Efficiency of Feed Utilization by Various Animal Species Fed Similar Rations

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ABSTRACT The relative response and efficiency of feed utilization by rats, chicks, pigs, sheep and steers were compared when two rations varying in fiber content made of the same natural feedstuffs were fed. Daily gains and feed intakes were characteristic of the species, considering the type of ration fed. Proportions of final body fat in rats and chicks were from 4 to 8%; in pigs 35 to 41%, and in ruminants from 21 to 26%. Body protein, however, was greatest in rats and chicks and lowest in pigs, sheep and steers. Body weight gain as fat was less than 10% in rats and chicks but from 40 to 50% in pigs, sheep and steers. The protein in liveweight gain was approximately 20% in rats, 29% in chicks, 10 to 12% in pigs and sheep and 18% in steers. Energy content of the weight gain was about 2,000 kcal/kg in rats and chicks but 4,500 to 5,200 in the larger animals. The total efficiency of feed utilization in terms of liveweight gain per unit of feed intake was lowest for ruminants; pigs and rats were intermediate, and chicks were the most efficient. Total efficiency of feed utilization measured as calories showed pigs to be most efficient, followed by sheep, steers, chicks, and rats in that order. Protein gain per unit of feed intake was greatest for chicks followed by rats, pigs, steers and sheep. Feed capacity played a major role in relative efficiency of feed utilization because less feed was used for maintenance when the animals gained faster. The correlation coefficient between feed capacity and efficiency of energy utilization in terms of energy gain was 0.91.

The efficiency at which animals utilize feed to produce human food has been an intriguing question discussed by many reviewers. The most recent reviews were those by Blaxter (1) and Kleiber (2), whereas Brody (3) has extensive discussions on this subject. However, few experiments have compared directly the efficiency of feed utilization by various animal species. Calculations based on independent and unrelated investigations yield efficiency comparisons, but variation between investigations in feed quality and chemical composition might result in less exact comparisons than desirable. Furthermore, species have often been compared when fed rations composed of different proportions of natural feedstuffs characteristic only for a particular species and hence the comparative nutritional aspects were lost because a second variable was introduced. Moreover, to the author's knowledge, direct comparative studies on body composition have not been reported in the literature.

The purpose of the research to be reported herein was to compare the total efficiency of feed utilization by rats, chicks, pigs, sheep and steers fed rations composed of the same natural feedstuffs. Body composition was studied to indicate its implication in the relative response of the various species to similar rations. The magnitude of the role played by food intake was determined. From information on why differences exist between species, it was hoped to indicate where emphasis would be most rewarding in improving the efficiency of feed utilization within any one species.

EXPERIMENTAL

Two rations, one higher in available energy (low fiber) and the other lower in available energy (high fiber), were compounded to supply nutritive requirements for the various animal species. For purposes of discussion these rations will be referred to as the low and high fiber rations. These rations were not necessarily the most suitable for each species but were chosen as a compromise so that the same proportion of feedstuffs could be fed to all animals and comparative utilization studied. Simple-stomach animals, for

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TABLE 1

Ration composition[1]

	High fiber	Low fiber
	%	%
Feedstuffs		
Alfalfa hay	30[2]	5
Barley grain	46.5	70.5
Soybean oil meal	15	15.0
Cottonseed oil meal	5	5
Tallow	2	2
Ca_2PO_4	1	2
NaCl	0.5	0.5
Chemical composition		
Crude protein	21.4	19.4
Ether extract	4.2	4.2
Crude fiber	13.6	7.6
Lignin	3.7	1.7
Ash	7.0	6.3
Gross energy, kcal/g	4.87	4.85

[1] For swine the following were added to each kg: 660 IU vitamin A; 3.6 mg Ca pantothenate; 2 g methionine; 11.74 mg chlortetracycline; and 200 mg $ZnCO_3$. For chicks and rats the following were added to each kg: 660 IU vitamin A; 374 IU vitamin D_3; 3.6 mg Ca pantothenate; 2 g methionine; 11.74 mg chlortetracycline; 200 mg $ZnCO_3$; 40 mg $MnCO_3$; 400 mg $CaCO_3$; and 10 g Ca_2PO_4 to the high fiber ration; and 660 IU vitamin A; 3.6 mg Ca pantothenate; 0.46 mg riboflavin; 2 g methionine; 374 IU vitamin D_3; 11.74 mg chlortetracycline; 200 mg $ZnCO_3$; 53 mg $MnCO_3$; and 3.7 g $CaCO_3$ to the low fiber rations. These additions provided the nutrient requirement recommended by the committees on Animal Nutrition of the National Research Council (see footnote 1 in text).

[2] For the steers, 10% Sudan hay replaced 10% of the alfalfa hay. The chemical analysis did not change.

example, would not have tolerated an all-roughage ruminant ration nor would ruminants have tolerated the highest energy rations fed to poultry. Natural feedstuffs from the same source (table 1) were the major components. The roughage was chopped through a 2-cm screen and mixed with steam rolled barley and other ingredients for the steers and sheep. For the pigs, screen size was reduced to 0.6 cm for the hay and the prerolled barley was ground through a 0.3-cm screen. The latter ration was processed further by grinding through a 0.2-cm screen for the rats and chickens to prevent ingredient selection by the animals. Additional vitamins, minerals and methionine were added to the basal ration to supply the requirements for these nutrients as suggested by the National Research Council.[1] These nutrients were not added to all rations because some species do not have a dietary requirement for them. Additions in all cases were at the expense of the total ration. The antibiotic, chlortetracycline, was added to the swine, rat, and chicken rations, and a 30-mg and 15-mg implant of stilbestrol was administered to each steer and sheep, respectively, at the beginning of the experimental feeding periods.

The animals were at various ages when started on experiment, but the ages chosen were representative of that used in many investigations. Ages at the beginning of the trials were: rats, 4 weeks; chicks, 1 day; pigs, 12 weeks; sheep, 5 months; and steers, 8 months. The steers, sheep and swine were castrate males of the Hereford, Hampshire × Corriedale cross, and Duroc breeds, respectively. The rats were uncastrate males from the Sprague-Dawley strain and the chicks were male white Plymouth Rocks.

Animals were distributed at random to experimental groups fed the low fiber ration, the high fiber ration and a representative group to be slaughtered for an estimation of initial body composition. Only enough steers were available, however, for the group fed the low energy ration and the group for initial body composition studies. All animals were fed individually. Some chickens did not adjust immediately to individual feeding; data from these chicks were not used.

Animals were housed in quarters maintained above their critical temperature. The rats and chicks were in screen-bottom cages in the rat colony maintained between 25.5 and 27.8°C. The sheep and pigs were housed in the same animal quarters, maintained on a concrete floor where the temperature ranged between 21 and 29°C. The steers were housed in outdoor wire pens with an earth floor and exposed to variations in temperature from 18.33 to 35°C. In this latter case, it was not expected that the temperature was so low as to cause the animal to burn body substance in order to keep warm; hence, comparisons of efficiencies of energy utilization should be valid between the various species.

[1] National Research Council, Committee on Animal Nutrition: Nutrient requirement of domestic animals — sheep, pub. 504 ('57); beef cattle, pub. 579 ('58); swine, pub. 648 ('59); poultry, pub. 827 ('60); laboratory animals (rats), pub. 990 ('62). National Academy of Sciences — National Research Council, Washington, D. C.

Body composition was determined on representative groups of animals of each species slaughtered at the initiation of the trials and on all remaining animals killed at the end of the experiment. Differences between the initial and final composition were used to calculate gain in energy and protein. Composition of the rats was determined as described by Meyer (4) applying the factors of 4.65 kcal/g of fat-free dry matter and 9.28 kcal/g of fat (5) to calculate caloric content. The composition of the chicks was determined by grinding and macerating the carcass, gastrointestinal tract contents and feathers separately. Ether extract, protein and ash were determined directly on the 3 carcass components. The factors suggested by Fraps and Carlyle (6), 5.66 kcal/g for protein and 9.35 kcal/g of ether extract, were used to estimate caloric content. Body composition of the sheep was determined by the method of Meyer (7) and for the steers as described by Garrett et al. (8).

The specific gravity technique was used to estimate the body composition of swine with some calculated formulas in the absence of a complete set of regression equations for this specie. Basically the methods described by Clawson et al. (9) were used, but a relationship between body fat and carcass specific gravity was not available. Through the courtesy of the authors their detailed data were used to calculate the following equation:

$$Y = 576.68 - 517.38\ X$$

where Y = percentage of body fat and X = the carcass specific gravity. The correlation coefficient was −0.94. From the data published by Mitchell (10) another equation was derived:

$$Y = 8.67 + 0.946\ X$$

where Y = per cent body water and X = carcass water percentage. The correlation coefficient was 0.95. These equations were used with those suggested by Clawson et al. (9) for the calculation of body composition.

RESULTS AND DISCUSSION

Daily gains and feed intake (table 2) were generally characteristic of the species, considering the type of ration fed. The pigs, even though equal to the sheep in initial weight, made much greater gains and consumed more feed than the sheep. The steers, the largest animals, consumed the most feed and made the greater daily gains.

Consumption of the low fiber ration by the simple-stomach species, rats, chicks and pigs, produced greater daily liveweight and energy gains than when the high fiber ration was consumed. Feed intake was somewhat lower for the rats and chicks fed the low fiber ration, whereas

TABLE 2

Animal response

	High fiber ration					Low fiber ration			
	Rats	Chicks	Pigs	Sheep	Steers	Rats	Chicks	Pigs	Sheep
Days fed	28	56	90	60	134	28	56	90	60
No.	7	8	6	6	8	7	9	6	5
Initial wt, kg	0.071	0.037	35.70	34.7	268	0.070	0.042	36.0	35.4
Daily gain, kg	0.0036	0.0136	0.608	0.272	1.29	0.0043	0.0145	0.726	0.209
Daily gain, kcal	7.10	27.49	2.691	1.218	5.933	8.75	31.48	3.715	1.032
Daily feed intake, kg	0.0139	0.0413	2.22	1.46	7.93	0.0132	0.0408	2.49	1.27
Final carcass wt, kg	—	0.532	58.6	26.5	265	—	0.571	68.2	26.4
Final carcass wt, %	—	66.6	64.8	52.4	60.0	—	68.0	68.0	54.9

the pigs consumed a larger quantity of the low fiber ration than that of the high fiber ration. The sheep, like the rats and chicks, consumed less daily feed when fed the low fiber ration than when fed the high fiber ration, but made a lower daily liveweight and energy gain. Carcass weight, however, for both groups of sheep was the same, indicating that differences between the response in liveweight gain was due to gastrointestinal tract fill.

The initial body composition (table 3) shows a great variation between species. The pigs initially contained about 27% body fat and were highest in energy but lowest in protein content. Sheep were next with 17% fat. The steers, however, although containing only 11% fat, had the largest quantity of protein relative to the fat content of all the species. Rats and chicks initially were very low in body fat and energy content but high in protein. This variation between the species cannot be attributed entirely to species variation since the physiological age of the animals varied greatly.

Variation in the final body composition was similar to that observed in initial body composition. Fat content increased with the high fiber ration with all animals except the chicks in which a decrease was manifest. Proportion of body fat in the rats and chicks was from 4 to 8%, in pigs from 35 to 41% fat, and in ruminants from 21 to 26%. Body protein, however, was greatest in rats and chicks but lower in pigs, sheep and steers.

Consumption of the low fiber ration compared with the high fiber ration produced a higher fat content in all cases except with the sheep where the difference was not significant. Conversely the protein content was lower in all animals fed the low fiber ration compared with those fed the high fiber ration.

Composition of the weight gain (table 3) revealed that at this age rats had approximately 10% fat in their weight gain and 20% protein with a total caloric content of approximately 2,000 kcal/kg. The chicks had between 4 and 6% fat in their liveweight gain with from 28 to 30% protein and a caloric gain of approximately 2,100 kcal/kg, which was very similar to that found for the rats.

Gains of swine represented from 40 to 50% fat and from 10 to 12% protein with a caloric content ranging between 4,400 and 5,100 kcal/kg. Sheep and steers produced approximately the same quantity of fat and had a caloric equivalent of weight gain similar to that observed with

TABLE 3

Body composition

	Initial body composition	Final body composition		Composition of weight gain	
		High fiber ration	Low fiber ration	High fiber ration	Low fiber ration
Protein, %					
Rats	20.8	20.0	19.8	19.5	19.2
Chicks	17.0	28.9	28.2	29.5	28.3
Pigs	14.8	13.3	12.1	12.3	10.4
Sheep	16.7	15.2	15.1	11.8	10.5
Steers	19.2	18.2	—	18.4	—
Fat, %					
Rats	2.8	6.6	7.6	9.2	10.4
Chicks	5.3	3.8	5.9	3.7	5.9
Pigs	27.6	35.1	40.9	40.0	49.6
Sheep	17.2	24.8	25.5	41.3	48.5
Steers	11.1	21.1	—	38.7	—
Energy, kcal/kg					
Rats	1,435	1,742	1,823	1,956	2,050
Chicks	1,460	1,990	2,150	2.087	2,186
Pigs	3,411	4,031	4,512	4,436	5,128
Sheep	2,541	3,168	3,268	4,530	5,283
Steers	2,133	3,023	—	4,589	—

the pigs. The steers, however, had a greater quantity of protein in their weight gain.

Consumption of the low fiber ration in all cases resulted in a greater fat and energy content of the liveweight gain than did consumption of the high fiber ration. As a consequence of the greater fat content, protein content of the weight gain was proportionately less for those fed the low fiber ration compared with those fed the high fiber ration.

The total efficiency of feed utilization (table 4) in terms of liveweight gain per unit of feed intake was the lowest for the ruminants, with the sheep being somewhat more efficient than the steers. The pigs were intermediate, whereas the chicks made the most efficient use of their feed for liveweight gain. Efficiency of the rats fell between that of pigs and chicks. With the exception of the sheep, all animals made more efficient use of the low fiber ration for liveweight gain than they did with the high fiber ration. The relative ranking efficiency of feed utilization was the same with both rations.

Calculating efficiency of feed utilization in terms of energy gained per unit of feed intake revealed a different picture than that obtained with liveweight gain. Here pigs were the most efficient, followed by sheep, steers, chicks and rats. A greater quantity of fat in the liveweight gain (table 3) made this difference apparent. Response was quite similar with the 2 rations, with the exception of that of the sheep. Sheep were more efficient, for example, than the chicks when fed the high fiber ration, but both produced about the same quantity of energy gain per unit of feed intake when fed the low fiber ration. Moreover, all simple-stomach species produced a greater quantity of energy gain per unit of feed intake with the low fiber ration than they did with the high fiber ration.

Protein gain was greatest per unit of feed intake for the chicks followed by the rats, pigs, steers and sheep. The low fiber ration allowed a greater production of protein per unit of feed intake with the rats and chicks, but a somewhat lower protein production by the pigs and sheep.

Feed capacity as defined by Kleiber (2) measures feed intake relative to metabolic body size. This can be considered as a measure of the feed intake relative to the maintenance requirement since basal metabolism and the maintenance requirement are proportional to 70 $W_{kg}^{3/4}$. In this case, with the high fiber ration the swine and steers had the greatest feed capacity followed by the sheep, chicks and rats. Intake of feed per unit of metabolic body size for all species with the exception of the swine was lower when the low fiber ration was fed. It was apparent that the feed capacity and efficiency of feed utilization in terms of energy gain were correlated ($r = 0.91$). This high correlation indicates that intake of food relative to the maintenance requirement was probably responsible for much of the differences found among species. This confirms comments made by Blaxter (1) and Kleiber (2).

TABLE 4

Efficiency of feed utilization

	High fiber ration					Low fiber ration			
	Rats	Chicks	Pigs	Sheep	Steers	Rats	Chicks	Pigs	Sheep
Total efficiency of feed utilization[1]									
Liveweight, g	261	327	274	185	163	322	356	292	160
Relative value, %	100	125	105	71	62	100	111	91	61
Energy gain, kcal	511	662	1,213	832	748	663	782	1,492	813
Relative value, %	100	130	237	162	146	100	119	225	123
Protein, gain, g	51	96	33	22	26	62	101	30	17
Relative value, %	100	188	65	43	51	100	163	48	27
Feed capacity, g[2]	68	80	99	88	96	61	76	105	77
Relative value, %	100	117	145	129	141	100	124	172	126

[1] Total gain/kilogram of feed consumed.
[2] Feed consumed/unit of metabolic body size ($W_{kg}^{3/4}$).

The steers and swine had approximately the same feed capacity with the high fiber ration, but the pigs made a much greater energy gain per unit of feed intake than the steers, indicating that the steers were less efficient in the utilization of the feed consumed. Differences, therefore occurred in the efficiency of total feed utilization other than that caused by variation in feed capacity.

That relative feed capacity (feed intake/$W^{3/4}$) plays a major role in total efficiency of feed utilization was apparent in our data. If the maintenance requirement is larger in proportion to the feed used for gain then animals become less efficient. If it is assumed that the net energy required for maintenance is approximately equal to $70 \times W^{3/4}$ (2) then the proportion of net energy used for maintenance can be calculated. The most efficient animal, the pig, used 31 and 37% of the net energy consumed for maintenance with the high and low fiber rations, respectively, whereas the ruminants, sheep and steers, utilized from 49 to 52% of the net energy for maintenance. These animals were intermediate in their energy gain per unit of feed intake. Chicks compared quite closely with the ruminants using 55 and 57%, respectively, of the net energy of the high and low fiber ration for maintenance, whereas the rat, the least efficient, used 66 and 69%, respectively, for maintenance. Fraps (11) reported that chickens stored 58% of the productive energy from corn meal, whereas rats stored only 32%, showing that chickens were more efficient than rats.

It is expected that digestibility will play an important role, particularly when high roughage rations are used. Blaxter (1) illustrated this point. For low fiber feeds, however, differences in digestibility might not be as great. Crampton (12) could find little difference between humans, rats, guinea pigs, sheep and swine when fed wheat in their rations. On the other hand, Thomke (13) showed that pigs and sheep digested the organic matter in oat grain to a similar extent; whereas, chickens were lower in their ability to digest the organic substances in oat grain. Furthermore, he showed that the regression coefficients differed between species and that sheep generally deviated from chickens and pigs in their response to increasing amounts of crude fiber.

Therefore, although feed capacity plays the major role as a factor influencing efficiency of feed utilization, digestibility and metabolic losses have further effects. Nonetheless, research to improve the efficiency of feed utilization of certain species would be the most rewarding in the area of food intake. Admittedly, differences in physiological ages were present in this experiment, although representative of that used in many investigations, and future research may indicate physiological age to be most important in food intake of growing animals.

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Aging and Food Restriction:

CHANGES IN BODY COMPOSITION AND HYDROXYPROLINE CONTENT OF SELECTED TISSUES[1]

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ABSTRACT Two groups of male chickens started at one day of age to be fed standard rations, one group being permitted to eat ad libitum whereas the other was restricted on the basis of 80% of the amount of feed consumed by the first group at a similar body weight. Throughout the 3-year study there was a significant difference in body weight between the restricted and nonrestricted groups. The carcass composition at the end of the 3-year period was the same for both groups. The hydroxyproline content of skin, liver, muscle, comb and aorta was studied and a peak in concentration was observed between 1 and 3 months of age. In the case of certain tissues (muscle and comb) a further increase occurred between 1 and 3 years. A difference between restricted and full-fed birds in hydroxyproline content was observed only for abdominal aortic tissue at the 6 and 12 months sampling periods, with the restricted birds showing a significantly higher hydroxyproline content. At the final sampling period (38 months) the full-fed birds had higher abdominal cholesterol concentrations than the restricted birds, but on a dry, fat-free basis, there was little difference in hydroxyproline of the aorta. This last observation suggested a difference in lipid content of the aorta at 6 and 12 months.

Caloric, or food restriction has been shown to extend the life span of rats and mice (1). Relatively little information is available from such studies regarding the body composition of animals restricted in their food intake. The "aging" of certain tissues in terms of collagen accretion has also received only scant attention.

The present investigation was undertaken as part of a larger effort to determine the effects of moderate food restriction on aging and aortic atherosclerosis in the male chicken. Gross body composition was studied, as was hydroxyproline (collagen) content of liver, skin, muscle, comb and aorta. The relationship of food restriction to atherosclerosis will be described in a separate communication.

EXPERIMENTAL

Two groups of 75 male Leghorn chicks started to be fed a standard starting ration at one day of age in September, 1959. During the course of the 38-month experiment,[2] 38 animals from each of the 2 groups were killed for hydroxyproline analysis of various tissues. Thirty-three birds from the full-fed group died during the experimental period, and 13 from the food-restricted group. Because of the much heavier mortality of the former group, 14 additional birds of the same age and from the same hatch, which had been maintained under the same conditions as those originally placed on experiment, were added at a later date to the unrestricted group. Table 1 shows the composition of the 2 rations that were used for this study. The starting ration was given to all animals until they reached a body weight of 900 g. At that time, they were fed the growing ration, which was continued for the remainder of the experiment. One group of birds was allowed to eat ad libitum, and the second (restricted) group was given an amount of food equal to 80% of that consumed by the full-fed group at the same body weight. During the period of rapid growth an approximation of body surfaces, namely (body weight)$^{0.7}$, was

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[2] The exact duration of the experiment was 37 months and 15 days. For simplicity we have rounded this to 38 months.

TABLE 1

Composition of rations used in this study

Ingredients	Amount	
	Starter[1]	Grower
	%	%
Ground corn	53.70	51.90
Ground barley	5.00	23.75
Dried whey	2.50	1.25
Corn distiller's solubles	2.50	2.50
Meat and bone meal (50% protein)	2.50	2.50
Fish meal (60% protein)	3.75	—
Alfalfa meal (17% protein)	2.50	3.75
Soybean oil meal (44% protein)	23.62	10.50
Dicalcium phosphate	1.00	0.90
Limestone	1.00	1.00
Trace mineral mix[2]	0.10	0.10
Salt	0.25	0.25
Molasses	1.00	1.00
Vitamins[3]	0.60	0.60

[1] The starter was given until the birds reached a body weight of 900 g, when they were given the grower ration for the remainder of the experiment.
[2] Delamix, product of the Limestone Corporation of America, Newton, N. J.
[3] Supplied per kg of diet: vitamin A, 550 IU; vitamin D_3, 550 IU; vitamin B_2, 2.75 mg; niacin, 16.5 mg; choline chloride, 576 mg; vitamin B_{12}, 6.6 μg; menadione, 2.2 mg; calcium pantothenate, 5.76 mg; procaine penicillin, 11 mg.

used as the basis of comparison; later the arithmetic average of $(\text{body weight})^{0.7}$ + (body weight) was used, insuring maintenance of a weight differential without weight loss by the restricted birds. Food consumption was recorded at all times, first on a group and later on an individual basis. The food allowance for the restricted birds was adjusted daily for the first 3 months, weekly from 3 months until the end of the first year, and monthly thereafter.

During the first 4 weeks the birds were housed in electrically heated cages; they were then transferred to unheated community cages, and at approximately 6 months of age to individual cages. At all times the cages were located in temperature-controlled rooms.

For the tissue analyses 8 to 10 birds from each group were used at the ages indicated in the tables of results. An appropriate amount of tissue from muscle (pectoralis major), skin, liver, and comb was removed and dried to constant weight in a forced draft oven at 85°C and a portion hydrolyzed with 6 N HCl at 120°C. Hydroxyproline (as a measure of collagen content) was determined subsequently by the method of Neuman and Logan (2). The aorta was removed in one piece and analyzed in the same manner as described for the other tissues, except that it was separated into thoracic and abdominal segments at some of the sampling periods.

Before the surviving birds were killed at the end of the experiment, the energy expenditure of 15 birds from each treatment was measured in an automated multiplace respirometer (3).

After food had been removed for 12 hours, the 18 full-fed and 24 restricted birds surviving for 38 months were killed with chloroform. The body cavity was opened and the aorta removed for separate analysis as were also small portions of the other tissues analyzed for hydroxyproline. The remainder of the carcass was dried in a forced draft oven at 100°C to constant weight. The carcasses were next ground individually in a power meat grinder and appropriate samples taken for nitrogen analysis by a micro-Kjeldahl digestion followed by a colorimetric nitrogen determination, using an Auto Analyzer (4). Other carcass samples were lipid-extracted twice with a mixture of chloroform-methanol in the proportions 2:1, followed by filtration and evaporation of solvent and weighing of the residue. The aortas of the birds killed at the end of the experiment were also lipid-extracted, and cholesterol determined on a portion of the chloroform-methanol extract by the colorimetric procedure of Zlatkis et al. (5). For the final analysis only, hydroxyproline content was determined on the dry, fat-free aortic tissue (for all prior analyses the tissues had not been fat-extracted).

RESULTS AND DISCUSSION

Body weight. Table 2 shows the body weights and food consumption values of the 2 groups of birds throughout the experiment. The restricted group maintained a significantly lower body weight at every weighing period. Furthermore, the restricted birds were much more uniform in body weight than those permitted to eat ad libitum. This may reflect the variation in food intake of the full-fed birds. For the full-fed birds, standard errors were sometimes 5 to 6 times as great as

TABLE 2

Monthly body weights and feed consumption values for full-fed and restricted birds

Year and month	Body weight		Feed consumption[1]	
	Full-fed	Restricted	Full-fed	Restricted
	g	*g*	*g*	*g*
1959				
9	37 ± 0.2[2]	37 ± 0.2	16.8	10.9
10	276 ± 2	159 ± 1	35.9	19.0
11	846 ± 37	393 ± 15	74.7	34.4
12	1298 ± 38	693 ± 38	88.7	47.5
1960				
1	1630 ± 23	977 ± 24	97.1	62.3
2	1825 ± 77	1275 ± 28	93.1	72.1
3	1901 ± 26	1489 ± 10	87.2	74.1
4	1865 ± 28	1503 ± 18	81.0	58.3
5	1914 ± 31	1612 ± 18	70.0	62.6
6	1933 ± 33	1710 ± 19	74.0	63.6
7	1927 ± 34	1756 ± 20	67.0	55.6
8	1958 ± 34	1695 ± 19	68.0	59.0
9	1932 ± 39	1731 ± 22	68.5	59.6
10	1886 ± 14	1717 ± 26	76.2	59.4
11	1921 ± 41	1719 ± 26	69.2	59.9
12	1919 ± 42	1708 ± 27	73.9	58.0
1961				
1	2001 ± 58	1772 ± 24	78.5	58.8
2	1822 ± 59	1831 ± 28	73.7	61.0
3	2036 ± 51	1865 ± 32	80.3	58.7
4	2009 ± 70	1876 ± 25	79.7	60.4
5/1	2059 ± 61	1909 ± 24	75.2	57.6
5/29	1870 ± 60	1735 ± 28	62.2	51.9
6	2452 ± 66[3]	1793 ± 31	87.4[3]	55.2
7	2565 ± 91	1921 ± 32	89.6	59.1
8	2500 ± 95	1818 ± 27	84.1	59.3
9	2483 ± 95	1956 ± 54	84.4	59.9
10	2521 ± 105	1841 ± 25	84.9	57.0
11	2535 ± 114	1919 ± 24	86.6	59.8
12	2561 ± 109	1953 ± 24	84.6	61.7
1962				
1	2550 ± 111	1932 ± 29	83.7	58.2
2	2541 ± 110	1905 ± 37	83.2	56.3
3	2547 ± 115	1953 ± 28	78.6	56.5
4/2	2473 ± 105	1952 ± 27	78.0	56.1
5/1	2560 ± 101	2046 ± 72	78.9	57.1
5/28	2451 ± 111	2048 ± 81	78.6	58.5
6	2441 ± 117	2010 ± 79	76.7	57.1
7	2448 ± 121	1993 ± 31	81.9	56.1
8	2518 ± 137	1994 ± 31	85.3	57.8
9	2480 ± 116	1921 ± 29	76.0	58.6
10/17	2368 ± 129	1899 ± 27	84.8	60.9
10/31	2261 ± 132	1832 ± 26		

[1] Average daily intake during month indicated.
[2] Mean value ± SE.
[3] The relatively large change from the previous value was occasioned by the addition of 14 birds of the same age and from the same hatch that had been maintained under the same conditions.

those noted for the restricted birds. In addition, a first plateau in body weight of approximately 1900 g was reached by the full-fed birds at 6 months, whereas the restricted birds had reached only their first plateau of 1700 g around 9 months of age. Peak body weights were, however, reached at approximately the same time by both groups (23 months).

Body composition and energy expenditure. Table 3 lists the body composition and oxygen consumption for the birds surviving at the end of 38 months on experiment. No differences were noted between the 2 groups of birds in any of the body components measured, nor were the oxygen consumption values significantly different from each other. In view of the

TABLE 3

Energy exchange and body composition of birds full-fed or restricted in their food intake

Measurement	Food intake[1]	
	Full-fed	Restricted
Oxygen consumption (l/kg/24 hr)	16.07 ± 0.63[2]	16.40 ± 0.70
Final body weight (g)	2261 ± 132	1832 ± 26
Moisture (%)	63.81 ± 0.34	63.77 ± 0.20
Nitrogen (% wet wt)	4.35 ± 0.04	4.37 ± 0.04
Nitrogen (% dry wt)	11.98 ± 0.33	12.06 ± 0.11
Lipid extract (% wet wt)	4.72 ± 0.51	4.28 ± 0.23
Lipid extract (% dry wt)	12.99 ± 1.38	11.70 ± 0.62

[1] For the oxygen consumption determinations 15 birds/treatment were used; for the composition analyses 15 full-fed and 24 restricted birds were used.
[2] Mean ± SE.

highly significant difference in body weight, these are important observations. It is generally assumed that food or caloric restriction will result in a reduced level of body fat. In the present study, after more than 3 years of moderate food restriction, the restricted birds were merely smaller in size without any change in the percentage composition. Recently Fabry et al. (6) made similar observations on intermittently starved and chronically underfed rats. They reported even a relatively greater lipogenesis for the retarded rats compared with the full-fed animals.

Hydroxyproline. Table 4 shows the hydroxyproline values (which may be taken as a measure of collagen concentration) for liver, skin, breast muscle, and comb. In the case of all 4 tissues a peak in hydroxyproline content appeared between 1 and 3 months of age. For muscle as well as for comb there was a further significant increase and a new peak at the final sampling period. There were no major differences between full-fed or restricted birds in hydroxyproline content of any of the tissues whose values are listed in table 4. However, the liver hydroxyproline content, compared with that of muscle or skin, showed a relatively small increase with age. The decline in hydroxyproline content in skin and liver tissue after 6 months may be a reflection of an increased fat content for these tissues. A similar observation for a decline with age in hydroxyproline content was made for aortic tissue (table 5).

Aorta hydroxyproline and cholesterol. The hydroxyproline content of aortic tissue (table 5) also reached a peak at 3 months as had the values for the other tissues examined (table 4). Also, the full-fed birds had significantly lower hydroxyproline concentrations in the abdominal segment than the restricted birds at the 6- and 12-month sampling periods. For both these periods the hydroxyproline concentration is shown as a percentage of dry weight. At the final (38-month) period, the values are expressed as a percentage of dry-defatted weight and are nearly the same. As suggested above for skin, the high 6- and 12-month values for the restricted group are probably a reflection of a higher fat content in the abdominal aorta of the full-fed birds. The cholesterol content of the abdominal aorta at 38 months bears out this contention (table 5).

TABLE 4

Hydroxyproline values of liver, skin, muscle and comb from restricted and full-fed chickens

Age	Food intake	
	Full-fed	Restricted
month	*% dry wt*	*% dry wt*
	Liver	
Day-old	0.12 ± 0.003[1]	0.12 ± 0.003
1	0.16 ± 0.002	0.17 ± 0.01
3	0.27 ± 0.002	0.25 ± 0.01
6	0.23 ± 0.01	0.21 ± 0.03
12	0.20 ± 0.02	0.18 ± 0.01
	Skin	
Day-old	2.33 ± 0.13	2.33 ± 0.13
1	4.11 ± 0.26	4.38 ± 0.44
3	5.24 ± 0.14	7.76 ± 0.26
6	8.16 ± 0.42	7.23 ± 0.36
12	6.60 ± 0.64	5.79 ± 0.38
38	6.45 ± 0.19	5.58 ± 0.23
	Muscle (pectoralis major)	
Week-old	0.23 ± 0.01	0.23 ± 0.01
1	0.20 ± 0.01	0.22 ± 0.01
3	0.34 ± 0.02	0.33 ± 0.02
6	0.36 ± 0.06	0.29 ± 0.02
12	0.33 ± 0.03	0.38 ± 0.02
38	0.56 ± 0.04	0.57 ± 0.03
	Comb	
1	3.7 ± 0.1	4.1 ± 0.4
3	5.9 ± 0.2	5.8 ± 0.2
6	5.8 ± 0.2	5.3 ± 0.2
12	5.1 ± 0.2	5.0 ± 0.2
38	6.3 ± 0.1	6.1 ± 0.1

[1] Mean ± SE.

TABLE 5

Hydroxyproline and cholesterol content of aortas from full-fed and food restricted chickens

Age	Full-fed			Restricted		
	Whole aorta	Thoracic segment	Abdominal segment	Whole aorta	Thoracic segment	Abdominal segment
month						
			Hydroxyproline (% dry wt)			
Day-old	2.0 ± 0.1[1]			2.0 ± 0.1		
1	2.4 ± 0.1			2.9 ± 0.3		
3	4.3 ± 0.2			3.8 ± 0.1		
6		4.0 ± 0.1	6.9 ± 0.3		3.9 ± 0.1	8.1 ± 0.2
12		4.0 ± 0.1	6.6 ± 0.3		3.7 ± 0.3	7.6 ± 0.1
			(% dry, defatted wt)			
38		2.9 ± 0.1	5.0 ± 0.1		3.1 ± 0.1	5.4 ± 0.1
			Cholesterol (% dry wt)			
38		0.81 ± 0.09	2.70 ± 0.50		0.83 ± 0.07	1.56 ± 0.22

[1] Mean ± SE.

The full-fed birds had nearly twice as much cholesterol as the restricted birds did.

GENERAL COMMENTS

Unexpectedly, moderate food restriction, as compared with ad libitum feeding, did not alter the percentage composition of the carcasses of the restricted animals; instead, they were merely smaller. This raises certain questions concerning reasons for the lower mortality of the food-restricted group. We stated in the experimental section that 13 of 75 birds started died on the restricted regimen, whereas 33 birds died[3] on the full-fed regimen. Obesity, in the usually accepted sense of excess body fat could not possibly have been an important factor contributing towards the increased mortality of the full-fed birds. However, the distribution of body lipid in the 2 groups may have differed. The difference in cholesterol content for the abdominal aorta between groups (table 5) lends support to this possibility.

The early peak in hydroxyproline concentration, which was noted at 3 months in most of the tissues examined, may reflect the fast growth rate of the chicken. In the rat Kao and McGavack (7) observed a gradual and continuous increase in aortic collagen content through 2 years of age. Skin collagen, on the other hand, reached a peak at 5 weeks of age. It is interesting to speculate whether and how the marked difference in collagen accretion of aortic tissue between rat and chicken might relate to the difference in susceptibility of these species to atherosclerosis.

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[3] The mortality figures may be misleading since of the 75 birds started on each regimen, 38 were killed for analyses during the study.

Some Effects of High and Low Sodium Intake During Pregnancy in the Rat

IV. GRANULATION OF RENAL JUXTAGLOMERULAR CELLS AND ZONA GLOMERULOSA WIDTH[1,2]

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ABSTRACT Histological examinations of the kidney and adrenal were made to determine whether sodium conservation during pregnancy in the rat is related to the degree of granulation of the juxtaglomerular cells of the kidney, considered indicative of renin secretion, and whether this, in turn, influences the width of the zona glomerulosa, considered indicative of aldosterone secretion. Pregnant animals receiving low, moderate and high levels of dietary sodium were studied along with nonpregnant controls. Determination of sodium and potassium retention and the concentration of sodium and potassium in plasma and muscle provided essential supporting data for interpretation of the histological findings. Both pregnancy and decreasing levels of sodium intake led to an increase in juxtaglomerular granulation and in zona glomerulosa width. In all but the low sodium pregnant group, in which there was evidence of sodium deficiency, an increase in juxtaglomerular granulation was accompanied by an increase in zona glomerulosa width and in the percentage of dietary sodium conserved by the kidney. The low sodium pregnant group had less juxtaglomerular granulation than the corresponding nonpregnant group or the other pregnant groups. Despite this degranulation, the low sodium pregnant animals had the widest zona glomerulosa and conserved the greatest percentage of dietary sodium. It is suggested that in this group juxtaglomerular degranulation was due to a rate of renin secretion exceeding the rate of production, thereby reducing the number of secretory granules present in the cells.

In recent investigations in this laboratory (1–3) on the effects of varying levels of dietary sodium during pregnancy in the rat, an adverse effect of a low sodium intake was noted. Despite an increase in the percentage of dietary sodium retained, the animals fed the low sodium diet had significantly reduced sodium retention and exhibited signs of a sodium deficiency including hyponatremia and reduced muscle sodium. In addition, these animals failed to show the decrease in hematocrit level noted in the other groups during pregnancy. Kirksey and Pike (1) suggested that in the low sodium group, the expected increase in blood volume due to pregnancy did not occur.

Alterations in the juxtaglomerular apparatus of the kidney indicating an inverse relationship between the level of sodium intake and the degree of granulation of the juxtaglomerular cells has been observed in a sodium deficiency by Hartroft and Hartroft (4) and by Tobian et al. (5). A direct correlation between the degree of granulation of the juxtaglomerular cells and the amount of extractable renin in the kidney has also been observed (6, 7). In addition, a direct relationship between the degree of juxtaglomerular granulation and the secretory activity and width of the zona glomerulosa has been noted (8–10). These observations led to the postulation by Hartroft and Hartroft (11) that the secretory product of the juxtaglomerular cells, presumably renin, acts as a trophic substance stimulating the secretion of aldosterone. Recently, Mul-

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[3] Taken in part from a dissertation submitted to The Graduate School, The Pennsylvania State University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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row et al. (12) noted that the injection of renin extracted from the kidney stimulated the secretion of aldosterone in hypophysectomized, nephrectomized dogs.

Tobian (13) hypothesized that the juxtaglomerular cells function as stretch receptors in the walls of the afferent arteriole of the glomerulus. Decreased blood volume has been associated with sodium deficiency (14–16) and, according to Tobian, may stimulate the secretory activity of the juxtaglomerular cells because of decreased stretch in the afferent arteriole.

Markedly increased secretion of aldosterone during pregnancy in humans has been reported by Jones et al. (17). Venning et al. (18) noted that in pregnancy, although the blood volume is increased, aldosterone excretion and sodium retention are markedly increased. They suggested that despite the increase in total vascular volume in pregnancy, the effective vascular volume may be reduced and may be responsible for stimulation of aldosterone secretion. The decreased effective vascular volume may result because of changes in fluid balance and an increase in the vascular bed. Thus increased granulation of the juxtaglomerular cells and an accompanying increase in sodium retention may occur in pregnancy in response to decreased distention of the afferent arteriole. Therefore, an investigation attempting to relate sodium intake during pregnancy with alterations in juxtaglomerular granulation and zona glomerulosa width was considered pertinent to the understanding of sodium regulation during pregnancy in the rat.

EXPERIMENTAL

Young adult female rats of the Sprague-Dawley strain were maintained with laboratory chow until regular estrous cycles were established. They were then divided into 6 experimental groups of 8 animals each. Three of the groups were mated and 3 served as nonpregnant controls. The composition of the basal ration was the same as that used previously (1). The basal ration was supplemented with 0.032, 0.32 and 3.2% sodium chloride for the low sodium, control and high sodium diets respectively. By analysis these diets contained 1.1, 6.0 and 53.5 mEq of sodium/100 g of diet. Approximately 6 days prior to the beginning of the experimental period the animals were fed the control ration. On the 1st day of the experiment, the nonpregnant group was subdivided: one-third continued to be fed the control diet and the remaining animals were equally divided between the high and low sodium levels. The pregnant animals were assigned to 1 of the 3 levels of sodium intake on the day mating was confirmed by the presence of sperm in the vaginal smear and were fed these diets for the 3-week gestation period. The nonpregnant groups were fed the experimental rations for a comparable 3-week period. Rations were provided ad libitum and weekly records of food intake were kept.

Procedures similar to those used previously (1, 2) were used to obtain data on hematocrit levels and plasma sodium and potassium concentrations on the 1st and 21st days of the experimental period.

