of age suffered a significantly higher incidence of liver necrosis than those weaned at 25 days of age. Genatosan "low-vitamin" casein completely prevented liver necrosis even in rats that were weaned at an early age. Glaxo "vitamin-free" casein and Glaxo "C" casein (unextracted) led to an incidence of acute liver necrosis more than twice as great as that found when Genatosan "low-vitamin" casein was employed.

Table 49

Management of Experimental Animals, Including Procedure for Rearing Rats Before Low-Protein Diets Were Given (1045)

	Expt. 1 July-Sept. 1951	Expt. 2* Oct. 1951-Jan. 1952	Expt. 3 Feb.-May 1953
Mothers' diet	Rowett Institute stock rat cubes + cow's milk	Rowett Institute stock rat cubes + cow's milk	Rowett Institute stock rat cubes + cow's milk
Litter	1st	1st	1st
High-protein diet	Contained 16 per cent. Glaxo "vitamin-free" casein	Contained 16 per cent. Genatosan casein "low-vitamin" content	Contained 16 per cent. Glaxo casein "C" unextracted
Age of young when high-protein diet first offered (days)	19	17	17
Age when weaned (days)	17 (group A) 19 („ B) 25 („ C)	17 (groups D, F, H) 25 („ E, G, I)	17 (group J) 20 („ K) 25 („ L)
Age when low-protein diet first offered (days)	38	38	38
Duration of experiment (days after feeding on low-protein diet)	72	73	72

Table 50

Age at Weaning and Incidence of
Acute Liver Necrosis in Expts. 1-3 (1045)

Expt	Group	Age when weaned (days)	Low-protein diet (L.P.D.)	No. of rats tested	No. dead with acute liver necrosis	Median survival time (days after eating L.P.D.)
1	A	17	Glaxo	12	11	10
	B	19	" vitamin-free "	12	10	13
	C	25	casein	12	9	28
2	D	17	Genatosan casein	10	0	...
	E	25	" low-vitamin " content	10	0	...
	F	17	Yeast-sucrose diet	10	8	16
	G	25		10	3	... *
	H	17	Yeast-maize-starch-	9	9	18
	I	25	sucrose diet	8	2	... †
3	J	17	Glaxo casein " C "	8	8	6
	K	20	unextracted	8	8	6
	L	25		8	4	... ‡

* One rat died on each of the following days after eating the low-protein diet; 19, 35, 46.
† One rat died on each of the following days after eating the low-protein diet; 20, 51.
‡ One rat died on each of the following days after eating the low-protein diet; 2, 12, 20, 26.

Anti-Goiter Effects

The anti-goiter effect of casein has stimulated considerable research concerning possible mechanisms.

1. Remington (1226), in 1937, discovered that vitamin-free casein added to a goitrogenic diet for rats reduced goiter severity in direct relation to the amount fed (See Table 51). Yeast was without effect on goiter incidence and liver worsened the condition. After analyzing samples of the casein used, however, the author concluded that the anti-goiter effect was due to its iodine content.

2. Van Middlesworth (1485) confirmed Remington's discovery (1226), using a "low iodine" casein in a goitrogenic diet for rats (See Table 52). The author suggested that the iodine requirement of the rat may be decreased by casein.

3. Axelerad et al. (0087) studied the effect of casein on goiter development in mice with a diet very low in iodine (4.6 μg./100 g).

One group of 9 C3H (Heston) male mice weighing 17-27 grams was placed on a liver-yeast-glucose-mineral salts goitrogenic diet; a second group was fed the same basal ration but with liver replaced by casein (16%)[a]. Both groups were allowed food and water ad libitum over a period of 74 days. Two-and-a-half hours before sacrifice on the last day, all animals were

[a]Acid-extracted vitamin test casein, Nutritional Biochemicals Corporation, Cleveland, Ohio

injected with 2 μc of radioactive iodine. The rate of radioactive-iodine uptake and degree of goiter were then determined at autopsy. The results presented in Table 53 suggested that the anti-goiter effect of casein was not due to the minute amount of iodine carried.

Table 51

Effect of Casein on Goiter Severity in Rats (1226)

DIET	327	328	329	330	COLONY
Wheat gluten	20	15	10	0	
Casein	0	5	10	20	Commercial dog biscuit
Corn meal	78	78	78	78	
$CaCO_3$	1	1	1	1	
NaCl	1	1	1	1	
Number of rats	10	10	10	10	10
Sex	4 M-6 F	4 M-6 F	5 M-5 F	3 M-7 F	5 M-5 F
Gain in weight (gm.)	51±2	68±4	74±4	67±4	120
Thyroid weight (mg.)	27.4 (19.1-37.7)	24.9 (18.2-36.1)	23.1 (18.2-27.9)	18.3 (15.1-21.1)	15.8 (11.0-20.2)
Thyroid weight per 100 gm. body weight (mg.)	23.9±1.0 (18.7-35.2)	18.9±0.6 (13.7-21.0)	17.1±0.5 (13.6-21.0)	14.1±0.4 (11.4-16.5)	8.8±0.4 (6.3-11.7)
Dry matter (%)	21.0	23.6	25.9	27.4	32.0
Iodine, dry basis (%)	0.0137	0.0297	0.0451	0.0595	0.2000
Total iodine (γ)	0.79	1.75	2.70	2.99	10.1
Degree of enlargement	2.4	1.9	1.7	1.4	

4. Van Middlesworth (1486) reinvestigated the goiter-preventing effect of casein using a preparation that contained no inorganic iodide and only a trace of organic iodine. The final concentration of iodine in the diet amounted to only 0.01 μg of I per gram. Analysis of the data obtained led the author to suggest that the active goiter-preventing principle in casein is produced or released when the casein-iodine combination is digested.

5. Nordsiek (1091) investigated the effect of casein on the goitrogenic action of soybeans in rats fed test diets containing various proportions of the two substances.

Diets designated 35 and 35 CAS (Table 54) contained 35% by weight of raw soybeans; those designated 60 and 60 CAS contained 60%. Corn oil (5%) and cellulose (6%) were included in diets 35 and 35 CAS. All diets

Table 52

Thyroid Weights of Rats Fed Different Diets for Progressively Increased Durations (1485)

Diet	Weeks on Diet	No. of Rats	Body wt. (g) ±S.D.*	Thyroid wt. (mg) ±S.D.*	Thyroid mg/100gm body wt. ±S.D.*
		Fed diet less than 4 mo			
Control (Purina Lab. chow)	8	8	153 ± 14	15 ± 1.7	9.8 ± 1.1
Cereal-G	6	9	175 ± 33	54 ± 18	31 ± 10
Cereal-G	12	9	202 ± 41	60 ± 12	29.7 ± 12
Cereal-G+25% casein	8	14	208 ± 43	31.4 ± 14	15.1 ± 4.1
Cereal-G+NaI (1.5 μg NaI per day)	8	6	152 ± 17	16 ± 3.6	10.5 ± 2.4
Cereal-G+NaI (3.0 μg NaI per day)	8	4	155 ± 17	15 ± 3.4	9.7 ± 2.8
		Fed diet 4 to 8 mo			
Control (Rockland Farms)	24	9	197 ± 44	22 ± 4.8	11 ± 1.9
26% casein (with sucrose)	16	5	315 ± 38	23 ± 4.3	7.3 ± 1.3
13% casein (with sucrose)	16	11	260 ± 43	18 ± 5.6	7.0 ± 1.8
Cereal-G+25% casein	20	6	336 ± 14	32 ± 8.8	10.7 ± 4.1
Remington	32	15	275 ± 18	39 ± 19	14 ± 7.1
Cereal-G	32	11	266 ± 27	192 ± 58	72 ± 20
		Fed diet 10 to 26 mo			
Control (Purina Lab. chow)	40-50	13	362 ± 38	27 ± 3.6	7.5 ± 0.98
Cereal-G	50	10	249 ± 57	135 ± 55	54.4 ± 22
Cereal-G+10% casein	50	2	370 ± 71	61 ± 9	17 ± 6
26% casein (with sucrose)	100	5	294 ± 36	26 ± 5.2	8.7 ± 1.7
Remington	80-100	6	438 ±118	231 ±146	52.8 ± 33
Cereal-G 3 mo. then Cereal-G+25% casein 2 mo. then Cereal-G 6 mo		7	329 ± 53	296 ±104	90.0 ± 53

*S.D. means standard deviation calculated from the range (R. B. Dean and W. J. Dixon, Anal. Chem. 23, 636 (1951).