Data on sodium retention were obtained for the 3rd week only, using stainless steel metabolism cages for collection of urine and feces. Since it was noted in preliminary studies that the animals restricted their food intake when first placed in metabolism cages, a 4-day preliminary period was allowed for adjustment to the cages before initiating the balance studies. Upon commencement of the balance studies, the urine from each animal was collected daily and transferred to a one-liter volumetric flask. The collecting funnel was washed with hot, 1% sulfuric acid and the washings were added to the volumetric flask. At the end of the experimental period, the urine and washings were made up to volume and filtered. An aliquot was retained for analysis. Feces were prepared for analysis as described previously (1). All sodium and potassium analyses were made in a Beckman DU flame photometer equipped with a photomultiplier attachment.

On the 22nd day of the experimental period, the animals were chloroformed and tissues removed for biochemical and histological investigation. The litters of pregnant animals were removed by abdominal section and the litter weight and number of live young recorded.

The wet and dry weights of the gastrocnemius muscle were obtained. The muscle was then acid-digested and diluted for analysis of sodium and potassium.

A slice approximately 0.32 cm in thickness was taken from the kidney at right angles to the longitudinal axis at the midpoint and was fixed in Helly's fluid for 48 hours. After washing in running water for 24 hours, the slice was embedded in paraffin and sectioned (4 μ). After the weight of the pair of adrenal glands was obtained, the adrenals were fixed as was the kidney slice and were sectioned (6 μ) at right angles to the longitudinal axis at the midpoint.

The kidney sections were stained by the method developed by Pitcock and Hartroft (19) involving the use of Bowie's stock solution and differentiation with a mixture of xylol and clove oil. After differentiation the juxtaglomerular granules were an intense blue in contrast with the bright red of the renal parenchyma. The slide labels of the kidney sections were masked and the slides coded to avoid any subjective influence in scoring the sections. Three sections from each animal were examined under high dry magnification and scored for juxtaglomerular granulation according to the method of Hartroft and Hartroft (4) in which the degree of granulation is expressed as a juxtaglomerular index. This index increases as the degree of granulation increases. The indices of 3 sections from each animal were averaged to give the final juxtaglomeular index for the animal.

The adrenal sections were stained with hematoxylin. For measurements on the adrenal, as on the kidney, the slide labels were masked and the slides coded. The width of the zona glomerulosa was measured at 4 positions in each of 3 sections from one adrenal from each animal. Measurements were made under low power magnification using an eyepiece micrometer calibrated with a stage micrometer. Measurements of the zona glomerulosa were based on the appearance of a relatively dense band of darkly stained nuclei lying beneath the capsule. However, this measurement was difficult in some sections because of a greater degree of hypertrophy of the cells. The zona glomerulosa in these sections could be measured more easily by darkening the field and considering not only the nuclei, but the fact that the cytoplasm in the zona glomerulosa had a more translucent appearance than in the other zones of the cortex. At the 4 positions at which measurements were taken a count was made of the number of cells comprising the width of the zona glomerulosa.

RESULTS AND DISCUSSION

To provide essential data for the interpretation of the histological findings, many of the procedures carried out in previous studies (1–3) on the effect of 3 levels of dietary sodium on pregnancy in the rat were repeated and the earlier observations confirmed.

The degree of granulation of the juxtaglomerular cells (table 1) increased significantly ($P < 0.01$) due to the effect of pregnancy. The juxtaglomerular index was 15.4 in the control nonpregnant group and increased to 25.3 in the control pregnant group. An increase in granulation also accompanied decreasing levels of sodium intake ($P < 0.01$). The increases in the nonpregnant groups were not as marked as those observed in nonpregnant animals by Hartroft and Hartroft (4) or by Tobian et al. (5). Marked differences in the experimental procedures employed appear to account, in part at least, for the differences. In this study mature animals were used, whereas growing rats were used by Hartroft and Hartroft (4). Because of the need to assure successful pregnancy, the lowest level of sodium used in this study (1.1 mEq/100 g of diet) was considerably higher than that used by the other investigators. In addition, in this study the experimental period was of necessity limited to the 3-week gestation period and

TABLE 1

Average juxtaglomerular index of the rat kidney

Dietary treatment	Nonpregnant	Pregnant
Na (mEq/100 g)		
1.1	16.1 ±7.7[1]	12.5±3.5
6.0[2]	15.4 ±5.2	25.3±6.1
53.5	11.1[3]±3.6	19.2±3.7

[1] SE of mean.
[2] Control groups.
[3] Mean for only 7 animals.

was shorter than the experimental periods usually utilized by the other investigators.

In the pregnant groups the typical decrease in granulation due to the effect of increasing levels of sodium intake was apparent when the average juxtaglomerular indices of animals fed control and high levels of sodium intake were considered. The juxtaglomerular index decreased from 25.3 in the control group to 19.2 in the high sodium group. However, the pregnant animals fed low sodium rations had a juxtaglomerular index of only 12.5 which was, in fact, lower than that of the nonpregnant animals fed the same level of sodium.

As reported previously (1–3) and confirmed in the present study, the pregnant animals fed the low sodium diet appeared to be deficient in sodium. Their food intake and weight gain were more limited and the average litter weight and maternal tissue gain (total weight gain minus litter weight) were smaller than in the other pregnant groups. They showed signs of lethargy and debility associated with sodium deficiency (20). Sodium was withdrawn from the muscle to a greater degree than in any other pregnant group (table 2). On the 21st day of the experimental period these animals had marked hyperkalemia (table 3) which has been observed by others in a sodium deficiency (20, 21). They were unable to maintain a plasma sodium concentration (table 3) within the range of the control pregnant animals. The decrease in plasma sodium due to pregnancy was much more marked in the low sodium group than in the control and high sodium groups. In addition, these animals failed to show the decrease in hematocrit due to pregnancy (table 3) which was noted in the other pregnant groups. Brown and Pike (22) associated the decrease in hematocrit value during pregnancy with an increase in blood volume. Therefore the unchanged hematocrit in the low sodium pregnant group suggests that the expected increase in blood volume did not take place. A failure to show the normal increase in blood volume in pregnancy may be associated with a sodium deficiency since a decrease in blood volume has been demonstrated in sodium deficiency states (16, 20).

The linear correlation between the juxtaglomerular index and the percentage of dietary sodium excreted in the urine (fig.

TABLE 2

Average sodium and potassium concentrations of gastrocnemius muscle

Dietary treatment	Nonpregnant		Pregnant	
	Na	K	Na	K
	mEq/kg	*mEq/kg*	*mEq/kg*	*mEq/kg*
Na (mEq/100 g)				
1.1	24.1 ± 1.5[1]	103.1 ± 1.3	13.1[2] ± 0.7	103.8 ± 3.4
6.0[3]	23.1 ± 1.8	102.8 ± 2.6	19.7 ± 0.9	103.0 ± 2.2
53.5	25.1 ± 2.1	103.7 ± 4.9	21.3 ± 1.0	100.8 ± 2.0

[1] SE of mean.
[2] Interaction, $P < 0.001$.
[3] Control groups.

TABLE 3

Average plasma sodium and potassium concentrations and hematocrit (21st day)[1]

Dietary treatment	Nonpregnant			Pregnant		
	Plasma		Hematocrit	Plasma		Hematocrit
	Na	K		Na	K	
	mEq/l	*mEq/l*	%	*mEq/l*	*mEq/l*	%
Na (mEq/100 g)						
1.1	149.0 ± 2.6[2]	5.3 ± 0.4	48.0 ± 2.1	132.6[3] ± 3.1	7.2[3] ± 0.6	48.8[3] ± 2.4
6.0[4]	151.7 ± 3.1	5.3 ± 0.4	47.3 ± 1.6	143.1 ± 1.8	5.2 ± 0.4	42.0 ± 1.7
53.5	150.1 ± 3.3	5.3 ± 0.4	49.0 ± 1.8	145.3 ± 4.3	5.2 ± 0.3	40.0 ± 3.1

[1] Values for 1st day for all groups were similar to values for nonpregnant groups on 21st day.
[2] SE of mean.
[3] Interaction, $P < 0.001$.
[4] Control groups.

1) was inverse ($r = -0.330$) and only slightly significant ($P < 0.05$) when all groups of animals were included. It appeared from a consideration of figure 2 that the low sodium pregnant animals constituted a distinct and separate grouping. When this group was omitted, the correlation between the juxtaglomerular index and sodium excretion for the remaining 5 groups ($r = -0.672$) was significant ($P < 0.001$). This inverse correlation between sodium excretion and the juxtaglomerular index is to be expected if, as hypothesized by Hartroft and Hartroft (11), increased granulation of the juxtaglomerular cells and, presumably, increased secretion of renin, lead to greater conservation of sodium by the kidney.

Despite the degranulation observed in the low sodium pregnant group, sodium was conserved to the greatest extent by these animals (table 4). Only 13.2% of the ingested sodium was excreted whereas the control pregnant animals excreted 23.5% and the nonpregnant controls 93.2%. According to the hypothesis of Hartroft and Hartroft (11), the circulating level of renin would have been greatest during the last week of the experimental period in the low sodium pregnant animals. Therefore it is presumed that the degranulation observed represented a rate of secretion exceeding the rate of accumulation. Such degranulation of secretory cells may indicate either marked inactivity or vigorous activity with a disproportionality between the rate of production and the rate of secretion (23, 24). In the control pregnant group a similar phenomenon may have occurred but to a lesser degree. It appears that these animals also constituted a separate and distinct grouping and that some degree of degranulation may have occurred because of an accelerated secretion rate.

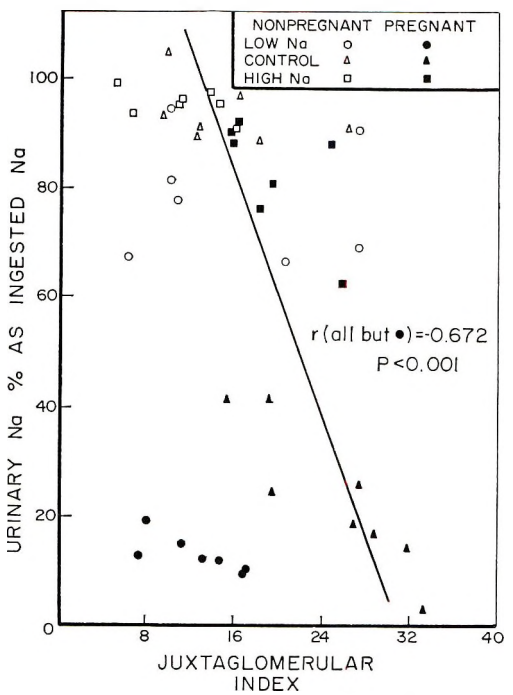

Fig. 1 Urinary Na as percentage ingested Na and juxtaglomerular index.

Neither Hartfroft and Hartroft (4, 25) nor Tobian et al. (5) noted degranulation of the juxtaglomerular cells after severe and lengthy sodium deprivation. However, when the retention of the nonpregnant and pregnant animals fed control rations of sodium were compared, it was noted that the nonpregnant animals retained 0.13 mEq of sodium in the final week, whereas the pregnant animals retained 4.97 mEq (table 4). Therefore it is suggested that the degree of deprivation imposed upon the pregnant animals fed the low sodium ration may have been considerably greater than that reported by other investigators.

Tobian (13) has suggested that the decreased blood volume noted in sodium deficiency acts as a stimulus for increased juxtaglomerular granulation because of the resultant decrease in stretch in the afferent arteriole. In the control and high sodium pregnant groups in this study, granulation of the juxtaglomerular cells was increased despite the fact that these groups showed indications of the increased blood volume typical of pregnancy. If, as suggested by Venning et al. (18), the effective vascular volume is actually reduced in pregnancy, Tobian's hypothesis could explain the increased granulation of the juxtaglomerular cells in normal pregnancy. In the pregnant animals fed the low sodium ration, indications were present which suggested the absence of the usual increase in blocd volume during pregnancy. Therefore, it is presumed that the

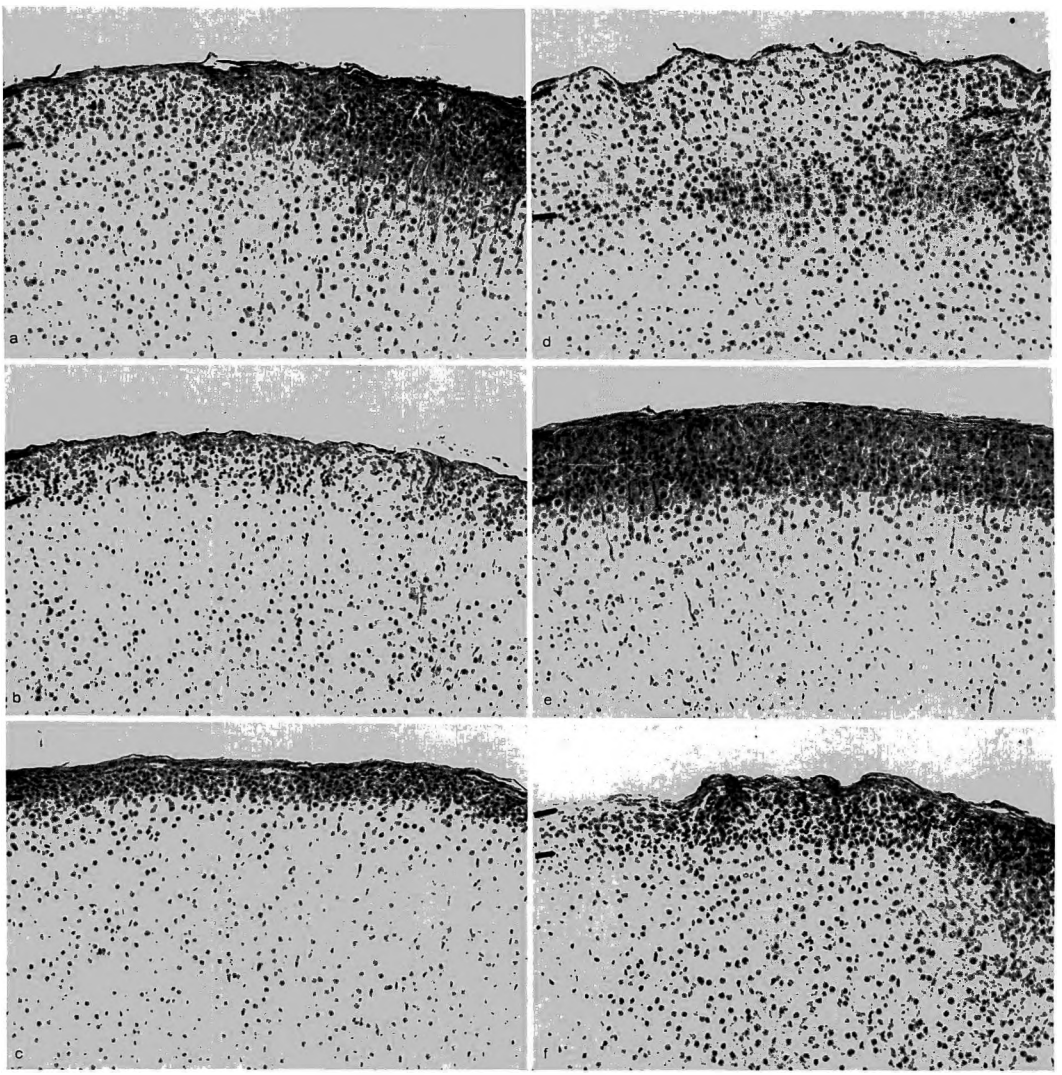

FIGURE 2

a Section from adrenal gland of a nonpregnant animal fed the low sodium ration. The zona glomerulosa and part of the zona fasciculata are shown. (low power)

b Section from adrenal gland of a nonpregnant animal fed the control ration. The zona glomerulosa and part of the zona fasciculata are shown. (low power)

c Section from adrenal gland of a nonpregnant animal fed the high sodium ration. The zona glomerulosa and part of the zona fasciculata are shown. The zona glomerulosa is extremely compact and well demarcated. Cytoplasm in the zona glomerulosa is more homogeneous than that in figures 2a and 2b. (low power)

d Section from adrenal gland of a pregnant animal fed the low sodium ration. The zona glomerulosa and part of the zona fasciculata are shown. The zona glomerulosa is poorly demarcated. The cytoplasm in the zona glomerulosa is abundant and coarsely vacuolar and there is evidence of an increase in the number of lipoid droplets. (low power)

e Section from adrenal gland of a pregnant animal fed the control ration. The zona glomerulosa and part of the zona fasciculata are shown. The zona glomerulosa is well demarcated. The cytoplasm is more abundant than that in the nonpregnant control animals. (low power)

f Section from adrenal gland of a pregnant animal fed the high sodium ration. The zona glomerulosa and part of the zona fasciculata are shown. The zona glomerulosa is well demarcated. (low power)

TABLE 4

Average sodium and potassium retention and urinary excretion (3rd week)

Dietary treatment	Nonpregnant				Pregnant			
	Retention		Urinary excretion		Retention		Urinary excretion	
	Na	K	Na	K	Na	K	Na	K
Na (mEq/100 g)	*mEq*	*mEq*	*% ingested*	*% ingested*	*mEq*	*mEq*	*% ingested*	*% ingested*
1.1	0.07[1] ± 0.11[2]	0.28[1] ± 0.72	78.3[1] ± 10.2	94.8[1] ± 5.2	0.66[1,3] ± 0.09	0.68[1,3] ± 1.09	13.2[1,3] ± 3.0	92.2[1,4] ± 9.6
6.0[5]	0.13[1] ± 0.23	−0.43[1] ± 0.66	93.2[1] ± 5.2	100.4[1] ± 5.2	4.97 ± 0.57	2.58 ± 1.15	23.5 ± 12.4	84.8 ± 6.1
53.5	0.92 ± 0.84	0.01 ± 0.47	95.0 ± 4.3	96.9 ± 3.3	10.46[1] ± 6.31	3.74[1] ± 1.82	82.3[1] ± 9.4	79.8[1] ± 8.4

[1] Mean for only 7 animals.
[2] SE of mean.
[3] Interaction, $P < 0.001$.
[4] Interaction, $P < 0.01$.
[5] Control groups.

effective vascular volume was further decreased and that the secretory activity of the juxtaglomerular cells was increased above that in the other pregnant groups.

Increase in the width of the zona glomerulosa has been associated with sodium deprivation (26–28) and with increased secretion of aldosterone (8, 9). Representative sections of the adrenal glands from each group of animals are shown in figure 2 (a–f). The zone increased in width (table 5) due to the effect of pregnancy ($P < 0.001$). This observation in pregnant animals is consistent with findings of other investigators who noted that during normal preganacy in humans the output of aldosterone is increased (17, 18, 29). The width of the zona glomerulosa also increased with decreasing levels of sodium intake ($P < 0.001$) and this increase was most apparent in the pregnant groups. There was a significant interaction ($P < 0.001$) between pregnancy and the effect of sodium intake. The pregnant group fed the low sodium diet had the widest zona glomerulosa of any group. These animals also had the heaviest adrenals (table 5). Eisenstein and Hartroft (8) and Hartroft and Eisenstein (9) associated not only an increase in the width of the zona glomerulosa but an increase in the weight of the adrenals with increased secretion of aldosterone.

There was an inverse correlation ($r = -0.863$) between the width of the zona glomerulosa and the percentage of ingested sodium excreted in the urine (fig. 3) which was significant ($P < 0.001$). The low sodium pregnant animals had the widest zona glomerulosa and the smallest excretion of sodium and presumably secreted the largest amount of aldosterone. In addition, they excreted a greater percentage of ingested potassium than either of the other pregnant groups. Increased potassium excretion may also be indicative of increased secretion of aldosterone (30, 31).

There was a significant increase in the number of cells comprising the width of the zona glomerulosa due to the effect of pregnancy ($P < 0.001$) and to decreasing levels of sodium intake ($P < 0.01$). The linear correlation between the increase in zone width and the

TABLE 5

Average adrenal weights and width of the zona glomerulosa

Dietary treatment	Nonpregnant				Pregnant			
	Weight		Width		Weight		Width	
Na (mEq/100 g)	*mg*	*mg/100 g body wt*	*μ*	*no. of cells*	*mg*	*mg/100 g body wt*	*μ*	*no. of cells*
1.1	64.56 ± 4.27[1]	24.97 ± 2.62	70.7 ± 4.4	7.7 ± 0.7	75.75[2,3] ± 4.57	24.38[2,3] ± 1.72	119.4[3] ± 15.4	9.2 ± 1.7
6.0[4]	66.07[2] ± 9.60	25.96[2] ± 4.46	65.4 ± 12.0	7.1 ± 0.9	60.26 ± 4.46	16.23 ± 1.73	99.9 ± 13.4	9.8 ± 1.2
53.5	65.08 ± 5.35	26.08 ± 2.79	66.1 ± 11.1	6.6 ± 0.8	54.10[2] ± 3.62	14.14[2] ± 0.82	68.4 ± 5.0	7.6 ± 0.5

[1] SE of mean.
[2] Mean for only 7 animals.
[3] Interaction, $P < 0.001$.
[4] Control groups.

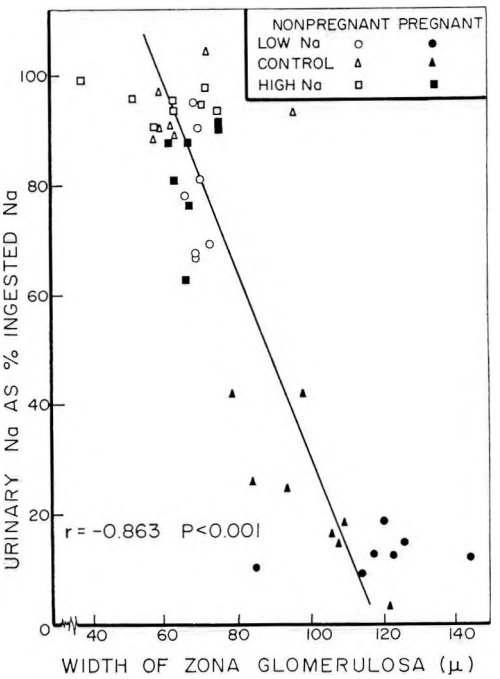

Fig. 3 Urinary Na as percentage ingested Na and width of zona glomerulosa.

number of cells comprising the width (fig. 4) was direct ($r = 0.951$) and significant ($P < 0.001$) and was indicative of hyperplasia. The histopathological evaluation of the adrenal sections indicated that in the low sodium pregnant group not only did a greater degree of cytoplasmic hypertrophy exist but that the cells contained more abundant lipoid droplets than in other groups. The investigations of Hartroft and Eisenstein (9) associated both cellular hypertrophy and an increase in the content of large lipoid droplets in the zona glomerulosa with increased aldosterone secretion.

Hartroft and Hartroft (25) observed a direct linear correlation between the width of the zona glomerulosa and the juxtaglomerular index. In this study the correlation ($r = 0.131$) between the width of the zona glomerulosa and the juxtaglomerular index was not significant when all groups were considered (fig. 5). However, it appeared that the low sodium pregnant animals, in which an increase in the width of the zona glomerulosa was not accompanied by an increase in the juxtaglomeru-

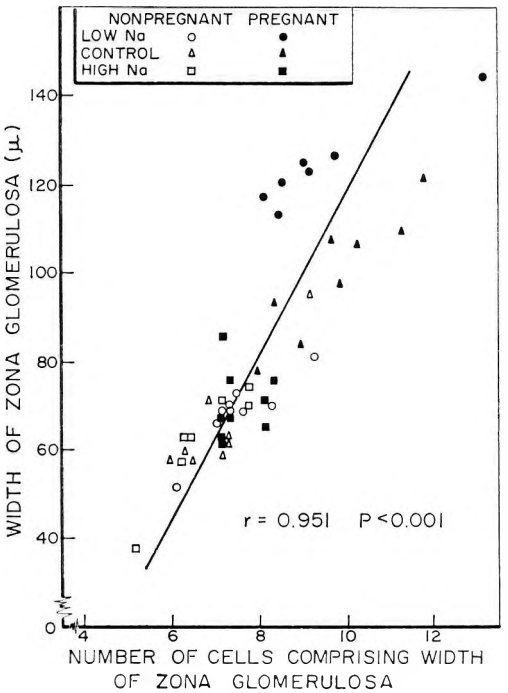

Fig. 4 Width of zona glomerulosa and number of cells.

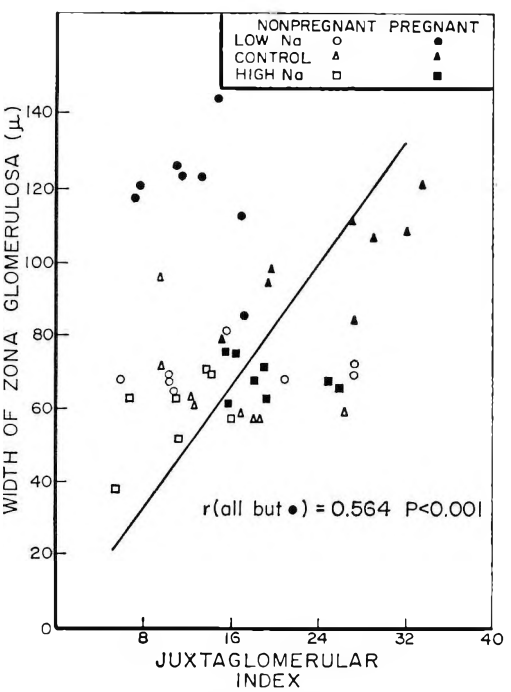

Fig. 5 Width of zona glomerulosa and juxtaglomerular index.

lar index, constituted a separate and distinct grouping. When this group was omitted, there was a direct correlation ($r = 0.564$) which was significant ($P < 0.001$). This correlation and the fact that pregnancy increased significantly both juxtaglomerular granulation and the width of the zona glomerulosa indicates that the mechanism regulating sodium conservation as postulated by Hartroft and Hartroft (11) is operative in pregnancy.

The increase in width of the zona glomerulosa in the low sodium pregnant group, despite degranulation of the juxtaglomerular cells, lends support to the suggestion that the degranulation in this group was due to a rate of secretion exceeding the rate of production, thereby reducing the number of secretory granules present in the cells.

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Utilization of Glycine Nitrogen at Various Levels of Glycine Intake[1,2]

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ABSTRACT Metabolism of approximately 20 mg of N^{15} from glycine, ingested as a single dose, was studied in each of 2 dogs, in 5 successive experiments wherein total intake of free glycine increased stepwise from 1.5 to 19.5 g/day. Output of N^{15} as total nitrogen, urea, and ammonia, as well as the amount in fibrinogen, were determined 6, 12, 24, and 48 hours after ingestion. Percentage of N^{15} retained after 24 hours, and the percentage observed in fibrinogen, were both inversely proportional to the logarithms of total free glycine intake. The 13-fold increase in glycine intake reduced the former percentage by about one third, and the latter by about two thirds. Rate of urea formation, expressed as milligrams of urea nitrogen per kilogram of body weight per hour, was a linear function of glycine intake during the most active period of catabolism (second 6-hour period after ingestion). Percentage of urinary ammonia originating from ingested glycine during the first two 6-hour periods increased linearly with glycine intake during the first 4 levels of supplementation, but not at the highest level. Glycine evidently had a sparing effect on formation of ammonia from other sources, but this effect was limited.

In some of the problems that have been solved by using N^{15}-labeled compounds, composition of the diet has not been a critical factor. For example, the classical demonstration that glycine and arginine are precursors of creatine (1) could probably be repeated under a wide variety of dietary conditions. On the other hand, quantitative studies on the utilization of N^{15} from individual amino acids and ammonia (2–5) led us to suppose that there might be a systematic relationship between total intake of a given amino acid and retention of N^{15} ingested in that form. Accordingly, we have studied the metabolism of a fixed amount of glycine-N^{15}, fed as a single dose, in each of 2 dogs, at 5 successively greater levels of total glycine intake. Incorporation of the heavy isotope into fibrinogen, rate and amount of urinary excretion, and its distribution between urinary ammonia, urea, and total nitrogen, were determined.

EXPERIMENTAL

Two normal adult mongrel bitches served as experimental animals. Their diet contained: (in per cent) casein,[3] 36.2; cracker meal of the type commercially prepared from wheat flour and water only, 36.2; corn oil,[4] 19.6; yeast,[5] 4.0; and Phillips-Hart (6) salt mixture, 4.0. Each dog also received 3 drops of a concentrate[6] of vitamins A and D daily. In preliminary studies, the amount of food required per day for maintenance of weight was found to be 150 g for dog no. 57, and 175 g for dog no. 79. Each animal was fed the required amount in a single meal daily at 9 AM. The nitrogen content of this basal diet was 5.85%.

Five 8-day experiments were performed on each dog. Intake of unlabeled glycine was uniform throughout the 8-day period. In successive experiments, the basal diet was supplemented with 1.2, 2.4, 4.8, 9.6, and 19.2 g of unlabeled glycine. Since the basal diet was estimated to contain 1.0 to 1.2 g of glycine, the glycine supplement varied from about 1 to 16 times the basal intake.

To the meal fed at 9 AM on the seventh day, 320 mg of N^{15}-labeled glycine was

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[2] Presented in part at the Annual Meeting of the American Society of Biological Chemists held in Atlantic City, April, 1963.
[3] National Casein Company, Chicago.
[4] Mazola, Corn Products Company, New York.
[5] Type 58, Atlantic Yeast Corporation, Brooklyn, New York.
[6] Oleum Percomorphum, Mead Johnson and Company, Evansville, Indiana.

added. Two lots of labeled glycine were used; one contained 21.65 mg and the other 19.22 mg of excess N^{15} in the amount fed. This difference did not affect the results, since retention, incorporation into fibrinogen, and excretion of N^{15} were all calculated as percentages of the amount ingested. After the 8-day period, glycine supplementation was stopped, and determinations of urinary nitrogen continued while the animal received the basal diet alone. Experiments were carried out at least 4 weeks apart, to allow nearly complete excretion of N^{15}. In the figures and tables which follow, free glycine ingested includes both labeled and unlabeled glycine, so that range of supplementation is from 1.52 to 19.52 g/day, or 13-fold.

Amounts of nitrogen excreted in urine as urea, ammonia, and total nitrogen, as well as plasma concentrations of fibrinogen, isolated as fibrin, and N^{15} enrichment of nitrogen from each of these sources, were determined 6, 12, 24, and 48 hours after ingestion of N^{15}. All experimental periods were terminated by catheterizing and washing the bladder. Analytical methods have been described previously (3).

RESULTS AND DISCUSSION

Utilization of ingested glycine nitrogen. The largest supplement of glycine fed represented an increase in nitrogen intake of about 40%. As shown in table 1, the dogs excreted an extra amount of urinary nitrogen approximately equal to the increased intake in all experiments. Significant gain in weight occurred during the 3 periods of highest glycine supplementation, indicating probable deposition of a major part of the carbon skeleton of the ingested glycine.

TABLE 1

Comparison of increases in nitrogen intake and urinary nitrogen output, at five levels of glycine supplementation

Increase in nitrogen intake (1)	Increase in urine nitrogen (2)	Difference (1) minus (2)	Wt gain during 8-day period
g/day	*g/day*	*g/day*	*kg*
0.28	0.26[1]	0.02[1]	0[1]
0.51	0.58	−0.07	0
0.96	1.00	−0.04	0.23
1.85	1.92	−0.07	0.28
3.64	3.37	0.27	0.34

[1] Numbers in the last 3 columns are averages for the 2 dogs.

Although the output of nitrogen in urine indicated that no net storage of this element occurred, N^{15} retention and incorporation into fibrinogen indicated extensive replacement of body nitrogen by ingested glycine nitrogen. As shown in figure 1, the percentage of ingested N^{15} which remained in the animals after 24 hours was inversely proportional to the logarithm of the dose of ingested glycine over the entire range of supplementation. This percentage was 52.5 at the lowest, and 34.5 at the highest level.[7]

The fact that the 24-hour retention of N^{15} from ingested glycine was depressed only one-third by a 13-fold increase in glycine intake may reflect the extent to which ingested glycine nitrogen replaced nitrogen in the various pools of the body. This appears reasonable, since the amount of extra nitrogen ingested could be accounted for as urinary nitrogen. Moreover, glycine is converted to serine and thence into cysteine, and its nitrogen is also transferred to other amino acids, so that many different pools are involved.

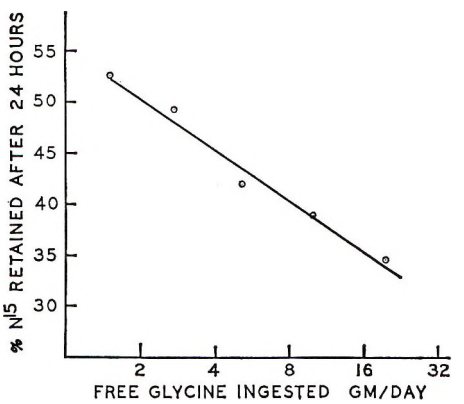

Fig. 1 Percentage of N^{15} from glycine retained after 24 hours decreased as glycine intake increased, and was related to the logarithm of glycine intake in a linear manner.

[7] During a period of several years, we carried out 11 experiments on dogs in which labeled glycine containing about 20 mg of excess N^{15} were added to the otherwise unsupplemented basal diet. Percentage of N^{15} retained after 24 hours averaged 58.4, with standard deviation ± 4.8. Thus the change from 52.5 to 34.5, besides being consistent, and an average for 2 animals, is 4 times the standard deviation observed for single experiments.

Incorporation of N^{15} into fibrinogen (isolated as fibrin) occurred quite rapidly, and followed a very uniform time course (fig. 2). In all experiments, excess of N^{15} reached a maximum 24 hours after ingestion of the isotope. The fraction of administered glycine nitrogen accounted for in total circulating fibrinogen at that time was calculated from the chemically determined concentration of fibrinogen and an estimate of total plasma volume.[8] Percentages so calculated, like those of N^{15} retained after 24 hours, were inversely related to the logarithm of the dose of glycine, as indicated in figure 3. Although percentage of ingested glycine nitrogen incorporated into fibrinogen decreased as glycine intake increased, the amounts incorporated increased with glycine intake in an almost linear manner (fig. 4) during the first 4 levels of supplementation, but not at the highest level. The amino acid composition of dog fibrinogen is not known, but at the upper levels of glycine feeding the ingested glycine must have provided a very substantial portion of the daily requirement, not only for glycine but also for serine needed for fibrinogen replacement. The level of plasma fibrinogen did not change during the course of an experiment.

Studies of Walter et al. (8), undertaken for precisely the same reason as ours, were carried out on rats, with intravenously injected glycine, and glycine-2-C^{14} tracer. We

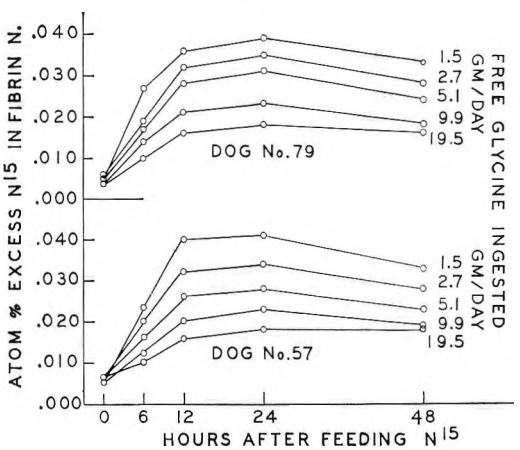

Fig. 2 Chart showing atom per cent of excess N^{15} found in fibrin nitrogen at various time intervals following ingestion of N^{15}-labeled glycine.

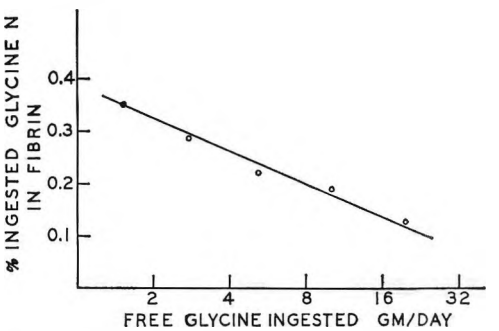

Fig. 3 Percentage of ingested glycine nitrogen incorporated into fibrinogen decreased as glycine intake increased, and was related to the logarithm of glycine intake in a linear manner.

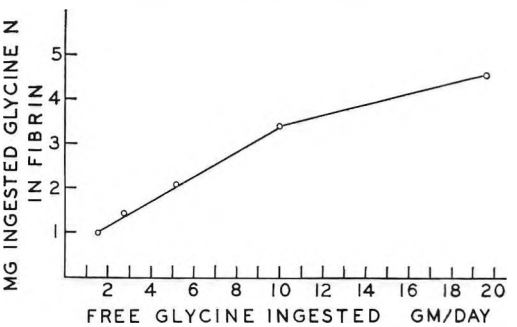

Fig. 4 Chart showing that the amount of glycine nitrogen incorporated into fibrin increased as glycine intake rose.

used dogs, which were fed a fixed amount of N^{15}-labeled glycine plus variable amounts of unlabeled glycine, added to a meal, and therefore absorbed gradually. Similarities between our results and those of the above investigators are therefore more unexpected than differences. In both studies, there is an inverse relationship between the dose of glycine and percentage of incorporation, with a change in the latter that is small in relation to change in the former. In the studies of Walter et al. (8) a 17,000-fold increase in dose of glycine reduced relative specific activity of various proteins or hemin by a factor of 2 to 4. In our experiments, the effect of glycine intake on percentage incorporated into fibrinogen was much larger, but still relatively small, and quite consistent. Thus the problem of differences in glycine intake, which motivated both studies, is not a serious one in either type of experiment.

[8] Plasma volume = 53.8 ml/kg body weight (7).

Each of the methods of labeling glycine has advantages and limitations. Strictly speaking, a study with N^{15}-labeled glycine is a study of metabolism of N^{15} from glycine, and one with glycine-2-C^{14} deals with metabolism of the second carbon of glycine. This makes little difference in instances where the whole glycine molecule is involved, that is, when glycine is converted to serine and the latter incorporated into protein, or when glycine itself is incorporated into protein, creatine, purines, and many other compounds. However, in the case of N^{15} labeling, the problem is complicated by transfer of nitrogen to many amino acids other than serine. The C^{14} from glycine, on the other hand, will appear in glucose, fat, and a whole series of intermediates involved in its oxidation to carbon dioxide.

The highest glycine dosage used by Walter et al. (8) was 250 mg in rats weighing 300 to 350 g, or about 0.77 g/kg. At this level, 62% of glycine-C^{14} was still in the body after 24 hours. Our largest dose of 19.5 g in dogs weighing 17 and 19 kg was of similar order, but N^{15} retained after 24 hours was only 34.5% of intake. That this figure is much smaller than the one for carbon, and that it varied systematically with glycine intake (fig. 1) suggests that removal and excretion of the nitrogen of glycine is a less involved process than metabolism of its carbon. Comparable experiments on this point in the same species seem indicated.

Urea formation. The most active period of catabolism, as judged by the amount of N^{15} appearing in the urine, was the second 6-hour period after ingestion of the meal. For this time period, the rate of urea formation from the glycine fed was calculated[9] and expressed as milligrams of urea nitrogen formed per kilogram of body weight per hour. Plotting these values against amounts of free glycine ingested yielded the linear relationship characteristic of a first-order reaction (fig. 5). The largest intake of glycine was still well below the maximal one that the dog is able to metabolize to urea, as determined by Handler et al. (9). In calculating rates of protein degradation and loss, Hoberman (10) assumed that deamination of amino acids in the whole animal follows first-order kinetics with respect to the size of the nitrogen pool. The present data appear to support this assumption, at least for the experimental conditions used. Similar observations on urea formation have been made by Duda and Handler (11), who injected increasing amounts of ammonium lactate into rats and measured urea formed after 20 minutes. In this case, also, urea formation was linear with respect to amounts of substrate injected, or like a first-order reaction.

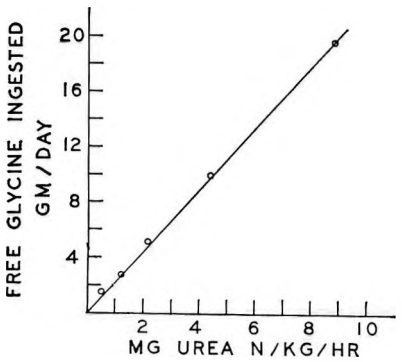

Fig. 5 A linear relationship, characteristic of a first-order reaction, was observed when urea formation during the second 6-hour period after the daily meal was calculated in milligrams per kilogram per hour and plotted against glycine intake.

Ammonia formation. Rates of excretion of urinary ammonia during the various experiments are shown in table 2. The rate was highest during the second 6-hour period after ingestion of the meal, and there was little or no correlation between the amount of glycine ingested and the rate of excretion of ammonia.

Percentages of urinary ammonia formed from free glycine nitrogen ingested were calculated[10] for the first and second 6-hour periods after feeding, and plotted against intake of free glycine (fig. 6). The relationship was linear, except at the highest level of supplementation. Apparently glycine had a sparing effect on formation of

[9] $\dfrac{\text{Urea } N^{15} \text{ output, in mg, for 6–12 hr period}}{N^{15} \text{ ingested, mg}} \times \dfrac{\text{Total free glycine N ingested, in mg}}{\text{wt of dog in kg}} \times 6$

[10] $\dfrac{\text{Ammonia } N^{15} \text{ output in mg}}{N^{15} \text{ ingested, in mg}} \times \text{Total free glycine N ingested in mg}$

$$\frac{\text{Urinary ammonia N in mg}}{\text{Total free glycine N ingested in mg}} \times 100$$

ammonia from other nitrogenous sources, but this effect was limited, so that the rapid initial increase in percentage of urinary ammonia coming from glycine did not continue beyond the fourth level of supplementation.

The limiting factor was probably not the capacity of the animals to form ammonia from glycine, since rates of ammonia excretion in the present study are much lower than those observed by Kamin and Handler (12) in fasting anesthetized dogs that received glycine intravenously. A possible explanation of our results is that the extent to which glycine can replace other sources of ammonia is more limited when it is ingested with a meal containing protein than when it is injected alone into a fasting animal. Supporting this interpretation is our observation that percentage of urinary ammonia formed from glycine nitrogen (fig. 6) was greatest during the first 6-hour period, whereas rate of ammonia excretion (table 2) was greatest during the next 6 hours.

TABLE 2

Rate of ammonia nitrogen production

Glycine added to diet	Dog no.	Hours after ingestion of diet		
		0–6	6–12	12–24
g/day		*mg/kg/hour*		
1.52	57	0.33	1.54	0.84
	79	0.41	1.63	1.69
2.72	57	0.44	1.42	0.79
	79	0.22	2.37	1.21
5.12	57	0.39	1.44	0.91
	79	0.57	1.81	1.25
9.92	57	0.37	1.35	0.80
	79	0.83	2.15	1.17
19.52	57	0.51	1.70	0.93
	79	0.73	1.87	1.42

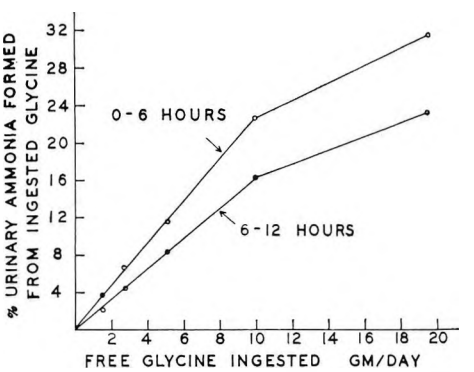

Fig. 6 Chart showing the relationship between percentage of urinary ammonia formed from glycine, and amount of free glycine ingested, during the first two 6-hour periods after the daily meal.

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Protection by Dietary Fat Afforded Serum γ_1-Globulin of X-Irradiated Rats[1]

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ABSTRACT A study was made to determine the influence of dietary fat or methyl linoleate on the response of the serum proteins of the rat to X-irradiation. Electrophoretic studies showed that X-irradiation caused a substantial decrease in the concentration of γ_1-globulin in the serum of rats fed a fat-deficient diet. This change did not occur in rats that had been fed the fat-free diet plus a daily supplement of methyl linoleate or a diet containing 15% cottonseed oil. The similar body weights of the linoleate-supplemented rats and the fat-deficient rats demonstrated that the protective effect of dietary fat was not due simply to a mechanical shielding of the blood-forming organs by a fatty layer. It is suggested that the protection against X-ray afforded by dietary linoleate or fat may be due to protection of the antibody-forming system.

Cheng et al. (1) have reported that fat in the diet affords protection against X-irradiation. Rats reared with a fat-free diet exhibited a higher mortality rate after exposure to X-ray than did rats reared with a diet containing 15% cottonseed oil. Subsequent experiments by Cheng et al. (2, 3) and Deuel et al. (4) confirmed this observation and demonstrated that the protective factor in cottonseed oil was probably an essential fatty acid, since administration of methyl linoleate to the fat-deficient rats reduced the mortality rate to that observed in the fat-fed rats.

These observations led us to initiate an investigation to determine whether protection from X-ray by a certain fatty acid could be correlated with any characteristics of blood serum proteins as determined by electrophoresis. Our results suggest that the presence of methyl linoleate in the diet of the rat protects the γ_1-globulin of the serum against the effect of X-irradiation.

METHODS

Weanling rats of the University of Southern California strain were fed a purified diet containing 0% fat but adequate in all other respects as demonstrated by Cheng et al. (1). Eight weeks after weaning they were equitably distributed into 6 groups — a, b, c, d, e and f — according to age, weight, sex and littermates. All groups continued to be fed the 0% fat diet until the body weights reached a plateau, after which the rats of groups d, e and f were given a supplement of methyl linoleate (100 mg/rat/day by stomach tube) for the duration of the experiment. Groups a, b and c received no supplement. From weaning throughout the experiment 3 additional groups of rats — g, h and i — were fed a diet suggested by Cheng et al. (1) containing 15% cottonseed oil.

Animals receiving all 3 diets were given X-irradiation: groups a, d and g received 600 r, groups b, e and h received 700 r, groups c, f and i received 800 r whole-body irradiation in a single dose. X-irradiation was performed 14 days following initiation of the methyl linoleate supplement in groups d, e and f, and approximately 14 days after the growth plateau had been reached in groups a, b and c. The groups fed 15% fat were irradiated at approximately the same age as the other groups. The number of rats studied with each diet and at each X-ray dosage is given in table 1. Several animals from each group were drawn to serve as controls. These were weighed and killed for serum samples before irradiation. At 4 days after irradiation approximately one half of each group were weighed and then killed for serum samples. At 7 days after irradiation the remainder were weighed and killed. (Preliminary experiments with rats fed com-

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TABLE 1

Grouping of rats[1] used in experiment

	Type of diet							
	0% Fat			0% Fat + linoleate			15% Fat	
	Group and no. of rats			Group and no. of rats			Group and no. of rats	
	Used		Died	Used		Died	Used	Died
Control	39			20			15	
600r, 4 days[1]	a 24		0	d 12		0	g 14	0
600r, 7 days	a 19		1	d 18		0	g 22	0
700r, 4 days	b 15		0	e 10		0	h 10	0
700r, 7 days	b 14		2	e 17		1	h 10	0
800r, 4 days	c 21		0	f 13		0	i 10	0
800r, 7 days	c 18		4	f 10		2	i 10	0
Totals	150		7	100		3	91	0

[1] Rats were killed 4 or 7 days after irradiation.

mercial laboratory chow[2] had shown that the effects of X-irradiation on blood serum proteins are as great after 7 days as after 14 days.) Blood serum of each group was studied by moving boundary electrophoresis, using a barbiturate buffer.[3] Serum from 4 or 5 rats was pooled for each electrophoretic run.

RESULTS

Figures 1, 2 and 3 show the differences in loss of weight and the changes in γ_1-globulin in the serum between rats fed 0% fat and those fed linoleate before and after they had been irradiated with 600 r, 700 r and 800 r. The various features of these figures are examined for significance by using the rank test of Wallis and Roberts.[4] They are discussed below.

At 600 r X-irradiation, there were no differences between the rats fed 0% fat and those fed linoleate, that proved to be significant in these tests. But as the radiation dosage was increased, significant differences appeared. At 4 days with 800 r, the rats fed 0% fat lost a significantly ($P = 0.007$) larger percentage of their original weight than the linoleate-fed rats. At 7 days with 700 r, the difference in percentage loss of weight was significant with $P < 0.002$. At 7 days and 800 r, the difference in percentage weight loss was significant at $P = 0.05$. The γ_1-globulin losses after X-irradiation were quite consistently more for the rats fed 0% fat than the linoleate-fed rats. Since in the measurements γ_1-globulin the serum of 4 or 5 rats was used to make one electrophoretic test, these statistical measurements were less numerous and less sensitive than those for weight comparisons. Nevertheless, a test grouping all γ_1- tests after radiation of 700 r and 800 r show the γ_1-globulin in the rats fed 0% fat was significantly less ($P = 0.03$) than for those of linoleate-fed rats.

The growth curves of the rats fed 15% cottonseed oil from weaning are not included for comparison, as these rats were considerably heavier than those in the other groups; hence, some of the difference might have been attributed to the shielding of the rat from X-ray by a fatty layer. The protective effect on the γ_1-globulin was greater with a 15% fat diet than with the linoleate diet. The linoleate diet was

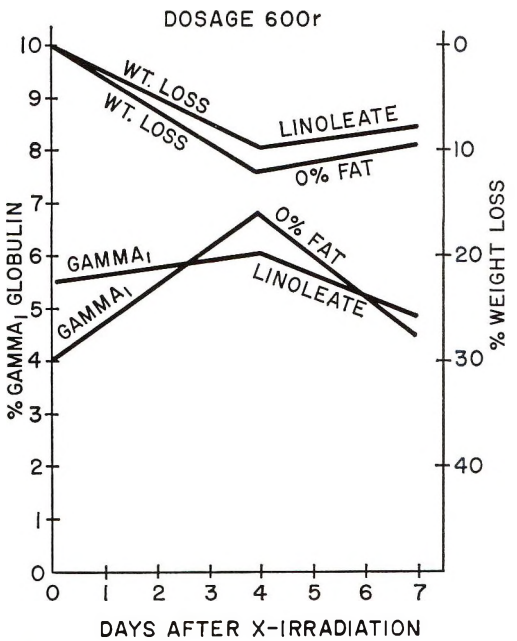

Fig. 1 Effect of irradiation on body weight and γ_1-globulin (600 r). At 4 and 7 days after rats were given a 600 r dosage of whole-body X-irradiation, there was no significant difference in either weight lost or in change in γ_1-globulin. The loss of weight from the zero to 4th day is significant.

[2] Purina Laboratory Chow, Ralston Purina Company, St. Louis.

[3] In grams, 24.75 barbital sodium; 27.82 NaCl; and 2.67 $CaCl_2$ made up to 6 liters; adjusted to pH 8.6.

[4] Wallis, W. A., and H. V. Roberts 1957 Statistics — a New Approach. The Free Press, Glencoe, Illinois, p. 594.

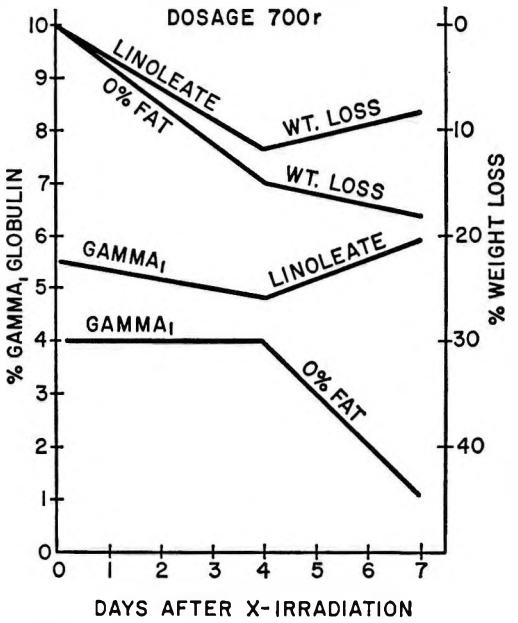

Fig. 2 Effect of irradiation on body weight and γ_1-globulin (700 r). With a 700 r full-body X-irradiation dosage, the rats fed 0% fat lost a significantly larger ($P < 0.002$) percentage of their original weight by the 7th day than the rats fed a linoleate diet.

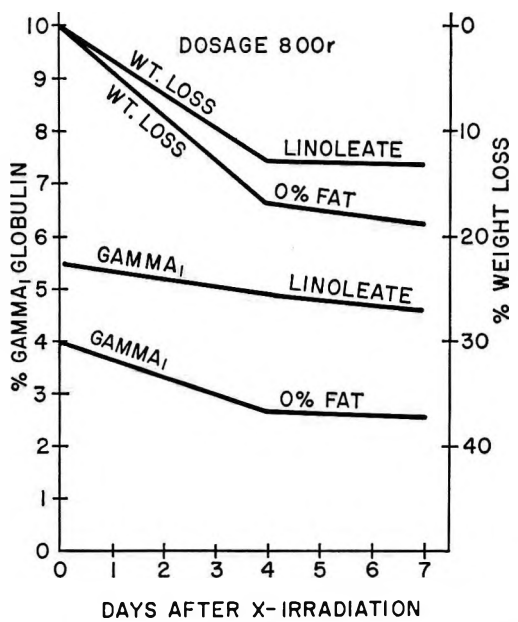

Fig. 3 Effect of irradiation on body weight and γ_1-globulin (800 r). With an 800 r full-body X-irradiation dosage, the rats fed 0% fat lost significantly more weight ($P = 0.05$ at 7 days) than the rats fed linoleate.

sufficient, however, to sustain the system producing γ_1-globulin.

All of the electrophoretic data are shown in table 2. Figure 4 shows the representative serum electrophoretic patterns for each diet group 7 days after irradiation with 800 r. Only one nonirradiated control is shown as the control patterns were similar for all diets. The chief differences in the patterns, aside from the decrease in albumin concentration after X-ray reported by Fischer et al. (5), Hohne et al. (6) and others, lie in the γ_1-globulin fraction. The pattern for the 0% fat group shows an almost complete disappearance of the γ_1-globulin after irradiation at 700 r and 800 r. The curve for X-rayed rats fed the linoleate supplement has a pattern similar to the control with slightly increased γ_1-globulin. The pattern for the rats fed 15% cottonseed oil differs from the 2 preceding ones: the γ_2-globulin fraction is almost completely absent, and the γ_1-globulin is maintained higher than in the controls.

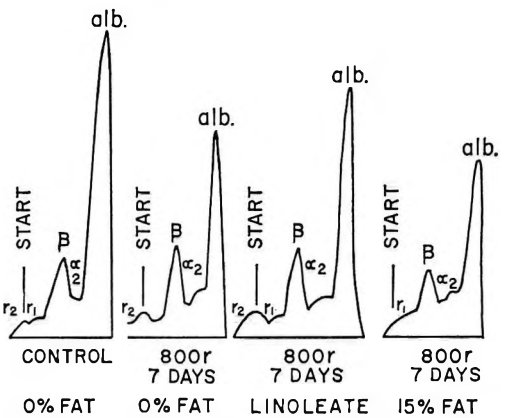

Fig. 4 Typical electrophoretic patterns. Graphs show the effect of X-irradiation on the serum electrophoretic patterns of rats fed diets with and without fat. The animals were killed 7 days following 800 r of irradiation.

In table 2 the 15% fat group shows an increase in γ_1-globulin at 600 r and 700 r and a return toward the normal proportion at 800 r. From these data it is concluded that the normal serum electrophoretic pattern was best maintained in the group supplemented with linoleate, and that stimulation of production of γ_1-globulin was greatest in the group receiving the 15% cottonseed oil diet.

TABLE 2

Changes in electrophoretic pattern of serum proteins of rats after whole body X-ray descending side

Group	No. of sets of rats[1,2]	Albumin + a_1-globulin	a_2-Globulin	β-Globulin	γ_1-Globulin	γ_2-Globulin
		%	%	%	%	%
0% Fat diet						
Control X-ray	9	66.4	8.1	13.1	4.0	9.0
a) 600r, 4 days[2]	5	56.0	11.3	14.6	6.8	11.2
600r, 7 days	5	56.1	9.8	17.0	4.5	12.6
	Mean	56.0	10.5	15.8	5.6	11.9
b) 700r, 4 days	3	63.5	9.7	16.3	4.0	6.5
700r, 7 days	3	53.0	13.4	21.5	1.2	10.9
c) 800r, 4 days	3	59.3	10.6	19.3	2.7	8.1
800r, 7 days	3	56.1	13.2	20.2	2.6	7.7
	Mean	56.5	11.7	19.3	2.6	8.3
Linoleate in diet						
Control X-ray	4	66.5	8.2	13.3	5.5	6.5
d) 600r, 4 days	3	59.7	9.6	15.8	6.1	8.8
600r, 7 days	3	60.7	9.7	15.1	4.8	9.6
	Mean	60.2	9.6	15.4	5.4	9.2
e) 700r, 4 days	2	63.3	9.3	13.5	4.8	9.2
700r, 7 days	3	55.0	10.4	17.7	5.9	10.9
f) 800r, 4 days	2	62.7	11.2	14.8	4.9	6.6
800r, 7 days	2	57.3	10.9	16.3	4.6	10.8
	Mean	59.6	10.4	15.6	5.0	9.4
15% Fat diet						
Control X-ray	4	65.6	8.4	12.8	6.5	6.7
g) 600r, 4 days	3	61.3	7.2	16.0	6.9	8.6
600r, 7 days	4	63.8	8.3	16.1	8.2	3.5
	Mean	62.5	7.7	16.0	7.5	6.0
h) 700r, 4 days	1	50.9	13.2	19.8	8.5	7.6
700r, 7 days	2	58.0	12.1	17.9	7.8	4.0
i) 800r, 4 days	2	61.7	9.0	16.3	8.0	5.3
800r, 7 days	2	57.5	11.0	20.3	5.4	5.6
	Mean	57.0	11.3	18.6	7.4	5.6

[1] Four or five rats in each set.
[2] Rats were killed 4 or 7 days after irradiation.

DISCUSSION

The results presented here, as well as the work of Cheng et al. (2, 3), demonstrate that the protective effect of dietary fat in X-irradiated rats is not due simply to a mechanical shielding of the blood-forming organs by a fatty layer. This is shown by the similar protection afforded by administration of methyl linoleate, in which case the body weights were greater than those of the fat-deficient rats.

In the present work, the protective effect of dietary fat or linoleate was evidenced by lesser loss of body weight and a more rapid recovery of body weight following X-irradiation and by less decrease in concentration of serum γ_1-globulin than that caused by X-ray in rats fed a fat-free diet.

It is commonly accepted that a large majority of antibodies are found in the γ_1-globulin fraction of the serum proteins. Raffel (7) has quoted literature in support of the view that there is a diminution of native defense after severe X-irradiation; the authors suggest that the prinicipal action of dietary fat or linoleate in X-rayed rats may be to protect the antibody-forming system.

ACKNOWLEDGMENTS

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Effects of Heat Treatment on the Metabolizable Energy Value of Soybeans and Extracted Soybean Flakes for the Hen[1]

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ABSTRACT The effects of graded levels of heat treatment of ground soybeans and extracted dehulled soybean flakes (autoclaving 10, 40, or 60 minutes at 107°C, or 120 minutes at 120°C) on their utilization by adult hens were studied by measurement of metabolizable energy and fat absorbability. In general, the effects observed were similar to those previously obtained in studies with young chicks. The metabolizable energy values of the unheated materials were markedly and significantly lower than those of the optimally heated samples. Prolonged heating reduced the energy value of extracted flakes, but had little or no deleterious effect on the energy value of ground soybeans. The metabolizable energy values of the optimally heated soybeans and extracted flakes for hens were in close agreement with those reported for chicks. Absorbability of dietary fat was impaired by unheated extracted flakes, and this effect was abolished by heat treatment. Absorbability of soybean oil in ground soybeans was also impaired to about the same extent, but was not consistently improved by heat treatment. The possible existence of a specific factor which interferes with fat utilization was discussed. Under the conditions of these experiments, egg production was reduced by feeding unheated soybeans or extracted flakes as the sole source of protein.

Fisher et al. (1) have reported that unheated extracted soybean flakes, when properly supplemented with vitamin B_{12} and methionine, can serve as the sole source of protein in the diet of the laying hen, supporting egg production equal to a commercially processed soybean meal. This observation is in contrast with the well-established growth retardation resulting from feeding raw soybean meal to chicks, and suggests that the chick and the hen may differ in their ability to utilize unheated soybean protein. Previous work from this laboratory (2) has shown that the heat treatment of soybeans and extracted soybean flakes necessary to achieve maximal growth when they are fed to chicks also is necessary for maximal metabolizable energy. The following studies were undertaken to determine the relationships between heat treatment and utilization of these materials by adult hens.

EXPERIMENTAL

Ground whole soybeans and extracted dehulled soybean flakes were given various heat treatments by autoclaving for 5, 10, 40 and 60 minutes at 107°C and for 120 minutes at 120°C. The composition of the soybeans and extracted dehulled soybean flakes, and the details of the treatments used were the same as described in our previous work with chicks (2).

The composition of the experimental diets is given in table 1. The ground whole soybeans and extracted dehulled soybean flakes served as the sole sources of protein in the 2 series of diets. In formulating the soybean flake diets, degummed soybean oil and cellulose were added in proportions to simulate the oil and hulls provided in the other diets by ground whole soybeans. The diets were formulated to supply 53 g of crude protein (N $\times$ 6.25) per 1000 kcal of metabolizable energy when utilization of diet energy was maximal, based on the energy values obtained in our previous studies with chicks. DL-Methionine was added to bring the

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TABLE 1
Composition of experimental diets

Component	Soybean flakes diet	Soybean diet
	%	%
Glucose[1]	38.52	38.52
Ground soybeans	—	41.36
Extracted dehulled flakes	31.93	—
Soybean oil (degummed)	8.11	—
Cellulose	1.32	—
Constant ingredients		
Methionine	0.10	
Corn oil	5.00	
Cellulose	5.00	
Dicalcium phosphate	4.00	
Limestone	3.00	
Salt	0.50	
Mineral mixture[2]	0.38	
Vitamin mixture[2]	1.12	
Antioxidant (BHT)[3]	0.02	
Chromium "bread"[4]	1.00	

[1] Cerelose, Corn Products Company, Argo, Illinois.
[2] Vitamin and mineral mixtures supply in milligrams or units per 100 grams diet: K_2HPO_4, 220; $MgSO_4$, 120; $FeSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 15; ZnO, 6; $CuSO_4 \cdot 5H_2O$, 1.5; NaI, 0.3; Na_2SeO_3, 0.02; thiamine, 1.0; riboflavin, 2.0; calcium pantothenate, 5; niacin, 15; folacin, 0.2; pyridoxine·HCl, 1.5; biotin, 0.05; vitamin B_{12}, 0.002; choline chloride, 84; vitamin A, 1000 USP units; vitamin D, 75 ICU; α-tocopheryl acetate, 11.0 IU.
[3] Butylated hydroxytoluene.
[4] Contains 30% Cr_2O_3.

total sulfur amino acid content of the diets to approximately 3.6% of the protein, and vitamin B_{12} was added at the level of 0.02 mg/kg.

Each diet was fed ad libitum for 14 days to 2 duplicate lots of 4 Single Comb White Leghorn hens maintained in individual wire-floor cages. There were 2 experimental periods. In the first, the 6 diets containing dehulled soybean flakes were fed. At the end of this period, diets containing the respective heat-treated ground whole soybeans were substituted for the soybean flake diets, and were fed to the same hens excepting in the case of the unheated diet. In the latter case, 2 new lots of 4 hens each were used because the raw soybean flake diet had caused one-half of the hens to cease egg production and begin molting.

During the second week of each experimental period, excreta were collected from each lot at 24-hour intervals on 4 successive days for the determination of metabolizable energy and fat absorbability. Chromic oxide was incorporated in each of the diets at a level of approximately 0.3% as an index substance to eliminate the need for quantitative collection of the excreta and quantitative measurement of diet intake. The methods of processing excreta, conducting chemical analyses for moisture, nitrogen, combustible energy, fecal fat and chromic oxide, and computing metabolizable energy and fat absorbability from these data, have been described previously (3–5). In calculating metabolizable energy values, the data were adjusted to a condition of nitrogen balance, based on nitrogen in the feed intake and the mixed excreta; the protein of the eggs produced did not enter into the calculations because it was considered to be retained nitrogen (synthesized protein) rather than excreted. The energy values obtained are designated as "nitrogen-corrected metabolizable energy" to distinguish them from values derived without this correction. For the purposes of the present work, this procedure has 2 advantages; it eliminates any effect of differences in egg production, and it makes possible the direct comparison of energy values for chicks and hens. All data are reported in terms of dry weight.

RESULTS

The effect of heat treatment on the metabolizable energy content of dehulled soybean flakes is shown by the data summarized in table 2. The metabolizable energy of the soybean flakes was calculated by subtracting from the metabolizable energy of each complete diet the value of glucose (3.64 kcal/g; reference 6), and the apparent value of the soybean oil and corn oil. The latter was computed from the apparent fat absorbability (table 3) and its gross energy value, 9.39 kcal/g.

The data show markedly lower utilization of raw soybean flakes as compared with flakes autoclaved 5 to 60 minutes. Overheating (120 minutes, 120°C) reduced utilization as measured by metabolizable energy. Analysis of variance (7) and application of Duncan's multiple range test (8) showed that the raw and overheated samples were significantly lower than the others ($P < 0.05$). No significant differences were found among the samples autoclaved 5 to 60 minutes at 107°C. The value observed for the raw

TABLE 2

Effect of heat treatment on metabolizable energy of extracted soybean flakes and ground soybeans

Heat treatment	Metabolizable energy			
	Soybean flakes		Soybeans	
	kcal/g dry matter		*kcal/g dry matter*	
None	1.96		2.79	
	1.92	1.94[1]	2.90	2.84
5 min, 107°C	2.61		3.42	
	2.78	2.70	3.53	3.48
10 min, 107°C	2.77		3.21	
	2.74	2.76	3.48	3.36
40 min, 107°C	2.82		3.47	
	2.89	2.86	3.34	3.40
60 min, 107°C	2.88		3.46	
	2.66	2.77	3.49	3.48
120 min, 120°C	2.34		3.73	
	2.50	2.42	3.68	3.70

[1] Underlined values are averages of duplicate lots; individual replicate values are in respective left columns.

flakes (1.94 kcal/g) and the mean value for the optimally heated samples (2.77 kcal/g) are in general agreement with the respective values obtained in our previous studies with chicks. No differences were apparent among the various heated meals, and fat utilization was uniformly high in all these diets. Since a low-fat diet was not included in this experiment, no direct estimation of endogenous fat excretion is available to correct these values to true absorbability. However, from previous work it is estimated that such a correction would increase the apparent absorbability about 2%, which would bring it to approximately 98% in the diets with heated flakes. This agrees well with other determinations which we have made of the absorbability of soybean oil by chickens.

Also summarized in table 2 are data showing the effect of heat treatment on the metabolizable energy content of ground soybeans. These values were estimated by subtracting from the metabolizable energy content of each complete diet the values for glucose and corn oil (8.8 kcal/g; reference 5). Samples autoclaved for 5, 10, 40 and 60 minutes were significantly higher in metabolizable energy value than the raw beans, again showing the beneficial effect of heat treatment. The data also showed that autoclaving soybeans for 2 hours at 120°C produced significantly higher metabolizable energy than the milder heat treatments. This unexpected increase was due entirely to increased fat absorbability, as shown by the data in table 3 and the computations summarized in table 4. Based on the observed total metabolizable energy of the soybean samples and the absorbability of the oil, the nonfat constituents showed essentially constant value in all of the heated samples, averaging 2.48 kcal/g dry matter (table 4). This estimated value is less than that observed for the soybean flakes, due to the presence of indigestible hull in the beans. It is comparable to the average value of 2.50 kcal/g previously observed with chicks for commercially processed 44% protein soybean meal, which has essentially the composition of extracted soybeans (9).

TABLE 3

Apparent absorbability of dietary fat

Heat treatment of soybean flakes or soybeans	Apparent absorbability[1]			
	Soyflake diets		Soybean diets	
	%	%	%	%
None	77		77	
	84	81[2]	80	78
5 min, 107°C	94		79	
	95	94	79	79
10 min, 107°C	98		71	
	96	97	77	74
40 min, 107°C	96		84	
	97	96	74	79
60 min, 107°C	98		85	
	94	96	84	84
120 min, 120°C	96		94	
	96	96	90	92

[1] Uncorrected for endogenous fat. From previous studies, it is estimated that such correction would increase apparent absorbability by 2%.

[2] Underlined values are averages of duplicate lots; individual replicate values are in respective left columns.

TABLE 4

Estimation of the utilization of the nonfat constituents of soybeans

Heat treatment of soybeans	Apparent absorbability of oil	ME[1] of oil per g beans	Observed ME of beans	ME of non fat portion	
				per g beans	per g
	%	*kcal*	*kcal/g*	*kcal*	*kcal*
None	78	1.42	2.84	1.42	1.76
5 min, 107°C	79	1.44	3.48	2.04	2.54
10 min, 107°C	74	1.35	3.36	2.01	2.50
40 min, 107°C	79	1.44	3.40	1.96	2.44
60 min, 107°C	84	1.53	3.48	1.95	2.42
120 min, 120°C	92	1.68	3.70	2.02	2.51

[1] ME indicates metabolizable energy computed as 0.195 g × 9.34 kcal/g × absorbability.

The relatively low absorbability of soybean oil in ground heated soybeans, compared with soybean oil added as such to the diet, in this experiment confirms previous observations with chicks (2, 10). Evidently, the physical state of the oil in the ground soybean is such that its utilization is impaired. The marked increase in fat utilization produced by prolonged heating suggests degradation of bean structure and the release of the oil. It was unexpected that the prolonged heating did not reduce utilization of the nonfat portion of the soybean, in contrast with the effect of this treatment on extracted flakes. This apparent greater resistance of soybeans to overheating (due perhaps to a protective effect of fat) was also noted in our studies with chicks.

A further experiment was conducted using the same procedures to confirm the effect of prolonged heating on the metabolizable energy of soybeans and extracted flakes, and to compare laboratory-prepared meal with a commercially processed 50% protein dehulled soybean meal. The data from this study are presented in table 5. They show that prolonged autoclaving of ground soybeans (2 hours, at 120°C) did not significantly reduce metabolizable energy below that of soybeans heated 40 minutes at 107°C. In this experiment, fat absorbability was similar for the 2 samples (81 and 79%, respectively). No explanation is evident for the discrepancy between the 2 experiments in the effect of prolonged heating on fat utilization; however, they agree in showing no reduction in the utilization of the nonfat constituents by prolonged heating of ground soybeans. The data in table 5 also show

TABLE 5

Comparison of laboratory-prepared and commercial soybean meal, and the effects of prolonged heating of flakes and beans

Heat treatment	Metabolizable energy			
	Soybean flakes		Soybeans	
	kcal/g		*kcal/g*	
Commercial soybean meal[1]	2.70			
	2.85	2.78[2]		
40 min, 107°C	2.80		3.30	
	2.79	2.80	3.35	3.32
2 hr, 120°C	2.67		3.30	
	2.71	2.69	3.27	3.28

[1] Dehulled, 50% protein.
[2] Underlined values are averages of duplicate lots; individual replicate values are in respective left columns.

only a moderate reduction in metabolizable energy value of extracted soybean flakes from prolonged heating. The reduction was less than observed in the previous experiment (table 2). The laboratory-processed soybean flakes sample (40 minutes, at 107°C) was similar to the commercially processed soybean meal in metabolizable energy. The value obtained here for the commercial meal was essentially identical with the mean value obtained with chicks for a large number of samples previously studied in our laboratory (9).

Presented in table 6 are data showing egg production for the 2 experimental periods, and for the preceding 7 weeks when the hens were fed a stock diet. Egg production was abruptly reduced from the pre-experimental level when the diets con-

TABLE 6

Egg production of hens fed experimental diets containing raw and heat-treated soybean flakes and soybeans

Heat treatment	Egg production[1]		
	Pre-experimental	Soybean flake diets	Soybean diets
	%	%	%
None	74	29	—
None	76	—	46
5 min, 107°C	72	64	64
10 min, 107°C	72	58	57
40 min, 107°C	66	56	50
60 min, 107°C	78	64	58
120 min, 120°C	70	60	45

[1] Egg production computed as eggs per 100 hen-days. Pre-experimental period 7 weeks; each experimental diet fed for 2 weeks.

taining either unheated soybeans or unheated flakes were fed. This was due in each case to cessation of production in 4 of the 8 hens about one week after commencing to feed the experimental diet. Analysis of variance and application of the Duncan multiple range test (7, 8) showed that the depressing effect of raw soybean flakes was significant ($P < .05$), but that there were no significant differences among the other treatments in the series of heated flakes. Similar analysis of the data obtained with unextracted soybeans showed that egg production supported by raw beans was not significantly lower than with heated beans at the 5% probability level. However, the use of a different group of hens for the raw soybean diet confounded the experimental design and made the direct comparison of production rate between this treatment and the others questionable. The abrupt decline from the pre-experimental level suggests that raw soybeans have a real effect on egg production under these conditions, similar to the effect of raw extracted flakes. Although the experimental period was too short to differentiate clearly among the heated meals, they did not appear to show any marked effects on egg production rate, with the possible exception of the soybean sample subjected to prolonged heating. The generally lower production rate during the experimental periods, as compared with the pre-experimental period, was due mainly to the gradual seasonal decline characteristic of chickens.

DISCUSSION

In general, the results of these investigations with adult hens parallel those we obtained in earlier studies with young chicks. Autoclaving markedly improved utilization of both soybeans and extracted flakes; the unheated materials interfered with fat utilization; and the utilization of soybean oil from ground soybeans was markedly poorer than if it were added to the diet as such.

The results reported by Fisher and coworkers (1) and the earlier work of others which they reviewed, indicated that the productive performance of laying hens fed raw soybean meal as the sole source of protein was not impaired. Under their conditions, there was no effect on hens analogous to the growth-retarding effect of raw soybean products on chicks. In our experiments, raw meal and beans caused a marked reduction in egg production, due mainly to cessation of production in one-half of the hens. This suggests 3 possible explanations. First, it is possible that our diets were more limiting in protein or methionine, and that the lower utilization of the unheated products produced a sudden protein deficiency. In the experiments of Fisher and co-workers there was an indication of reduced egg production when raw meal was fed in a low protein diet (12%). Also, the best relative performance of hens fed raw soybean meal in their studies occurred when the diet contained 0.3% added methionine. Second, it is possible that the materials used in our studies contained a higher concentration of inhibitory substances. Finally, it may be that the relatively high level of fat in our diets was a determining factor, since the unheated products interfered with fat utilization as well as with utilization of nonfat constituents. It is also possible that the relatively short duration of our experiments may have magnified the effect on egg production since the hens did not have time to adapt to the raw products; however, the precipitation of feather molting in one-half of the hens indicates that adaptation and recovery probably would have required a relatively long time.

Part of the poor nutritional value of the unheated materials was due to impaired fat utilization, which was particularly evi-

dent in the comparison of raw and heated extracted flakes. Utilization of fat from ground soybeans was of the order of 80% absorbability, which was similar to that in the presence of raw flakes; however, heat treatment of ground soybeans did not consistently improve fat utilization. Other studies in this laboratory (10) have shown that flaking soybeans prior to heat treatment markedly improves fat utilization by the chick, suggesting a physical barrier to fat digestion which is ruptured by flaking but not by grinding. Our present work suggests the further possibility that there may be interference with fat utilization by a specific substance (or substances), other than the growth inhibitor, which is more heat-labile in flaked soybeans than in ground soybeans. First, the absorbability of fat from ground heated soybeans is similar to that obtained in the presence of unheated flakes. Second, the improvement in fat utilization by prolonged autoclaving in one experiment (table 3) may indicate destruction of the interfering substance by severe treatment; even this treatment was not consistently effective.

Other evidence for a difference in heat sensitivity between soybeans and extracted flakes is also available. Prolonged heating of ground soybeans did not impair utilization of the nonfat constituents, whereas the same treatment applied to extracted flakes produced a marked reduction in metabolizable energy. This suggests that the presence of soybean oil or some other factor associated with the structure of the "intact" soybean exerts a protective effect against overheating. In our earlier studies with chicks, overheating under these same conditions reduced the utilization of both extracted flakes and ground soybeans; however, the effect was less with the ground soybeans, which may have been due to the protective effect suggested above.

Quantitative comparisons between young chicks and adult hens show identical values for commercially produced dehulled soybean meal (2.78 kcal/g). Hens show the same value for laboratory-autoclaved extracted soybean flakes (2.80 kcal/g) but the value of this material for chicks is substantially lower (2.58 kcal/g). This may reflect a fundamental difference between commercial processing and laboratory autoclaving, as we have suggested previously. Hens and chicks show identical values for ground heated soybeans (mean values of 3.41 kcal/g). In general, it seems valid to conclude that the values of raw and heated soybean products are quite similar for young chicks and adult hens, but that the processing conditions required for optimal utilization by hens are somewhat less narrowly confined.

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Thiamine Deficiency in Rabbits[1]

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ABSTRACT Limited studies by other investigators suggested that thiamine is not a dietary essential for the rabbit. To determine whether this is true, rabbits were fed a purified diet in which the thiamine level was varied and the thiamine antagonist, neopyrithiamine, was added. In the absence of the antagonist, mild ataxia of the hindquarters occurred in about one-half of the animals that received the thiamine-free diet. All rabbits fed the thiamine-free diet showed a decreased urinary thiamine excretion; the level of thiamine in the livers and brains was reduced. However, the rabbits fed this diet gained as much weight as those fed the same diet supplemented with the vitamin. When weanling rabbits were fed a diet containing neopyrithiamine there was no reduction in rate of body weight gain prior to the appearance of symptoms. The rabbits developed neurologic symptoms between the 18th and 57th day of the experiment. They showed severe ataxia, flaccid paralysis, coma and death within 3 days of onset unless treated. These symptoms were reversed by the administration of thiamine. Neopyrithiamine increased urinary thiamine excretion 10- to 20-fold over that of the controls; it produced a very significant reduction in the level of brain thiamine but had no effect on hepatic, cecal or fecal thiamine levels. The appearance of neurologic symptoms in rabbits fed a thiamine-free diet for prolonged periods and the effects produced by neopyrithiamine suggest that dietary thiamine is required by the rabbit.

There are only 2 reports of attempts to produce a thiamine deficiency in rabbits and both suggested that thiamine is not a dietary essential for this species. About 25 years ago Passmore (1) fed a purified-type diet supplemented with autoclaved yeast to 3 adult rabbits for 40 days without producing signs of thiamine deficiency. Baglioni (2) fed 2 rabbits autoclaved feedstuffs for 165 and 245 days at which time inanition developed. The inanition probably was not due to a thiamine deficiency since urine collected from these animals prevented the appearance of beri-beri in pigeons fed polished rice. The limitations of the earlier work led us to investigate the rabbits' requirement for thiamine more extensively.

At the start of this work there was no satisfactory purified diet which, when fed to rabbits, produced maximal growth. Although a soybean meal ration produced good growth in rabbits (3), this could not be used since soybean meal contains an appreciable amount of thiamine. It was observed that the purified guinea pig ration developed by Reid and Briggs (4) produced growth in rabbits which was only slightly less than that of the soybean meal ration. This diet could be prepared thiamine-free and for that reason was used in all experiments.

A preliminary attempt was made to produce a deficiency by feeding 7 weanling rabbits the preceding diet free of thiamine. These animals gained weight (21 g/day) as rapidly as 5 controls (25 g/day) fed the same diet supplemented with 1.6 mg of thiamine/100 g. Three of the 7 animals fed the thiamine-free diet developed mild ataxia; one animal on the 66th day, another on the 113th and the third on the 146th day.

EXPERIMENTAL

New Zealand white male rabbits, 3 to 4 weeks of age, weighing between 300 and

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400 g, were housed in a room maintained at 24°C. The animals were kept in individual metal cages equipped with 1.25-cm mesh screen floors. Urine and feces were collected in the same cage after the pan for sawdust was replaced by a collecting pan, from which the urine was drained by tube to a bottle containing 5 ml of glacial acetic acid and 5 ml of toluene. The 24-hour urine samples were stored in a refrigerator until analyzed. Fecal pellets were collected on 0.3-cm mesh screen placed under the 1.25-cm mesh screen and above the urine-collecting pan. The 24-hour fecal samples were kept frozen until analyzed. The rabbits were provided with food and water ad libitum. Urine and feces were collected throughout the 78 days of the experiment.

The basal ration[6] was that devised by Reid and Briggs (4) for guinea pigs, except that thiamine was omitted. Analysis of this diet for thiamine was negative; based on the limits of the method, it may be stated that the diet contained no more than 2 μg of thiamine/100 g. A total of 20 weanling rabbits was used. Of these, 7 were fed the basal thiamine-free diet; another 7 were fed the thiamine-free diet, but with neopyrithiamine added at 0.5 mg/100 g. Six other rabbits were fed the basal diet with thiamine hydrochloride added at a level of 0.1 mg/100 g. The diet of one-half of these rabbits also was supplemented with neopyrithiamine at the level of 0.5 mg/100 g.

The animals continued to be fed their respective diets for 78 days. At autopsy, the brains and livers were removed, washed in saline and dried with a paper towel. A sample of cecal contents was taken. All samples were frozen until thiamine analyses were performed. Thiamine was determined by the thiochrome method as described by Mickelsen and Yamamoto (5). It was established in preliminary studies that the antagonist, neopyrithiamine, did not interfere with the analyses, even at levels of 10 times greater than thiamine.