Table 53

Inhibition of Development of Low Iodine Goitre by Casein (0087)

Composition of Diets								
Casein	Extracted liver	Dried brewer's yeast	Glucose	$CaCO_3$	NaCl	Iodine content of diet	Thyroid weight*	I^{131} uptake*
						μg./100 gm.	mgm/left lobe	% injected do per left lobe
-	16	10	72	1	1	3.5	12.0 ± 1.2	43.6 ± 3.1
16	-	10	72	1	1	4.6	0.8 ± 0.1	23.0 ± 3.0

*Mean ±Standard error.

Table 54

Effects of Casein on Soybean Goitrogenesis

Effects on Thyroid Gland Parameters of Feeding Soybean Diets *ad libitum* to Male Sprague-Dawley Rats for 7 Weeks Starting When Weaned at 3 Weeks of Age with Weights Averaging 48 g, Avg values, 10 rats/group. (1091)

Diet code	60MEAL*	35	35CAS	60	60CAS
Iodine in diet, μg/g	.005	.005	.003	.003	.003
Food consumed, g	601	460	637	603	590
Wt gained, g	194	95	208	165	187
Thyroid wt, mg/100 g					
Fresh	19.9	18.8	17.1	27.4	20.8
Stand. dev.	4.0	3.3	4.1	6.9	2.9
Dry	4.31	5.16	4.06	6.05	4.85
Stand. dev.	.05	.78	.09	1.23	.73
Iodine in pooled thyroids, % of dry wt	.0063	.0150	.0180	.0033	.0110

* Control.

contained 4% of U.S.P. XIII No. 2 salt mixture (no iodine salts) and 2.2% of a complete vitamin mixture in glucose. Casein (purified, Nutritional Biochemicals Corp.) was incorporated at a level of 17% in diet 35 CAS and 15% in diet 60 CAS. The remainder of each diet was made up of corn starch. The control diet also contained soybean meal protein but the material had been extracted previously at a temperature known to destroy much of the goitrogenic acticity of the material. The level of iodine in the various diets, by analysis, was only 0.003 to 0.005 μg/g. The author points out that this amount is less than 2% of the rat's requirement.

Weanling male albino rats of the Sprague-Dawley strain, 3 weeks old and weighing 48 g, in groups of 10, were placed on the various diets *ad libitum* for 7 weeks and food consumption and bodyweight recorded. At the end of the 7th week, the animals were sacrificed, and the wet and dry weights of the thyroid glands were determined. The results are presented in Table 54.

The author concluded that prevention of the goitrogenic effect of raw soybeans by casein was *not* due to the minute amount of iodine present in the preparation.

6. Giedosz (0475) studied the effect of casein on iodine metabolism in the mouse using the radioactive-iodine technique. One group of 6 white mice (specifications not mentioned) was given methyl thiouracil (MTU)

orally at a level of 1500 mg/kg BW daily for 28 days. A second group received 100,000 mg/kg BW of casein in addition to MTU. A control group of the same number was left untreated. On the last day of the experiment, 3 microcuries of free radioiodine (I^{131}) in physiological saline were injected s.c. into each animal. The following observations were made: radioactivity of the thyroid gland in vivo after 1, 3, 6, and 24 hours; total and protein-bound I^{131} in the serum, and I^{131} concentration in the urine, after 24 hours; the I^{131} content of the thyroid at autopsy. The results are presented in Tables 55 and 56.

According to the author, the data obtained indicated that MTU antagonized the action of thyrotrophic hormone as evidenced by a decrease in the amount of iodine in the thyroid after 24 hours (Table 55) and a diminution of the total amount of protein-bound iodine (Table 56). (It is known that the thyrotrophic hormone increases the amount of iodine in the thyroid gland and the formation of active organic iodine compounds (0475)). Casein enhanced the effect of MTU on the thryotrophic hormone by causing a larger decrease in protein-bound iodine (Table 56).

Table 55

Radioactivity of the Thyroid (0475)

In vivo					Skeletonized	
animals & their number	time after giving I^{131}				I^{131} in the entire thyroid	I^{131} in 1 mg of thyroid
	1^h	3^h	6^h	24^h		
control 6	23.6 ±1.7	25.7 ±2	31.0 ±5.5	33.0 ±2.3	32.4 ±2.33	1.6 ±0.38
Fed methylo-thiouracil 8	47.5 ±1.9	42.5 ±1.1	38.7 ±2.8	31.6 ±2.3	31.2 =2.1 (thyroid enlarged)	0.59 ±0.09
fed methylo-thiouracil and casein 6	35.2 ±1.3	32.9 ±1.1	31.5 ±1.1	23.6 ±0.6	23.0 ±0.44	0.44 ±0.1

Casein differed from methyl thiouracil in its effect on iodine metabolism in the mouse, however, in the following respects: (1) casein increased the excretion of iodine in the urine whereas MTU had the opposite effect (Table 56) and (2) MTU caused enlargement of the thyroid gland, casein did not (Table 55).

Table 56

Radioactivity of the Blood and Urine (0475)

Animals & their number	Total I^{131} in the serum	I^{131} combined with protein serum	Ratio I^{131} nonorganic to organic in the serum	I^{131} in the urine %
control 6	546±101	382±69.8	0.42±0.04	29±3.16
fed methylo-thiouracil 6	818±78.4	177±35.5	3.6 ±1.14	24±2.76
fed methylo-thiouracil and casein 6	637±113	143±24.4	3.4 ±0.4	34±1.1

Other Effects

Reports on the effect of casein on a few other disorders appeared to be noteworthy.

A. Rats

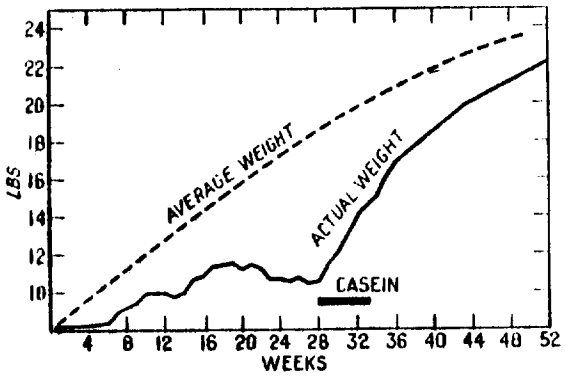
1. Suzuki (1413) compared the effect of casein and vitamin B (Paranutrin) supplements on the nutritional value of three commercial dextro-maltose formulas for rats. Six albino rats from the same litter were used in the study (qualifications not given).

Addition of Paranutrin in amounts up to 1 g daily failed to correct the nutritional deficiency of any of the formulas. On the other hand, supplementation with casein at levels of 11-25%, caused a dramatic increase in the growth of the test animals.