RESULTS

The data in figure 1 show that the omission of thiamine from the ration did not influence the rate of weight gain. Similarly the addition of neopyrithiamine to the ration did not influence the rate of gain of the rabbits, at least prior to the appearance of gross symptoms. Animals fed the neopyrithiamine rations lost weight only during the terminal 3 days preceding death.

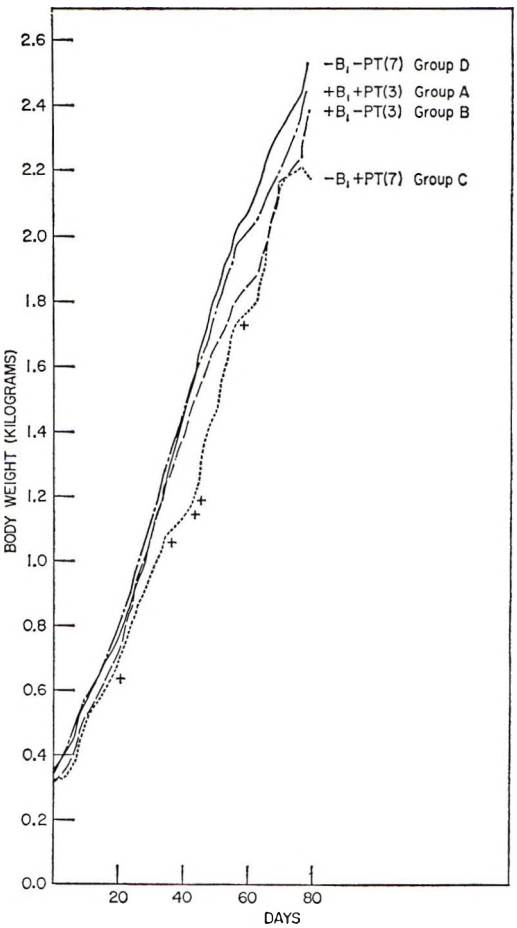

Fig. 1 Mean weight of rabbits fed diets with and without thiamine (Vitamin B_1 and neopyrithiamine (PT); ( ) number of animals started on experiment; + represents death of one animal.

[6] This ration contains: (in per cent) vitamin-free casein, 30; sucrose, 9.3; corn oil, 7.3; potassium acetate, 2.5; magnesium oxide, 0.5; salts, 6; powdered cellulose (Cellophane Spangles, Rayon Processing Co., Pawtucket, R. I.), 15; cornstarch, 20; glucose monohydrate (Cerelose, Corn Products Co., Argo, Ill.), 9.4. Vitamins were added to the glucose monohydrate to give the following levels/100 g of diet: (in milligrams) vitamin D_3, 0.004; vitamin B_{12}, 0.004; thiamine, 1.6; riboflavin, 1.6; pyridoxine, 1.6; vitamin A acetate, 0.6; α-tocopheryl acetate, 2.0; 2-methyl-1,4-naphthoquinone, 0.2; Ca pantothenate, 4.0; folic acid, 1.0; biotin, 0.06; niacin, 20.0; and (in grams) choline chloride, 0.2; inositol, 0.2; and ascorbic acid, 0.2.

None of the rabbits fed the basal thiamine-free ration or the thiamine-supplemented rations, showed any neurological symptoms during the 78 days of the study. However, 6 of the 7 rabbits fed this thiamine-free diet, but with antagonist added, developed gross and spectacular symptoms after 18 to 57 days (table 1). The symptoms consisted of ataxia, flaccid paralysis, convulsions, coma and death. The rabbits lay on their sides, unable to rise; they vigorously thrashed their feet, and showed marked head retraction; their sclera and conjunctiva were inflamed. Death usually intervened no longer than 3 days after the first onset of symptoms.

Two rabbits were given thiamine intraperitoneally when symptoms appeared. One of these (no. 82) was comatose when the first injection was given. Within 30 minutes it was conscious and attempted to stand. A total of 3 mg of thiamine was given within 24 hours, at which time the rabbit was normal except that it held its head slightly to one side; it redeveloped typical symptoms and died 23 days later. On autopsy there was evidence of brain hemorrhage. The other rabbit (no. 80) was given 1 mg of thiamine intraperitoneally when ataxia was well established. Four hours later all signs of the deficiency had disappeared. Of the 7 animals in this group, only one had not developed typical thiamine deficiency symptoms by the end of the experimental period, although a weight loss of 250 g in the final 2 days suggested that a typical onset was about to occur. All adverse gross effects of the antagonist were completely prevented when the diet was supplemented with 1 μg of thiamine/g.

Urinary thiamine excretion was increased by neopyrithiamine when it was added to either the basal or thiamine-supplemented rations (table 2). The lack of significance for the urinary thiamine ex-

TABLE 1

Appearance of deficiency symptoms in neopyrithiamine-fed rabbits receiving a thiamine-free diet

Animal no.	Appearance of symptoms	Symptoms			Death
		Ataxia	Paralysis	Coma	
	days				*days*
77	33	+	+	+	34
78	—	—	—	—	78 killed
79	18	+	+	+	19
80	28	+	+[1]	—	78 killed
81	38	+	+	+	42
82	21	+	+	+[2]	44
83	57	+	+	+	58

[1] Treated with 1 mg of thiamine, intraperitoneal; full recovery.
[2] Treated with 3 mg of thiamine, intraperitoneal; full recovery. Symptoms reappeared on the 44th day.

TABLE 2

Thiamine content of excreta and tissues of rabbits after 78 days on experiment

No. of rabbits	Diet supplement		Thiamine in				
	Thiamine HCl	Neopyrithiamine	Urine[1]	Feces[2]	Cecal content	Brain	Liver
	μg/g	*μg/g*	*μg/24 hours*	*μg/g dry wt*	*μg/g dry wt*	*μg/g dry wt*	*μg/g dry wt*
3	1	5	21.0 ± 4.9[3]	1.8 ± 0.69[3]	5.2 ± 0.76[3]	3.8 ± 0.41[3]	5.4 ± 1.1[3]
3	1	0	0.9 ± 0.18	2.3 ± 0.73	2.1 ± 0.50	7.9 ± 0.24	7.0 ± 0.42
7	0	5	7.9 ± 1.1	0.8 ± 0.10	3.3 ± 0.48	2.2 ± 0.15	4.6 ± 0.59
7	0	0	0.6 ± 0.09	1.1 ± 0.10	3.4 ± 0.97	6.7 ± 0.31	4.6 ± 0.53

[1] Five to 8 urine collections for each animal.
[2] Three fecal collections were made for each animal.
[3] Mean ± SE.

cretion of the rabbits fed the basal and supplemented rations (without antagonist) is probably due to the low level of dietary thiamine supplement used in this experiment. (In the preliminary studies mentioned in the Introduction, there was a significantly greater thiamine excretion by the rabbits receiving the thiamine-added ration; however, the thiamine supplement was 16 times greater than that used in the experiment herein reported.)

Fecal thiamine levels were not affected by the antagonist (table 2). On the other hand, dietary thiamine doubled the fecal thiamine excretion although this failed to be of any statistical significance.

Neopyrithiamine produced a highly significant decrease in the thiamine level of the brains, whether or not the rabbits had received thiamine (table 2). The rabbits fed the basal thiamine-free ration had lower brain thiamine levels than did those receiving the thiamine-supplemented ration, whether the antagonist was present or not.

In contrast with brain, the liver thiamine level was not altered by the antagonist. However, rabbits fed the thiamine-free ration had lower liver thiamine levels than those fed the thiamine-supplemented diet.

DISCUSSION

In most species of animals, a deficiency of thiamine is associated with anorexia and marked weight loss. Under our experimental conditions this did not occur in the rabbit. Weanling rabbits fed a simple thiamine-free diet did not show change in the rate of weight gain. After receiving the thiamine-free diet for extended periods (more than 66 days) about one-half of the animals showed a mild ataxia. Rabbits fed this diet had lower brain and liver thiamine levels than those of controls; however, fecal, cecal and urinary excretion of thiamine were the same.

The rabbit regularly secures some thiamine by ingestion of its soft feces, and this can be approximated. The so-called "soft" feces have a composition very similar to that of the cecal contents (6–8). Our results indicate that when rabbits are fed a thiamine-free ration, the cecal contents had 3 μg of thiamine/g of dry matter. Rabbits excrete about 15 g of soft feces daily, all of which is consumed. Since one-half of the soft feces is solid, approximately 20 μg of thiamine/day are available to the rabbit from this source. Undoubtedly, this source of endogenous thiamine can modify the interpretation of the results reported on these animals. No attempt was made to prevent coprophagy in this experiment. If the rabbit requires any thiamine for growth, it is an amount considerably less than that needed by the rat, which has been shown to be about 1 μg/g of diet (9). Essentially normal growth is observed in the rabbit (fig. 1) when the only source of this vitamin is the 20 μg/day provided by the soft feces. Since the food intake is about 65 g/day, if thiamine is needed for growth, then this need is satisfied by no more than 0.3 μg thiamine/g of diet.

There is no evidence in any of our data that dietary thiamine has any relation to growth of rabbits. This is emphasized by the results which show that the antagonist, neopyrithiamine, produces marked neurological symptoms and death, but does not have any effect on the growth rate. Since thiamine administration corretced the neurological symptoms and prevented death, it is obvious that a true thiamine deficiency was produced even though growth was normal.

The level of thiamine in the brain was reduced markedly by neopyrithiamine. This was not, however, to the levels proposed by DeCaro et al. (10, 11) as essential for the development of neurological symptoms in rats and mice. This is additional evidence that the rabbit differs from the rat in its metabolism of thiamine.

The antagonist produced about a 15-fold increase in urinary thiamine excretion of the rabbits; this agrees with the results reported for the rat by DeCaro et al. (10, 12). The levels of thiamine in the brain and liver of rabbits fed the deficient diet are in the lower range of those values reported for control rats (13). The lack of any effect of neopyrithiamine on the liver thiamine level is in agreement with that observed in rats and mice (11, 12, 14). In the latter animals, the liver thiamine levels were maintained even when the animals died of neopyrithiamine toxicity.

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Adaptation of Rats to Diets Containing Ethionine or Excess Leucine[1]

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ABSTRACT Rats fed a diet containing a sublethal level of ethionine lose weight initially, then adapt to the diet after 6 or 7 days and grow at a rate comparable to that of the control group for at least 3 weeks. Rats fed a high leucine diet also adapt to the diet after 6 or 7 days, but the rate of growth after adaptation is below that of the ethionine-fed group, although the initial weight loss is less. The growth-retarding effects of ethionine and excess leucine were additive. Rats that had become adapted to the high leucine diet lost their ability to grow rapidly with this diet when they were fed the control diet for only one day. Rats that had become adapted to the diet containing ethionine retained their ability to grow rapidly with this diet when they were fed the control diet for as long as 3 days, but lost it when they were fed the control diet for 5 days.

In a previous study of leucine-isoleucine and valine antagonism the growth of rats fed a high leucine diet was severely retarded for a few days, but after 5 or 6 days, if the level of leucine was not too high, the animals adapted to the diet and grew relatively well (1). Subsequently, in experiments to determine whether the growth-retarding effects of ethionine and excess leucine were additive, we observed that rats also adapted within a few days to diets containing moderate amounts of ethionine and thereafter grew quite well. Experiments illustrating this and pointing up some differences between the response of rats to ethionine and to a high intake of leucine are described in this paper. A preliminary account of this work has been presented.[4]

EXPERIMENTAL

Male Holtzman rats weighing between 80 and 100 g were used throughout. They were housed in individual, suspended cages with screen bottoms and fed ad libitum. Each group consisted of 6 rats and the average initial weights of the groups in each experiment differed by less than one gram. Fresh food was provided every day and body weights of the animals were recorded at a regular time daily. The basal diet used was similar to that described earlier (1). It contained: (in per cent) casein, 9; salt mixture, 5; corn oil and fat-soluble vitamins, 5; water-soluble vitamin supplement, 0.25; choline chloride, 0.15; and sucrose as the carbohydrate to make 100. Amino acid supplements were added at the expense of sucrose as indicated in the results.

RESULTS

The changes in body weight of young rats fed the basal diet to which 0.25% (group 1), 0.5% (group 2) or 1% (group 3) of DL-ethionine was added are shown in figure 1. Rats fed the diets containing 0.25% or 0.5 % of DL-ethionine lost weight during the first few days of the experiment but after 6 to 7 days resumed a rate of growth almost identical with that of the control group (not shown in this figure). The group fed the diet containing 1% of DL-ethionine did not gain after the first 7 days of severe weight loss but did maintain their weight subsequently. Two of the 6 rats in this group died on the 9th and the 15th days of the experiment.

In another experiment 2 groups of rats were fed the basal diet to which 0.3% of DL-methionine was added and 1% or 2%

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[2] Present address: The Institute for Cancer Research, Philadelphia, Pennsylvania.
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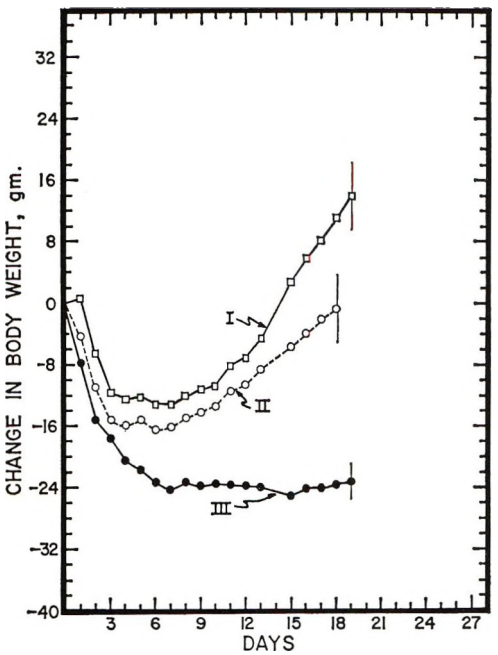

Fig. 1 Effect of various dietary levels of ethionine on the growth of young rats fed a diet containing 9% of casein. Group I, 0.25% DL-ethionine; group II, 0.5% DL-ethionine; group III, 1.0% DL-ethionine.

of DL-ethionine. The group receiving 1% of ethionine lost about 17 g during the first 6 days, then gained at nearly the same rate as the control group until the 23rd day when the experiment was ended. The group receiving 2% of ethionine lost about 32 g during the first 8 days, but maintained the lower weight thereafter until the end of the experiment with 2 of 6 rats having died during this period.

Growth curves for rats fed the basal diet supplemented with 0.3% of DL-methionine (group 1) and also with 5% of L-leucine (group 2), 1% of DL-ethionine (group 3) or both L-leucine and DL-ethionine (group 4) are shown in figure 2. Group 3, receiving ethionine, originally lost about 3 times as much weight as group 2, receiving leucine; both groups started to gain weight after 6 days, but the rate of gain of the group receiving ethionine was greater than that of the group receiving leucine. Group 4, receiving both ethionine and leucine, lost much more weight than either of the other groups. The effects of ethionine and excess leucine were additive for the first week. On the 22nd day of this experiment, groups 2, 3, and 4 were fed the basal diet supplemented with methionine (diet 1) for 3 days, and then on the 25th day of the experiment each group was offered its original diet again for another 12 days. When group 2 was fed the high leucine diet during this second period it lost as much weight as it had originally and again required 6 or 7 days to adapt to the high leucine intake. When group 3 received the diet containing ethionine during the second period it lost only a small amount of weight for 2 days, then resumed a rate of growth equal to that observed originally after the 7th day. Group 4, receiving both ethioine and excess leucine, lost weight for 7 days and gained slightly thereafter, but the weight loss during the second period was just one-half that observed originally. These results suggested that rats previously fed ethionine remained adapted to ethionine for 3 days when they were fed the control diet, but

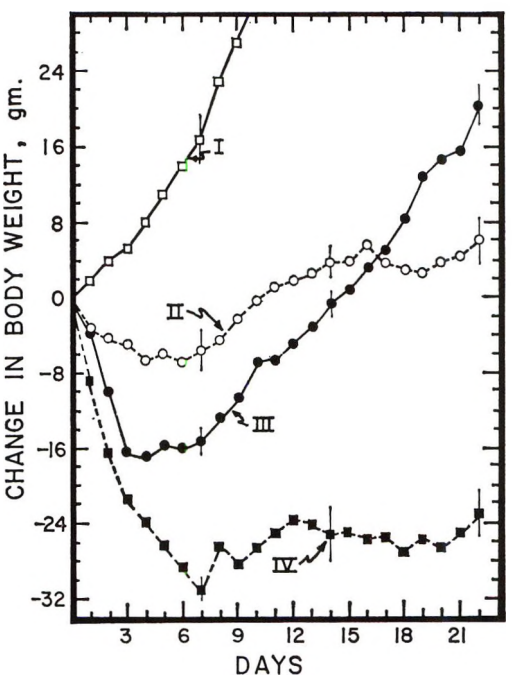

Fig. 2 The effect of dietary additions of ethionine and leucine on the growth of young rats fed a diet containing 9% of casein and 0.3% of DL-methionine. Group I, no additions; group II, 5% L-leucine; group III, 1% DL-ethionine; group IV, 5% L-leucine and 1% DL-ethionine.

that rats previously fed excess leucine did not retain their ability to tolerate a high leucine intake.

To study this in more detail 9 groups of rats were fed the basal diet supplemented with 0.3% of DL-methionine for 4 days. One group received this basal diet for the next 4 weeks. Four of the remaining groups received the high leucine diet for 12 days (period 1); then, the excess of leucine was omitted for 1, 2, 3, or 5 days as indicated in figure 3; and following this, each group was again fed the high leucine diet for another 12 days (period 2). Growth curves for period 1 and period 2 for each group fed the high leucine diet are shown in figure 3. The other 4 groups were fed the basal diet containing 0.3% of DL-methionine and 1% of DL-ethionine for 12 days (period 1); then, the ethionine was omitted for 1, 2, 3, or 5 days as indicated in figure 4; and following this each group was again fed the diet containing ethionine for another 12 days (period 2). Growth curves for period 1 and period 2 for each group fed the diet containing ethionine are shown in figure 4.

In this study in which all rats were fed the low protein diet (control diet) for 4 days prior to the beginning of the experiment the groups fed the 5% leucine diet did not lose weight as in the previous experiment in which the 5% leucine diet was offered immediately. Allowing the rats to adjust to the basal low protein diet did not appear to affect their response to the diet containing 1% DL-ethionine.

Rats fed the 5% L-leucine diet for a second 12 days after having received the control diet for one day grew at a rate comparable to their original rate of growth (fig. 3). Rats fed the high leucine diet for a second 12 days after having received the control diet for 2, 3, or 5 days grew even less than they had originally (fig. 3). Each group that received the basal diet for 1, 2, 3, or 5 days after being fed the 5% L-leucine diet for 12 days gained more weight than the control group during the same interval (fig. 3). This and the fact that the animals were heavier during the sec-

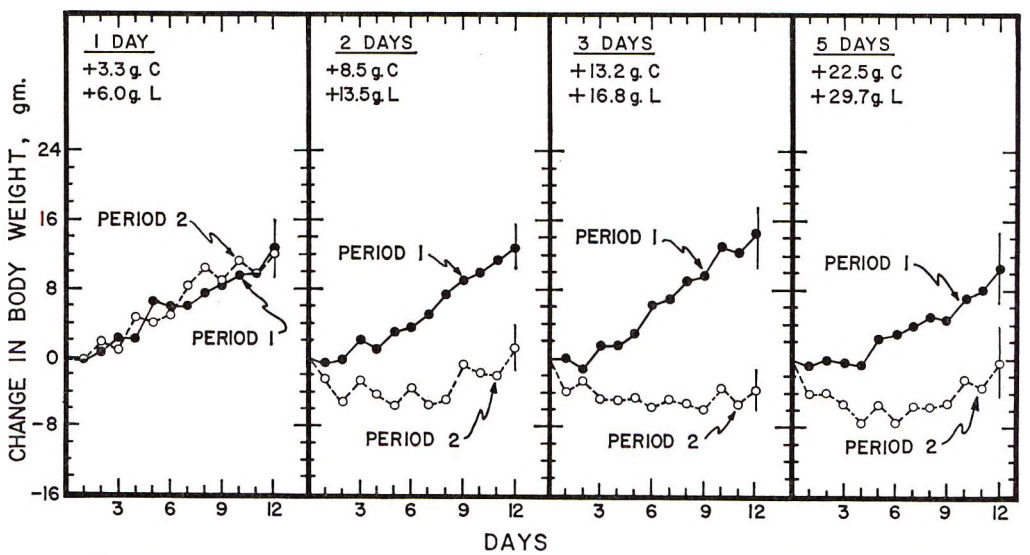

Fig. 3 Loss of adaptation to a dietary excess of leucine by rats fed a control diet for various intervals after receiving a high leucine diet. The 4 groups were fed the diet containing 9% of casein, 0.3% of DL-methionine, and 5% of L-leucine for 12 days (period 1 ●——●); then the leucine was omitted for 1, 2, 3, or 5 days as indicated; then the diet containing excess L-leucine was fed for another 12 days (period 2 ○——○). The gain in body weight of each group during the 1, 2, 3, or 5 days it was fed the control diet is shown at the top of each graph followed by the letter *L*. The gain in body weight during the same interval of a control group that received the basal diet throughout the experimental period is followed by the letter *C*. The vertical lines represent the standard errors of the means.

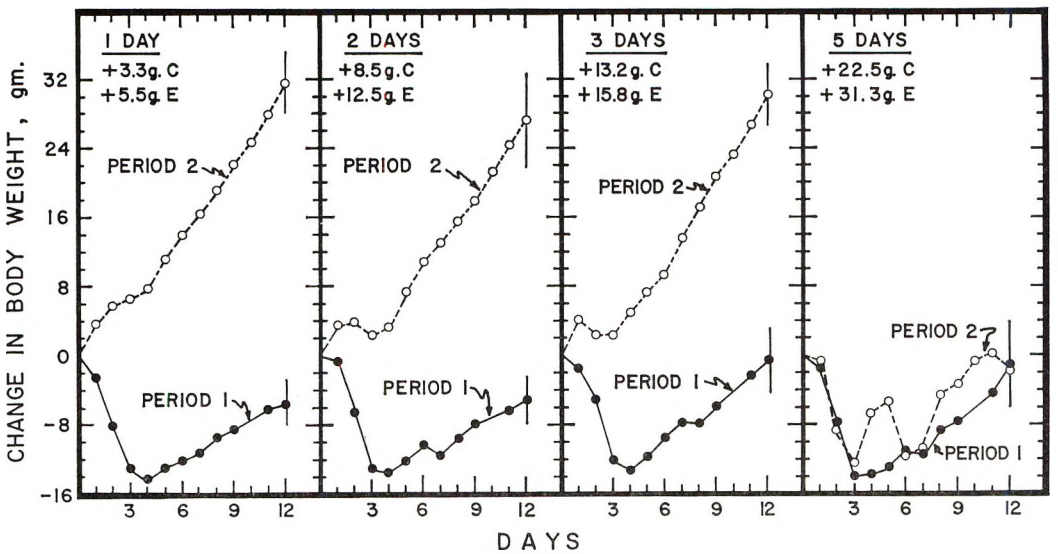

Fig. 4 Retention of adaptation to ethionine by rats fed a control diet for various intervals after receiving a diet containing ethionine. The 4 groups were fed a diet containing 9% of casein, 0.3% of DL-methionine, and 1% of DL-ethionine for 12 days (period 1 ●——●), then the ethionine was omitted for 1, 2, 3, or 5 days as indicated; then the diet containing DL-ethionine was fed for another 12 days (period 2 ○——○). The gain in body weight of each group during the 1, 2, 3, or 5 days it was fed the control diet is shown at the top of each graph followed by the letter *E*. The gain in body weight during the same interval of a control group that received the basal diet throughout the experimental period is followed by the letter *C*. The vertical lines represent the standard errors of the means.

ond period when they were fed the 5% L-leucine diet may explain the slower adaptation of these groups during period 2 than during period 1.

The results for the groups fed ethionine differ from those for the groups fed excess leucine. Rats fed the 1% DL-ethionine diet for a second 12 days after having received the control diet for one day continued to grow at about the same rate as the control group (fig. 4). After showing a very slight weight loss for 2 or 3 days, the groups receiving the control diet for 2 and 3 days also continued to grow at rates similar to those observed toward the end of the first period. Only rats fed the 1% DL-ethionine diet for a second 12 days after having received the control diet for 5 days lost as much weight as they had lost originally (fig. 4).

DISCUSSION

Although adaptation to excess leucine is lost by rats receiving the control diet for only one day (fig. 3), rats allowed to adapt to a diet containing ethionine retain their ability to grow with this diet after receiving the control diet for 3 days. They do, however, lose this ability if they are fed the control diet for 5 days (fig. 4). The mechanism of adaptation of rats to high leucine and ethionine-containing diets is not known but the results of these experiments suggest that 2 different mechanisms are involved.

The effects of ethionine and excess leucine on growth were additive (fig. 2), suggesting that each acts by a separate mechanism in causing growth depression. Previously ethionine was shown to inhibit liver regeneration more than could be accounted for by the depression it caused in food intake, whereas excess leucine did not (2).

Since rats fed diets containing ethionine lose weight severely during the first few days and then grow at a relatively fast rate after 6 or 7 days, provided the ethionine-to-methionine ratio is not too high, this information should be taken into account in metabolic studies in which ethionine is used.

It should be emphasized that the word "adaptation" used here applies only to the growth response of rats during a relatively short experimental period. Continuous feeding of sublethal doses of ethionine to rats results in profound pathological alterations of the liver characterized by striking proliferation of ductular cells in 5 to 7 weeks (3–5), in growth retardation in 2 to 3 months (6), and in production of hepatocarcinoma in 8 to 9 months (7).

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Influence of Age on Utilization of Raw Soybean Meal by Chickens[1]

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ABSTRACT The effects of raw soybean meal in chickens of different ages were studied in 3 separate experiments. In one experiment, raw and heated soybean meal diets were fed to chicks at various ages up to 12 weeks of age for a period of 2 weeks. In the other experiment, the experimental diets were fed to one-day-old chicks for 8 weeks. In the last experiment, the experimental diets were fed to 30 week-old laying hens for 8 weeks. Criteria used were growth, feed efficiency, pancreas weights, nitrogen retention and egg production. Growth of chickens that were 6 weeks of age or more before being fed raw soybean meal was not affected by the active principle in the raw meal that depresses growth of younger chicks. Pancreatic hypertrophy was evident in chicks that showed no growth depression, but was greatly reduced in birds at 12 weeks and completely absent in hens fed raw soybean meal. The egg production, feed utilization and nitrogen retention of hens fed raw meal was no different than that of the hens fed heated soybean meal diet. Results support the concept that raw soybean meal has an active fraction which cannot be fully digested by the young chick because of the lack of specific enzyme(s) and that a part of this protein is absorbed which acts in a positive manner to produce pancreatic hypertrophy. This active fraction is hydrolyzed by enzymes produced by the older birds, hence produces no physiological effects which are observed in young chicks.

Since almost all nutritional studies on raw soybean meal have been conducted with very young animals, no definite information is available on the effects of raw soybean meal in chickens of different ages. Carver et al.[2] reported excellent egg production by hens fed a 13% protein all-mash diet containing either raw or autoclaved soybean meal. Fisher et al. (1) reported that egg production by hens was unaffected by feeding raw soybean meal. Saxena et al. (2), confirming these reports, noted excellent egg production and feed efficiency and no pancreatic hypertrophy in hens fed a diet in which all protein was from raw soybean meal. Bornstein et al. (3) reported that the susceptibility of chicks to the detrimental effects of raw soybean meal decreased with age. Alumot and Nitsan (4) reported that up to 3 weeks of age, proteolytic activity in the intestines of the chicks fed raw soybean meal was almost completely inhibited, but beginning with the 4th week the proteolysis increased, approaching that of the controls at 6 weeks of age. Nesheim et al. (5) reported that the dietary raw soybean meal depressed fat absorption in chicks at 2 weeks of age but not at 4 weeks of age. They emphasized that the marked effect of raw meal on fat absorption was probably a true age difference and not merely an adaptation to raw meal feeding.

The purpose of the present study was to investigate the influence of age of chickens on the utilization of raw soybean meal. Results of such a study are reported in this paper.

EXPERIMENTAL

Three experiments were conducted. Male New Hampshire × Delaware chicks were used in experiments 1 and 2, and adult Single Comb White Leghorn hens[3] were used in experiment 3. The chicks were housed in electrically heated battery brooders until 4 weeks of age. They were then transferred to rearing batteries for the rest of the experimental period. The laying hens were housed in individual wire cages and were exposed to 14 hours of

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[1] Scientific Paper no. 2296. Washington Agricultural Experiment Stations, Pullman. Project 1678. This investigation was supported, in part, by funds from Medical and Biological Research by State of Washington Initiative no. 171.

[2] Carver, J. S., J. McGinnis, C. F. McClary and R. J. Evans 1946 Poultry Sci., 25: 399 (abstract).

[3] Hatched on June 4, 1962 and 30 weeks of age when placed on experiment.

TABLE 1
Composition of basal diet

	%
Soybean brew flakes[1]	50.0
Cellulose[2]	2.0
Glucose monohydrate	33.4
Corn oil	10.0
Limestone	1.0
Dicalcium phosphate	2.0
Iodized sodium chloride	0.5
Mineral-vitamin mixture[3]	0.5
DL-Methionine	0.6
Protein, calculated	25.0

[1] Especially processed low temperature desolventized soybean flakes, obtained from Archer-Daniels-Midland, Minneapolis.
[2] Solka Floc, Brown Company, Berlin, New Hampshire.
[3] Premix supplied the following per kilogram of diet: (in grams) potassium (KCl), 2.5; (in milligrams) manganese. ($MnSO_4 \cdot H_2O$), 55; zinc ($ZnSO_4 \cdot 7H_2O$), 44; iron ($FeSO_4 \cdot 7H_2O$), 20; copper ($CuSO_4$), 2; cobalt ($CoCl_2 \cdot 6H_2O$), 1; magnesium ($MgSO_4 \cdot 7H_2O$), 440; riboflavin, 3.3; Ca pantothenate, 9.3; niacin, 26.4; thiamine, 1.76; folic acid, 0.55; pyridoxine·HCl, 2.8; choline, 1320; biotin, 0.09; menadione, 2.2; oleandomycin, 22; (in micrograms) vitamin B_{12}, 9; and vitamin D_3, 440 ICU; vitamin A, 4400 IU; vitamin E, 5 IU.

light daily. Chicks and the hens were maintained in ventilated laboratories having a temperature regulated at approximately 22°C.

Composition of the experimental diet for chicks is shown in table 1. The experimental diet for the hens, containing 34% raw soybean flakes, was the same as used previously (2). Soybean brew flakes[4] were heated in a steam atmosphere in thin layers in an autoclave for 30 minutes at approximately 102°C to prepare the heated soybean meal. Chickens were weighed at the beginning and at the end of each experimental period and the feed consumption was recorded for that period. Three chicks from each group were randomly selected, weighed individually and then killed for the purpose of pancreas measurements. Pancreas weights were obtained on 6 hens from each treatment at the end of the 8-week experimental period in the same manner. The entire pancreas was rapidly removed, freed of extraneous tissue and immediately weighed. The weight of the pancreas was expressed in milligrams per 100 g body weight. Feed and water were supplied ad libitum. Data were analyzed statistically using Fisher's *t* test according to Snedecor (6).

Experiment 1 was conducted to determine the influence of age of chicks on their sensitivity to the deleterious effects of raw soybean meal. At one day of age, 2 groups of 5 chicks started to be fed each of the 2 experimental diets and a sufficient number for the remainder of the experiment were fed a stock mash diet. After 2, 4, 6, 8, and 10 weeks of age, 20 chicks were randomly assigned to 4 groups of 5 chicks each and changed to the experimental diets. Two groups received raw soybean meal diet while the other 2 received the heated soybean meal diet for 2 weeks.

To test the effects of a change in the dietary treatments on pancreatic hypertrophy, at the end of 6, 8, 10, and 12 weeks, one group of chicks receiving raw soybean meal was fed the heated soybean meal diet for 72 hours, after which time the pancreas weight was determined. Similarly, at 6 and 12 weeks of age, one group of chicks receiving heated meal was fed raw meal diet for 72 hours. Body weight and pancreas weights were recorded at the time of killing.

Experiment 2 was conducted to test the effects of prolonged feeding of raw soybean meal to chicks. Each experimental diet was fed to 4 groups of 10 chicks from hatching to 8 weeks of age. At 4 and 8 weeks of age, pancreas measurements on 8 chicks from each treatment were obtained. Electrophoretic analysis was conducted on blood serum samples of 4 chicks from each of the treatments at 2, 4, 6, and 8 weeks of age. Serum protein was determined by the micro-Kjeldahl method, using selenium as a catalyst.

Experiment 3 was conducted to study the utilization of raw soybean meal by the laying hen. Twenty-four laying hens were housed in individual wire cages and were fed each of the 2 experimental diets for 8 weeks. At the end of the experimental period excreta were collected for two 48-hour periods from 3 hens selected randomly from each treatment. Total nitrogen was determined on feed and fecal material by the Kjeldahl method and the nitrogen retention for egg formation and other body functions, was calculated. Data on body weight, pancreatic hypertrophy,

[4] Especially processed low temperature desolventized soybean flakes obtained from Archer-Daniels-Midland, Minneapolis.

egg production and feed consumption were obtained.

RESULTS

Results of experiment 1 (table 2) showed that rate of gain and feed efficiency were essentially the same when the chicks were 6 weeks of age before they were fed the experimental diets based on raw or heated soybean meal. Pancreatic hypertrophy was, however, observed in all chicks fed raw soybean meal diet regardless of age when placed on experiment. The extent of hypertrophy was not as great when chicks

TABLE 2

Influence of age on utilization of raw soybean meal by the chick

Age[1] of chicks	Growth rate		Feed efficiency		Pancreas weights	
	Heated	Raw	Heated	Raw	Heated	Raw
weeks	*g/chick/day*		*feed/gain*		*mg/100 g body wt*	
0–2	8.5	4.2[2]	1.60	2.10[2]	560	840[2]
2–4	19.0	14.7[2]	1.77	2.55[2]	500	900[2]
4–6	27.8	23.1[3]	1.97	2.40[2]	271	625[2]
6–8	32.5	32.7	2.06	2.10	278	480[2]
8–10	31.5	30.8	2.98	2.93	274	414[2]
10–12	24.2	23.3	4.02	4.09	285	354[3]

[1] Heated and raw soybean meal diets were fed only during these age periods. Prior to this period the chicks were either just hatched or were fed a stock diet.
[2] $P < 0.01$, compared with appropriate treatment with heated soybean meal.
[3] $P < 0.05$, compared with appropriate treatment with heated soybean meal.

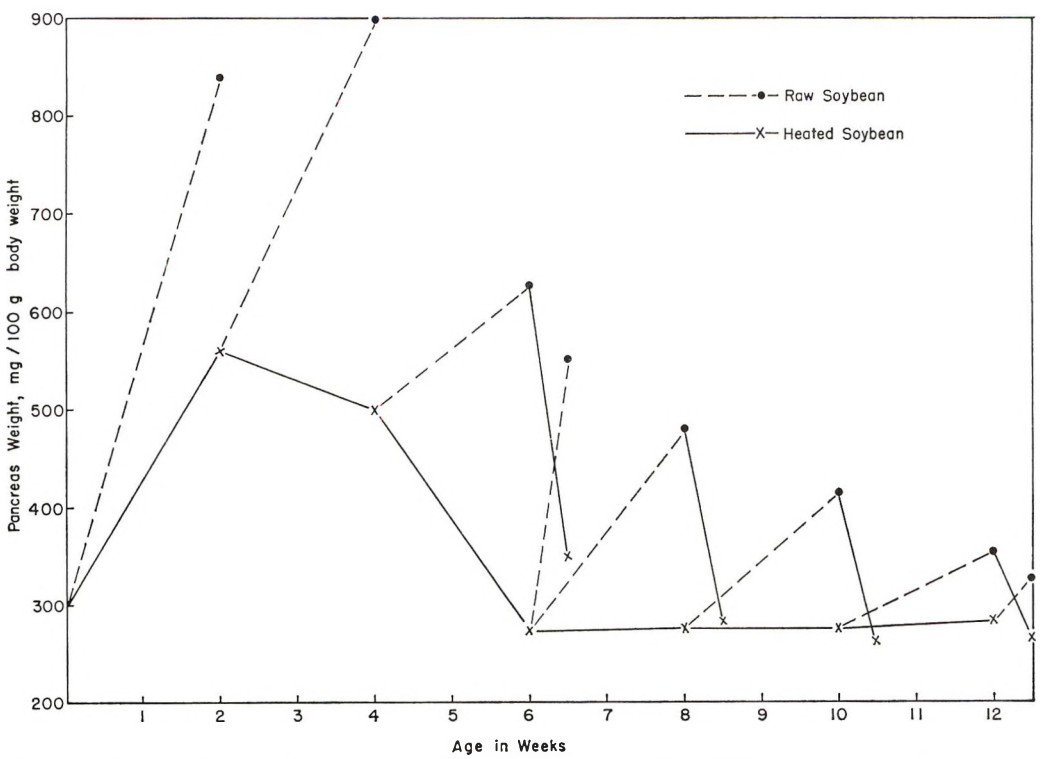

Fig. 1 Pancreatic weight as influenced by feeding raw and heated soybean meal to chicks at various ages. Pancreatic hypertrophy resulted when chicks were fed raw soybean meal. Chicks receiving raw meal, at 6, 8, 10 and 12 weeks, when fed heated soybean meal diet had "normal" pancreatic weights within 72 hours. Chicks receiving heated meal, at 6 and 12 weeks when fed raw meal showed hypertrophied pancreas within 72 hours, showing that pancreatic hypertrophy was reversible.

were 8 and 10 weeks of age as it was when they were zero, 2, 4, and 6 weeks of age when starting to be fed the experimental diets.

At 6, 8, 10, and 12 weeks of age, chicks that had been fed raw soybean meal for the previous 2 weeks and had marked pancreatic hypertrophy, showed normal pancreatic weights within 72 hours after changing them to the heated soybean meal diet. Conversely, at 6 and 12 weeks of age, chicks that had been fed the heated soybean meal diet exhibited pancreatic hypertrophy within 72 hours after receiving the raw soybean meal diet (fig. 1).

The results of experiment 2 (table 3) showed that at 8 weeks of age, chicks fed the raw meal weighed 181 g ($P < 0.01$) less than those fed the heated soybean meal diet. However, the average gain for the 6- to 8-week period was essentially the same in both the treatments. Chicks fed raw soybean meal showed marked pancreatic hypertrophy at the end of 8 weeks. No differences were observed between the electrophoretic patterns of serum protein of chicks on the 2 treatments at various ages. Also, there were no differences in the amount of serum protein between the treatments.

Results of experiment 3 (table 4) showed that raw soybean meal had no effect on weight gains and did not cause pancreatic hypertrophy when fed to laying hens. No differences were observed in egg production by hens fed raw meal and those fed heated soybean meal diets. Nitrogen retention by hens fed raw meal was essentially the same as that obtained with birds fed heated soybean meal diets. No difference was noted in efficiency of feed utilization between the 2 treatments.

DISCUSSION

Results of the experiments reported in this paper show clearly that although raw soybean meal causes marked growth depression, lower feed efficiency, pancreatic hypertrophy and poor nitrogen retention in very young chicks, it was completely ineffective in causing any of these deleterious effects in older birds. Growth of chickens that were 6 weeks of age or more before being fed raw soybean meal was not affected by the active principle in the raw soybean meal that depresses growth

TABLE 3

Average weight, feed efficiency and pancreatic hypertrophy in chicks fed raw soybean meal from zero to 8 weeks of age

Age	Heated soybean meal			Raw soybean meal		
	Avg wt	Feed efficiency	Pancreas	Avg wt	Feed efficiency	Pancreas
weeks	*g*	*feed/gain*	*mg/100 g body wt*	*g*	*feed/gain*	*mg/100 g body wt*
0	43	—	300	43	—	300
2	159	—	—	94[1]	—	—
4	410	1.98[2]	440	288[1]	2.49[1,2]	807[1]
6	805	—	—	610[1]	—	—
8	1156	2.80[3]	289	975[1]	2.93[1,3]	500[1]

[1] $P < 0.01$, compared with appropriate treatment with heated soybean meal.
[2] Feed efficiency zero to 4 weeks.
[3] Feed efficiency 4 to 8 weeks.

TABLE 4

Utilization of raw soybean meal by laying hens

Soybean meal treatment	Body weight		Egg production	Feed efficiency	Nitrogen retention[1]	Pancreas wt
	Start	End				
	g	*g*	%	*g feed/egg*	%	*mg/100 g body wt*
Raw	1842	1875	85.5	123	47.7	274
Heated	1877	1935	86.4	124	48.4	254

[1] Represents nitrogen not excreted in urine and feces and would include nitrogen used in egg formation.

of younger chicks. Pancreatic hypertrophy, on the other hand, was still very evident in chicks that showed no growth depression, but was greatly reduced in birds at 12 weeks and completely absent in hens fed raw soybean meal for 8 weeks. Poor nitrogen retention in chicks fed raw soybean meal has been reported previously (7, 8). Results of this study showed that hens fed raw meal retained an equivalent amount of nitrogen as the ones fed the heated soybean meal diet. Feeding raw soybean meal did not affect either the total serum protein or the albumen-to-globulin ratios as determined by paper strip electrophoresis. This was true of all ages that were studied. The egg production by the hens fed raw soybean meal was no different than that of the hens fed heated soybean meal. Furthermore, no difference was noted in the efficiency of feed utilization between the 2 treatments. All these observations show that as the chick grows older, it becomes less sensitive to the factors in raw soybean meal that cause the marked physiological changes in the very young chick.

Unpublished results from this laboratory indicate that a pancreatic homogenate from young chicks has a lower activity for hydrolyzing a polysaccharide in barley than a comparable preparation from hens. As chicks grow older the activity of the pancreatic homogenate increases. This observation plus those reported in this paper and elsewhere showing that older birds are less affected by raw soybean meal than young chicks suggests that a deleterious fraction is present in raw soybean meal that escapes digestion in young chicks but is susceptible to hydrolysis by enzymes produced in the older birds. Possibly some of the fraction of raw soybean meal that is not digested by young chicks is absorbed and causes the pancreatic hypertrophy through some unknown manner as a result of blocking the release of the enzyme in the young chick. We have obtained evidence in our laboratory (9) which shows that young chicks fed raw meal failed to release the pro-enzyme material in response to intraperitoneal injection of pilocarpine. Histological examination of the pancreas of such chicks showed an accumulation of the zymogen material in the acinar cells. Whether hypertrophy is due to an accumulation of zymogen or to excessive secretion, as has been shown to be the case in rats by Haines and Lyman (10), it appears very likely that once the stimulus has been removed the target organ should return to normal. That such was the case has been amply demonstrated in this study (fig. 1).

It could, therefore, be hypothesized that raw soybean meal has an active fraction (protein) which cannot be fully digested by the young chick because of the lack of specific enzyme(s) and also that part of this protein is absorbed which causes the growth depression and pancreatic hypertrophy by interfering with the mechanism in the young chick which regulates the release of enzymes from the pancreas.

That pancreatic hypertrophy is not caused by a severe deficiency of an amino acid was shown in an experiment in our laboratory in which a lysine deficiency in chicks caused poor growth but had no effect on the pancreas.[5] Furthermore, amino acid supplementation of the diet of young chicks (11) and rats (12) containing raw soybean meal failed to overcome completely the growth depression and had little or no effect on pancreatic hypertrophy observed. It appears, therefore, that raw soybean meal contains a fraction (protein) which acts in a positive manner, as opposed to being deficient in some nutrient, to produce the observed effects in chicks. Apparently, therefore, some of the protein that is not digested acts as a stimulant for the target organ (pancreas).

Work is now in progress to determine whether a fraction of raw soybean meal which markedly depresses chick growth is digested differently by pancreatic homogenates from chicks and hens.

ADDENDUM Following submission of this manuscript, a paper entitled "The Influence of Age of Chicks on their Sensitivity to Raw Soybean Oil Meal," by S. Bornstein and B. Lipstein, appeared in Poultry Science, *21* (i): 61, 1963.

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Lipid Metabolism of Young Female Rats Fed Diets Varying in Fat and Calories[1,2,3]

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ABSTRACT The effect of diet on rate of synthesis of several lipids was studied using young female rats. Dietary variations were 1 or 20% cottonseed oil and 20% animal fat (lard and butter in equal amounts). Young adult and weanling animals were fed these diets for 4, 7 or 12 weeks, at which time each was given Na-1-C^{14}-acetate. Amounts of serum and liver lipids and isotope incorporation were determined. Cholesterol synthesis was enhanced, whereas noncholesterol lipids were depressed by the higher amounts of fat. At 10 weeks the animals fed the higher level of fat had very high cholesterol activities with only slightly larger concentrations of cholesterol in the livers and serum. When food intake was restricted the rate of cholesterol metabolism was related to the amount of dietary or stored fat utilized for energy.

Several experiments have shown that cholesterol synthesis is a basic necessity in the organism and is physiologically controlled. In 1954 Hutchens et al. (1) using acetate-1-C^{14} incorporation in the intact rat showed that cholesterol specific activity declined after 96 hours of fasting to about 9% of initial values; however, this rate was maintained for 240 hours. During the entire time the total quantity of liver cholesterol remained constant. Rate of cholesterol synthesis is also affected by dietary variations (2–5). Brice and Okey (6) observed that acetate-2-C^{14} incorporation into cholesterol by rats was only one-half as great with 5 as with 40% dietary fat. Stage of development as a factor influencing lipid metabolism is illustrated in an investigation by Schwenk and Joachim (7) which showed that livers of rat fetuses synthesized cholesterol at 20 times the rate of the maternal livers.

The purpose of the present investigation was to study the effect of diets varying in kind and amount of fat on acetate-1-C^{14} incorporation into liver and serum lipids of rats of 2 ages. The alteration in lipid metabolism brought on by utilization of body fat was observed in rats given diets restricted in calories.

EXPERIMENTAL

A low-fat and a 20% fat diet were used. The percentage composition of the low-fat diet fed to adult rats was: lactalbumin,[5] 10; salt mixture (Jones and Foster),[5] 4; cottonseed oil,[6] 1; vitamin mixture,[7] 5; and the remainder corn dextrin.[8] For the 20% fat diet, cottonseed oil was substituted for the same weight of corn dextrin. Diets were prepared at 3 to 4 week intervals and the bulk stored at −20°C. Fresh food was placed in each cage daily for the animals fed ad libitum. The thiobarbituric acid test (TBA) (8) was used to determine rancidity. Neither storage for a week in the refrigerator nor exposure to room temperature and air for 24 hours caused an appreciable TBA value.

These diets were fed to 11-week-old Holtzman female rats born in the laboratory and reared with laboratory chow.[9] After 2 weeks, one-half of each group of

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[3] This report is taken from a thesis submitted by Jacqueline Dupont in partial fulfillment of the requirements for the Ph.D. degree.

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[5] Jones, J. H., and C. Foster, J. Nutrition 24: 245, 1942, obtained from Nutritional Biochemicals Corporation, Cleveland.

[6] Furnished by HumKo Products, Memphis, Tennessee.

[7] Supplying the following vitamins per 10 g of diet (approximate daily consumption): (in μg) thiamine·HCl, 100; riboflavin, 100; pyridoxine·HCl, 100; niacin, 500; Ca pantothenate, 500; 2-methyl-1-4-naphthoquinone, 40; folic acid, 40; biotin, 2; vitamin B_{12}, 0.6; calciferol, 1; vitamin A acetate, 30; (in mg) *p*-aminobenzoic acid, 10; inositol, 20; choline·Cl, 40; α-tocopherol, 1. Ascorbic acid was added to the vitamin mixture in the amount of 100 mg/kg as an antioxidant.

[8] Fisher Scientific Company, New York.

[9] Ralston Purina Company, St. Louis.

20 animals were fed restricted diets and the 4 groups continued for 2 additional weeks. The calorie allowance was one-half the average daily consumption of the previous 11 days for each rat. The diets contained doubled quantities of lactalbumin, salt mixture and vitamins so that only fat and carbohydrate were reduced. Animals on caloric restriction were fed twice daily.

In a second series, 2 groups of 16 weanling females were fed the above diets except that all contained 20% lactalbumin. An additional group of 24 rats was fed a diet containing 20% fat as an equal mixture of lard [10] and butter.[11] One-half of each of these groups were fed the diets ad libitum for 7 weeks and the remainder 12 weeks.

At the end of each feeding period the following procedure was used for isotope incorporation studies. In the evening, 8 hours before the scheduled time of injection, the food was removed. After 6 hours the food was replaced and left for 2 hours. Then each rat was given an intraperitoneal injection of approximately 20 μc of 1-C^{14} Na-acetate in 1 ml physiological saline. The food was again removed and the animal left with free access to water for 6 hours. Each animal was then injected intraperitoneally with thiopental sodium and a blood sample was taken by heart puncture. The liver was bled, excised and immediately weighed, minced and the fat extracted by the procedure of Folch et al. (9) using a Waring Blendor. Blood was centrifuged and serum fat extracted by the same method.

Total fat of livers was determined by evaporating an aliquot of the chloroform methanol extract, drying at 100°C for 30 minutes, and weighing. Total cholesterol of serum and livers was determined with the color reagent of Zak et al. (10) after lipid extraction and saponification. Lipid phosphorus was determined by the method of Chen et al. (11) and phospholipids calculated by assuming P to be 3.7% of the molecular weight. Samples of lipid extracts were plated in 3.2-cm planchets and counted in a proportional counter[12] to determine C^{14} content. Corrections were made for self-absorption of C^{14}. Cholesterol was precipitated with digitonin according to Sperry and Webb (12), plated on filter paper using slight suction, and counted as described. Self-absorption was negligible in the digitonide precipitates.

RESULTS AND DISCUSSION

Food consumption and weight gains were similar for the various diets. The animals restricted in calories almost maintained their weights, whereas their counterparts fed ad libitum continued to gain slightly.

Table 1 shows the gross lipid composition of the livers of the rats. The 3 groups consuming cottonseed oil diets ad libitum had larger livers than other groups and, with the exception of the 15-week-old rats fed from weaning, the livers were also fatter than those of comparable groups fed other diets. The greater quantity of lipid was shown in all classes. Both the 15-week-old groups fed cottonseed oil ad libitum for 4 weeks and those fed from weaning had higher phospholipids (28 and 39% higher), cholesterol (50 and 54% higher) and glycerides (66 and 92% higher) than low-fat counterparts. The differences were statistically significant with $P < 0.001$ for the short-term group and 0.005 for those fed from weaning. Those fed the lard-butter diet had values for the 3 lipid components which were not different from those of the groups fed cottonseed oil, and were greater than the values for animals fed low-fat diets for each component (P's < 0.025).

The 10-week-old rats fed cottonseed oil from weaning had fatty livers. The values for all lipid components were greater than for the lard-butter group at either age, and greater than either fat group at the later age (all comparisons, $P < 0.001$) except for phospholipids.

Table 2 shows the C^{14}-acetate incorporation data for liver lipids. The percentage of the injected dose appearing in liver lipids is shown in column 1. Calculation was made of the proportion of the lipid label which was in cholesterol (column 2). Specific activity is given as disintegrations per minute, per milligram of material. The 3 groups fed low-fat ad libitum had 5 to 6% of total lipid C^{14} in cholesterol and 4 to

[10] Purchased in local retail market.
[11] See footnote 10.
[12] Nuclear-Chicago.

TABLE 1

Distribution of liver lipids

Age of rats and diet	Liver wt	Total lipid	Cholesterol	Phospholipid	Glyceride (by difference)
	g	*%*	*mg*	*mg*	*mg*
			Experiment 1		
15 Weeks					
Low-fat	6.29 ± 0.20[1]	5.2 ± 0.20	19.2 ± 1.03	193 ± 6.8	187 ± 16.7
Low-fat[2]	6.66 ± 0.22	6.3 ± 0.15	20.8 ± 1.28	204 ± 6.4	124 ± 7.6
20% CSO[3]	7.17 ± 0.19	8.1 ± 0.34	28.9 ± 2.22	247 ± 7.4	311 ± 20.1
20% CSO[2,3]	7.52 ± 0.20	6.3 ± 0.22	25.5 ± 1.83	237 ± 7.7	213 ± 16.0
			Experiment 2		
10 Weeks					
Low-fat	5.43 ± 0.12	5.6 ± 0.20	12.7 ± 0.75	153 ± 4.6	136 ± 9.4
20% CSO[3]	7.93 ± 0.20	8.9 ± 0.26	29.6 ± 1.43	274 ± 4.8	403 ± 25.5
20% L-B[4]	6.72 ± 0.18	6.5 ± 0.25	17.1 ± 0.59	213 ± 7.9	205 ± 16.0
15 Weeks					
Low-fat	5.63 ± 0.22	5.9 ± 0.21	14.6 ± 1.71	184 ± 14.5	134 ± 13.5
20% CSO[3]	7.25 ± 0.23	7.4 ± 0.38	22.5 ± 1.60	255 ± 10.3	257 ± 29.2
20% L-B[4]	6.42 ± 0.11	7.0 ± 0.23	19.1 ± 0.98	228 ± 11.3	202 ± 14.8

[1] SD.
[2] Calories reduced during last 2 weeks.
[3] Cottonseed oil.
[4] Lard-butter.

TABLE 2

Incorporation of acetate-C^{14} into liver lipids and ratio of C^{14}-cholesterol to total lipid C^{14}

Age of rats and diet	Total activity[1]		Specific activity	
	Incorporation	C^{14}-cholesterol-to C^{14} total lipid ratio	Non-cholesterol	Cholesterol
	%		*dis/min/mg × 10^{-2}*	
		Experiment 1		
15 Weeks				
Low-fat	0.69	0.05 ± 0.01[2]	8.1 ± 2.2	6.7 ± 1.5
Low-fat[3]	1.77	0.03 ± 0.003	23 ± 2.2	13 ± 1.6
20% CSO[4]	0.49	0.14 ± 0.02	3.9 ± 2.7	12 ± 2.3
20% CSO[3,4]	0.82	0.18 ± 0.01	8.0 ± 5.8	28 ± 4.7
		Experiment 2		
10 Weeks				
Low-fat	0.54	0.06 ± 0.01	7.9 ± 2.0	8.8 ± 1.5
20% CSO[4]	0.47	0.28 ± 0.04	2.3 ± 0.2	21 ± 3.5
20% L-B[5]	0.44	0.20 ± 0.04	3.8 ± 0.5	25 ± 5.6
15 Weeks				
Low-fat	0.52	0.06 ± 0.01	7.1 ± 2.5	7.0 ± 0.7
20% CSO[4]	0.43	0.12 ± 0.01	3.3 ± 0.5	9.0 ± 1.1
20% L-B[5]	0.37	0.13 ± 0.04	3.5 ± 0.6	12 ± 2.0

[1] % of injected dose.
[2] SD.
[3] Calories reduced during last 2 weeks.
[4] Cottonseed oil.
[5] Lard-butter.

5% of the liver lipid was cholesterol. The specific activities were, therefore, similar, indicating that a relatively constant relationship existed in the partition of acetate between fatty acid and cholesterol cycles. The feeding of fat altered this relationship. Incorporation of acetate into noncholesterol lipids (fatty acids) was considerably less when fat was fed than in its absence. The total activity (% incorporation) was about two-thirds the maximal values observed and specific activity was about one-half. These differences existed despite the much larger pool of lipids in the liver, as-

sociated with fat feeding. The suppression of fatty acid synthesis by ingested fat has been reported by Hill et al. (5), and Brice and Okey (6). The former group found that within an hour after ingestion of oil *de novo* synthesis of fatty acids was decreasing. These investigators also showed that synthesis of cholesterol began to increase within 12 hours after ingestion of oil and in 72 hours had increased 400%. The present results are in accord with those observations. In both experiments the 15-week-old rats had greater total activity of cholesterol with dietary fat than without ($P < 0.005$). The specific activity of cholesterol, however, was not statistically different between dietary groups of the rats fed from weaning to 15 weeks of age. This contrasts with 10-week-old rats and with those fed only 4 weeks. In both shorter periods cholesterol specific activity doubled with fat feeding. It appears that the young female rats required some time to make an adjustment to large amounts of fat in the diet and the younger animals were less able to make a rapid adjustment than older animals.

The data shown for the series of rats subjected to caloric restriction reveal relationships between cholesterol activity and calories derived from fat. Because of changes in efficiency of utilization of energy when calories are restricted which were not determined in this study, only approximations may be made of energy derived from fat. It may be assumed that the low-fat ad libitum diet provided adequate energy from carbohydrate and therefore, recycling of stored fat would depend entirely on the needs of the whole organism. Reduction of the dietary source of energy to approximately 50 to 60% of normal consumption would entail changes in cycling of carbohydrates and lipids with a probable increase in utilization of fat for energy. The situation with 40% of caloric intake as fat would be quite different with respect to utilization of fat for energy, and reduction of dietary energy supply would entail utilization of fat for energy in much greater proportion than any of the other diets presented. If one considers the above series to present a comparison of fat as the source of small, moderate or large proportions of the calories used for energy, the incorporation of acetate into cholesterol is directly related to that proportion. The cholesterol specific activity value for the low-fat ad libitum diet supplying small quantities of fat for energy was 670; a moderate supply of fat, either from diet or from low-fat-restricted calorie treatment, resulted in values of 1190 and 1300, respectively, and

TABLE 3

Serum lipids and the ratio of C^{14}-cholesterol to total lipid C^{14}

Age of rats and diet	Serum lipids		C^{14}-cholesterol-to-C^{14} total lipid ratio
	Cholesterol	Phospholipids	
	mg/100 ml	*mg/100 ml*	
	Experiment 1		
15 Weeks			
Low-fat	94 ± 7.1[1]	148 ± 10.6	0.15 ± 0.02
Low-fat[2]	116 ± 6.0	164 ± 7.3	0.13 ± 0.01
20% CSO[3]	120 ± 8.4	164 ± 8.2	0.39 ± 0.03
20% CSO[2,3]	120 ± 6.9	167 ± 5.2	0.43 ± 0.02
	Experiment 2		
10 Weeks			
Low-fat	90 ± 7.7	168 ± 15.2	0.24 ± 0.06
20% CSO[3]	123 ± 10.1	208 ± 13.9	0.88 ± 0.06
20% L-B[4]	110 ± 18.6	217 ± 9.1	0.73 ± 0.06
15 Weeks			
Low-fat	68 ± 3.3	154 ± 7.3	0.24 ± 0.04
20% CSO[3]	109 ± 10.3	199 ± 13.5	0.55 ± 0.05
20% L-B[4]	92 ± 3.5	173 ± 8.6	0.47 ± 0.02

[1] SD.
[2] Calories reduced during last 2 weeks.
[3] Cottonseed oil.
[4] Lard-butter.

a large proportion of energy derived from a diet containing 40% fat calories and fed in restricted amounts resulted in a value of 2800 dis/min/mg. Total activity presented the same relationship, the values for the 3 comparisons being: small, 0.029, moderate, 0.060 and 0.073 and large, 0.156.

Serum lipid analyses are shown in table 3. In each series the presence of fat in the diet resulted in greater concentrations of cholesterol and phospholipids in the serum. Caloric restriction with the low-fat diet produced serum lipids similar to those of animals fed fat. Cholesterol synthesis also differed between animals fed diets with and without fat. The proportion of acetate appearing in cholesterol compared with fatty acids was much more varied in serum than in liver. In the young rats (10-week-old) 73 and 88% of the lipid label was in cholesterol when lard-butter and cottonseed oil diets were fed, in contrast with 24.0% for the corresponding low-fat group. At 15 weeks of age the percentages were 47, 55 and 24, respectively, for the animals fed from weaning. A comparable difference was shown in the adult rats fed for 4 weeks.

The total and specific activities of serum lipids were in the same relationship to diet as they were in the liver lipids. The age-diet relationship is shown in figure 1. The values for specific activity of cholesterol are similar to those for liver cholesterol. Figure 2 illustrates the relationship between cholesterol specific activity and the proportion of calories obtained from fat. The serum cholesterol responded even more significantly than liver cholesterol. It is also shown that young female rats

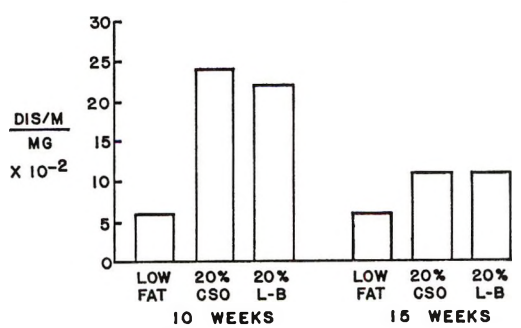

Fig. 1 Specific activity of serum cholesterol of rats of 2 ages when dietary fat was varied.

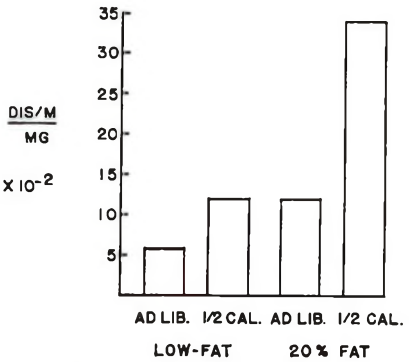

Fig. 2 Specific activity of serum cholesterol of rats when fat calories were varied.

exhibited very rapid rates of metabolism of cholesterol when fat was included in the diet and that a relationship exists between utilization of energy from fat and rate of metabolism of cholesterol.

ACKNOWLEDGMENT

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A Study of the Hypocholesterolemic Activity of the Ethyl Esters of the Polyunsaturated Fatty Acids of Cod Liver Oil in the Chicken

I. EFFECT ON TOTAL SERUM CHOLESTEROL

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ABSTRACT This report is concerned with the polyunsaturated fatty esters of cod liver oil and their effect on total serum cholesterol in hypercholesteremic chickens. An ethyl ester fraction of cod liver oil (I no. 315) was found 4 times as effective as natural cod liver oil (I no. 160) in lowering total serum cholesterol in the hypercholesterolemic chicken. Increasing the iodine number of the ethyl ester preparation to 374 increased the hypocholesterolemic activity approximately twofold. An ethyl ester fraction of menhaden oil was found equal in hypocholesterolemic activity to an equivalent ethyl ester fraction of cod liver oil. The hypocholesterolemic effect of the polyunsaturated fatty esters is related to the degree of unsaturation of the fatty esters fed. This hypocholesterolemic effect of the fish oils was obtained only during the period that they were fed. When the oils were withdrawn from the ration, hypecholesterolemia returned. Dietary hypercholesterolemia diminished with age.

For several years, reports (1–8) have indicated that the fatty acids of vegetable oils will reduce hypercholesterolemia in man. Other and more recent articles (9–13) have shown that marine oils, which contain more of the highly unsaturated nonessential fatty acids, possess similar if not greater hypocholesterolemic serum activity. Recently, purified linoleic acid (12, 14, 15), arachidonic acid (11, 16), and fractions containing polyunsaturated marine fatty acids[2] (13) have been used in the study of hypercholesterolemia. In the work herein reported the effect of various whole oils and highly unsaturated fish oil fractions on the serum cholesterol of hypercholesterolemic chickens has been studied.

EXPERIMENTAL

Arbor Acres White Rock or White Leghorn chicks were used in these studies. Initially, both sexes were represented in each treatment, but later we restricted our studies to cockerels. The birds were raised in electrically heated wire-floor battery brooders, and at 4 weeks of age they were transferred to larger unheated cages whose environmental temperature was 22°C.

In table 1 is listed the experimental chicken ration used. Coconut oil with an iodine number of 10 (Wijs method) was

TABLE 1

Experimental chicken ration NR116

Glucose monohydrate[1]	39.98
Soy protein[2]	25.23
Coconut oil	19.41
Salt mix[3]	4.85
Cellulose[4]	2.91
Diatomaceous earth[5]	2.91
Cholesterol	1.94
Vitamin mix[6]	0.97
DL-Methionine	0.68
Sodium taurocholate	0.49
Glycine	0.29
Choline chloride	0.29
Butylated hydroxytoluene	0.048

[1] Cerelose, Corn Products Company, Argo, Illinois.
[2] ADM C-1 Assay Protein, Archer-Daniels-Midland, Minneapolis.
[3] Salt mix: (g/kg ration) $CaHPO_4$, 21.51; $CaCO_3$, 14.92; KH_2PO_4, 8.76; NaCl, 6.00; $MgSO_4$, 2.50; $FeSO_4 \cdot 7H_2O$, 0.333; $MnSO_4 \cdot H_2O$, 0.333; $ZnCO_3$, 0.100; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.006; $CuSO_4 \cdot 5H_2O$, 0.0026; KI, 0.0026; $CoCl_2 \cdot 6H_2O$, 0.0017.
[4] Cellu Flour, Chicago Dietetic Supply House, Chicago, Illinois.
[5] Micro-Cel, Johns-Manville Products Corporation Manville, New Jersey.
[6] Vitamin mix: (mg/kg ration) inositol, 250; *d*-α-tocopheryl succinate, 50; nicotinamide, 30; thiamine·NO_3, 20; Ca pantothenate, 20; riboflavin, 10; pyridoxine·HCl, 4.5; vitamin A acetate, 1.720; folic acid, 0.8; menadione, 0.4; biotin, 0.15; vitamin D_3, 0.020; cyanocobalamim, 0.015.

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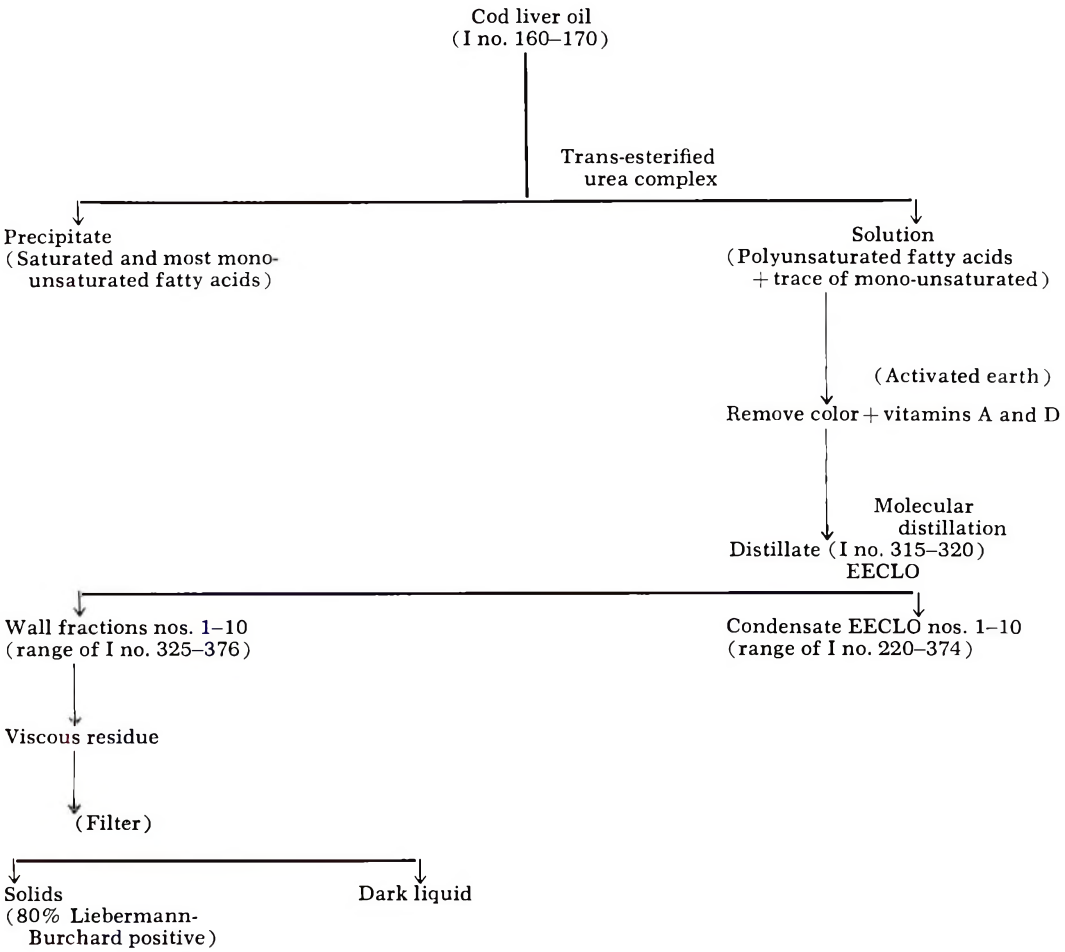
[1] Present address: Health Research Institute, Fairleigh Dickinson University, Madison, New Jersey.

[2] Peifer, J. J., and W. O. Lundberg 1961 Effect of dietary fats and marine oil fractions on cardiovascular tissues. Federation Proc., 20: 93 (abstract).

TABLE 2

Procedure for the preparation of the ethyl ester fractions of the polyunsaturated fatty acids of cod liver oil



the source of saturated fat. This basic ration (NR116) was used in all our experiments in which we studied the hypocholesterolemic effect of an oil, with the test oil substituting for an equivalent amount of coconut oil; in this way all rations were kept isocaloric.

The birds received ration NR116 until 6 or 7 weeks of age, at which time they were bled from a wing vein, weighed, and randomly fed the test ration for 7 days. At the end of this 7-day test period, they were again bled and weighed, and their feed consumption recorded. The percentage change in total serum cholesterol between the initial and seventh day cholesterol value was used as our criteria in evaluating the hypocholesterolemic activity of an oil.

Cholesterol analysis[3] was a modification of the Albers and Lowry (17) fluorometric procedure. Iodine numbers were determined by the Wijs method as given by Hawk et al. (18). The alkaline isomerization determinations were carried out using the procedure of Herb and Riemenschneider (19).

The preparation of the different ethyl ester fractions of cod liver oil (EECLO) is outlined in table 2. Fraction EECLO

[3] Kahn, S. G., and H. Yacowitz 1958 Aspects of fluorometric analysis for total cholesterol in serum and tissue. Federation Proc., 17: 991 (abstract).

and condensate EECLO fractions 1, 3, 7 and 10 were studied for hypocholesterolemic activity. The fractions were free of vitamin A and vitamin D as determined by spectrophotometric (20) and radioactive phosphorus methods (21), respectively. Infrared analysis showed no peroxides, sterols, or trans-configurations present in the EECLO fractions assayed for hypocholesterolemic bio-activity. The fatty acid composition of the cod liver oil distillate (I, no. 315–320) and its comparable mehaden oil preparation as analyzed by gas chromatography[4] are presented in table 3. The oil preparations were similar except that the cod liver fraction contained approximately 4% more pentaenes and the menhaden oil had slightly more than 3% tetraenes. Their iodine values were very nearly identical. To all ethyl ester preparations was added 0.05% butylated hydroxytoluene (BHT) to insure stability of the polyunsaturated fatty esters.

TABLE 3

Gas-liquid chromatographic analysis

Fatty acids[1]	% Composition of the ethyl esters	
	Cod liver oil	Menhaden oil
14:0	0.31	1.52
14:	0.12	0.08
14:0 br	—	0.11
15:0	—	0.18
15:0 br	0.19	0.65
15:0 br	0.16	0.63
Unknown	1.08	0.56
16:0	0.50	1.44
16:1	6.47	8.52
16:3	1.29	4.39
16:4	1.69	4.40
16:0 al	0.37	0.20
17:0	0.57	(trace)
17: br	1.10	0.45
Unknown	—	0.13
18:0	—	0.21
18:1	6.82	5.01
18:2	3.31	2.32
18:2	0.40	0.21
18:3	2.21	2.30
18:4	10.38	9.74
Unknown	0.43	0.42
19:0 br?	0.52	0.49
19: un?	0.79	0.78
20:1	3.79	1.78
20:2	0.59	0.11
20:2?	0.40	0.27
20:3	2.09	0.12
20:4	1.82	2.79
20: un	0.47	1.86
20:5	32.03	28.79
Unknown	2.33	1.87
20: un	0.87	0.78
22:4	1.32	1.16
22:5	1.54	0.60
22:5	—	1.79
22:6	13.76	13.34

[1] 20:5 = chain length carbons:5 double bonds; br = branched chain; ? = questionable; al = aldehyde; un = unsaturated fatty acid of unknown structure.

RESULTS AND DISCUSSION

I. Effect of polyunsaturated fatty esters on total serum cholesterol of hypercholesterolemic chickens. Table 4 shows that coconut oil produced greater hypercholesterolemia than butter, which prompted us to use it in our NR116 ration. Other investigators (22, 23) have reported a similar hypercholesterolemic effect from feeding coconut oil.

An effective way to diminish total serum cholesterol in a hypercholesterolemic chicken was to withdraw fat from the ration (table 4); however, 10% cod liver oil reduced the serum cholesterol to the same extent as a fat-free ration. Equivalent amounts of safflower, linseed, corn, and whale oils or the elimination of an equal amount of coconut oil (10%) from the ration were not as effective as cod liver oil in decreasing total serum cholesterol. Even at 2% of the ration, cod liver oil was more effective in reducing total serum cholesterol than an equivalent amount of safflower oil.

It seemed pertinent to determine whether there was a difference in activity between a fish liver oil and a fish body oil. Table 5 presents results obtained when 2% cod liver oil was added to ration NR116 and compared with a 2% menhaden oil supplementation. The data indicate that both marine oils reduce serum cholesterol in hypercholesterolemic chickens. Body weight gain and feed intake were similar in treated and control groups. The decrease in total serum cholesterol, therefore, could not be attributed to a decrease in exogenous cholesterol intake. Various doses of cod liver oil were tested in the chicken (table 6), and a nonlinear dose response was obtained. Increasing the cod liver oil concentration of the ration resulted in less efficient hypocholesterolemic activity.

[4] Through the courtesy of Dr. E. H. Ahrens and Dr. J. W. Farquhar of the Rockefeller Institute, New York.

TABLE 4

Effect of various oils on serum cholesterol in the chicken

Treatment[1]	No. of birds[2]	Total serum cholesterol			Body weight			Feed consumption
		Initial	7 Day	Change	Initial	7 Day	Change	
		mg/100 ml	*mg/100 ml*	%	*g*	*g*	%	*g/bird/day*
NR116	8	2087 ± 140[3]	1992 ± 173[3]	− 4.5	541 ± 25[3]	693 ± 33[3]	28.3	44.6
NR116 − 10% Coconut oil[4]	8	2206 ± 317	1705 ± 183	− 20.7	501 ± 26	635 ± 34	27.0	42.9
NR116 + 10% corn oil	8	2103 ± 237	1320 ± 225	− 37.2	516 ± 36	676 ± 48	31.0	46.4
NR116 + 10% Safflower oil	8	1795 ± 180	1092 ± 110	− 39.4	541 ± 38	718 ± 41	41.1	46.4
NR116 + 10% Linseed oil	8	1542 ± 156	1180 ± 108	− 24.7	504 ± 27	665 ± 33	32.0	55.4
NR116 + 10% Whale oil	8	2062 ± 274	1118 ± 164	− 45.9	543 ± 16	685 ± 28	26.3	42.9
NR116 + 10% Cod liver oil	8	3085 ± 314	1149 ± 198	− 62.8	496 ± 37	652 ± 54	31.2	41.1
NR116 − 20% Coconut oil[5]	8	2112 ± 197	805 ± 78	− 60.9	502 ± 18	655 ± 23	30.4	48.2
NR116	8	1922 ± 149	2295 ± 223	19.4	1181 ± 44	1338 ± 72	13.2	89.3
NR116 + 20% Butter[6]	8	1550 ± 218	1715 ± 266	10.6	1147 ± 72	1320 ± 79	15.1	89.3
NR116 + 10% Safflower oil	8	1356 ± 209	1246 ± 218	− 8.1	1228 ± 100	1400 ± 133	14.0	89.3
NR116 + 10% Butter[6] + 10% safflower oil	8	1458 ± 181	1442 ± 235	− 1.1	1192 ± 95	1394 ± 110	16.9	89.3
NR116 + 10% Cod liver oil	8	1825 ± 193	1156 ± 198	− 36.7	1186 ± 75	1356 ± 95	14.3	89.3
NR116 + 10% Butter[6] + 10% cod liver oil	8	1590 ± 139	1131 ± 205	− 28.9	1236 ± 52	1397 ± 55	13.0	89.3
NR116	10	2430 ± 276	2077 ± 265	− 14.5	1894 ± 89	1969 ± 89	4.0	64.3
NR116 + 2% Safflower oil	10	1613 ± 227	1255 ± 244	− 22.2	1983 ± 116	2127 ± 141	7.3	74.3
NR116 + 2% Cod liver oil	10	1956 ± 271	1078 ± 337	− 44.9	2007 ± 73	2211 ± 83	10.2	82.8

[1] Pre-fed the basal ration NR116 from one day of age. An equal amount of coconut oil was removed from the ration when a test oil was included.
[2] An equal number of male and female Arbor Acres White Rock birds were used in each treatment.
[3] Mean ± SE.
[4] 10% Coconut oil only.
[5] Fat-free ration.
[6] Butter was salt free.

TABLE 5

Comparison of the effect of cod liver oil and menhaden oil on serum cholesterol in the chicken

Group	No. of birds[2]	Treatment[1]			Body weight			Feed consumption	
		0–7 Days	7–22 Days	22–29 Days	Initial	29 Days	Change	0–7 Days	22–29 Days
					g	*g*	%	*g/bird/day*	
A	20	NR116	NR116	NR116	785 ± 25[3]	1140 ± 35[3]	45.2	49	63
B	20	NR116 + 2% cod liver oil	NR116	NR116 + 2% menhaden oil	769 ± 18	1159 ± 30	50.7	51	61
C	19	NR116 + 2% menhaden oil	NR116	NR116 + 2% cod liver oil	812 ± 22	1173 ± 32	44.5	50	66

Total serum cholesterol

Group	Initial	7 Days	22 Days	29 Days	Change		
					0–7 Days	7–22 Days	22–29 Days
	mg/100 ml	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	%	%	%
A	1847 ± 113[3]	1807 ± 121[3]	2169 ± 140[3]	2221 ± 153[3]	0.4	20.0	2.4
B	1689 ± 81	1302 ± 87	2032 ± 92	1439 ± 112	−22.9	56.2	−29.2
C	1742 ± 133	1118 ± 93	1622 ± 118	1271 ± 114	−35.7	44.8	−21.9

[1] Fed pre-test diet (NR116) from one day to 10 weeks of age. An equal amount of coconut oil was removed from the ration when a test oil was included.
[2] White Leghorn cockerels.
[3] Mean ± SE.

TABLE 6

Effect of cod liver oil on serum cholesterol in the chicken

Treatment[1]	No. of birds[2]	Body weight			Feed consumption	Total serum cholesterol		
		Initial	7-Day	Change		Initial	7-Day	Change
		g	*g*	%	*g/bird/day*	*mg/100 ml*	*mg/100 ml*	%
NR116	9	970 ± 70[3]	1082 ± 74[3]	11.5	59	1724 ± 283[3]	2047 ± 228[3]	18.7
NR116 + 1% cod liver oil	9	800 ± 41	898 ± 46	12.3	48	1377 ± 278	1247 ± 203	− 9.4
NR116 + 2.5% cod liver oil	9	846 ± 54	908 ± 54	7.3	48	1333 ± 128	1119 ± 152	−16.0
NR116 + 5% cod liver oil	10	1028 ± 78	1107 ± 82	7.7	55	1087 ± 160	845 ± 116	−22.3
NR116 + 10% cod liver oil	9	1041 ± 64	1128 ± 68	8.4	56	1214 ± 185	870 ± 165	−28.3
NR116 + 20% cod liver oil	8	998 ± 70	1048 ± 88	5.0	53	1095 ± 199	659 ± 105	−39.8

[1] Fed pre-test diet (NR116) from one day to 12 weeks of age. Cod liver oil replaced an equal amount of coconut oil.
[2] White Leghorn cockerels.
[3] Mean ± SE.

The hypocholesterolemic activities of the ethyl esters of cod liver oil (EECLO) and the ethyl esters of menhaden oil (EEMO) are shown in table 7. A similar study is outlined in table 8 in which EECLO and EEMO were fed at 3 different levels to hypercholesterolemic chickens. Here the experimental feeding regimen was as follows: test rations for 7 days, basal ration NR116 for 14 days, test rations with equal amounts of the 2 oils interchanged for 7 days, basal NR116 for 14 days, and finally test rations for 7 days with oils administered in reverse dose order. The data both in tables 7 and 8 illustrate the hypocholesterolemic action of the 2 oil preparations. The hypocholesterolemic effect of the fish oils was obtained only during the period in which they were fed, and hypercholesterolemia returned when the fish oils were withdrawn from the rations. The results indicate that EEMO and EECLO were comparable in activity. The average percentage of changes in total serum cholesterol from pre-treatment levels for birds fed the basal ration (NR116) plus 2% cod liver oil or the basal ration plus 0.5% EECLO were − 18.0% and − 23.5%, respectively. These data represent the average of 4 separate experiments in which the 2 treatments were compared, and where a total of 40 birds were fed 2% cod liver oil and 100 birds were fed 0.5% EECLO. From this and from the previous studies with complete fish oil, it appeared that 0.5% EECLO was equivalent in hypocholesterolemic potency to 2% whole cod liver oil. This was a fourfold increase in hypocholesterolemic activity. The EECLO fraction (I, no. 315) represented approximately 30% of the original cod liver oil.