B. Humans

1. Gibbens (0472) treated 61 breast-fed infants suffering from diarrhea, with calcium caseinate (Plasmon powder) with dramatic results (See Figs. 5 and 6). Two to three teaspoonsful twice or three times daily usually resulted in an improvement in 2 to 3 days. Doses as high as 10-15 g daily have been used by other European physicians. The author reported also that casein has been used successfully as a supplemental food for premature and debilitated infants, as well as for chronic suppuration and a number of other miscellaneous conditions.

Figure 5



Weight chart in Case 1. (0472)

Figure 6

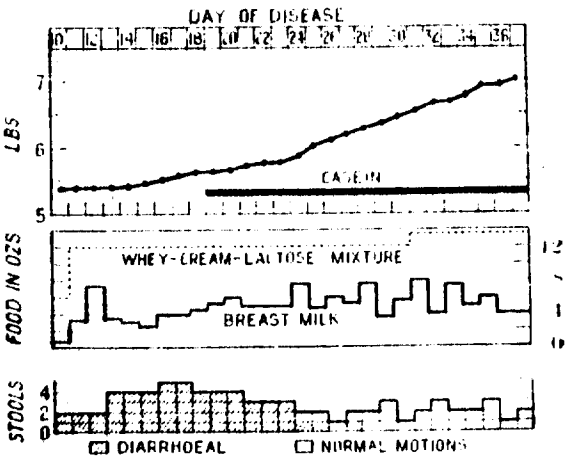


Chart showing increase of weight and improvement of stools under treatment (0472)

2. Jekat *et al.* (0679) compared the biological values of casein with whole milk, a commercial powder, cheese, lactalbumin, and whole egg, in five human test subjects (19-23 years of age) in balance experiments of 21 to 25 days. The data obtained, arranged for comparative purposes, are listed in Table 57.

Table 57

Biological Value of Casein (0679)

Food	Biological Value
Whole egg protein - Standard	100
Casein	72
Milk	80
Lactalbumin	124
Commercial milk powder ('Nesmida')[a]	122
Swiss cheese	83
Edamer cheese	85
Egg and milk	119
Lactalbumin and potatoes	134

[a]Made by the Nestle firm

BIOCHEMICAL ASPECTS

I. Breakdown

The effect of heat and other agents on casein have been studied by a number of workers with somewhat conflicting results.

A. Rats

1. Goldblatt et al. (0489) determined the effect of heat on the nutritive value of casein for piebald black and white rats when fed at a level of 20% as the protein source in a basal ration containing wheat starch, crisco, vegex (for vitamin B), lemon juice (for vitamin C), a standard salt mixture (McCallum #185), and 150 mg of cod liver oil daily. Sixteen animals (11 males, 47 g and 5 females, 46 g) constituted each group.

The casein (Technical, Merck) used in the diet for Group I was heated at 105-110°C for 36 hours (three 12-hour periods) in a thin layer (raked every hour) in a room with forced air draft. The preparation used for Group II was heated in like manner at 125-130°C. Neither heat treatment changed the nutritive value of casein significantly as judged by the weight gain of the rats employed, according to the authors. Both groups grew normally.

2. Greaves et al. (0514) also studied the effect of dry heat (100-140°C) on the biological value of commercial casein (acid) with, and without, amino acid supplements, in feeding experiments with groups of young rats (28 days old) consisting of both sexes. The data are presented in Fig. 7.

Heating at 130°C caused a loss in growth value of 11% after 1/2 hour and 18% at the end of 2 hours. At 140°C for 1/2 hour, the loss was 19%. Biological value was not affected at 100°C up to 8 hours and at 120°C up to 2 hours. Lysine was found to be the first amino acid damaged at 140°C, and histidine the second. The loss in nutritive value of the heated casein (140°C) could be restored by supplementation with these amino acids.

3. Baldwin et al. (0099) found that autoclaving casein with dextrose for 15 minutes at 120°C caused considerable loss in nutritive value on the basis of animal growth tests and microbiological assay. Feeding experiments with young (28-29 day) weanling albino rats indicated that the greater part of the loss was due to destruction of arginine, lysine, histidine, methionine, and threonine. The author pointed out that this phenomenon is of considerable practical importance in the preparation of dietetic formulas.

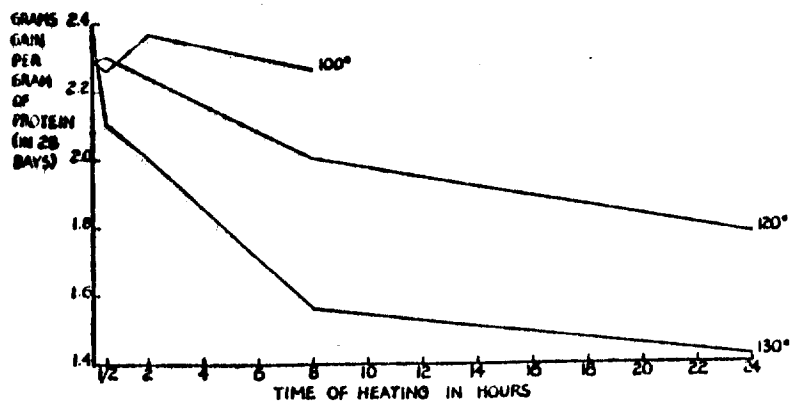


Figure 7

Decrease in gain in body weight per gram eaten by young rats produced by varying periods of heating casein at 100°, 120° and 130°. (0514)

B. Guinea Pigs

Luz et al. (0911) investigated the effect of heat on the allergenicity of the major milk proteins using several very sensitive assay techniques: gross anaphylaxis, Schultz-Dale & Prausnitz-Kuster techniques, passive cutaneous anaphylaxis, and gel precipitation.

The data obtained indicated that casein and β-lactoglobulin were more heat-stable than the other milk proteins. Casein withstood boiling for one hour and was the only protein that showed antigenicity after 120°C for 15 minutes. All three allergens (casein, α-lactalbumin, and β-lactoglobulin) survived the evaporated milk manufacturing heat treatment process in an antigenically-active form. The authors concluded that "heat-denatured" milk was not a safe substitute for milk-sensitive patients except for those with a pure bovine albumin allergy.

C. Rabbits

Seibert (1339) in a study of protein fevers found that casein prepared with the use of pyrogen-free water did not cause febrile reactions when injected into rabbits. On storage under non-sterile conditions, however, the material might become pyrogenic. The products of bacterial growth, therefore, rather than casein itself represented the cause of febrile reactions previously attributed to casein.

Pasteurization, acid precipitation, drying, milling and other processing factors do not destroy spore-forming bacteria in skim milk and in the casein prepared from it. Bacillus cereus has been found in commercial casein and in vivo studies in experimental animals have indicated the probability of toxic products connected with its presence (1339).

D. Humans

Ramshaw et al. (1204) isolated and identified the compounds associated with the "gluey" off-flavor of stored commercial casein and sodium caseinate by means of gas chromatography and mass spectrometry. Most of the objectionable flavor was found in the non-acid steam-volatile fraction which contained a variety of compounds (See Table 58). Results of organoleptic tests by a panel of experts indicated that the "gluey" flavor was due mainly to the n-alkanols and furan derivatives formed. Further study of processing and storage conditions led to the conclusion that these compounds originated from lipid oxidation and non-enzymic "browning", a reaction between lactose and casein (1204). (Commercial casein may contain up to 2% each of lactose and fat (1204).