The EECLO was further fractionated by molecular distillation (table 2). Ten condensates were collected, and 4 were bioassayed in the chicken, these being the original EECLO fraction and condensates 1, 3, 7, and 10. Table 9 describes the polyunsaturated fatty acid composition of these EECLO fractions, and shows the results obtained when these condensates were fed to chickens at the 0.5% level of the total ration. The fraction with the lowest iodine number (EECLO-1) did not appreciably influence the total serum cholesterol of the chickens. The condensate with the high-

TABLE 7

Comparison of the effect of the ethyl esters of cod liver oil and of menhaden oil on serum cholesterol in the chicken

Group	No. of birds[2]	Treatment[1]			Body weight			Feed consumption	
		0–7 Days	7–20 Days	20–27 Days	Initial	27-Day	Change	0–7 Day	20–27 Day
					g	*g*	%		
A	10	NR116	NR116	NR116	467 ± 22[3]	894 ± 36[3]	91.4	39	36
B	9[4]	NR116 + 0.125% EECLO	NR116	NR116 + 0.125% EEMO	448 ± 16	843 ± 12	88.2	42	52
C	10	NR116 + 0.5% EECLO	NR116	NR116 + 0.5% EEMO	434 ± 15	845 ± 27	94.7	40	53
D	10	NR116 + 2% EECLO	NR116	NR116 + 2% EEMO	430 ± 15	818 ± 34	90.2	39	42
E	10[4]	NR116 + 0.125% EEMO	NR116	NR116 + 0.125% EECLO	439 ± 19	779 ± 71	77.4	35	40
F	10	NR116 + 0.5% EEMO	NR116	NR116 + 0.5% EECLO	435 ± 13	842 ± 28	93.6	39	43
G	10	NR116 + 2% EEMO	NR116	NR116 + 2% EECLO	432 ± 19	851 ± 44	97.0	38	42

Total serum cholesterol

Group	Initial	7 Days	20 Days	27 Days	Change		
					0–7 Days	7–20 Days	20–27 Days
	mg/100 ml	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	%	%	%
A	1960 ± 117[3]	1914 ± 127[3]	1818 ± 150[3]	1874 ± 85[3]	− 2.3	− 0.5	3.1
B	2144 ± 179	1860 ± 143	2175 ± 116	2142 ± 105	− 13.2	16.9	− 0.2
C	1988 ± 196	1524 ± 186	1805 ± 195	1353 ± 152	− 23.3	18.4	− 25.0
D	1897 ± 205	1169 ± 70	1774 ± 193	1256 ± 98	− 33.1	51.8	− 29.2
E	2130 ± 189	1904 ± 196	2189 ± 206	2296 ± 232	− 10.6	15.0	4.9
F	1919 ± 173	1474 ± 157	2094 ± 151	1536 ± 129	− 23.6	42.1	− 26.6
G	2204 ± 313	1411 ± 102	2116 ± 222	1267 ± 160	− 36.0	50.0	− 40.1

[1] Fed pre-test diet (NR116) from one day to 6 weeks of age; EECLO = ethyl ester of cod liver oil; EEMO = ethyl esters of menhaden oil. An equal amount of coconut oil was removed from the diet when a test oil was included.

[2] White Leghorn cockerels.

[3] Mean ± SE.

[4] During the 20 to 27 day treatment period, group B had only 8 birds and group E had 9 birds.

TABLE 8

Comparison of the effect of the ethyl esters of cod liver and of menhaden oil on serum cholesterol in the chicken

Group	No. of birds[2]	Treatment[1]					Body weight			Feed consumption		
		0–7 Days	7–21 Days	21–28 Days	28–42 Days	42–49 Days	Initial	49 Days	Change	0–7	21–28	42–49
							g	*g*	%	*g/bird/day*		
A	8	NR116	NR116	NR116	NR116	NR116	470 ± 10[3]	1151 ± 39[3]	144.9	43	52	58
B	10[4]	NR116 + 0.125% EECLO	NR116	NR116 + 0.125% EEMO	NR116	NR116 + 2% EEMO	439 ± 12	1111 ± 41	153.1	29	44	53
C	10	NR116 + 0.5% EECLO	NR116	NR116 + 0.5% EEMO	NR116	NR116 + 0.5% EEMO	457 ± 13	1253 ± 39	174.2	31	46	50
D	10[4]	NR116 + 2% EECLO	NR116	NR116 + 2% EEMO	NR116	NR116 + 0.125% EEMO	457 ± 13	1163 ± 62	154.5	30	46	57
E	9[4]	NR116 + 0.125% EEMO	NR116	NR116 + 0.125% EECLO	NR116	NR116 + 2% EECLO	438 ± 11	1126 ± 58	157.1	35	54	63
F	9	NR116 + 0.5% EEMO	NR116	NR116 + 0.5% EECLO	NR116	NR116 + 0.5% EECLO	433 ± 17	1129 ± 58	160.7	36	47	51
G	10[4]	NR116 + 2% EEMO	NR116	NR116 + 2% EECLO	NR116	NR116 + 0.125% EECLO	431 ± 15	1150 ± 47	166.8	31	56	62

Total serum cholesterol

Group	Initial	7 Days	21 Days	28 Days	42 Days	49 Days	Change				
							0–7 Days	7–21 Days	21–28 Days	28–42 Days	42–49 Days
	mg/100 ml	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	%	%	%	%	%
A	1936 ± 135[3]	1698 ± 194[3]	1391 ± 185[3]	1469 ± 175[3]	1649 ± 183[3]	1542 ± 162[3]	−12.3	−18.1	5.6	12.3	− 6.5
B	2040 ± 112	2004 ± 151	2042 ± 267	1667 ± 148	1753 ± 121	1257 ± 142	− 1.7	1.8	−18.4	5.2	−28.5
C	2103 ± 131	1647 ± 100	2142 ± 124	1483 ± 117	1876 ± 145	1477 ± 137	−21.7	30.1	−32.1	26.5	−21.3
D	2027 ± 81	1081 ± 103	1473 ± 130	925 ± 116	1443 ± 172	1297 ± 153	−46.7	36.3	−37.1	56.0	−10.1
E	1949 ± 123	1704 ± 119	1700 ± 124	1512 ± 90	1524 ± 133	1062 ± 102	−12.6	0.0	−10.5	1.1	−30.5
F	1959 ± 90	1468 ± 60	1582 ± 133	1190 ± 102	1576 ± 118	1308 ± 97	−25.1	7.8	−24.8	32.4	−17.0
G	2137 ± 170	1328 ± 118	1688 ± 126	1041 ± 120	1492 ± 212	1465 ± 195	−37.8	27.1	−38.3	43.3	− 1.8

[1] Fed pre-test diet (NR116) from one day to 6 weeks of age; EECLO = ethyl esters of cod liver oil; EEMO = ethyl esters of menhaden oil. An equal amount of coconut oil was removed from the diet when test oil was included.

[2] Mean ± SE.

[3] White Leghorn cockerels.

[4] From the 21st day on groups B and D had 9 birds and groups E and G had 8 birds.

TABLE 9

A comparison of hypocholesterolemic effects of several ethyl ester fractions of cod liver oil

Treatment[1]	Iodine no. of EECLO[2]	No. of birds[3]	Change in serum total cholesterol
			%
NR116		10	− 6
NR116+0.5% EECLO	315	10	−13
NR116+0.5% EECLO-1	220	10	− 5
NR116+0.5% EECLO-3	283	10	−19
NR116+0.5% EECLO-7	347	10	−17
NR116+0.5% EECLO-10	374	10	−24

[1] Pre-fed ration NR116 for 14 weeks. An equal amount of coconut oil was removed from the ration when an EECLO fraction was included. (EECLO = ethyl esters of cod liver oil.)
[2] Alkaline isomerization values for ethyl ester fractions of cod liver oil.

Fraction	Iodine no.	Dienoic	Trienoic	Tetraenoic	Pentaenoic	Hexaenoic
		%	%	%	%	%
EECLO	315	6.1	8.2	10.4	38.0	25.0
EECLO-1	220	5.5	3.0	13.1	12.3	3.0
EECLO-3	283	7.0	3.6	17.0	26.6	7.2
EECLO-7	347	5.1	6.9	8.5	49.1	20.5
EECLO-10	374	5.9	6.2	3.8	38.3	45.1

[3] 14-week old cockerels were used.

est iodine number (EECLO-10) promoted a twofold decrease in the total serum cholesterol when compared with the original ethyl ester fraction (EECLO). It appears that the unsaturation of the oil is the determining factor affecting the ability of a fish oil to lower serum cholesterol. The greatest increase in potency, fourfold, was gained in the concentration of the original cod liver oil into the first ethyl ester fraction (EECLO). This activity was improved approximately twofold in a fraction in which the iodine number was increased from 315 to 374.

Examination of the polyunsaturated fatty acid composition of the four EECLO fractions, shown in table 9, indicates that no one polyunsaturated fatty acid was solely responsible for the hypocholesterolemic activity of cod liver oil. The data rather suggest that an increase in unsaturation increases hypocholesterolemic activity; however a level of concentration can be reached where a further increase in the unsaturation of an oil does not cause a parallel decrease in total serum cholesterol. Thus there appears to be a limit to the amount of reduction in serum cholesterol that can be obtained with polyunsaturated fatty ester feeding. Beyond this point an increased consumption of unsaturated fatty esters or an increase in iodine number of the fatty ester does not appear to promote further reduction of the total serum cholesterol of a hypercholesterolemic chicken.

II. Effect of age on serum cholesterol in hypercholesterolemic chickens fed polyunsaturated oils. Forty White Leghorn chicks (20 males and 20 females) were fed ration NR116 from day of hatch to 2 months of age, and were then started on an unsaturated-saturated oil feeding program. An equal number of male and female birds were fed according to either a cod liver oil or safflower oil program. The effect of long-term alternate feeding of polyunsaturated and saturated oils to these chickens is shown graphically in figure 1. Total serum cholesterol diminished with time regardless of whether the birds were fed the polyunsaturated oils. The effect was observed in both male and female birds. This confirms Rodbard's report (24, 25) in which he observed a decrease in plasma cholesterol at 20 weeks in chicks fed a 2% cholesterol diet. Adamson and co-workers (26) have also reported this phenomenon. The polyunsaturated oils, however, continued to exert a hypocholesterolemic action until 112 days, although the extent of activity was not as pronounced as their initial effect. Alternate saturated oil feeding (coconut oil)

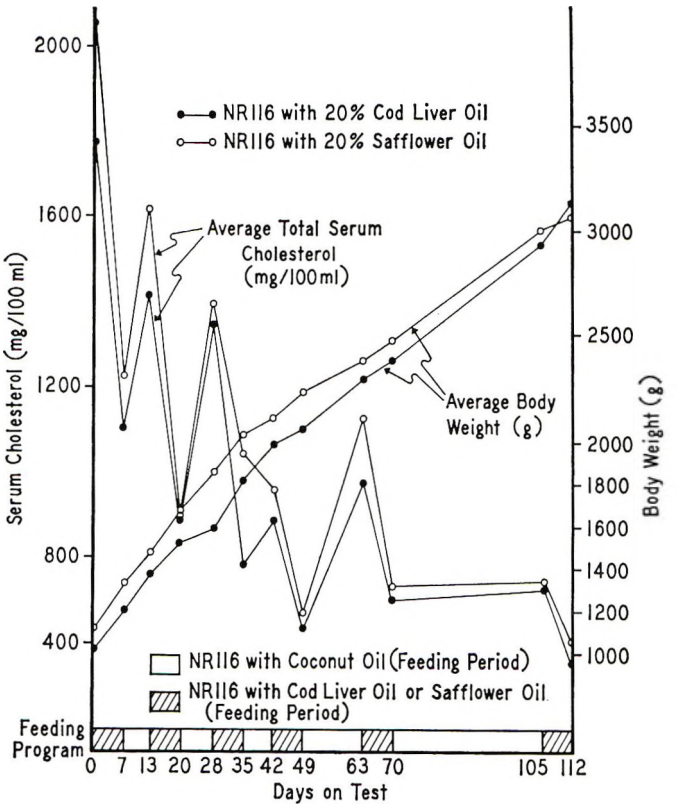


Fig. 1 The effect of long-term alternate feeding of polyunsaturated and saturated oils on total serum cholesterol of hypercholesterolemic chickens.

stimulated an increase in total serum cholesterol, but the amplitude of each successive peak, in all but one period, fell below the total serum cholesterol value of the previous saturated oil feeding period. The rate of growth of both the safflower and cod liver oil groups was similar and essentially parallel.

This reduction in total serum cholesterol, which occurs with age in hypercholesterolemic birds, may be due to a homeostatic mechanism. During this process of lowering the blood level, there is evidence to indicate that the excess cholesterol in the blood is deposited in the tissues (27).

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A Study of the Hypocholesterolemic Activity of the Ethyl Esters of the Polyunsaturated Fatty Acids of Cod Liver Oil in the Chicken

II. EFFECT ON SERUM AND TISSUE CHOLESTEROL AND AORTIC AND CORONARY ATHEROSCLEROSIS[1]

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ABSTRACT The polyunsaturated fatty acid esters of cod liver oil or menhaden oil were fed to both hypercholesterolemic and normocholesterolemic chickens. Serum phospholipid and serum, adrenal, abdominal aorta, and intestinal wall cholesterol concentrations were lowered in the hypercholesterolemic chicks. Serum glutamic-oxaloacetic transaminase and liver, spleen, brain, and thoracic aorta cholesterol concentrations did not differ from the values obtained in birds fed the basal diet. Only serum phospholipid and cholesterol were reduced in normocholesterolemic birds fed the fatty esters. The incidence of atherosclerosis in the aortic and coronary vessels of birds fed the polyunsaturated fatty acid esters, did not differ from their respective controls. The hypocholesterolemic effect of the polyunsaturated fatty esters was observed in 90-day-old chicks; however, after 400 days the effect was not seen.

The unsaturated fatty acids of vegetable and marine oils have been shown to possess hypocholesterolemic activity when fed to chicks made hypercholesterolemic by the feeding of cholesterol and saturated fat (1–6). It has been reported that the feeding of the polyunsaturated vegetable oils does not affect the development of aortic atherosclerosis (5, 7); however, Feigenbaum and co-workers (8) have reported greater atherosclerotic involvement in birds fed coconut oil and cholesterol than in those given diets supplemented with corn oil plus cholesterol. In this study, we report the effect of 2 polyunsaturated fish oil preparations, with iodine numbers of approximately 315, on serum and tissue cholesterol values and on aortic and coronary atherosclerosis.

EXPERIMENTAL

White Leghorn cockerels were used in this study. The birds were raised in electrically heated Wahmann brooders and at 4 weeks of age they were transferred to larger unheated cages where the environmental temperature was 22°C.

The hypercholesterolemic chick ration (NR116) fed in this study was described in our previous report (4). This purified ration contained a very high percentage of coconut oil plus cholesterol and sodium taurocholate which we (4) and other workers (9) had found to promote extremely high levels of serum cholesterol. The normocholesterolemic ration fed differed from ration NR116 in that cholesterol and sodium taurocholate were not incorporated into ration NR118. The 2 withdrawn ingredients were replaced by an equivalent amount of glucose monohydrate.[3] The test rations were started at one day of age. The polyunsaturated fish oil preparations were added to the experimental rations as 0.5% of the diet, substituting for an equivalent amount of coconut oil; thus all rations were kept isocaloric. A conventional growing ration was fed to another group of chicks. This ration consisted of: (expressed as % of ration) corn, 47.00; soybean oil meal (50% protein), 35.00; animal fat, 6.00; fish solubles (50% solids), 2.50; whey (50% lactose), 2.50; alfalfa, 2.00; CaH PO_4, 1.75; Ca CO_3, 1.60; DL-methionine, 1.00;

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[3] Cerelose, Corn Products Company, Argo, Illinois.

NaCl, 0.50; commercial trace mineral mix,[4] 0.10; vitamin mix,[5] 0.05; choline chloride, 0.05. The data obtained from these birds would be representative of normal birds.

The 2 polyunsaturated fish oil preparations were ethyl esters of the polyunsaturated fatty acids of cod liver oil (EECLO) and menhaden oil (EEMO). Their iodine numbers were determined by the Wijs method as given by Hawk et al. (10), and ranged between 315 to 320. The method of preparation of these fatty ester fractions, as well as their fatty acid compositions, was described in a previous paper (4). They were composed primarily of tetraenoic, pentaenoic, and hexaenoic fatty acids. To insure the stability of the polyunsaturated fatty esters, 0.05% butylated hydroxytoluene (BHT) was added to the preparations.

The following treatments were used: ration NR116 (hypercholesterolemic basal ration); ration NR116 + 0.5% ethyl esters of the polyunsaturated fatty acids of menhaden oil (EEMO); ration NR116 + 0.5% ethyl esters of the polyunsaturated fatty acids of cod liver oil (EECLO); ration NR118 (normocholesterolemic basal ration); ration NR118 + 0.5% EEMO; and ration NR118 + 0.5% EECLO. At the start of these studies there were 20 birds per ethyl ester treatment and 40 chicks were fed each basal diet. All the birds receiving the ethyl ester preparations and one-half the number of birds in each of the basal groups were killed at 90 days. The remaining chicks in each of the basal ration fed groups were divided into 2 treatments. One-half of the birds continued to be fed their respective basal ration, and the remaining half received the basal ration plus 0.5% EECLO. These birds were continued on test for another 300 days.

When the birds were killed the aorta was removed by dissection from the junction at the heart and its bifurcation into the iliac arteries. The adventitia was stripped off and the aorta split longitudinally and then divided into thoracic and abdominal sections at the point where the aorta coincides with the sixth vertebra. The aortic score was derived by multiplying the percentage of the area covered by the lesions and the severity of the lesions present. The latter was scored from 1 to 4 with increasing severity using the criteria outlined by Horlick and Katz (11). Tissue sections were taken from the lower third of formalin-fixed hearts, embedded in paraffin and stained with hemotoxylin-eosin. All arteries with a diameter of 100 μ or more were examined for lesions. Additional sections were frozen, stained with Oil red O, and all vessels with a clearly defined lumen and wall studied for fat deposition. The coronary score was the percentage of these arteries that exhibited any plaque formation. Frozen liver sections were stained with Oil red O and examined for sudanophilic material.

Total cholesterol was determined by a modification[6] of the Albers and Lowry (12) fluorometric procedure and was adapted to both blood and tissue samples. Intestinal contents were removed by flushing with distilled water and the various organs excised for analysis. Serum glutamic-oxalacetic transaminase (SGO-T) was determined by the Reitman and Frankel (13) procedure, and serum phospholipid phosphorous by the modified Youngberg method for lipid phosphorous (10).

RESULTS AND DISCUSSION

Body and organ weights

Three-month feeding period. In table 1 are presented the results on body weights, expressed as percentage of body weight. With but one exception, no significant differences exist between the ethyl ester fed birds and their respective control. The adrenal mean weight value for the chicks fed ration NR116 + 0.5% EECLO was significantly lower ($P < 0.01$) than that of the NR116 control group. Differences were observed between those birds given NR116 and NR118. The increased liver, adrenal, and aortic weights observed for the birds fed ration NR116, when compared with NR118, may be explained by the reduced body size of birds fed NR116 combined with an increase in their organs' tissue cholesterol and fat content (1, 14).

[4] Delamix, Limestone Corporation of America, Newton, New Jersey.

[5] Vitamin mix: (mg/kg ration) *d*-α-tocopheryl succinate, 50; nicotinamide, 33.8; Ca pantothenate, 5.5; riboflavin, 3.3; menadione, 2.2; vitamin A acetate, 2.752; vitamin D_3, 0.01875; cyanocobalamin, 0.015.

[6] Kahn, S. G., and H. Yacowitz 1958 Aspects of fluorometric analysis for total cholesterol in serum and tissue. Federation Proc., *17*: 991 (abstract).

TABLE 1

Effect of feeding 0.5% ethyl esters of cod liver oil or ethyl esters of menhaden oil on body and organ weights[1] (weights as percentage of body weight)

Treatment[2]	No. of birds[3]	Avg body wt	Liver	Spleen	Adrenal	Heart	Thoracic aorta	Abdominal aorta	Total feed consumed/ bird	Feed efficiency[4]
		g							*kg*	
NR116	16	1048	3.51 ± 0.13[5]	0.195 ± 0.017	0.0084 ± 0.0004	0.550 ± 0.026	0.0267 ± 0.0013	0.0056 ± 0.0004	3.35	3.34
NR116 + 0.5% EEMO	13	1118	3.38 ± 0.22	0.166 ± 0.013	0.0075 ± 0.0004	0.482 ± 0.026	0.0251 ± 0.0016	0.0054 ± 0.0004	3.65	3.38
NR116 + 0.5% EECLO	16	1144	3.28 ± 0.12	0.197 ± 0.009	0.0064 ± 0.0002	0.517 ± 0.019	0.0218 ± 0.0006	0.0049 ± 0.0002	3.37	3.05
NR118	18	1275	2.38 ± 0.07	0.218 ± 0.017	0.0055 ± 0.0003	0.561 ± 0.017	0.0192 ± 0.0005	0.0040 ± 0.0002	3.20	2.58
NR118 + 0.5% EEMO	19	1295	2.49 ± 0.09	0.238 ± 0.016	0.0060 ± 0.0002	0.526 ± 0.018	0.0194 ± 0.0007	0.0036 ± 0.0002	3.38	2.69
NR118 + 0.5% EECLO	14	1339	2.43 ± 0.09	0.259 ± 0.011	0.0051 ± 0.0003	0.574 ± 0.016	0.0183 ± 0.0007	0.0045 ± 0.0002	3.65	2.80
NR109	10	1399	1.74 ± 0.07	0.233 ± 0.022	0.0047 ± 0.0002	0.487 ± 0.022	0.0191 ± 0.0021	0.0051 ± 0.0004	—	—

[1] Three-month feeding period.

[2] Rations: NR116 = hypercholesterolemic; NR118 = normocholesterolemic; NR109 = conventional; EECLO, EEMO = ethyl esters of cod liver oil and menhaden oil, respectively.

[3] Three-month-old White Leghorn cockerels.

[4] Grams of feed per gram of gain.

[5] Mean ± SE.

TABLE 2

Effect of feeding 0.5% ethyl esters of cod liver oil or ethyl esters of menhaden oil on tissue cholesterol and serum cholesterol, phospholipid and glutamic-oxaloacetic transaminase[1]

Treatment[2]	No. of birds[3]	Total cholesterol									Serum P.L.[5]	C/P[6]	Serum GO-T[7]
		Liver	Spleen	Adrenal	Brain	Duodenum	Ileum[4]	Thoracic aorta	Abdominal aorta	Serum			
		mg/g	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/100 ml*	*mg/100 ml*		U/*ml*
NR116	16	31.24	7.43	88.17	10.60	8.22	11.38	9.91	6.16	2090	647	3.23	270
NR116+ 0.5% EEMO	13	29.47	7.97	68.57[8]	12.94	5.82[9]	9.07	9.73	4.06[8]	1350[8]	460[8]	2.93	297
NR116+ 0.5% EECLO	16	32.54	6.25	68.88[8]	12.43	6.11[9]	8.74[9]	8.08	4.08[8]	1258[8]	430[8]	2.93	264
NR118	18	3.07	2.42	41.15	11.75	3.05	3.55	1.85	2.49	157	335	0.47	143
NR118+ 0.5% EEMO	19	2.96	2.82	37.17	10.20	3.05	3.25	1.91	2.49	131[8]	230[8]	0.57	150
NR118+ 0.5% EECLO	14	2.69	1.82[9]	35.06	11.01	3.10	3.28	1.74	2.28	132[8]	257[8]	0.51	133
NR109	10	3.76	3.24	28.92	10.74	2.05	2.50	2.09	4.69	76	—	—	—

[1] Three-month feeding period.
[2] Rations, NR116 = hypercholesterolemic; NR118 = normocholesterolemic; NR 109 = conventional; EECLO, EEMO = ethyl esters of cod liver oil and menhaden oil, respectively.
[3] Three-month-old White Leghorn cockerels.
[4] Ileum, first 25 cm only.
[5] Phospholipid (phospholipid phosphorus × 25).
[6] Cholesterol/phospholipid.
[7] Serum glutamic-oxaloacetic transaminase.
[8] $P < 0.01$ (highly significant).
[9] P between 0.01 and 0.05 (significant).

TABLE 3

Effect of long-term feeding 0.5% ethyl esters of cod liver oil on body and organ weights[1] (weights as percentage of body weights)

Treatment[2]	No. of birds[3]	Avg body wt	Liver	Spleen	Adrenal	Heart	Thoracic aorta	Abdominal aorta	Feed efficiency[4]
		g							
NR116	7	1772	2.39 ± 0.32[5]	0.090 ± 0.046	0.0092 ± 0.0013	0.412 ± 0.023	0.0215 ± 0.0033	0.0159 ± 0.0048	6.44
NR116 + 0.5% EECLO	6	1615	4.15 ± 0.92	0.086 ± 0.053	0.0120 ± 0.0008	0.514 ± 0.053	0.0233 ± 0.0029	0.0147 ± 0.0040	10.84
NR118	5	1858	1.46 ± 0.09	0.086 ± 0.002	0.0072 ± 0.0004	0.479 ± 0.048	0.0187 ± 0.0017	0.0061 ± 0.0006	9.53
NR118 + 0.5% EECLO	6	2010	1.69 ± 0.26	0.164 ± 0.062	0.0070 ± 0.0006	0.443 ± 0.022	0.0170 ± 0.0029	0.0071 ± 0.0004	8.37

[1] Thirteen-month feeding period.
[2] Rations, NR116 = hypercholesterolemic; NR118 = normocholesterolemic; EECLO = ethyl esters of cod liver oil.
[3] Thirteen-month-old White Leghorn cockerels.
[4] Grams of feed per gram of gain.
[5] Mean ± SE.

Feed efficiency values, defined as feed consumed per unit weight gain, were similar for all groups within a dietary treatment. This implies that all birds receiving cholesterol ingested similar amounts of this material, and that the differences in cholesterol values observed between groups fed the NR116 ration (table 2) were not due to variation in cholesterol intake.

Birds fed the conventional ration (NR 109) had smaller livers than chicks fed ration NR118. This may be due to the lower fat content of this diet (1).

Thirteen-month feeding period. No significant differences exist between the birds fed EECLO and their control group (table 3). Differences were found between birds fed rations NR116 and NR118 in respect to liver, adrenal, and abdominal aorta weights. These differences were previously noted at 3 months age (table 1) and were probably due to increased cholesterol and fat deposition in these body tissues.

Serum cholesterol

Three-month feeding period. The ethyl ester preparations promoted highly significant ($P < 0.01$) reductions in serum cholesterol (table 2) regardless of whether the birds were fed cholesterol. This occurred despite the oil preparation representing only 0.5% of the ration. Normal birds fed the conventional ration had the lowest total serum cholesterol values. This may have been due to the low fat and cholesterol content of ration NR109.

Thirteen-month feeding period. After 13 months on test, serum cholesterol values were found to be no different between EECLO-supplemented birds and their basal control group (table 4). This contrasts sharply with the significant differences found at 3 months (table 2), but serum cholesterol, particularly in hypercholesterolemic birds, declines with age perhaps due to a homeostatic mechanism (4) which decreases abnormally elevated serum cholesterol levels. We are not able to explain otherwise why the hypocholesterolemic activity of EECLO was not apparent at 13 months.

Serum phospholipid

Three-month feeding period. Serum phospholipid, calculated by multiplying

TABLE 4

Effect of long-term feeding of 0.5% ethyl esters of cod liver oil on tissue and serum cholesterol[1]

Treatment[2]	No. of birds[3]	Liver	Spleen	Adrenal	Brain	Intestine		Aorta		Serum
						Duodenum	Ileum[4]	Thoracic	Abdominal	
		mg/g	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/g*	*mg/100 ml*
NR116	7	25.28	15.95	100.63	9.71	15.40	17.80	12.21	30.35	935
NR116 + 0.5% EECLO	6	29.14	16.30	81.75	10.03	11.40	18.90	7.52	25.55	990
NR118	5	3.96	3.50	77.17	10.36	3.50	3.55	2.21	4.30	122
NR118 + 0.5% EECLO	6	4.81	3.78	54.27[5]	11.01	3.70	4.90	1.95	3.22	128

[1] Thirteen-month feeding period.
[2] Rations, NR116 = hypercholesterolemic; NR118 = normocholesterolemic; EECLO = ethyl esters of cod liver oil.
[3] Thirteen-month-old White Leghorn cockerels.
[4] Ileum, first 25 cm only.
[5] *P* between 0.01 and 0.05 (significant).

phospholipid phosphorous by 25, was very significantly ($P < 0.01$) reduced with ethyl ester feeding (table 2). In this respect, plus the fact that they were increased with cholesterol feeding, serum phospholipid values paralleled serum cholesterol. There were no differences within a dietary treatment for calculated cholesterol: phospholipid (C/P) values (table 2). The C/P values indicated that cholesterol feeding of chicks caused a much greater increase in serum total cholesterol than in serum phospholipid; the differences were approximately five- to sixfold. If a low C/P plays an important role in reducing the incidence of atherosclerosis,[7] then feeding chicks cholesterol should stimulate atherogenesis, which did occur (table 5 and table 6) (15).

Serum glutamic-oxaloacetic transaminase (SGO-T)

Three-month feeding period (SGO-T). The SGO-T data given in table 2 show that no differences exist in the activity of this enzyme between groups within a dietary treatment. Feeding cholesterol did increase SGO-T levels. This phenomenon may have resulted from the increased fat and cholesterol concentration of the liver, an abnormal liver condition having been reported to increase SGO-T values (16,17).

Liver cholesterol

Three-month feeding period. Feeding cholesterol greatly increased liver cholesterol (table 2), and ethyl ester feeding did not prevent the accumulation; neither did it promote an increase in cholesterol deposition. Although the birds fed ration NR 109 had more cholesterol per gram of liver than NR118 fed chicks, their smaller liver weights made total liver cholesterol concentrations similar.

Thirteen-month feeding period. Liver cholesterol concentration (table 4) in cholesterol-fed birds did not change from the values observed in 3-month-old birds (table 2). Values from chicks fed ration NR118, however, did indicate an increase in liver cholesterol. This increase may be due to the high fat and essential fatty

[7] Ladd, A. T., A. Kellner and J. W. Correll 1949 Intravenous detergents in experimental atherosclerosis, with special reference to the possible role of phospholipids. Federation Proc., *8:* 360 (abstract).

TABLE 5

Effect of feeding 0.5% ethyl esters of cod liver oil or ethyl esters of menhaden oil on the aortic score of chicks[1]

Treatment[2]	No. of birds[3]	Aortic score	
		Thoracic	Abdominal
NR116	16	46 ± 9[4]	28 ± 6
NR116 + 0.5% EEMO	13	55 ± 12	38 ± 7
NR116 + 0.5% EECLO	16	44 ± 9	37 ± 6
NR118	18	2.5 ± 0.08	35 ± 7
NR118 + 0.5% EEMO	19	4.4 ± 1.5	29 ± 7
NR118 + 0.5% EECLO	14	1.5 ± 0.05	22 ± 5
NR109	10	1.0 ± 0.0	1.0 ± 0.0

[1] Three-month feeding period.
[2] Rations, NR116 = hypercholesterolemic; NR118 = normocholesterolemic; NR109 = conventional; EECLO, EEMO = ethyl esters of cod liver oil and menhaden oil, respectively.
[3] Three-month-old White Leghorn cockerels.
[4] Mean score ± SE.

TABLE 6

Effect of feeding 0.5% ethyl esters of cod liver oil or ethyl esters of menhaden oil on the coronary score of chicks[1]

Treatment[2]	No. of birds[3]	Avg % damaged coronary arteries	
		H-E stain[4]	Fat stain[5]
NR116	9	20.2 ± 4.6[6]	39.9 ± 6.0
NR116 + 0.5% EEMO	7	33.7 ± 6.5	49.8 ± 5.6
NR116 + 0.5% EECLO	8	19.5 ± 5.3	32.2 ± 6.0
NR118	9	0	0
NR118 + 0.5% EEMO	9	0	0
NR118 + 0.5% EECLO	7	0	0
NR109	10	0	0

[1] Three-month feeding period.
[2] Rations, NR116 = hypercholesterolemic; NR118 = normocholesterolemic; NR109 = conventional; EECLO, EEMO = ethyl esters of cod liver oil and menhaden oil, respectively.
[3] Three-month-old White Leghorn cockerels.
[4] H-E stain, hemotoxylin eosin.
[5] Fat stain, Oil red O.
[6] Mean score ± SE.

acid free ration fed (18). The polyunsaturated fatty esters of cod liver oil did not prevent this condition of an essential fatty acid deficiency.

Spleen cholesterol

Three-month feeding period. Ethyl ester feeding did not inhibit the accumulation of cholesterol into the spleens of cholesterol fed chicks (table 2). The EECLO preparation did promote a significant ($P < 0.05$) reduction in spleen cholesterol concentration per gram of tissue in chicks fed ration NR118; however, total spleen cholesterol concentration for this group did not differ greatly from that of its control group.

Thirteen-month feeding period. The concentration of cholesterol in the spleens of 13-month-old roosters (table 4) was greater than the values found in 3-month-old cockerels (table 2). Birds fed ration NR116, in particular, doubled their spleen cholesterol concentration. The EECLO preparation did not inhibit the increase in cholesterol accumulation in the spleen.

Brain cholesterol

Three-month-feeding period. The cholesterol concentration of brain per gram of tissue (table 2) was constant regardless of the ration fed. Our values correspond to those obtained by Dam and co-workers (2). Even birds fed ration NR109 did not differ in this respect. Davison and co-workers (19, 20) have reported that cholesterol-4-C^{14}, when incorporated into the brain tissue of freshly hatched chicks or young rabbits persisted for longer than a year in the central nervous system, although it disappeared from other organs in a relatively short time. McMillan et al.

(21) showed that intracisternally injected acetate was incorporated more rapidly and to a greater extent into the brain cholesterol of younger than of older rats. They also noted that intracisternal administration was much more effective than the intraperitoneal route in labeling brain cholesterol. However, the radioactivity of liver cholesterol was substantially the same for these 2 routes of administration. It is apparent that a blood brain barrier to exogenous cholesterol exists and that a large portion of the brain cholesterol is derived from *in situ* endogenous synthesis. This would explain the absence of increased brain cholesterol in cholesterol feeding.

Thirteen-month feeding period. As observed at 3 months (table 2), brain cholesterol values did not differ between any of the groups, regardless of the ration fed (table 4). The concentration of cholesterol in the brain was found to be the same in 3-month and 13-month-old birds.

Adrenal cholesterol

Three-month feeding period. Our chick data (table 2) substantiate the results reported (14, 22) in the rat that increased fat in the ration increases adrenal sterol content. The addition of cholesterol to the ration (NR116) accentuated this effect. Alfin-Slater and co-workers (18) have shown that essential fatty acid deficiency in the rat promoted an increase in adrenal cholesterol levels. The basal experimental rations (NR116 and NR118) fed to our birds were very low in essential fatty acids. A combination of high saturated fat, low levels of essential fatty acids, and cholesterol feeding contributed to the very high adrenal sterol values obtained in our birds. Feeding the polyunsaturated ethyl esters prevented, to some extent, the accumulation of cholesterol in the adrenal of birds fed ration NR116. The nonessential polyunsaturated fatty acids may function in a similar capacity in adrenal metabolism as the essential fatty acids. Since adrenal cholesterol is reported to be 80 to 90% esterified (23), it is not unlikely, as has been suggested by Sinclair (24), that polyunsaturated cholesterol esters are necessary for proper sterol metabolism.

Thirteen-month feeding period. The results in table 4 suggest that cholesterol feeding for 3 months will saturate the adrenal with cholesterol, since further feeding of ration NR116 did not significantly change adrenal cholesterol levels from the 3-month value of 88.17 mg/g (table 2). The 0.5% EECLO supplementation did not reduce adrenal cholesterol once the organ became saturated. Adrenal cholesterol was increased in birds fed ration NR118 for 13 months; however EECLO supplementation significantly ($P < 0.05$) retarded the deposition of cholesterol in the adrenal of these birds. The polyunsaturated fatty esters may function in the adrenal in a capacity similar to the essential fatty acids in preventing as rapid an accumulation of cholesterol as was observed in the NR118 control group.

Intestinal wall cholesterol

Three-month feeding period. Two segments of the intestine were analyzed, the duodenal loop and the succeeding 25-cm segment of the ileum. Cholesterol feeding greatly increased the tissue cholesterol content of these segments of the intestine. Both the EEMO and EECLO preparations significantly reduced ($P < 0.05$) the cholesterol concentration of the duodenal loop of birds fed ration NR116. Tissue cholesterol content of the first 25-cm section of the ileum was significantly ($P < 0.05$) lower with EECLO feeding, whereas the concentration in an equivalent section of the intestine of EEMO-supplemented birds approached being significantly lower than that of control chicks fed ration NR116. The feeding of the ethyl ester preparations did not significantly affect the concentration of cholesterol in the intestinal wall of birds fed ration NR118. We interpret these results as an indication of a reduction in exogenous cholesterol absorption under conditions of polyunsaturated fatty acid ester feeding.

Thirteen-month feeding period. Birds fed ration NR118 did not show a change from their 3-month intestinal cholesterol values (table 4). Cholesterol concentrations increased in both the duodenal and ileum segments of birds fed ration NR116, but the significant differences observed at 3 months (table 2) with EECLO feeding were absent at 13 months.

Aortic cholesterol

Three-month feeding period. Feeding cholesterol influenced the cholesterol content of the aorta (table 2). This has been shown by Dam and co-workers (2) and by Feigenbaum et al. (8). At 3 months of age, the thoracic aorta possessed a greater cholesterol concentration than the abdominal aorta in birds fed ration NR116. The reverse was true in birds not fed cholesterol. The slightly higher cholesterol values in the aortae of birds fed ration NR109 as compared with those fed NR118 may be due to the absorption of the natural plant sterols present in a conventional chick-growing ration. Ration NR118 was essentially sterol-free. Only in chicks fed ration NR116 did EEMO or EECLO supplementation significantly reduce aortic cholesterol concentration, and this reduction was restricted to the abdominal section of the aorta. A comparison of birds fed NR116 and NR118 for 3 months, suggests that elevated serum cholesterol may influence aortic cholesterol concentration.

Thirteen-month feeding period. The pattern of cholesterol concentration in aortic tissues of birds fed ration NR116 was changed at 13 months (table 4). Thoracic cholesterol did not significantly increase over that of the 3 month value (table 2) but the abdominal aortic cholesterol concentration increased approximately five- to sixfold. The EECLO did not significantly inhibit this accumulation of cholesterol into abdominal aortic tissue as it appeared to do at 3 months age. The difference in protein composition existing between abdominal and thoracic aortic tissue (25) may be one contributory factor for what appears to be a greater diffusion of serum cholesterol into the abdominal aorta. The apparent increase in abdominal cholesterol of 13-month-old birds fed ration NR118 was not significantly different from the values obtained at 3 months.

Aortic score

Three - month - feeding period. Mean aortic score values (table 5) indicated that treatment with EEMO or EECLO did not reduce the severity of atherosclerosis. Recently Fisher et al. (26) reported that the unsaturated fatty acid fraction from corn oil was ineffective in preventing atherosclerosis in chickens. Our data demonstrate that feeding cholesterol to chicks promotes atherogenesis, but the degree of atherosclerosis was not related to the cholesterol concentration of the aorta, as shown by the lack of difference in aortic score between abdominal aortae of birds fed ration NR116 and the 2 ethyl esters. This confirms the observation of Feigenbaum and co-workers (8). At 3 months, aortic atherosclerosis was slightly greater, although not significantly, in the thoracic than in the abdominal aortae of birds fed cholesterol. Birds fed ration NR118 had atheromatae only in the abdominal aorta, not in the thoracic aorta. These observations may explain the conflicting reports that have been published. Katz and Stamler (11) reported that exogenous cholesterol feeding initiated a more severe atherosclerosis in the thoracic aorta, but Fisher and co-workers (27, 28) and Siller (29, 30) maintain that the abdominal segment becomes more severely lesioned in the chicken. Our results from 3-month-old cockerels indicated that both situations occur depending on whether exogenous cholesterol was fed.

At 3 months of age, birds fed a conventional ration (NR109) had not formed gross aortic lesions.