Table 58

Composition of the Volatile Non-Acid Extract* from Stored Casein (1204)

Compound	Retention time, min		MS	Odour confirmation	% in extract
	Ap L	C 1540			
Ethyl acetate	2·4	2·0	+	+	1·5
2,3 Butadione	2·4	.	+	+	1·5
3-Methyl butanal	4·3	3·2	+	+	1·5
n-Pentanal	4·6	3·5	+	+	1·0
Dimethyl disulphide	6·9	5·5	+	+	4·0
Butanol	.	14·0	+	.	0·5
Pentanol	7·6	18·5	+	.	4·5
Hexan-2-one	7·9	.	+	.	tr
Hexanal	8·3	12·5	+	+	6·5
Toluene	8·6	.	+	.	1·0
Heptan-2-one	14·5	34·2	+	+	1·4
Heptanal	15·0	14·8	+	+	4·2
Furfural	15·0.	34·2	+	+	1·4
Hexanol	.	28·8	+	.	2·5
Ethyl benzene	15·0	.	†	.	tr
Dimethyl benzene	16·5	.	†	.	tr
Methional	18·5	.	†	+	tr
Methyl furyl ketone	20·2	37·0	+	.	0·5
Octan-2-one	23·6	23·0	+	+	1·4
Methyl furfural	24·0	.	+	.	1·4
Benzaldehyde	23·8	38·2	+	+	34·5
Octanal	24·6	23·5	+	+	2·1
Benzonitrile	24·8	.	+	.	0·5
Terpene	31·0	.	†	.	tr
Nonan-2-one	32·3	31·0	+	+	2·4
Acetophenone	33·0	46·0	+	.	0·5
Nonanal	33·7	31·3	+	+	8·3
Phenylacetaldehyde	34·7	.	+	+	0·5
Decan-2-one	40·7	.	+	.	1·4
Decanal	42·3	.	+	.	1·0
α-Terpineol	44·2	49·5	+	+	1·2
Naphthalene	46·3	50·3	+	.	1·3
Undecan-2-one	48·9	44·5	+	.	1·4
Undecanal	50·4	.	†	.	0·5
Methylnaphthalene	55·5	.	†	.	tr
Dodecan-2-one	56·5	.	+	.	0·5
Dodecanal	58·0	.	†	.	0·5
Tridecan-2-one	63·5	56·7	+	.	2·5
Tridecanal	65·0	.	†	.	tr
Trimethylnaphthalene	73·0	.	†	.	tr
Benzthiazole	.	63·0	+	+	2·5
Phenol	.	68·2	+	+	1·5

* See p. 216. † Not positively identified by MS but spectra similar
+ Positive identification in MS.

E. *In Vitro* Studies

1. Lea *et al.* (0848) studied the "browning" reaction in freeze-dried sodium caseinate with glucose, stored at 37°C and 75% relative humidity. After one month under these conditions a brown color had developed and the product was less soluble than originally. Chemical analysis revealed that 90% of the lysine, 70% of the arginine, 30% of the histidine, 50% of the methionine, and about 33% of the tyrosine had reacted with the carbohydrate.

2. Ramshaw and coworkers (1203, 1204) observed the so-called "browning" or Maillard reaction in commercial casein in storage without additives.

3. The same authors (1205) determined that the off-flavor of ultraviolet-treated casein was due to indole, skatol, benzyl methyl sulfide, and unsaturated aldehydes, that were formed during the irradiation.

II. Absorption-Distribution

Some dietary casein *per se*, in a fully-antigenic form, is absorbed from the gastrointestinal tract and distributed in the body to an extent sufficient to induce a hypersensitive state (0490, 0975). Most of the casein consumed, however, is digested; the resultant amino acids are readily absorbed from the intestinal tract and distributed for metabolic purposes (1412).

A. Rats

Peraino *et al.* (1147) fed male albino rats, weighing approximately 200 g, test diets containing varying concentrations of casein, carbohydrate, and fat to determine the effect on stomach emptying time and absorption of nitrogen from the gastrointestinal tract. The data are presented in Table 59 and Figs. 8, 9, and 10.

The data obtained show that the protein-free diet emptied most rapidly from the stomach. Casein, at levels of 15% and 30% delayed stomach emptying with normal feedings but not when reduced amounts of food were fed (Figs. 8 and 10).

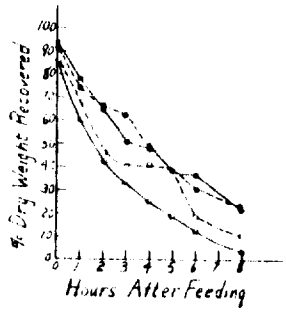
The quantity of nitrogen that disappeared from the upper gastrointestinal tract per unit time was statistically greater when the diet contained 50% casein than when the concentration was only 15% (Fig. 9). Previous studies showed that this disappearance was due to absorption and not by passage into the large intestine.

Table 59

Composition of Experimental Diets (1147)

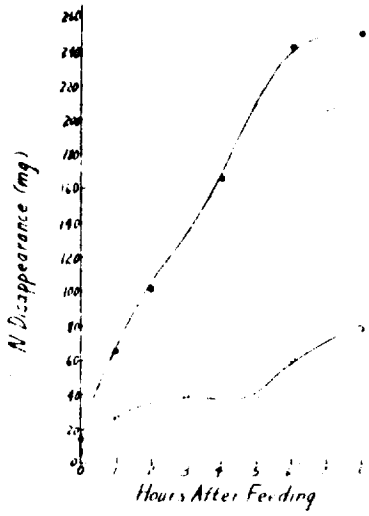
Diet	% casein	mg N/g	Type of carbohydrate	% fat
A	0	<1	Dextrin*	5 corn oil
B	0	<1	Sucrose	5 corn oil
C	15	21	Dextrin	5 corn oil
D	15	21	Sucrose	5 corn oil
E	30	42	Dextrin	5 corn oil
F	30	42	Sucrose	5 corn oil
G	50	70	Dextrin	5 corn oil
H	50	70	Sucrose	5 corn oil
I	15	20	Dextrin	25 lard
J	15	20	Sucrose	25 lard
K	15	20	Dextrin	50 lard
L	15	20	Sucrose	50 lard
M	0	<1	Dextrin	50 lard

*Autoclaved cornstarch.



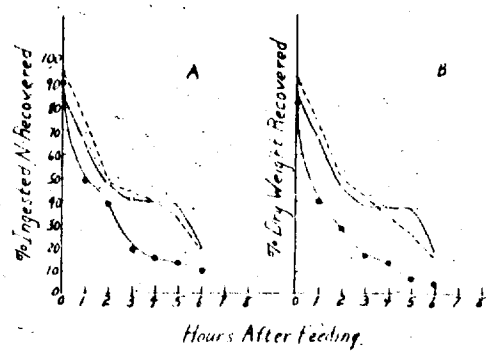
The effect of dietary protein level on the rate of emptying of total solids from the stomach. The diets contain dextrin as the carbohydrate. Diet A ○ ○ (protein free), diet C ○ ○ (15% of casein), diet E ● ● (30% of casein), diet G ● ● (50% of casein).

Figure 8 (1147)



The effect of dietary protein level on the rate of disappearance of nitrogen from the gastrointestinal tract between the cardiac sphincter and ileocecal junction. The diets contain dextrin as the carbohydrate. Diet C ○ ○ (15% of casein), diet G ● ● (50% of casein).

Figure 9 (1147)



The effect of level of food intake on the rate of gastric emptying of diet (
1.5 g fed ● —●, 3.0 g fed ○ — ○, 5.0 g fed ○ — ○

Figure 10 (1147)

B. Rabbits

Jones et al. (0688) followed the disappearance of casein from the circulation in rabbits after i.p. injection. The time of appearance of precipitin antibodies in the blood and casein in the urine were determined also. One animal received 0.6 g of casein dissolved in N/20 NaOH (300 mg/kg BW); the other was injected with 0.5 g (250 mg/kg BW). The data are presented in Table 60.

The results obtained indicated that casein absorbed from the peritoneal cavity was not excreted unchanged through the kidneys in quantities sufficient to be detected; rather, it remained in the circulation for a considerable period of time, 12 to 13 days at least. Antibodies appeared in the blood as early as 7-8 days after intraperitoneal injection.

Crystalline egg albumin, in contrast to casein was excreted unaltered and relatively rapidly via the kidney from the bloodstream. Eighteen hours after injection, this protein could not be detected in the circulation but was present in relatively high concentration in the urine. Egg albumin antibody appeared in the circulation 8 to 10 days after injection.

C. Dogs

Denton et al. (0320) determined the concentration of amino acids in the blood plasma of dogs at varying intervals after feeding casein, zein, or beef. The results are presented in Table 61.

When casein or beef were fed, the concentration of amino acids in the portal vein increased rapidly (See Table 61). With zein, however, a decrease occurred prior to the expected rise in amino acid concentration in the portal vein circulation. The authors concluded that the amino acids from casein and beef entered the portal vein within a narrow enough period to allow maximal utilization.

Table 60

The Behavior of Casein and Its Antibody in Rabbits 3 and 4. (0688)

Rabbit No.	Days after injection	Test of serum for casein in		Test of urine for casein in			Test of serum for antibody			
							Dilutions of antigen			
		1.0 cc.	0.1 cc.	1.0 cc.	0.5 cc.	0.1 cc.	1:100	1:200	1:400	1:800
3	1	+*	−	−	−	−	−	−	−	
	2	+	−	−	−	−	−	−	−	
	3	+	±	−	−	−	−	−	−	
	4	+	±	−	−	−	−	−	−	
	6	+	?	−	−	−	−	−	−	
	7	+	?				−	±	±	
	8	+	?				+	+	+	
	9	+	−				+	+	+	
	10	+	−				+	+	+	
	11	±	−				±	+	+	+
	13	±	−				±	+	+	+
	14	±	−				±	+	+	+
	16	−	−					±	+	+
4	1			−	−					
	2			−	−					
	3	+		−	−		−	−	−	
	4	+		−	−		−	−	−	
	5	+		−	−		−	−	−	
	7	+					−	−	−	
	8	+					−	−	−	
	9	+					±	±	±	
	10	±					±	+	+	
	11	±					+	+	+	
	12	±					+	+	+	
	14	−					+	+	+	

* + indicates a well defined turbidity; ±, a slight turbidity.

Table 61

Concentration of Amino Acids in Blood Plasma after Feeding Casein to Dog 102A (0320)

The figures are in micrograms per ml. of plasma.

Amino acid	0 hr.		1 hr.		2.5 hrs.		5 hrs.	
	Portal vein	Radial vein	Portal vein	Radial vein	Portal vein	Radial vein	Portal vein	Radial vein
Arginine	29.6	29.9	37.4	30.4	33.2	21.9	30.7	21.7
Histidine	6.4	6.8	18.4	9.5	16.4	10.5	14.6	11.4
Isoleucine	9.0	8.8	41.4	12.4	26.2	21.0	19.5	10.5
Leucine	10.4	10.6	41.6	14.7	30.8	14.4	21.4	17.3
Lysine	10.7	12.0	31.5	18.9	30.0	16.3	25.9	20.4
Methionine	7.7	7.2	17.7	9.0	16.9	10.8	15.3	13.4
Phenylalanine	7.4	8.1	27.0	10.9	21.5	9.9	17.4	13.6
Threonine	21.2	21.7	38.4	26.6	30.4	22.4	32.4	21.4
Tryptophan	15.2	16.4	21.6	20.8	22.7	15.9	22.4	21.3
Valine	12.4	11.8	44.4	17.6	33.8	18.0	32.4	22.8

III. Metabolism and Excretion

Casein is regarded as a "complete" protein nutritionally in that it contains the proper kinds of amino acids in the right amounts for adequate nourishment and growth (1412). Following digestion, casein amino acids are readily absorbed from the intestinal tract and metabolism studies indicate a utilization of about 98.5% (1412).

A. Dogs

Zamychkina (1591) studied the digestion, absorption, and distribution of casein-I^{131} *in vitro* and in the intact dog. Casein-I^{131} (5,000-10,000 pulses/min/ml) in phosphate buffer pH 7.6 was mixed with pancreatic and intestinal juice from dogs with chronic fistulas in the *in vitro* studies. After incubation at 37.5°C for varying periods of time, radioactivity of the protein-free trichloric acid filtrate was measured. The nature of the hydrolytic products was determined by paper chromatography.

In vivo experiments were conducted with 4 intact dogs and 3 animals with Schiff or Schwann type gall gladder fistulas which were given casein-I^{131} in milk and water at a level of 100-200 pulses/min/g BW (a few drops of Lugol's solution were given also to each dog to prevent accumulation of I^{131} in the thyroid). After varying periods of time, the radioactivity of blood samples and bile was determined. The study extended over a period of 8-10 months.

In vitro experiments showed that digestion of casein by a mixture of pancreatic and intestinal juices took place very rapidly; during the first 2-5 minutes, 20-25% of the protein was hydrolyzed. Digestion for 10-12 minutes resulted in 60-80% hydrolysis and after 20-30 minutes, 95-98% of the sample was digested.

Digestion of casein-I^{131} took place very rapidly *in vivo* also. Shortly after the preparation was fed to dogs, radioactivity was detected in the bloodstream and reached a maximum in about 2 hours, indicating relatively rapid absorption of digestion products from the gastrointestinal tract.

Hydrolytic products of the digested casein were rapidly excreted in the bile. Five to 10 minutes after administration, the radioactivity of bile samples was 200% of the administered value/g BW. The maximal bile radioactivity level (500-700%) was reached after 30-60 minutes and maintained for a few hours after which it gradually decreased to 10-50% by 24 hours. During the development of pathological processes in the liver, a slower elimination of casein digest products was noted.

B. Cattle

1. Lofgreen et al. (0895) gave 4 calves (Jersey, Holstein, and Guernsey strains), 9-10 weeks of age, a single feeding of a diet made with casein-P^{32} and determined the rate of digestion and distribution of metabolites in comparison with results on 4 similar calves fed inorganic P^{32} in the diet (Table 62). Results obtained are presented in Figs. 11 and 12.

Casein digestion in the calf begins soon after ingestion, as indicated by detection of P^{32} in the blood stream within 15 minutes. The process continues over a relatively long period of time, however, and the maximum blood level of radioactive phosphorus was not reached until 13-23 hours after feeding.

Analysis of various organs and tissues of animals sacrificed 24 hours after being fed casein-P^{32} indicated that the liver and kidneys had a rapid rate of phosphorus exchange whereas that of the brain was low. In the gastrointestinal tract, the rumen, omasum, and jejunum, showed the most rapid rates of exchange.

2. Derrig et al. (0324) investigated the effect of sodium caseinate infused rumenally or abomasally on milk production, milk composition, N utilization, amino acid levels, and amino acid uptake by the mammary gland in the dairy cow. This particular investigation was part of a general study designed to determine the nutrients which may be limiting milk production factors in high-producing dairy cows.