Thirteen-month feeding period. Feeding of EECLO for the last 10 of 13 months did not reduce or inhibit the severity of atherosclerosis (table 7). Atherosclerosis continued to develop in all birds irrespective of the diet fed, although it continued to be much more severe in the cholesterol-supplemented birds. The level of cholesterol in the aorta did not parallel the severity of atherosclerosis. The thoracic and abdominal aortae were equally affected in birds fed ration NR116. Birds given NR 118 again showed greater plaque formation in the abdominal aorta with only very slight involvement in the thoracic section; however the differences were not statistically significant.

Coronary score

Three-month feeding period. Mean coronary scores are shown in table 6. The feeding of EEMO or EECLO did not prevent fatty infiltration of the coronary ves-

TABLE 7

Effect of long-term feeding of 0.5% ethyl esters of cod liver oil on the aortic score of chicks[1]

Treatment[2]	No. of birds[3]	Aortic score	
		Thoracic	Abdominal
NR116	7	179 ± 50[4]	195 ± 35
NR116 + 0.5% EECLO	6	114 ± 56	169 ± 26
NR118	5	13 ± 12	36 ± 15
NR118 + 0.5% EECLO	6	19 ± 16	97 ± 19

[1] Thirteen-month feeding period.
[2] Rations, NR116 = hypercholesterolemic; NR118 = normocholesterolemic; EECLO, EEMO = ethyl esters of cod liver oil and menhaden oil, respectively.
[3] Thirteen-month-old White Leghorn cockerels.
[4] Mean score ± SE.

sels of NR116 fed birds. Chicks fed ration NR118 or NR109 were free from coronary damage, so determined by this method, at 3 months of age. The results indicated that cholesterol feeding was necessary for the development of experimental coronary atherosclerosis.

Liver histology

Three-month feeding period. The feeding of ration NR116 promoted moderate-to-heavy infiltration of the liver cells with stainable fat as well as mild-to-heavy deposition of fat droplets outside the hepatic cells. When cholesterol was eliminated from the ration (NR118) a reduction in extracellular and intracellular stainable liver fat was observed. Supplementation of either ration with 0.5% polyunsaturated fatty esters did not influence the amount of stainable fat in the liver.

GENERAL DISCUSSION

A severe atherosclerotic condition develops when hypercholesterolemia is induced by cholesterol supplementation; however, from the data presented, a significant change in the serum total cholesterol level of the chicken is not necessarily reflected by a parallel change in aortic or coronary atherogenesis. This is also evident when polyunsaturated fatty esters of cod liver oil or menhaden oil are fed. Although EEMO and EECLO lower serum cholesterol, they do not influence aortic or coronary atherogenesis. This observation may be true for many other hypocholesterolemic compounds, and it raises the question whether the measurement of serum cholesterol alone is a valid criterion in the evaluation of anti-atherosclerotic compounds.

Apparently, under conditions of cholesterol loading certain organs have the ability to accumulate great amounts of this material, whereas other tissues maintain a constant cholesterol level. The polyunsaturated fatty acids appear to influence tissue cholesterol only when a well-established excess of cholesterol is present in the body.

The hypocholesterolemic action of the polyunsaturated fatty esters is lost after prolonged feeding in older chickens. Exactly why this occurs is not known. We can only speculate that the chicken develops homeostatic conditions which negate the hypocholesterolemic activity of the polyunsaturated fish oil.

In these studies, continuous ingestion of 0.5% ethyl ester was well tolerated by the chicken. It is doubtful whether large amounts of the oils would have been more efficacious, since highly significant reductions in serum cholesterol were obtained at 3 months with this low level. It is also questionable that the feeding of larger amounts of fish oils would prolong their hypocholesterolemic action.

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Counteracting the Growth Retardation of Raw Soybean Meal with Extra Protein and Calories[1]

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ABSTRACT Day-old or 3-week-old chicks were given raw or heated soybean diets alone or supplemented with extra protein or corn oil or both. The one-day-old chicks did not respond well to protein, amino acid, or corn oil supplementation when the diet contained more than 15% raw soybean protein. On the other hand, 3-week-old chicks responded very well to supplementation of raw soybean diets with protein or corn oil, or both. With 27% raw soy protein and 15% additional corn oil, growth equivalent to that of 27% heated soy protein without extra corn oil was obtained. Thus, the growth depression of raw meal could be overcome through protein and calorie supplementation. At equivalent levels of energy and protein intake, however, the raw soybean diets were inferior to heated soybean diets indicating that the nutritional defect responsible for the growth depression on raw soybean diets affects energy and protein utilization.

The nature of the nutritional defect responsible for the poor growth of experimental animals given raw soybean meal in comparison with heated soybean has continued to elude the investigator. Earlier suggestions that the presence of trypsin inhibitor [chickens (1)] or of a toxic principle [rats (2)] might be responsible for the growth depression have been generally discarded. Rackis et al. (3) working with rats, recently concluded that "all raw [soybean] fractions contain widely different levels of trypsin inhibitor activity as measured chemically; and, there appears to be no direct relationship between trypsin inhibitor activity, growth inhibition, and pancreatic hypertrophy." In previous work from this laboratory with chicks (4, 5) the importance of a toxic principle in raw soybeans was ruled out when we showed that increasing levels of raw soybean meal improved rather than further depressed growth.

In 1958 Fisher and Johnson (5) showed that supplementation of a 15% protein raw soybean diet with a balanced mixture of essential amino acids would overcome most of the growth retardation observed in the chick. This was confirmed by Booth et al. (6) and also by Borchers (7), all working with rats. Recently, the work of Saxena et al. (8) suggested that amino acid supplementation of raw soybean meal might be effective only at suboptimal protein levels, since these workers showed only a partial effectiveness at an optimal level. These workers (8) therefore concluded that amino acid availability does not account for all of the growth-retarding activity of raw soybean meal.

It occurred to us that another interpretation of the inability of Saxena et al. (8) to overcome the growth retardation at higher protein levels may have been due to an altered calorie-to-protein ratio. This contention is supported by the work of Renner and Hill (9) and of Nesheim et al. (10) who have shown impaired fat absorption and energy utilization in chickens fed raw soybean meal. It was the purpose of the present investigation to study caloric density in relation to protein and amino acid supplementation of raw soybean diets. Our results indicate that 3-week-old, but not one-day-old chicks, can overcome the growth-retarding activity of raw soybean meal through proper supplementation of the diet with extra protein and calories.

GENERAL PROCEDURE

Fast-growing male or female crossbred chicks started to be fed experimental diets

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either at one day or at 3 weeks of age. In all but one experiment duplicate lots of 7 chicks were given the experimental diets for 2 weeks. Food and water were supplied ad libitum, and the chicks were reared in electrically heated batteries. The basal ration used in all experiments supplied in per cent: mineral mix,[2] 4.9; corn oil, 1; choline chloride (70% concentrate), 0.3; vitamins,[3] 0.25; DL-methionine,[4] 0.3; and glucose monohydrate to 100. The variable ingredients included fat-extracted soybean meal[5] (raw or heated), an amino acid mixture, isolated soybean protein, menhaden fish meal, fiber, and corn oil. The proportions of these materials are listed in the tables of results. The chickens placed on experiment at 3 weeks of age were given a standard starting ration from one day until the start of the experiment.

In experiment 2 we also measured oxygen consumption of representative birds selected from those on raw or heated soybean regimens. These measurements were carried out in an automated multiplace respirometer (11).

EXPERIMENTAL AND RESULTS

Experiment 1. This was designed to check the contention of Saxena et al. (8)

[2] For composition see Summers and Fisher (12).
[3] For composition see Summers and Fisher (12). The vitamins were generously supplied by Merck Sharp and Dohme, Rahway, New Jersey and Commercial Solvents Corporation, New York.
[4] A higher level of methionine was no more effective than the level used in these studies. The methionine was generously supplied by Dow Chemical Company, Midland, Michigan.
[5] The raw and heated meals were generously supplied by A. E. Staley Company, Decatur, Illinois.

TABLE 1

Growth response of one-day-old chicks to raw and heated soybean meal alone or in combination with other nitrogen supplements

Soybean meal protein[1]	Supplements		2-Week body wt[3]	Gain-to-feed ratio
	Protein[1]	Corn oil[2]		
%	%	%	g	
Raw, 15			109 ± 6	0.45
Raw, 25		3	136 ± 9	0.53
Raw, 32		7	138 ± 8	0.50
Heated, 15			161 ± 5	0.58
Heated, 25		3	182 ± 5	0.66
Heated, 32		7	182 ± 6	0.70
Raw, 15	isolated soy,[4] 12		148 ± 7	0.53
Raw, 15	fish,[5] 12		156 ± 10	0.56
Raw, 15	albumin,[6] 12		122 ± 5	0.50
Raw, 25	isolated soy, 12	3	158 ± 6	0.53
Raw, 25	fish, 12	3	158 ± 11	0.57
Raw, 25	albumen, 12	3	134 ± 10	0.47
Heated, 15	isolated soy, 12		181 ± 7	0.68
Heated, 15	fish, 12		190 ± 6	0.66
Heated, 15	albumen, 12		152 ± 4	0.58
Heated, 25	isolated soy, 12	3	187 ± 5	0.74
Heated, 25	fish, 12	3	200 ± 5	0.73
Heated, 25	albumen, 12	3	141 ± 9	0.54
Raw, 15	amino acids,[7] 12		149 ± 8	0.55
Raw, 25	amino acids, 12	3	143 ± 9	0.57
Raw, 25	amino acids, 20	3	126 ± 7	0.45
Heated, 15	amino acids, 12		164 ± 12	0.69
Heated, 25	amino acids, 12	3	182 ± 9	0.73
Heated, 25	amino acids, 20	3	156 ± 9	0.73

[1] N × 6.25.
[2] In addition to the quantity used in the basal ration.
[3] Mean values with standard errors for duplicate lots of 7 male chicks.
[4] ADM Assay Protein C-1, Archer-Daniels-Midland, Minneapolis.
[5] Supplied as menhaden fish meal.
[6] Egg albumen, Henningsen, Inc., New York.
[7] For composition see Fisher and Johnson (5).

that supplementation of optimal (25%) levels of raw soybean protein with additional protein or amino acids does not effectively overcome the growth retardation of chicks. In this experiment, increasing levels of raw or heated soybean protein, alone or in combination with other proteins or an amino acid mixture[6] were compared. The results (table 1) confirm the observation of Saxena et al. (8), since no appreciable improvement in growth was obtained when the basal diet, providing 25% of raw soybean protein was supplemented with protein or an amino acid mixture. These results also confirm our previous observations of a marked growth improvement when a 15% raw soy protein diet is similarly supplemented. Although supplementation of either raw or heated soybean meal with fish and isolated soy protein was very effective, poor growth was obtained with egg albumen. This is in agreement with our previous observations, but an explanation continues to elude us. The addition of a quantity of the amino acid mixture larger than that which was effective at the 15% level of raw soybean, depressed growth severely at the 25% level. A similar, although less severe, depression was also observed when the same amount of amino acids was added to heated soybean. This suggested that caloric intake might be limiting protein utilization and the next experiment was designed to evaluate dietary caloric density in relation to raw soybean supplementation with extra protein.

Experiment 2. In experiment 2, which is outlined in table 2, available caloric density of the diets was decreased through the addition of 10% fiber or increased by addition of 15% corn oil. This level of corn oil was used, since Nesheim et al. (10) had noted a defect in fat absorption in chicks fed raw soybean meal. A change was also made in the composition of the

[6] For composition see Fisher and Johnson (5).

TABLE 2

Growth response and oxygen consumption of one-day-old chicks given raw or heated soybean diets in combination with other protein supplements and different energy levels

Soybean meal protein[1]	Supplements		2-Week body wt[3]	Gain-to-feed ratio	Oxygen consumption
	Protein[1]	Other[2]			
%	%	%	*g*		*ml/g/hr*
Raw, 15	isolated soy,[4] 12		158 ± 6	0.68	
Raw, 15	isolated soy, 12	fiber,[5] 10	144 ± 5	0.62	
Raw, 15	isolated soy, 12	corn oil, 15	158 ± 10	0.80	
Heated, 15	isolated soy, 12		184 ± 6	0.92	
Heated, 15	isolated soy, 12	fiber, 10	176 ± 5	0.75	
Heated, 15	isolated soy, 12	corn oil, 15	193 ± 7	1.03	
Raw, 15	isolated soy + fish,[6] 6		142 ± 7	0.65	
Raw, 15	isolated soy + fish, 6	fiber, 10	115 ± 6	0.49	
Raw, 15	isolated soy + fish, 6	corn oil, 15	139 ± 12	0.63	33.2
Heated, 15	isolated soy + fish, 6		166 ± 8	0.74	
Heated, 15	isolated soy + fish, 6	fiber, 10	185 ± 6	0.80	
Heated, 15	isolated soy + fish, 6	corn oil, 15	222 ± 6	1.18	27.4
Raw, 25			139 ± 7	0.49	29.1
Heated, 25			179 ± 8	0.78	28.6

[1] N x 6.25.
[2] The corn oil additions were supplementary to the amount provided by the basal diet.
[3] Mean values with standard errors for duplicate lots of 7 female chicks.
[4] ADM Assay Protein C-1, Archer-Daniels-Midland, Minneapolis.
[5] Solka Floc, Brown Company, Berlin, New Hampshire.
[6] Supplied as menhaden fish meal.

diet common to all groups by using a combination of 12% isolated soy protein (as supplied by the manufacturer, not heat-treated) and 15% of raw or heated soybean protein. This made it physically possible to incorporate larger amounts of carbohydrate into the diet than if soybean meal with its poorly utilized carbohydrates (13) were used as the sole source of protein. As in experiment 1, one-day-old chicks were used.

The results in table 2 show that 15% corn oil did not improve growth of one-day-old chicks given raw soybean, but slightly improved the response of those given heated soybean meal. The addition of supplemental fish protein (6%) depressed growth at all energy levels for the raw soybean diets, but it improved growth with the heated soybean diets containing fiber or corn oil. This suggested that the chicks receiving heated soybean meal could utilize the additional protein because extra calories were being provided. The chicks fed the raw soybean diets, however, could not utilize the extra protein, presumably because the energy from corn oil was only partially available due to poor absorption (10). That calories are more limiting for raw than for heated soybean was further substantiated by the remarkably high gain-feed ratios for the 2 groups receiving heated soybeans (at 2 protein intakes) supplemented with corn oil. On the other hand, an improvement in gain-to-feed ratios due to corn oil, was shown only at the lower protein level when raw soybean was given. The oxygen consumption values for 2 paired groups of chicks given raw or heated soybean (table 2, last column) are similar, although slightly higher for the birds consuming raw meal. If the values were to be expressed on a surface area basis ($W^{0.67}$) or on the basis of $W^{0.75}$ the difference would disappear completely. Thus, a change in basal metabolic rate is probably not involved in the inferior utilization of corn oil by chicks given raw soybeans, and Nesheim's observation of poor absorption is most likely the major defect. Since Nesheim et al. (10) had found that the absorption defect was essentially alleviated after 2 weeks of age, the next 2 experiments were carried out with 3-week-old birds.

Experiment 3. This was designed to test the utilization by 3-week-old chicks of either raw or heated soybean meal with and without corn oil supplementation of diets essentially the same as those used in experiment 2. The growth retardation due to raw soybean was completely counteracted by the combination of extra protein and corn oil (table 3). This clearly indicates that 3-week-old chicks given extra corn oil utilized a raw soybean, high proten diet much better than one-day-old birds given the same diet. Although corn oil supplementation of the raw soybean diet produced such a striking response, both in growth and in the gain-to-feed ratio, only a small improvement was obtained with the heated soybean diet. This indicates the importance of extra calories for chicks given raw soybeans, and may explain the failure of Saxena et al. (8) to counteract the retardation of raw soybeans in high protein diets.

Experiment 4. The last experiment (table 4) was similar in design to experiment 1 (table 1), except that 15% corn oil was a major variable and the study was carried out with 3-week-old instead of one-day-old birds. The results in table 4 show the marked response to both calorie and protein supplementation of a raw soybean diet. The chicks getting 27% raw soybean protein in the presence of 15% corn oil grew as well as those receiving 27% heated soybean protein in the absence of corn oil. Also, the efficiency of food utilization (gain-to-feed ratio) for these groups is similar, thus pointing up the poor caloric efficiency of the raw soybean diets. The heated soy groups that received additional protein and corn oil grew at a faster rate than their raw soybean counterparts. This is not unexpected and should be interpreted to mean that the utilization of equivalent levels of protein and energy is always inferior with raw soybean diets. Nevetheless, the growth retardation of raw meal can be overcome by the chicken at low or high levels of protein (15 as well as at 25%). Were it physically possible to incorporate still more protein into a raw soybean diet without sacrificing calories, it should be possible to obtain equivalent growth to that shown in experiment 4 for

TABLE 3

Growth response of 3-week-old chickens to raw or heated soybean meal at two energy levels

Soybean meal protein[1]	Supplements		5-Week body wt[4]	Gain-to-feed ratio
	Isolated soy[2]	Corn oil[3]		
%	%	%	g	
Raw, 15	12		461 ± 20	0.37
Raw, 15	12	15	548 ± 14	0.60
Heated, 15	12		546 ± 11	0.52
Heated, 15	12	15	562 ± 5	0.70

[1] N × 6.25.
[2] ADM Assay Protein C-1, Archer-Daniels-Midland, Minneapolis; amount listed represents N × 6.25.
[3] Supplementary to the amount in basal diet.
[4] Mean values with standard errors for 10 male chickens/group; birds had been given a standard starting ration for 3 weeks before being fed experimental diets; average starting weight at 3 weeks: 220 g.

TABLE 4

Growth response of 3-week-old chickens fed raw or heated soybean meal in combination with different calorie and protein supplements for two weeks

Soybean meal protein[1]	Supplements		5-Week body wt[3]	Gain-to-feed ratio
	Protein[1]	Corn oil[2]		
%	%	%	g	
Raw, 15			529 ± 15	0.31
Raw, 21			594 ± 36	0.36
Raw, 27			622 ± 12	0.39
Raw, 15		15	576 ± 18	0.42
Raw, 21		15	627 ± 20	0.49
Raw, 27		15	660 ± 15	0.47
Heated, 15			650 ± 10	0.45
Heated, 21			661 ± 14	0.49
Heated, 27			652 ± 13	0.49
Heated, 15		15	599 ± 14	0.46
Heated, 21		15	692 ± 26	0.63
Heated, 27		15	731 ± 21	0.68
Raw, 15	isolated soy,[4] 12	15	656 ± 13	0.56
Raw, 15	fish,[5] 12	15	687 ± 14	0.58
Heated, 15	isolated soy, 12	15	730 ± 14	0.68
Heated, 15	fish, 12	15	752 ± 32	0.67

[1] N × 6.25.
[2] Supplementary to the amount in basal diet.
[3] Mean values with standard errors for duplicate lots of 7 male chickens; birds had been given a standard starting ration for 3 weeks before being fed experimental diets; average starting weight at 3-weeks: 305 g.
[4] ADM Assay Protein C-1, Archer-Daniels-Midland, Minneapolis.
[5] Supplied as menhaden fish meal.

the heated soybean group supplemented with extra protein and corn oil.

GENERAL COMMENTS

These studies indicate that with 3-week-old chicks, the growth retardation from raw soybean meal can be counteracted. This was achieved at suboptimal as well as at high protein levels by supplementation not only with extra protein but also with additional calories. The inability of one-day-old chicks to overcome the growth retardation at high protein levels (25%) is apparently due to an energy deficiency which is not alleviated by extra fat. This nutrient is apparently not well absorbed at that age by birds given raw soybean meal (10). In our opinion the differential utilization of corn oil by one-day-old versus 3-week-old birds weakens the possibility

that its effectiveness in the latter instance is due to constituents other than calories. Our studies do not, however, rule out this possibility. Dam et al. (14) have reported significant growth increases by increasing the vegetable oil content of poultry rations.

These studies explain the nature of the nutritional defects responsible for the growth retardation due to raw soybean diets. This knowledge should prove useful in the elucidation of the mechanism(s) underlying these defects. In the present study no such attempts were made.

Our observations on the oxygen consumption of one-day-old chicks given either raw or heated soybean meal (table 2) show no important difference in contrast with the differences reported by Saxena et al. (15). Since there are large differences in body weight between chicks fed either raw or heated soybean meal, it is possible that the magnitude of the difference reported by Saxena et al. would diminish if their values were expressed on the basis of metabolic size ($W^{0.67}$ or $W^{0.75}$).

As a consequence of the effectiveness of extra calories with high protein, raw soybean rations, we re-examined the report of Hill et al. (16) in which amino acid supplementation was shown ineffective in correcting the depression of raw soybean meal in a 30% protein diet. Since the diets used by Hill et al. were not supplemented with extra calories, our earlier suggestion (5) that the amino acid mixture used may have been imbalanced is probably the less likely explanation for the failure to overcome the growth retardation of raw soybeans.

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Effects of Long-term Feeding of a Fat-free Diet to Laying Hens

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ABSTRACT Three groups, each containing 15 Single Comb White Leghorn pullets were used for a 40-week study. Group 1 received a 20% protein semi-purified diet containing, by analysis, 0.05 to 0.07% fat. Group 2 received the same diet plus 5% lard. Group 3 received a 16.9% protein conventional layer mash. Egg production was poorer with the semi-purified diets than with the conventional layer mash. Adding lard to the semi-purified diet did not improve egg production. Fertility was similar in all 3 groups, but hatchability was poorer in group 1. By the end of 40 weeks, the period of time required for the eggs of group 1 to hatch was prolonged 24 to 36 hours. The lack of fat in the diet of group 1 resulted in a severe depletion of linoleic and arachidonic acids in the fat of the plasma, yolk and some of the body tissues and an increase in C_{20} triene content of the fat of these tissues. However, at the end of the 40-week period, the fat in the breast and thigh muscle still contained appreciable amounts of linoleic and arachidonic acids. The C_{20} triene found in the fat of group 1 was not observed in the fat of groups 2 and 3.

Cruickshank (1) reported that the composition of the body fat of the hen was changed by ingestion of saturated and unsaturated fatty acids, whereas egg fat was changed only by ingestion of unsaturated fatty acids. These observations were confirmed by Feigenbaum and Fisher (2), who suggested that in case of a dietary deficiency of polyunsaturated fatty acids, body depot stores are utilized for synthesis of egg fat. Radioisotope studies by Murty et al. (3), with laying hens have shown that linoleic acid is not synthesized from acetate. Mead (4), in a review of the metabolism of essential fatty acids, points out that linoleic acid is not formed to any appreciable extent by the animal body, but that arachidonic acid can be synthesized from linoleic acid and acetate. Since linoleic acid does not appear to be synthesized by the laying hen and body tissue stores may be utilized for egg fat formation, the feeding of fat-free diets over an extended period of time should result in a linoleic acid deficiency in laying hens. The object of this experiment was to: 1) determine the extent of depletion of polyunsaturated fatty acids in the tissues and eggs of laying hens fed a fat-free[1] diet for a 40-week period; and 2) study the effects of the depletion of polyunsaturated fatty acids on egg production, body weight maintenance, egg weight, fertility and hatchability of eggs laid during the 40-week period.

METHODS

Forty-five Single Comb White Leghorn pullets (commercial strain) were used for this experiment. They were reared in floor-pens with standard starting and growing mashes from hatch until housing time. The pullets were placed in individual cage laying batteries in a constant temperature room, held at 21°C. The composition of the diets is given in table 1.[2] For the first 6 weeks after housing, the pullets were fed diet 371. At the end of the 6-week period, the pullets were divided into 3 groups of 15 birds on the basis of egg production and body weight, and fed the experimental diets for a 40-week period. Group 1 was fed the fat-free diet 1, group 2 was fed the fat-free diet plus 5% lard added at the expense of glucose monohydrate (diet 2), and group 3 continued to be fed diet 371. Diets 1 and 2 were of a

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[1] Contained 0.05 to 0.07% fat.

[2] The authors are indebted to Central Soya, Decatur, Indiana, for the Promine R, to the Dow Chemical Company, Midland, Michigan, for the 2,2-dimethoxypropane, and Commercial Solvents Corporation, New York, for zinc bacitracin.

TABLE 1
Composition of diets

	No. 1	No. 2	No. 371
	%	%	%
Ground yellow corn	—	—	62.8
Soybean oil meal, 50% protein	—	—	19.0
Isolated soybean protein[1]	22.6	22.6	—
Alfalfa meal, 17% protein	—	—	5.0
Menhaden fish meal	—	—	2.0
Ground limestone	—	—	6.4
Steamed bone meal	—	—	3.5
Mineral mix[2]	9.0	9.0	—
6% Manganized salt[3]	—	—	0.5
Iodized salt	—	—	0.2
Dried corn fermentation solubles[4]	—	—	0.5
Vitamin and antibiotic mix[5]	1.0	1.0	—
Zinc bacitracin, 25 g/454 g	—	—	0.025
Vitamin A, 10,000 USP/g	—	—	0.075
Vitamin D_3, 15,000 ICU/g	—	—	0.005
Choline chloride	0.2	0.2	—
DL-Methionine	0.4	0.4	—
Glycine	0.5	0.5	—
Lard	—	5.0	—
Ground cellulose	5.0	5.0	—
Glucose monohydrate	61.3	56.3	—
	100.0	100.0	100.0
% Protein, calculated	20.0	20.0	16.9

[1] Promine R, Central Soya, Decatur, Indiana.
[2] Mineral mix supplied the following minerals of total diet: (in per cent) $CaCO_3$, 4.50; $Ca_3(PO_4)_2$, 3.20; K_2HPO_4, 0.45; NaCl, 0.50; $MgCO_3$, 0.20; $FeC_6H_5O_7 \cdot 5H_2O$, 0.06; $ZnCO_3$, 0.0125; KI, 0.004; $CuC_4H_6O_4 \cdot H_2O$, 0.004; $MnSO_4 \cdot H_2O$, 0.030; $Na_2SeO_4 \cdot 10H_2O$, 0.00005; $Na_2MoO_4 \cdot 2H_2O$, 0.0006; H_3BO_3, 0.001; $CoSO_4 \cdot 7H_2O$, 0.0002.
[3] Six parts $MnSO_4 \cdot H_2O$ and 94 parts NaCl.
[4] Contains 227 mg of riboflavin/454 g.
[5] The mix supplies the following vitamins and an antibiotic in milligrams per kilogram of diet: thiamine·HCl, 100; riboflavin, 16; D-calcium pantothenate, 25; pyridoxine·HCl, 10; biotin, 0.6; folic acid, 4; menadione, 5; niacin, 100; inositol, 100; vitamin B_{12} (0.1%), 15; vitamin A (500,000 USP/g), 36; vitamin D_3 (200,000 ICU/g), 8; α-tocopherol (1360 IU/g), 40; zinc bacitracin, 10; extended to 1% of the diet with ground cellulose.

semi-purified type using isolated soybean protein[3] as the only protein in the diet. A preliminary study had shown that feed consumption of hens fed the semi-purified diet was approximately 20% lower than that of those fed the control diet 371. In order that all groups would have approximately the same dietary protein intake, the semi-purified diets were formulated to contain 20% protein as compared with 16.9% protein in diet 371. Before preparation of the diets, the isolated soybean protein was extracted for 7 days with 95% methyl alcohol using a large continuous extractor of the type similar to that described by Menge et al. (5). Diet 1 (fat-free) was periodically analyzed for fat content by the acid hydrolysis method of the AOAC (6) and contained 0.05 to 0.07% crude fat. The semi-purified diets were pelleted using the method of McWard and Scott (7). Since the pelleting process involved drying overnight in a forced draft oven at 30°C and some vitamin destruction could occur, a weekly vitamin supplement was given to the pullets. This supplement, administered, via gelatin capsule, was equivalent to the amount of vitamins supplied by the consumption of 907 g of the semi-purified diet. Feed and water were supplied ad libitum. Twice a week the pullets were artificially inseminated with 0.05 ml pooled New Hampshire male semen. Records were kept on feed consumption, egg production, body weight maintenance, egg weight, fresh yolk weight, fertility and hatchability.

Once every 28-day period a 3-ml blood sample was collected in a heparinized tube, via wing vein puncture, from each pullet. After centrifuging, one milliliter of plasma from each pullet was pooled according to experimental groups and analyzed for fatty acid content.

[3] Promine R, Central Soya, Decatur, Indiana.

Daily weighings were made of all eggs laid during the experimental period. Eggs laid during one week of each 28-day period were broken out and the yolks freed of albumen and pooled by group, filtered through cheesecloth, freeze-dried and analyzed for fatty acid content. For one week of each 28-day period, eggs were broken out daily, the yolk rolled on a paper towel to remove all adhering albumin, and weights obtained on the fresh yolk. The remainder of the eggs laid during each 28-day period were incubated for fertility and hatchability studies. All eggs were candled at 6 days of incubation and the infertile eggs and those containing dead embryos broken out and examined.

At the end of the 40-week period, the feed was removed for 16 hours, the hens were killed and tissue samples (table 2) from each group were pooled for fatty acid analysis. The plasma and tissue samples were stored under nitrogen at − 20°C until analysis was performed.

The tissue, egg yolk, and plasma were saponified with 30% KOH (4 ml/g tissue) and ethyl alcohol (8 ml/g of tissue) at 50°C for 16 hours under nitrogen. The sterols were removed, the potassium salt of the fatty acids acidified, and taken up in petroleum ether (bp 30 to 60°C). They were methylated overnight at room temperature with 2-2-dimethoxypropane and 2% dry hydrogen chloride in superdry methanol (8).

Fatty acid analysis were made by gas-liquid chromatography at 200°C using a hot wire detector and a one-millivolt recorder. A 244-cm by 0.6-cm copper column packed with 20% diethylene glycol succinate on chromosorb W (60 to 80 mesh, acid and alkali washed) was used for the analyses. Helium was used as the carrier gas and flow rates (ca. 40 to 70 ml/min) were adjusted to give maximal peak separation. Commercial methyl esters of highest purity available were used for calibration and detector response determination. Samples containing unknown fatty acid peaks were hydrogenated by the method of Farquhar et al. (9) and chromatographed on apiezon L and diethylene glycol succinate columns. All analyses were made in duplicate. All solvents used for gas chromatography sample preparation were redistilled before use.

Crude fat was determined on the freeze-dried egg yolks from eggs of the 3 groups laid during the pre-experimental, 21st and 37th week by the acid hydrolysis method of the AOAC (6).

Duncan's multiple range test (10) was used for statistical analysis of the data.

RESULTS

The effects of the various dietary treatments on body weight maintenance, egg production, egg weight and yolk weight are shown in figure 1. All of the pullets continued to gain weight during the 40-week period. The average gain in weight at the end of this period was 266, 287 and 301 g/bird, for groups 1, 2 and 3, respectively. Feed consumption decreased sharply during the 17th week when a new mix of feed was fed to group 3. Replacing the feed with another mix from a new supply of ingredients resulted in a rapid return to normal feed consumption. There was a high incidence of 12- to 14-day embryonic mortality in eggs laid by this group during this period, but normal hatchability occurred in all subsequent hatches. The average feed consumption during the 40-week period was 96, 93 and 123 g/bird/day for groups 1, 2 and 3, respectively.

The egg production of groups 1 and 2 decreased sharply during the first 10 weeks of the experiment and reached a plateau at approximately 50% where it remained for the next 20 weeks. During the 30- to 40-week period, egg production of these 2 groups was below 50%. There was no significant difference in egg production between group 1 and group 2, but there was a highly significant difference between group 3, fed the control diet, and groups 1 and 2 fed the semi-purified diet.

Egg production data of individual birds of both groups 1 and 2 showed that some of the birds maintained production equivalent to that obtained in group 3, whereas others produced very poorly. Although several of the birds of groups 1 and 2 laid continuously and achieved 70% egg production for the 40-week period, the majority laid for a short time and then molted. After molting they would lay for a short

TABLE 2

Distribution of fatty acids in the fat of tissues of laying hens[1]

Fatty acid[2]	Retention time[3]	Heart			Kidney			Spleen		
		Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
		%	%	%	%	%	%	%	%	%
14:0	0.40	0.93	1.04	0.83	0.54	0.54	0.56	0.75	0.60	0.59
15:0[4]	0.52	2.23	2.70	2.29	1.84	2.18	2.10	2.88	2.85	3.13
16:0	0.63	18.75	19.28	18.61	18.80	20.35	18.33	20.75	21.72	22.24
16:1	0.73	5.34	3.76	3.21	4.42	2.47	1.98	2.56	1.62	1.49
17:0[4]	0.82	0.86	1.02	0.77	0.65	1.10	1.13	3.60	3.59	3.70
?	0.91	—	—	—	0.93	0.89	1.08	3.26	1.41	1.39
18:0	1.00	11.92	11.61	11.46	13.88	15.01	15.29	16.62	16.28	17.10
18:1	1.17	42.28	38.63	29.14	46.06	37.60	25.54	34.37	31.04	22.12
18:2	1.43	6.47	10.82	24.03	2.99	8.45	20.48	0.91	3.86	10.68
18:3	1.83	0.54	0.40	0.68	0.40	0.29	0.46	0.74	0.45	0.38
?	2.45	—	—	—	—	1.22	2.38	—	—	0.92
20:3[4]	2.67	4.28	—	—	4.65	—	—	5.54	—	—
20:4	3.03	6.85	10.73	8.98	4.84	10.35	10.02	11.59	16.56	15.97

[1] Each value represents average of duplicate determinations.
[2] First number is chain length, second is number of double bonds.
[3] Relative to methyl stearate chromatographed on diethylene glycol succinate.
[4] Identification is tentative.

period and molt again. Although the egg production of groups 1 and 2 was similar, the eggs laid by group 2 were significantly heavier ($P < 0.01$) than those laid by group 1. Also, the weight of the eggs laid by group 3 was significantly heavier ($P < 0.01$) than those laid by group 2.

A similar trend in the difference in yolk weights of the 3 groups was observed (see fig. 1). A highly significant difference in yolk weight existed between groups 1 and 3, but not groups 1 and 2 or groups 2 and 3.

The average total fat content of yolks of eggs laid by the 3 groups during the pre-experimental period was 59.4%. The total fat content of the yolks during the 21st week for groups 1, 2 and 3 was 56.3, 56.9, and 58.3%, respectively, and for the 37th week was 56.4, 55.0 and 58.0%, respectively. This difference in total fat for groups 1 and 2 fed the semi-purified diets, and group 3 fed the practical-type diet was statistically significant ($P < 0.01$).

The average fertility for the 40-week study was 89, 87, and 89% for groups 1, 2 and 3, respectively. The hatchability of group 1 was not as good as either group 2, or 3. The average hatchability for the experimental period was 82, 90 and 93% for groups 1, 2 and 3, respectively. Lateness in hatching was observed in group 1 after feeding the experimental diet for 20 weeks. As the experiment progressed beyond this period an increase in number of eggs that were in the pipped[4] stage was noted at hatching time. In the last or 10th hatch, 36% of the fertile eggs of group 1 pipped but had not hatched after 22 days of incubation in contrast to the completed hatching of the other two groups. The hatching of group 1 was delayed approximately 24 to 36 hours.

All of the eggs that did not hatch were broken out and examined. The few abnormalities noted were mainly in group 1. However, there was no consistency in the type of abnormalities observed in this group.

The fatty acid content of the plasma fat for the 3 groups is shown in figure 2. No graphs are given for myristic and palmitic acid because the levels of these fatty acids remained relatively constant during the 40-week period for all 3 groups. The average level and standard deviation of myristic and palmitic acid in the plasma fat for the 3 groups for the 40-week period was $0.44 \pm 0.07\%$ and $24.80 \pm 0.97\%$, respectively. The linolenic acid content of the plasma fat, which did not exceed 1% of the total, had considerable fluctuation between consecutive analyses. The linolenic acid level decreased slightly during the experiment for groups 1 and 2, but no significance was attached to the decrease because of the small amount present and

[4] Beak of embryo breaking through the shell at hatching time.

TABLE 2 (*Continued*)

Distribution of fatty acids in the fat of tissues of laying hens[1]

Liver			Skin			Thigh muscle			Breast muscle		
Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3	Group 1	Group 2	Group 3
%	%	%	%	%	%	%	%	%	%	%	%
0.58	0.45	0.44	0.78	1.00	0.76	0.82	1.02	0.98	1.00	1.05	1.18
—	—	—	0.28	0.22	0.19	1.15	1.39	1.34	3.73	4.75	4.90
21.46	21.47	21.23	19.96	19.87	18.68	20.40	20.91	20.77	23.20	22.86	22.86
5.21	3.36	3.06	9.39	7.30	6.30	7.55	5.81	4.85	4.70	3.25	3.04
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
9.20	12.47	12.34	3.92	4.04	3.53	7.36	7.97	7.51	9.68	9.68	9.30
60.27	52.92	47.64	61.58	59.35	46.33	53.32	50.70	37.20	42.24	42.96	31.87
0.44	3.60	11.90	3.68	7.70	23.32	6.22	8.87	24.18	8.08	8.82	20.08
0.22	0.26	0.32	0.40	0.57	0.88	0.43	0.40	0.97	0.39	0.54	0.74
—	—	—	—	—	—	—	—	—	—	—	—
1.00	—	—	—	—	—	0.94	—	—	1.88	—	—
1.18	5.20	2.56	—	—	—	1.82	2.92	2.20	4.47	6.10	6.03

the fluctuation of this fatty acid in the plasma fat of group 3 (control).

In general, the greatest changes in plasma fatty acid levels occurred during the first 4 weeks. In group 1, a sharp decrease in linoleic and stearic acids, a rapid increase in oleic and palmitoleic acids, and a gradual decrease in arachidonic acid occurred during this period. During the next 36 weeks the levels of linoleic and arachidonic acid in the plasma fat decreased very slowly and at the end of the experimental period were reduced to 0.26% and 0.40%, respectively. Oleic and palmitoleic acid levels increased slightly during this interval, whereas the stearic acid level continued to decrease.

At the end of the first 4-week period a new peak appeared in the chromatograms of the plasma methyl esters of group 1. A comparison of the chromatograms made before hydrogenation and after hydrogenation of the methyl esters showed this peak to be a 20-carbon-chain fatty acid. From the retention times of the fatty acid methyl ester on apiezon L and diethylene glycol succinate columns, it was tentatively identified as the C_{20} triene which had been reported by others (11–13) to appear when diets low in essential fatty acids are fed. The level of this fatty acid increased slowly and comprised only 1% of the total fatty acids at the end of the 40-week period.

Although the changes in plasma fatty acids in group 2 were not as great as those of group 1, the trend was the same except that the arachidonic acid level remained constant after a slight initial depression and no C_{20} triene was detected in the plasma of group 2. Except for the slight decrease in the arachidonic acid level during the first 4 weeks, group 2 maintained a level of plasma arachidonic acid that was similar to that of group 3. The level of all plasma fatty acids of group 3 remained relatively constant during the 40-week period.