Seven mid-lactation Holstein cows (fistulated), on a hay-protein-mineral salts-vitamin diet, were used in a 21-day experiment during which sodium caseinate was infused for three 7-day periods into the rumen, abomasum, and rumen again. Approximately 5 liters of a 9% (w/v) solution of sodium caseinate was infused each 24-hour period (900 mg/kg BW). Milk production and the other parameters of the study were determined by standard methods (See original article for details).

A significant increase in milk yeild, milk protein and N content, and dry matter digestibility resulted when sodium caseinate was infused into the abomasum. Also, a general increase in the concentration of all essential amino acids and most of the non-essential amino acids in the blood was detected.

The authors concluded that the data suggested the sequence of limiting essential amino acids for the lactating dairy cow is: phenylalanine, methionine, lysine, threonine, leucine, isoleucine, histidine, valine, and arginine (See original article for details).

Table 62

Description and Treatment of Experimental Animals (0895)

CALF NO.	BREED[1]	AGE	WEIGHT	P^{32} ADMINISTERED	
				AMOUNT	METHOD
		days	*kg*	*μc*	
3	J	71	42.2	293	Fed in labeled casein
4	J	56	48.1	2760	Fed as inorganic salt
5	H	63	57.2	26.5	Fed in labeled casein
6	H	67	78.5	260	Injected as inorganic salt
7	H	68	68.5	72	Fed in labeled casein
8	G	63	51.5	60	Fed in labeled casein
9	H	67	73.5	1000	Fed as inorganic salt
10	G	67	45.4	1000	Injected as inorganic salt

[1] Guernsey, Holstein and Jersey are abbreviated as G, H and J, respectively.

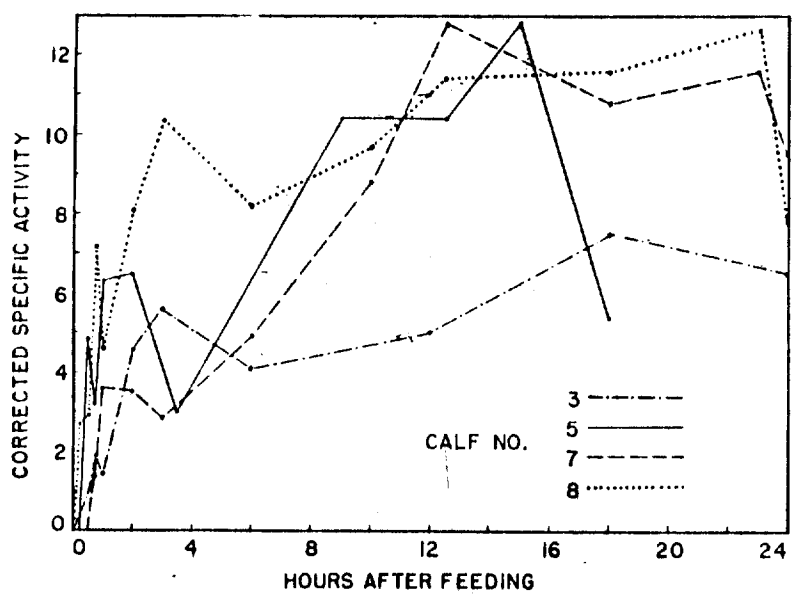


Figure 11 (0895)

Levels of P^{32} in the blood stream of calves fed labeled casein.

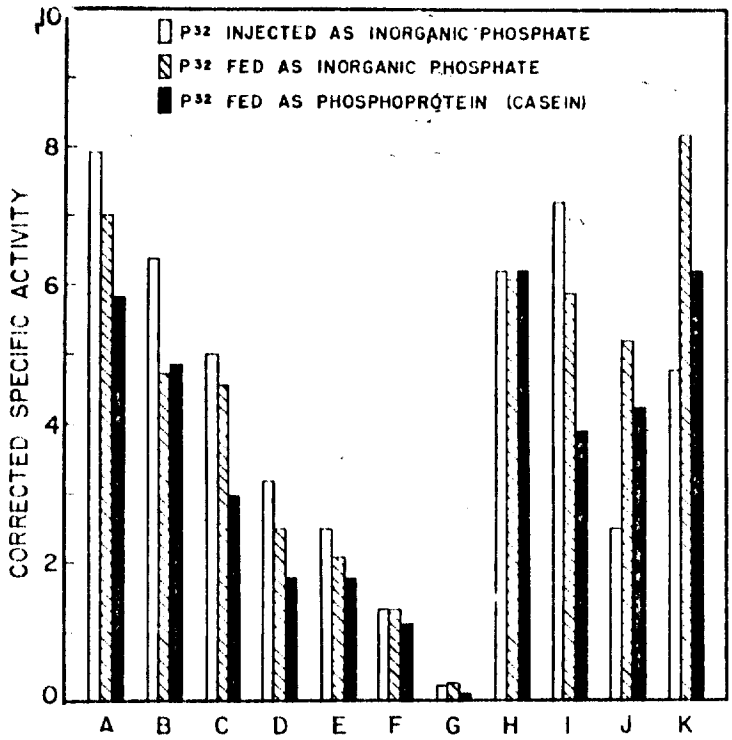


Figure 12 (0895)

Distribution of P^{32} in representative tissues of calves fed either labeled casein or labeled inorganic phosphate or injected with labeled inorganic phosphate. A, liver; B, kidney; C, heart; D, lung; E, testes, F, skeletal muscle; G, brain, H, rumen; I, omasum; J, abomasum; K, jejunum.

IV. Effects on Enzymes and other Biochemical Parameters

Rats

1. Westerfield et al. (1533) studied the effect of high, medium, and low concentrations of casein in a basal diet with, and without, added p-dimethylaminoazobenzene, on xanthine oxidase in various tissues of the rat.

Three groups of Sprague-Dawley rats weighing 175-200 g were fed diets containing 8, 12, or 21% casein (See Table 63) for a period of 18 weeks. Three additional groups of 39 rats each were placed on the same diets to which had been added 0.06% of p-dimethylaminoazobenzene. At the end of 1, 2, and 4 months, six rats from each group were sacrificed for enzyme analysis and histologic study. The data are presented in Table 63 and Fig. 13.

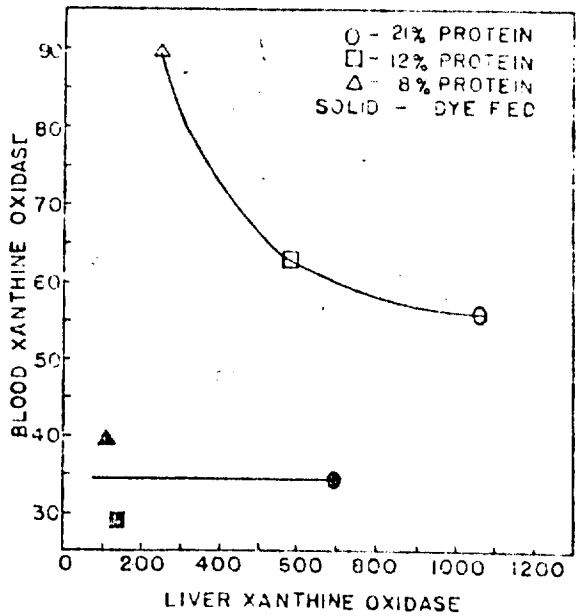
Table 63

The Xanthine Oxidase Activity of Tissues of Rats Fed Diets of Different Protein Content With and Without p-dimethylaminoazobenzene (1533)

Diet	Body wt. (gm.) Start	Body wt. (gm.) End	Xanthine oxidase activity (mean ± S.E.)* Liver	Kidney	Lung	Spleen	Intestine	Blood
			(On diet 4 weeks)					
21 per cent Protein	188	332	1312 ± 123					60 ± 3
" + Dye	182	267	730 ± 142					35 ± 6
12 per cent Protein	184	267	718 ± 158					67 ± 5
" + Dye	184	233	175 ± 56					32 ± 4
8 per cent Protein	183	243	318 ± 68					91 ± 4
" + Dye	181	191	49 ± 21					40 ± 7
			(On diet 8–9 weeks)					
21 per cent Protein	193	356	926 ± 42	222 ± 20	295 ± 24	461 ± 41	82 ± 36	49 ± 3
" + Dye	188	324	768 ± 117	65 ± 36	366 ± 46	610 ± 98	145 ± 69	25 ± 2
12 per cent Protein	181	324	413 ± 87	156 ± 18	261 ± 42	368 ± 46	15 ± 15	54 ± 7
" + Dye	191	227	105 ± 25	10 ± 10	168 ± 48	481 ± 65	146 ± 56	18 ± 7
8 per cent Protein	184	233	335 ± 48	160 ± 55	350 ± 38	370 ± 57	74 ± 26	87 ± 9
" + Dye	178	201	114 ± 30	7 ± 7	225 ± 36	435 ± 77	81 ± 41	19 ± 8
			(On diet 16–18 weeks)					
21 per cent Protein	186	449	990 ± 34	198 ± 29	189 ± 28	454 ± 22	79 ± 31	58 ± 1
" + Dye	178	395	616 ± 80	7 ± 7	165 ± 21	431 ± 87	29 ± 19	42 ± 2
12 per cent Protein	182	381	630 ± 61	247 ± 19	255 ± 20	435 ± 67	37 ± 29	67 ± 5
" + Dye	192	312	122 ± 15	46 ± 19	190 ± 24	625 ± 40	45 ± 45	35 ± 4
8 per cent Protein	177	253	82 ± 48	200 ± 27	244 ± 26	421 ± 57	54 ± 28	91 ± 10
" + Dye	202	208	129 ± 33	81 ± 19	249 ± 41	644 ± 69	150 ± 36	[illegible] ± 10

* Tissue xanthine oxidase activity in c. mm. O_2 per gram dry wt. per hour. Blood activity in µg. xanthine oxidized per cc. blood per hour. Each figure is the mean for six rats.

Figure 13
(1533)



The relationship between blood and liver xanthine oxidase in rats maintained on three different diets, with and without p-dimethylaminoazobenzene.

Each point represents the average of eighteen rats sacrificed after 1, 2, or 4 months on the diets. Liver xanthine oxidase units = c. mm. O_2 per gram dry liver per hour; blood xanthine oxidase units = µg. xanthine oxidized by 1 cc. blood per hour.

Casein caused a slight non-specific increase in the activity of fructokinase, hexakinase, glucokinase, and aldoreductase. The effects, however, were considerably less than those exerted by the various sugars tested, i.e., glucose, fructose, and sucrose.

V. Drug Interaction

A. Bacteria

Price et al. (1181) found that the anti-bacterial effects of neomycin and dihydrostreptomycin on Pseudomonas aeruginosa in vitro were markedly inhibited by casein (Pfanstiehl), hydrolyzed casein, or calcium caseinate at a concentration of 2.8% in the nutrient medium. Oxytetracycline, chlorotetracycline, and polymyxin B, on the other hand, were not affected.

B. Rats

Kornberg et al. (0780) studied the effect of casein, urea, sodium chloride, potassium chloride, and sodium bicarbonate on the kidney-damaging effect of sulfadiazine in the rat.

Albino rats of the Wistar and Osborne-Mendel strains, litter mates of the same sex and weight, were placed on test diets containing 1% sulfadiazine and varying concentrations of casein and the other test substances. After an experimental period of 30 days, the animals were sacrificed and the kidneys examined for the degree of damage.

Severe renal lesions resulted from feeding 1% sulfadiazine in a low casein diet (10%). At a casein level of 30%, however, the amount of kidney damage was significantly reduced, even in animals on a restricted water intake. Urea, sodium bicarbonate, and sodium chloride were effective in this respect also.

C. Rabbits

Nath et al. (1067) found that casein added as a supplement to a cystine-methionine-deficient diet protected rabbits for 40 days from hyperglycemia induced by daily doses of 50-150 mg/kg of sodium acetoacetate. The condition was prevented completely by a 2% supplement of methionine. The authors concluded, therefore, that the protective action of casein was due actually to its content of methionine. A deficiency of -SH compounds appears to be essential for acetoacetate-induced diabetes in experimental animals.

VI. Consumer Exposure

Casein and caseinates are widely employed in the food industry for a variety of purposes.

As foods, caseins are regarded as "complete" proteins with the proper amino acid composition for adequate nutrition and growth. Large quantities are used in the preparation of infant formulas, children's foods, diets for the elderly, sick & convalescents, diabetic foods, diets for the obese, calf starters & other animal feeds (1412).

Casein and caseinates are also excellent emulsifiers due to their ability to bind water and emulsify fats. At least 26 goods on the U. S. market contain casein or caseinates. Typical examples are: baked goods, ice cream, powdered toppings, coffee creamers, cereals, egg white substitutes, soup tablets, sausage, luncheon meats, etc. (See also Tables 66, 67, 68 and 69 for other examples). Casein is employed also as a wine clarifier (1034, 1412).

Sodium and calcium caseinates are the salts of casein most widely used today (1412). They are used mainly in the food and pharmaceutical fields as nutrients, binders, emulsifiers, texturizers, and whipping agents. Both are white or nearly white powders with a clean bland flavor (1412).

Estimated daily average and maximum intakes of casein, sodium caseinate, and calcium caseinate, as reported in the Comprehensive GRAS survey are given in Tables 64 and 65.

Table 64

Potential Daily Intakes from Milk Products
Based on Food Consumption by Eaters Only (0420)*

Age Group	Casein Potential Daily Intake, Mg.		Calcium Caseinate Potential Daily Intake, Mg.	
	Average	Maximum	Average	Maximum
0-5 Mo.	13,050	39,655	2,198	4,770
6-11 Mo.	32,825	139,860	5,528	16,823
12-23 Mo.	21,100	107,625	3,553	12,946
2-65+ Yr.	14,200	54,390	2,391	6,542

*From NAS/NRC Comprehensive Gras Survey, 1972, Table 15, Page 19.

Table 65

Estimated Daily Intake of Sodium Caseinate from all Foods Based on Food Consumption by Total Sample (0421)*

Age Group	Possible Daily Intake, Mg.	
	Average	Maximum
0-5 Mo.	289	700
6-11 Mo.	3,726	12,952
12-23 Mo.	4,820	11,301
2-65+ Yr.	6,948	14,336

*From NAS/NRC Comprehensive GRAS Survey, 1972, Table 13, Part A, p. 170

Foods in which sodium caseinate is used at the maximum level are milk products 9%, imitation dairy products and breakfast cereals 4.5%, and cheese 4% (0418).

The total 1970 poundage of sodium caseinate reported to NAS and FEMA was 13,231,488 lbs. (0417).

Other data reported in the NAS/NRC Comprehensive GRAS Survey are given in Tables 66, 67, and 68.

A considerable amount of casein is imported annually. In 1972, 105,401,000 lbs. was imported from abroad. The range over the past ten years was 87,878,000 (1963) to 135,288,000 (1970) (1474).

Medicinally, casein is used in non-specific protein therapy (1388), in the form of casein-iron complex for anemia, and combined with tannin ("caseinum tannetum") for treating diarrhea (1412). Combinations with bismuth, mercury, and silver have been used locally to treat infections (1412).

Casein is also one of the substances that migrate to foods from food packaging materials due to its wide use in the manufacture of synthetic textile fibers, paper coatings and glazes, and so forth (1388, 1412).

Table 66. Possible Daily Intakes of Sodium Caseinate Based on Food Consumption by Total Sample (0421)

FOOD CATEGORY NO.	NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
01	BAKED GOODS(R)	5	0-5 MO.	77.204920	102.182850	91.535320
			6-11 MO.	576.765420	1176.238140	686.810920
			12-23 MO.	1237.547850	2039.115540	1473.669100
			2-65+ YR.	3115.441560	4627.747740	3709.860560
02	BREAK CERLS(R)	*	0-5 MO.	26.769240	75.846140	26.769240
			6-11 MO.	594.923420	2660.009920	594.923420
			12-23 MO.	1164.461940	2270.923940	1164.461940
			2-65+ YR.	892.[illegible]	2311.077120	[illegible]
05	MILK PRODS(R)	7	0-5 MO.	137.255020	[illegible]	489.101720
			6-11 MO.	1470.557120	7072.249630	5651.842267
			12-23 MO.	1264.363350	4109.962720	4936.364800
			2-65+ YR.	930.868850	2842.095780	3577.388700
06	CHEESE(R)	*	0-5 MO.	**********	3.940100	**********
			6-11 MO.	106.[illegible]	962.1[illegible]	[illegible]
			12-23 MO.	307.320000	874.[illegible]	307.320000
			2-65+ YR.	370.340000	929.840000	370.340000
07	FROZN DAIRY(R)	4	0-5 MO.	9.432600	38.673660	32.[illegible]
			6-11 MO.	89.6[illegible]	243.025560	216.[illegible]
			12-23 MO.	136.929440	314.32[illegible]	3[illegible]
			2-65+ YR.	241.474560	581.691020	[illegible]
10	MEAT PRDES(R)	4	0-5 MO.	8.778160	21.034240	[illegible]
			6-11 MO.	156.279920	419.6[illegible]	[illegible]
			12-23 MO.	227.278120	350.5[illegible]	227.2[illegible]
			2-65+ YR.	590.007040	979.080560	590.007040
11	POULTRY(R)	*	0-5 MO.	10.924700	50.259620	11.729900
			6-11 MO.	85.212660	288.412000	91.492440
			12-23 MO.	144.208040	402.[illegible]	155.[illegible]
			2-65+ YR.	281.657260	716.[illegible]	302.[illegible]
17	CONF FROST(R)	*	0-5 MO.	**********	.200000	**********
			6-11 MO.	.200000	.400000	.200000
			12-23 MO.	.400000	1.400000	.400000
			2-65+ YR.	.600000	1.600000	.600000
19	SWEET SAUCE(R)	*	0-5 MO.	5.397790	7.185320	7.181[illegible]
			6-11 MO.	16.163370	55.073830	21.5[illegible]
			12-23 MO.	46.694100	136.400680	62.240[illegible]
			2-65+ YR.	122.123240	321.471471	162.70[illegible]
20	GELATIN PUD(R)	*	0-5 MO.	20.674000	27.909800	[illegible]
			6-11 MO.	132.313600	401.075600	250.211840
			12-23 MO.	142.650600	347.323200	269.759640
			2-65+ YR.	210.874800	542.692500	398.775120
21	SOUPS(R)	*	0-5 MO.	.199240	1.494300	.199240
			6-11 MO.	23.211460	72.423740	23.211460
			12-23 MO.	34.667760	95.734920	34.667760
			2-65+ YR.	31.579540	84.178900	31.579540
22	SNACK FOODS(R)	*	0-5 MO.	**********	.612520	**********
			6-11 MO.	2.449680	6.736520	5.372440
			12-23 MO.	6.736620	18.985020	25.024210
			2-65+ YR.	7.961460	22.655040	27.210430
23	BEV TYPE I(R)	*	0-5 MO.	2.063280	3.094920	2.063280
			6-11 MO.	19.515190	66.709690	19.515190
			12-23 MO.	46.595740	* 139.701250	46.595740
			2-65+ YR.	89.402800	236.738690	89.402800
27	GRAVIES(R)	*	0-5 MO.	.396000	1.188000	[illegible]
			6-11 MO.	5.544000	15.444000	[illegible]
			12-23 MO.	14.256000	40.392000	21.[illegible]
			2-65+ YR.	32.868000	84.346000	49.000000
28	IMIT DAIRY(R)	12	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	47.222140	77.[illegible]	62.[illegible]
			12-23 MO.	26.[illegible]	114.682340	[illegible]
			2-65+ YR.	30.[illegible]	80.[illegible]	40.[illegible]
48	SEAS FLAVRS(R)	*	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	.200000	**********
			12-23 MO.	**********	.400000	**********
			2-65+ YR.	.200000	1.000000	[illegible]
ALL CATEGORIES		34	0-5 MO.	288.567250	428.66[illegible]	259.[illegible]
**********			6-11 MO.	3725.827680	12952.3[illegible]	9257.[illegible]
**********			12-23 MO.	4819.988720	11301.22[illegible]	9031.[illegible]
**********			2-65+ YR.	8546.250200	14339.77[illegible]	10[illegible]

Table 15 - Potential Daily Intakes of Additional NAS & FEMA Substances (Group IV) per Food Category Reported, Based on Food Consumptions by Eaters Only. (0420)

Food Category No.	Name	Substance Name (Survey No.)	No. of Firms	Potential Daily Intake, Mg., Per Category (Age)	Average	High A	High B	Very High
05	Milk Prods (R)	Calcium Caseinate NAS 0556	*	0-5 mo.	2197.62	4769.93	2197.62	4769.93
				6-11 mo.	5527.73	16823.16	5527.73	16823.16
				12-23 mo.	3553.24	12945.75	7553.24	12945.75
				2-65+ yr.	2391.28	6542.34	2391.28	6542.34
		Casein NAS 0422		0-5 mo.	13050.00	28325.00	18270.00	39655.00
				6-11 mo.	32825.00	99900.00	45955.00	139860.00
				12-23 mo.	21100.00	76875.00	29540.00	107625.00
				2-65+ yr.	14200.00	38850.00	19880.00	54390.00
28	Imit. Dairy (R)	Casein NAS 0422		0-5 mo.	000.00	000.00	000.00	000.00
				6-11 mo.	672.30	846.45	672.30	846.45
				12-23 mo.	336.15	607.50	336.15	607.50
				2-65+ yr.	234.90	688.50	234.90	688.50

Table 68 continued

Food Category		No. of Firms	Sodium Caseinate Potential Daily Intake, Mg., Per Category				
			Average	Age	High A	High B	Very High
28	Imit Daily (R) P. 256	*	000.000000	0-5 mo.	000.000000	000.000000	000.000000
			559.919660	6-11 mo.	704.959090	741.253080	933.264420
			279.959830	12-23 mo.	505.951500	370.626540	669.807000
			195.634580	2-65+ yr.	573.411700	258.992040	759.114600
48	Seas Flavors (R) P. 281	*	* * * *	0-5 mo.	* * * *	* * * *	* * * *
			0.400000	6-11 mo.	0.800000	0.500000	1.000000
			0.600000	12-23 mo.	1.000000	0.750000	1.250000
			2.000000	2-65+ yr.	4.000000	2.500000	5.000000

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