The fatty acid content of egg yolk fat is shown in figure 3. The levels of myristic and palmitic acid in the yolk fat were not affected by dietary treatment. The average level and standard deviation of these 2 fatty acids for the 3 groups during the 40-week period was $0.49 \pm 0.04\%$ myristic acid and $24.98 \pm 0.26\%$ palmitic acid. The level of linolenic acid was similar to that in the plasma fat, and decreased slightly for groups 1 and 2 during the experiment. Dietary treatments 1 and 2 resulted in an increase in monounsaturated and a decrease in polyunsaturated fatty acids of the yolk fat similar to the changes that occurred in the plasma fatty acids. Feeding the semi-purified diets resulted in a definite lowering of stearic acid in the yolk fat. The level of the stearic acid in yolk fat of both groups 1 and 2 was significantly lower ($P < 0.01$) than that for group 3. The yolk fat from eggs laid by group 1 during the 37th week contained

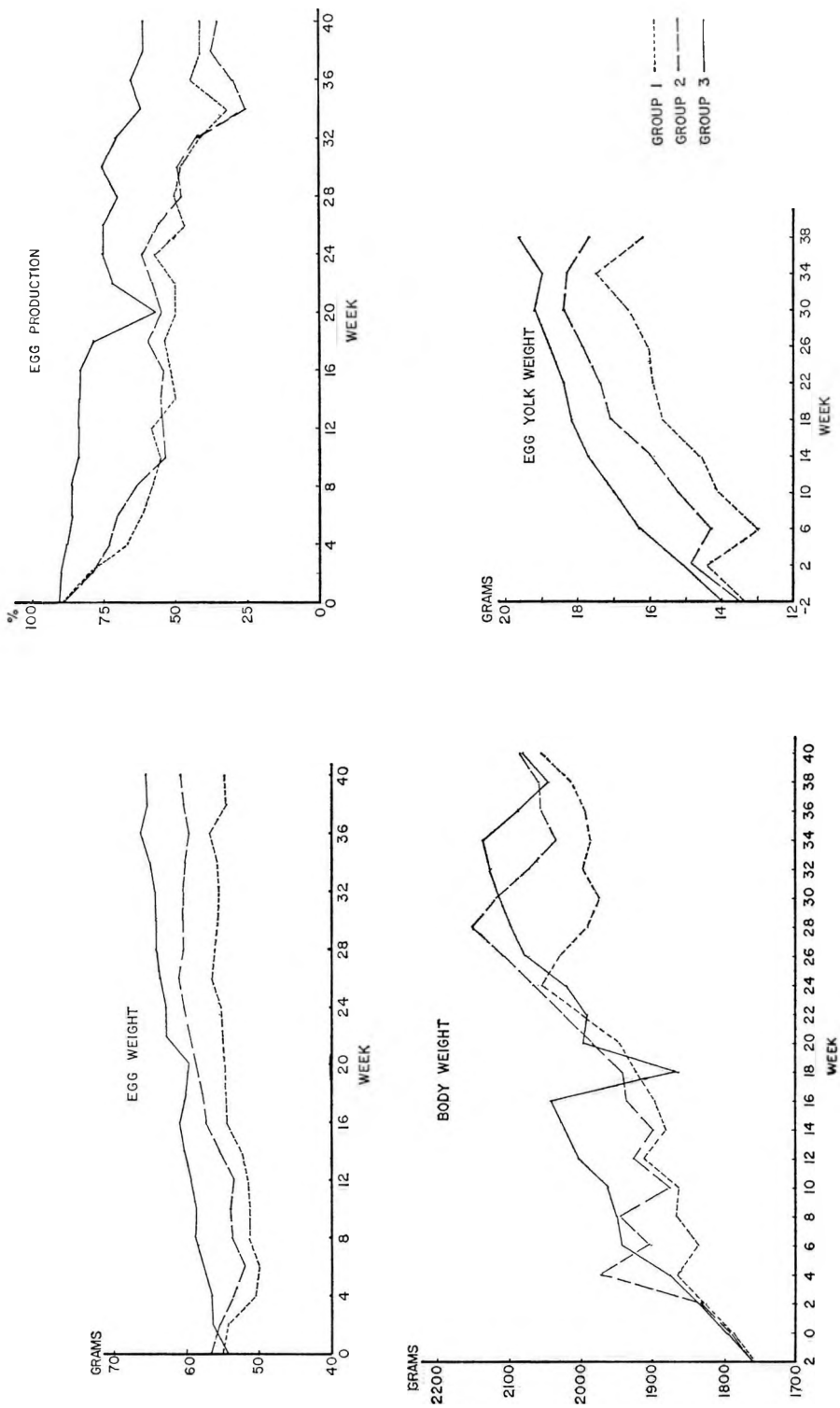

Fig. 1 Effect of dietary treatments on egg weight, yolk weight, body weight, and egg production.

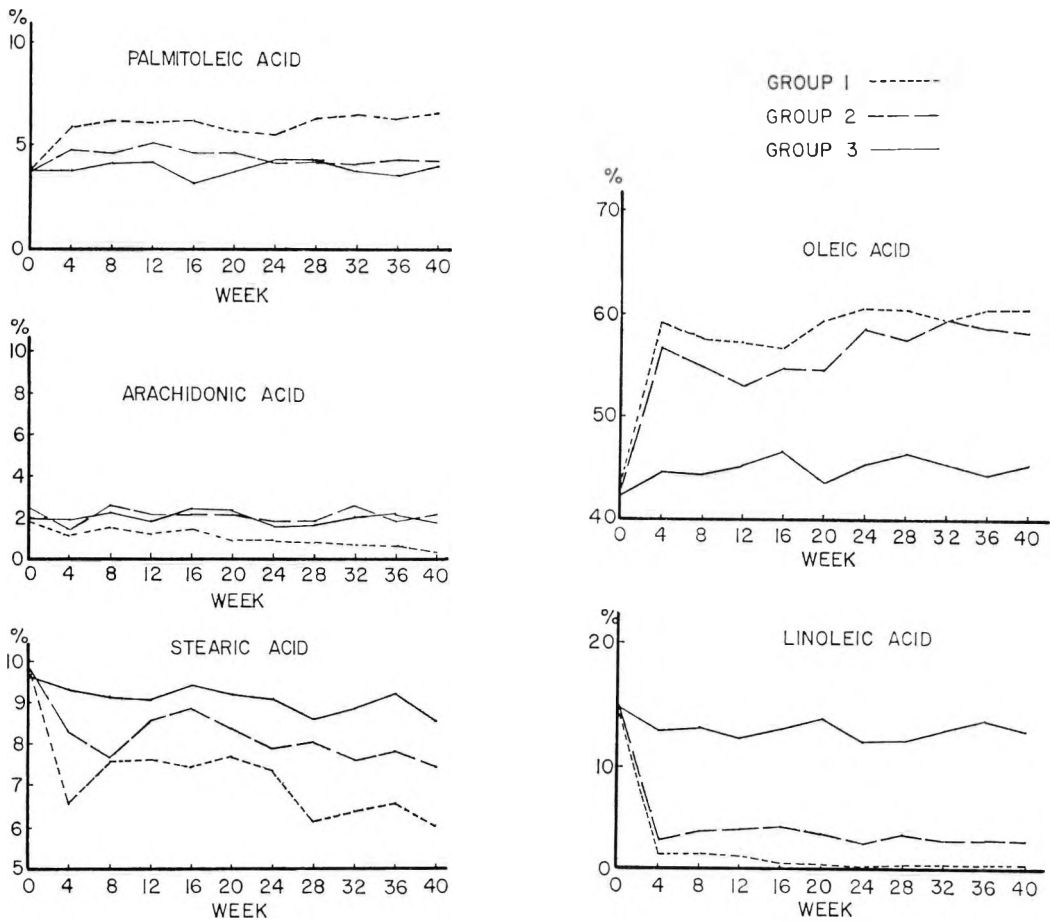

Fig. 2 Effect of diet on the fatty acid content of the plasma fat.

approximately 1% C_{20} triene. No traces of the C_{20} triene were detected in the yolk fat of either group 2 or 3.

The levels of fatty acids in the tissues of the 3 groups at the end of the 40-week period are shown in table 2. Feeding the fat-free diet to group 1 for 40 weeks resulted in a substantial, but not complete depletion of linoleic acid from the tissue fat. The greatest reduction in linoleic acid level occurred in the fat from spleen and liver. The tissue fat level of arachidonic acid was also reduced, but not as severely as for linoleic acid. Again, the C_{20} triene was only detected in the tissue fat of group 1. None of the dietary treatments affected the levels of palmitic or stearic acid in fat of tissues of the 3 groups. Four fatty acids were detected in some of the body tissue fats that were not observed in the plasma or egg yolk fat. From log retention volumes of the samples, before and after hydrogenation on apiezon L and diethylene glycol succinate, 2 of these fatty acids were tentatively identified as C_{15} and C_{17} saturated fatty acids. The latter is probably identical to the C_{17} saturated fatty acid reported by Wheeler et al. (14) to be present in trace amounts in egg yolk.

DISCUSSION

Machlin et al. (15) observed that the depot fat of laying hens fed a diet extremely low in essential fatty acids still contained relatively large amounts of linoleic acid at the end of a 12-week depletion

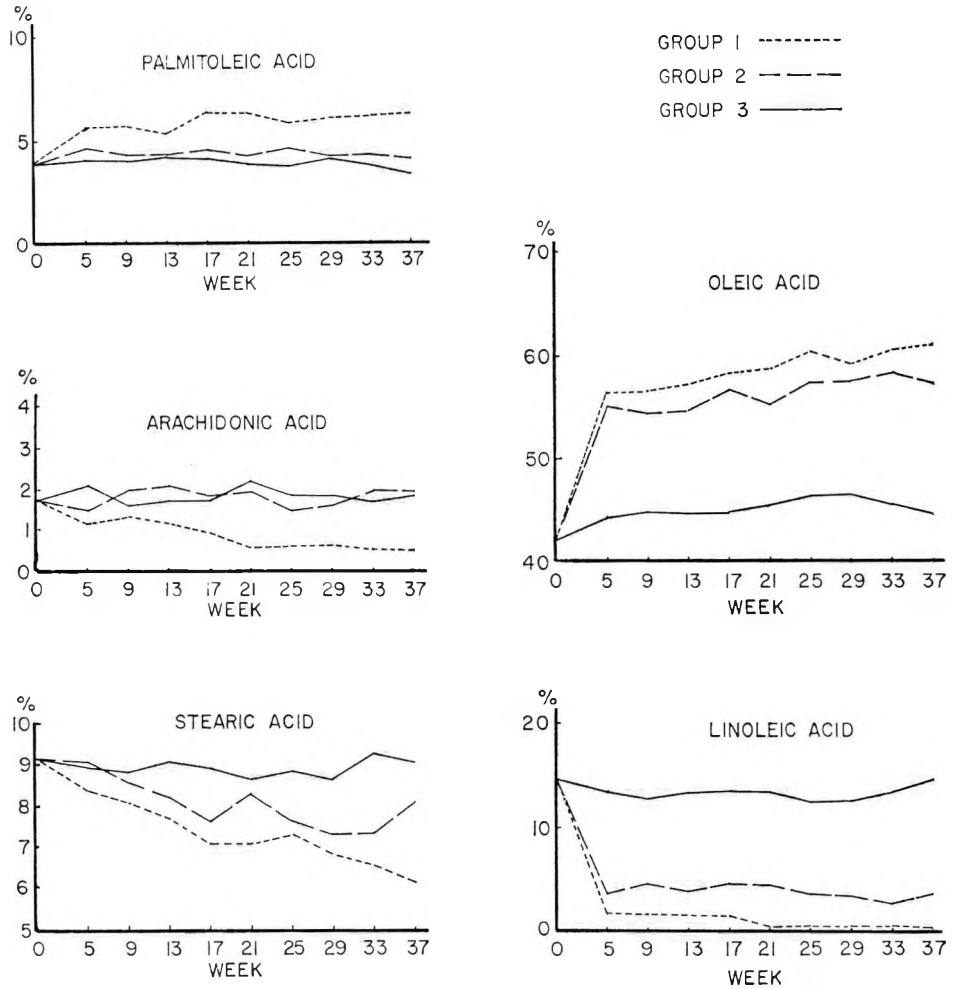

Fig. 3 Effect of diet on the fatty acid content of the egg yolk fat.

period. They suggested that very long periods of time would be required to deplete laying hens of essential fatty acids before egg production or hatchability would be affected. In our study it was found that even after a 40-week depletion period, substantial amounts of linoleic and arachidonic acid still remained in the fat of some of the tissues. Apparently linoleic acid was being utilized to maintain tissue arachidonic acid levels since linoleic acid depletion was more severe than arachidonic acid depletion.

According to calculations based on daily feed intake and levels of polyunsaturated fatty acids in the dietary fat, the daily intake of these fatty acids was estimated for the hens fed diets 1, 2, and 371. The average intake on a per bird per day basis was 36 mg for hens fed diet 1, 510 mg for hens fed diet 2, and 1,780 mg for hens fed diet 371. The daily intake of 36 mg of polyunsaturated fatty acids for group 1 was not sufficient for maintaining adequate levels of polyunsaturated fatty acids in the fat of the body tissues or the fat of the eggs. The inadequate intake of polyunsaturated fatty acids resulted in poor egg production, small egg size, and prolongation of time required for hatching of the incubated eggs. The daily intake of 510 mg of polyunsaturated fatty acids by hens of group 2

resulted in maintenance of arachidonic acid levels in body tissue fat and egg fat, increased egg weights, and a normal incubation period. However, linoleic acid levels in the body tissue fat and egg yolk fat were lower than those of group 3. Although the polyunsaturated fatty acid intake of group 2 was approximately 14 times greater than that of group 1, the egg production was no better than that of group 1. Therefore it appears that the daily intake of 510 mg of polyunsaturated fatty acids was not adequate to meet the essential fatty acid requirement of the laying hen for maintenance of good egg production. However, the possibility exists that the poor egg production of groups 1 and 2 may have been caused by a lack of some dietary ingredient not in the semi-purified diet but contained in the practical-type diet (exclusive of essential fatty acids).

Although Machlin et al. (15) reported that the C_{20} triene level in tissue fat of hens fed an essential fatty acid-deficient diet for 12 weeks did not increase, the results of the present study show a gradual increase in the C_{20} triene content of both plasma and egg yolk during the 40-week period. This material was not detected in the plasma and yolk fat of groups 2 and 3 during the entire experimental period, whereas after feeding the fat-free diet to group 1 for 4 weeks the level of the C_{20} triene in the fat of the plasma and yolk was 0.4 and 0.5%, respectively. When the experiment was terminated at 40 weeks, the level of the C_{20} triene had increased to about 1% of the total fatty acids in both plasma and yolk fat and to 4 to 5% in the fat of the heart, kidney, and spleen of group 1.

Although the presence of C_{20} triene in the tissue fat of group 1 did not have an apparent detrimental effect on egg production or body weight maintenance, the gradual increase in the level of the triene in egg yolk fat may be responsible for the delayed hatching of the eggs laid by group 1. In the early studies on essential fatty acid deficiencies, Evans et al. (16) reported that long-term feeding of fat-free diets to female rats resulted in a characteristic 1- to 3-day prolongation of gestation.

Godfrey (17) reported that poor hatchability occurred with small eggs. The late hatching of the eggs of group 1 could not be due to egg size since the average weight of the eggs of this group was greater during the 20- to 40-week period when the delayed hatch occurred than during the 4- to 16-week period when the eggs hatched out within the normal incubation period.

The addition of lard to the diet resulted in a significant increase in egg weights. Jensen and McGinnis (18) reported that the weight of eggs laid by hens fed purified diets could be increased by adding corn oil to the diet, and Treat et al. (19) observed that adding animal fat or a blend of animal and vegetable fat to the diet resulted in increases in egg weight.

Although the palmitic acid level of the plasma, tissue and egg yolk fat remains relatively constant, the stearic acid content may vary considerably. A large range of values for stearic acid content of egg yolk have been reported. Machlin et al. (15) reported 8% stearic acid in the yolk fat of hens fed a purified diet containing hydrogenated coconut oil, and 10% stearic acid when safflower oil replaced hydrogenated coconut oil. In the studies of Wheeler et al. (14), the stearic acid content of egg yolk fat ranged from 8.1 to 16.1%. Evans et al. (20) reported that egg oil from eggs of hens fed the basal diet contained 10.4% stearic acid, whereas egg oil contained 22.2% stearic acid when the diet contained 2.5% cottonseed oil and 26.3% stearic acid when the diet contained *Sterculia foetida* seed. Privett et al. (21) reported that the crude lipid of eggs obtained from hens fed a standard laying mash contained 14% stearic acid. In our studies we found that the egg yolk fat of eggs laid by the hens of group 3 fed diet 371 contained 8.9% stearic acid, whereas the stearic acid content of egg yolk fat from groups 1 and 2 fed the purified diet was 6 to 7%. These changes in the stearic acid content of the egg yolk fat suggest that stearic acid as well as the monenoic and dienoic acids can be altered by dietary methods.

Reiser (22) reported no difference in the total fat content of the egg yolk when hens were fed a low fat or 4% cottonseed

oil-supplemented purified diet. We have observed similar results in our studies using a fat-free and a 5% lard-supplemented semi-purified diet. In this study, no differences in total fat content of the egg resulted from supplementing the fat-free diet with 5% lard. However, the eggs from both groups fed the semi-purified diets had a lower fat content than those from group 3 fed the practical-type laying diet.

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Effect of Increments of Tryptophan and Niacin on Growth and on the Concentrations of Blood and Liver Pyridine Nucleotides[1]

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ABSTRACT Weanling rats fed a diet containing 6% of casein and 12% of gelatin exhibited a growth response to increments of tryptophan in the diet up to 0.06% of the diet. Blood and liver pyridine nucleotide concentrations also increased with increments of tryptophan. Niacin stimulates the growth of rats when the diet contains suboptimal levels of tryptophan, but has no effect when the level of tryptophan is adequate. Niacin (2.5 mg) caused a substantial increase in blood pyridine nucleotides which was not related to the adequacy of the diet as evidenced by gain in weight. Excess quantities of both niacin and tryptophan in the diet, which did not further increase growth, caused liver and blood pyridine nucleotides to increase.

In a previous study on the utilization of niacin and tryptophan as precursors of liver pyridine nucleotides in the rat, a basal diet was used (1) that was devoid of niacin and low in tryptophan, but which supported growth when either niacin or tryptophan was added. Although rats fed the deficient diet grew very little and apparently developed a niacin deficiency, their liver pyridine nucleotide concentrations were not depressed and a level (2.5 mg/100 g of diet) of niacin which prevented the deficiency condition did not increase pyridine nucleotide concentration. On the other hand, an amount of tryptophan which stimulated growth to about the same extent also increased liver pyridine nucleotide concentrations in some animals.

The relative effectiveness of either niacin or tryptophan as precursors of pyridine nucleotides appears to depend upon the particular conditions used in each experiment. It has been reported (2) that niacin and tryptophan were equally effective as precursors of liver pyridine nucleotides in rats when a range of equimolar quantities of each was added to a diet that was devoid of protein and lacking in niacin; but when niacin and tryptophan were added at physiological levels to diets which were devoid of both niacin and tryptophan, tryptophan was a more effective precursor (3). Burch et al. (4) in studies with rats noted a greater response in both blood and liver pyridine nucleotides to dietary niacin than to tryptophan, but in their investigations only one level of each nutrient was tested. In other studies (5) it was concluded that in young rats previously depleted with a niacin-free, tryptophan-free diet, a supplement of tryptophan is used first for protein synthesis and only subsequently as a precursor of blood pyridine nucleotides, and that niacin under these conditions does not cause an increase in blood pyridine nucleotides.

The investigations reported below were undertaken to study the response of both liver and blood pyridine nucleotide concentrations to graded levels of niacin and tryptophan in the diet, and to determine to what extent pyridine nucleotide concentrations in blood would reflect adequacy of intake of niacin and tryptophan.

EXPERIMENTAL

Male weanling rats of the Holtzman strain weighing 40 to 50 g were used in

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these experiments. They were housed in individual, suspended cages and were fed the basal diet for a period of 2 to 3 days. The animals were then separated into groups of 5 so that the average weights of the groups did not differ by more than one gram. The groups of rats were fed the experimental diets ad libitum and were weighed 3 times weekly during the 2-week experimental period.

The percentage composition of the basal diet was as follows: casein, 6.0; gelatin, 12.0; DL-methionine, 0.3; salt mixture, 5.0 (6); corn oil containing fat-soluble vitamins, 5.0; water-soluble vitamins (less nicotinic acid) in sucrose, 0.25 (6); choline chloride, 0.15; and dextrin to 100. Increments of niacin and L-tryptophan were added to the diets as indicated.

The high level of gelatin in this diet causes an amino acid imbalance which is prevented by additional tryptophan and is partially alleviated by niacin (1). This means of producing a severe combined niacin and tryptophan deficiency broadens the growth range over which the effects of niacin and tryptophan supplements can be studied.

At the end of the 2-week experimental period, blood was obtained directly from the tail of the rat and oxidized pyridine nucleotides were determined in whole blood by the method of Levitas (7). The animals were then decapitated and the livers removed for analysis. Oxidized liver pyridine nucleotides were determined by the method of Feigelson et al. (8), which involved homogenization of the liver in trichloroacetic acid, adsorption of the extracted pyridine nucleotides on charcoal, elution with pyridine and spectrophotometric determination of the eluate at 340 mμ, after reduction with hydrosulfite. When serum was required for the determination of N'-methylnicotinamide, the blood was obtained from the rats by heart puncture. N'-methylnicotinamide was then determined by the method of Levitas (7). Diphosphopyridine nucleotide (98% purity) was obtained from a commercial source.[5] Standards were included with each series of determinations.

RESULTS

Effects of graded levels of tryptophan on growth

The effects of increasing increments of L-tryptophan on the growth of rats fed the basal diet with or without niacin are shown in figure 1. Each point represents the average weight gain of 15 to 20 rats. When niacin was omitted from the diet, each increment of L-tryptophan up to 0.06% stimulated growth, but further increments caused only small increases in growth.

When niacin (2.5 mg/100 g diet) was included in the basal diet, growth was greater with each level of supplemental L-tryptophan from zero to 0.05% than it was when niacin was omitted. When the dietary level of tryptophan approached adequacy, the addition of niacin did not alter the growth response. With niacin in the diet the rate of growth approached the maximum with supplements of 0.05 to 0.06% of L-tryptophan. When the L-tryptophan supplement was almost sufficient for maximal growth, considerable overlapping of the values for the niacin-supplemented and nonsupplemented groups was observed; thus, with higher tryptophan

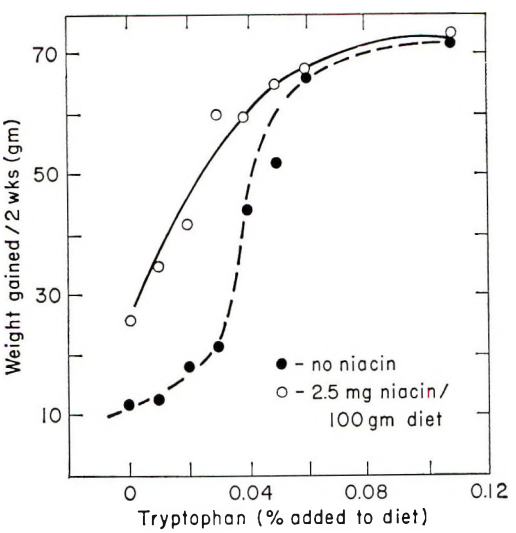

Fig. 1 Effect of increasing increments of tryptophan on the growth of rats fed a diet containing 6% of casein and 12% of gelatin with and without niacin.

[5] Sigma Chemical Company, St. Louis.

intakes the growth responses of the individual animals appeared to be influenced more by the quantity of food they consumed than by the level of tryptophan in the diet.

Effects of increments of niacin and tryptophan on growth and on liver and blood pyridine nucleotides

In another experiment, increments of niacin (zero, 2.5 mg, 10 mg, and 250 mg/100 g diet) were added to diets which contained inadequate supplements of tryptophan (0.0% and 0.02% L-tryptophan) and supplements adequate for nearly maximal growth (0.06% L-tryptophan). The results of the growth studies and the blood and liver pyridine nucleotide analyses are shown in figure 2.

Growth response (fig. 2A). When the tryptophan content of the diet was suboptimal (diets supplemented with zero and 0.02% of L-tryptophan) the addition of 2.5 mg of niacin /100 g of diet gave a marked growth response. The groups receiving the supplement of 0.06% of L-tryptophan responded very little to niacin. Increments of niacin up to 250 mg/100 g were no more effective than 2.5 mg in stimulating growth with any of the levels of tryptophan tested.

Blood pyridine nucleotides (fig. 2B). The addition of 2.5 mg of niacin/100 g of diet substantially increased the blood pyridine nucleotide concentrations when the tryptophan supplement was inadequate (0 and 0.02%), but not when it was nearly sufficient (0.06%) for maximal growth. Supplements of 10 mg and 250 mg of niacin/100 g of diet further increased blood pyridine nucleotide concentrations regardless of the dietary level of tryptophan. When 0.5% of tryptophan was added to the diet, the excess of tryptophan over the requirement for growth was large and blood pyridine nucleotide concentration increased, but not as much as when 250 mg of niacin was added per 100 g of diet.

In the method of Levitas, which was used to determine blood pyridine nucleotides, N'-methylnicotinamide is measured in addition to pyridine nucleotides; therefore, the N'-methylnicotinamide concentrations of the blood sera were determined separately. N'-Methylnicotinamide has been found to occur mostly in blood serum, whereas pyridine nucleotides usually occur in red blood cells. There were only small differences (from 3 to 6 μg/ml) among the N'-methylnicotinamide concentrations of the groups receiving zero, 2.5 mg or 10 mg of niacin/100 g of diet. The groups with 250 mg of niacin/100 g of diet showed an increase up to 8 or 10 μg/ml, but this was much too small to account for the large increase in the con-

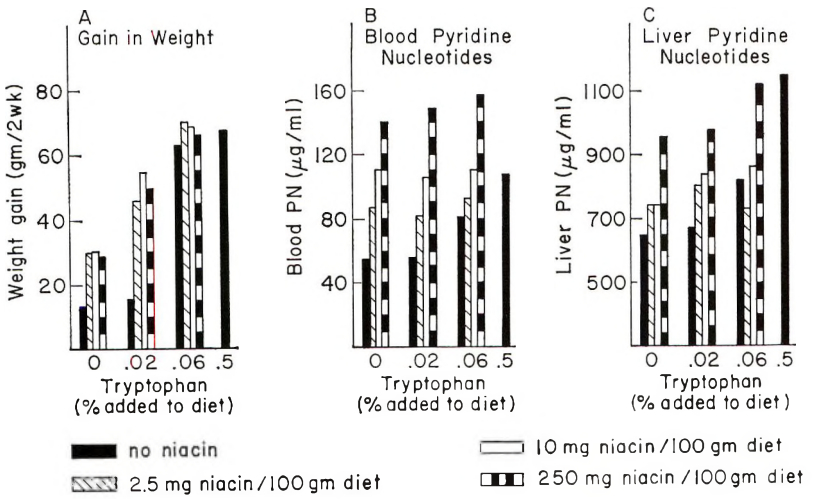

Fig. 2 Effect of increasing increments of niacin and tryptophan on growth and on blood and liver pyridine nucleotides.

centrations of pyridine nucleotides in whole blood.

Liver pyridine nucleotides (fig. 2C). Liver pyridine nucleotide concentrations also increased when 2.5 or 10 mg of niacin were added per 100 g of diet, to diets that were not supplemented or were supplemented with only 0.02% of tryptophan. When the level of tryptophan in the diet was adequate for normal growth, however, liver pyridine nucleotide concentrations did not increase with the smaller supplements of niacin. In all cases the 250 mg/100 g level of niacin caused a substantial increase in liver pyridine nucleotides; so did the addition of 0.5% of L-tryptophan. Thus niacin increased blood pyridine nucleotide synthesis at any level at which it was added, but increases in liver were most marked when a great excess was added.

Food intake. When niacin was added to diets containing suboptimal levels of tryptophan, there was an increase in food intake and consequently in tryptophan intake. Therefore, it seemed important to try to differentiate between the direct effect of niacin and its indirect effect in stimulating tryptophan intake. Values for tryptophan intake and niacin intake for each of the groups reported in figure 2 are given in table 1. Despite the higher food intakes of rats in groups 2, 3 and 4, receiving niacin but no additional tryptophan, they ingested an average of only 2.6 mg of L-tryptophan/g of weight gained; whereas group 1 which received no niacin ingested 3.9 mg of tryptophan/g of weight gained. Similar results were obtained for rats fed the diets supplemented with 0.02% of L-tryptophan; here again, groups 6, 7 and 8 which received niacin ingested 2.5 mg of tryptophan/g of weight gained, whereas the group receiving no niacin (group 5) ingested 4.3 mg of tryptophan/g of weight gained. These calculations indicate that the greater growth and higher blood and liver pyridine nucleotide concentrations of rats receiving niacin supplements were not a result of higher tryptophan intakes per gram of gain. When the diets contained 0.06% of supplementary L-tryptophan all groups had tryptophan intakes of between 2.7 and 3.1 mg/g of weight gained, with that of the group receiving no niacin being as low as any. If this amount of tryptophan is sufficient for full growth in the group receiving no niacin (fig. 2), an excess of niacin would be available for pyridine nucleotide synthesis in rats that received niacin as well as tryptophan.

TABLE 1

Niacin and tryptophan consumption of rats fed diets containing 6% of casein and 12% of gelatin supplemented with different amounts of L-tryptophan and niacin[1]

Group	Supplements		Intake/rat/day			Tryptophan/gain
	Tryptophan	Niacin	Tryptophan from casein	L-Tryptophan from supplement	Niacin	
	%	*mg/100 g*	*mg*	*mg*	*mg*	*mg/g*
1	0	0	3.9	0	0	3.9
2	0	2.5	5.4	0	0.19	2.5
3	0	10.0	5.8	0	0.83	2.7
4	0	250.0	5.2	0	18.5	2.5
5	0.02	0	3.6	1.0	0	4.3
6	0.02	2.5	6.2	1.8	0.22	2.4
7	0.02	10.0	7.2	2.1	1.03	2.4
8	0.02	250.0	7.6	2.1	27.2	2.7
9	0.06	0	6.5	5.6	0	2.7
10	0.06	2.5	7.8	6.7	0.28	2.7
11	0.06	10.0	8.2	7.0	1.17	3.1
12	0.06	250.0	7.7	6.6	27.2	3.0
13	0.5	0	7.4	53.0	0	12.6

[1] The casein contained 14% nitrogen; tryptophan content calculated from tables (Block, R. J., and D. Bolling 1951. The amino acid composition of proteins and foods, ed. 2. Charles C Thomas, Springfield, Illinois).

DISCUSSION

The growth curves obtained in this study (fig. 1) with rats fed a diet containing between 17 and 18% of protein supplemented with 0.06% of L-tryptophan (total tryptophan content 0.13%) suggest that the requirement of the rat for this amino acid may be much lower than was suggested by Oesterling and Rose (9) and closer to the estimate of Rao et al. (10).

In agreement with the results of Oesterling and Rose (9), the addition of niacin to the diet had little if any effect on the tryptophan requirement of the rat for normal growth, but did stimulate growth when the tryptophan level was suboptimal. Since the absence of the vitamin had no measurable effect on the tryptophan requirement when the diet contained 0.13% of tryptophan, Oesterling and Rose suggested that the quantity of tryptophan transformed to niacin must be small. However, in the present experiments, even when there was no niacin in the diet, and when the tryptophan level was low, the concentration of pyridine nucleotides in the liver was still substantial.

Similar results have been observed previously in rats maintained with diets in which a severe niacin deficiency was induced by an amino acid imbalance (1); in rats maintained with protein-free, niacin-free diets (2); and in rats maintained with tryptophan-free, niacin-free diets for long periods of time (3). In none of these studies did liver pyridine nucleotide concentrations decline very much below the value for rats fed adequate diets. These observations suggest that some tryptophan is always converted to niacin, or that a certain quantity of pyridine nucleotides is firmly bound to protein in the liver and the concentration per gram of liver does not decrease. Brown et al. (11) concluded from studies with human subjects that a certain amount of tryptophan was always catabolized via the kynurenine pathway to nicotinic acid. They indicated that this pathway was functioning even when the tryptophan content of the diet was so low that the subjects were in negative nitrogen balance, because, although the excretion of metabolites that arise via this pathway declined to between one-half and one-third of the amount found in the urine of subjects on normal diets, it did not cease.

Duncan and Sarett (12) had shown, using human subjects, that blood pyridine nucleotides increased much more rapidly when niacin was ingested in large amounts than when tryptophan was ingested. Further, Costabile et al. (13) using in vitro studies showed that red blood cells were unable to utilize tryptophan to form pyridine nucleotides, but would readily incorporate niacin. In the present studies, niacin supplementation resulted in a substantial increase in blood pyridine nucleotide concentration regardless of the tryptophan content of the diet. Since niacin can be so readily converted to blood pyridine nucleotides, it is not unexpected that pyridine nucleotide concentration of blood cannot be used as an indicator of the adequacy of tryptophan intake.

Although Burch and co-workers (4) obtained larger increases in liver and blood pyridine nucleotide concentrations from niacin than from an equimolar quantity of tryptophan, the quantity of tryptophan in the diet (16 mg/day) was probably just adequate for growth, whereas the niacin (5 mg/day) was in excess of the quantity required for growth.

It is well known that excess niacin and tryptophan in the diet are converted to N^1-methylnicotinamide as this product has been measured in the urine of animals in response to doses of niacin and tryptophan. The direct conversion of nicotinamide to N^1-methylnicotinamide has been shown by Cantoni (14), but to date no direct conversion of niacin to nicotinamide has been demonstrated. From the work of Handler (15) and subsequently Langan et al. (16) it appears likely that niacin is first metabolized to DPN, then to nicotinamide and finally to N^1-methylnicotinamide which is excreted as such. Thus the increases in pyridine nucleotides in response to large doses of tryptophan and niacin may be due to increased catabolism of tryptophan, via the kynurenine pathway, to niacin; and to metabolism of niacin to nicotinamide and N^1-methylnicotinamide with DPN as an intermediate.

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Proceedings of the Twenty-seventh Annual Meeting of the American Institute of Nutrition

SHELBURNE HOTEL, ATLANTIC CITY, NEW JERSEY
APRIL 16–20, 1963

COUNCIL MEETINGS

The American Institute of Nutrition Council met on Sunday evening, April 14, and Monday morning and evening, April 15. Formal actions of the Council were reported at the Institute business meetings and are included in the following minutes.

SCIENTIFIC SESSIONS

A full 5 days of scientific sessions on nutrition and related sciences were held as part of the annual meetings of the Federation of American Societies for Experimental Biology. A total of 249 abstracts was submitted to the Institute for inclusion on the scientific program. Of these, 19 were transferred to programs of other societies and intersociety sessions. Seventy-six abstracts were received by transfer from other societies. A nutrition program of 26 half-day sessions was arranged and published in the March-April issue of *Federation Proceedings*, Part 1. In addition, 5 half-day intersociety sessions on atherosclerosis and 5 half-day symposia were held. The symposia were as follows:

Research Approaches to Amino Acid Nutrition
Basis for Expressing Amino Acid Requirements and Efficiency of Protein Utilization by Poultry
Feeding Patterns and Their Biochemical Consequences
Fallout, Food, and Man
Food and Nutritional Problems in Prolonged Space Travel

BUSINESS MEETINGS

Business meetings were held on Tuesday, April 16, and Thursday, April 18, attended by approximately 200 and 160 members, respectively. Dr. Leo C. Norris, president, presided at both meetings.

I. *Minutes of 1962*

The minutes of the 1962 meeting, as published in *The Journal of Nutrition*, 78: 120, 1962, were approved.

II. *Election*

The Secretary transmitted the sealed ballots to the Tellers' Committee, Dr. Samuel Tove, chairman, and Dr. Kendall W. King. At the second business meeting the committee reported the election results from 504 ballots received as follows:

Effective July 1, 1963:
President: Dr. Grace A. Goldsmith
President-Elect: Dr. R. W. Engel
Councilor (three-year term): Dr. D. Mark Hegsted
Effective May 1, 1963:
Editorial Board, Journal of Nutrition (four-year term):
Dr. G. M. Briggs
Dr. R. M. Forbes
Dr. J. Hirsch
Dr. E. E. Howe

The names of individuals with the 10 highest number of votes for suggested members of the Nominating Committee were submitted to the President. (See complete list of officers and committees at the end of these proceedings.)

III. *Constitutional Amendments*

By over two-thirds of all votes cast, the following amendments to the constitution and by-laws were adopted:

A. By a vote of 457 for to 10 against, Article V, Section 1, has been changed to read as follows:

> *Section 1.* Membership. Election of Nominating Committee. A. The Nominating Committee shall consist of five members to be elected annually from a slate of five sets of two names with due consideration to representation of different scientific areas of nutrition. The slate shall be selected by the three Councilors with the senior Councilor serving as Chairman of the selection group. Members who have served on the Nominating Committee for two consecutive years shall be ineligible for re-election until after a lapse of one year. B. The incoming President shall designate one of the five selected members to be Chairman of the Nominating Committee.

B. By a vote of 465 for to 0 against, Article III, Section 2 has been changed to read as follows:

Section 2. Council. The President, the Past-President, the President-Elect, Secretary, Treasurer, and three additional Councilors, one of whom shall be elected annually to serve a term of three years, shall constitute an eight-member Board of Trustees and shall be known as "The Council." The President-Elect shall be considered as a Vice-President and serve in the absence of the President.

C. By a vote of 451 for to 10 against, Article VII, Section 3, and Article V, Section 2, have been changed to read as follows:

1. Article VII, Section 3

Section 3. Official Journals of the Institute. A. The editorial responsibility for each official journal of the Institute shall be vested in an Editor, Associate Editor (if appointed), and in an Editorial Board. B. Remains as is. C. New members of each Editorial Board, and persons filling vacancies, shall be members of the Institute and shall be appointed by the Council on recommendations of the Editor who will seek suggestions from the Editorial Board. The term of service of Editorial Board members is for four years beginning on May 1 (except for persons filling vacancies). D. Remains as is.

2. Article V, Section 2

Section 2. Nomination of Officials. A. The Nominating Committee shall make at least one nomination for the offices of President-Elect and Councilor and for the offices of Secretary and Treasurer, when applicable. Any member of the Institute may submit nominations to the Nominating Committee for its consideration along with those nominations made by the members of the Nominating Committee. Parts B and C remain the same.

D. By a vote of 459 for to 4 against, Article VII, Section 2, has been changed to read as follows:

Section 2. Publications Committee. The management of the official publications of the Institute shall be under the supervision of a Publications Committee. This committee shall be appointed by the incoming President with approval of the Council. It shall advise the Council on matters of publication management, designation of each official journal of the Institute, and shall negotiate for the approval of the Council, agreement with publishers of each official journal.

IV. *Membership Status*

The Secretary reported that as of April 10, 1962, there were 874 members of the Institute — 807 active, 57 retired and 10 honorary members, this being a net increase of 62 members since last year. Eight members retired during the year. The Clinical Division reported a total membership of 141.

Members present at the business meeting stood for a moment of silence in memory and in recognition of the following members of the Institute who had passed away since the last meeting:

Clifford W. Duncan, October 25, 1962
*R. Adams Dutcher, April 20, 1962
*Conrad A. Elvehjem, July 27, 1962
Arild E. Hansen, October 16, 1962
*Grace MacLeod, November 16, 1962
*Irvine McQuarrie, September 9, 1961
Russell F. Miller, February 24, 1963
Marjorie M. Nelson, January 28, 1963
P. Mabel Nelson, February 19, 1963

* Charter members.

Appropriate resolutions which had been received for deceased members were read and approved. Resolutions were read in honor of charter members, Drs. Dutcher, Elvehjem, MacLeod and McQuarrie, and are included in the minutes as per custom.

RESOLVED. That the American Institute of Nutrition, assembled at Atlantic City, New Jersey, in its Annual Meeting, April 16, 1963, place in its minutes for permanent record this statement of deep regret and sorrow at the passing of one of its most distinguished members, Raymond Adams Dutcher, and further

RESOLVED. That high tribute be paid to Dr. Dutcher, a charter member of the American Institute of Nutrition, for his outstanding accomplishments as an administrator, as a teacher and as a scientist. In the course of his 30-odd years as an administrator, Dr. Dutcher assembled and organized one of the few biochemistry departments whose graduates are accredited by the American Chemical Society. As a teacher he was outstanding and became the author of three textbooks in his chosen field. As a researcher, he was one of the pioneers in the vitamin field. In addition to his many responsibilities, he became the author or co-author of over 100 technical contributions reporting on various phases of his biochemical or nutritional researches.

RESOLVED. That the American Institute of Nutrition, assembled at Atlantic

City, New Jersey, in its Annual Meeting, April 16, 1963, place in its minutes for permanent record this statement of deep regret and sorrow at the passing of one of its most distinguished members, Conrad A. Elvehjem, and further

That high tribute be paid to Dr. Elvehjem for his extraordinary productivity and his outstanding contributions to nutritional biochemistry, to our knowledge of mineral requirements, of amino acid balance, of the vitamin B complex, and in particular of nicotinic acid;

for his services as an effective research advisor to the 89 graduate students who obtained their Ph.D. degrees under his direction;

for his services to this Society as a member of the Editorial Board of the Journal, as Councilor and as President;

for his services to his University as a successful administrator, as Chairman of the Biochemistry Department, as dean of the Graduate School and finally as President;

for his public service in governmental and scientific bodies of many kinds including the Committee on Food Composition, the Food and Nutrition Board of which he was Chairman, the National Science Foundation and many others.

His list of honors is a long one, and includes the two highest awards within the province of this Society — the Osborne-Mendel Award and the Mead-Johnson Award. In magnitude and quality, his record is not likely to be duplicated.

RESOLVED. That the American Institute of Nutrition recognize the loss of Dr. Grace McLeod on November 16, 1962, at the age of 84 years. She was a charter member of the Institute.

She served on the Editorial Board of the *Journal of Nutrition* for a period of years and was co-author of several leading textbooks on nutrition.

Professor MacLeod was a member of the Nutrition Staff of Teachers College, Columbia University, for a period of 25 years. She was known for her outstanding research in the field of energy metabolism. As a teacher, she was beloved and respected by her many students who continue to contribute through their research and teaching to the further development of the science of nutrition.

It is further resolved that this statement be entered in the minutes of this meeting and a copy sent to the family.

RESOLVED. That the American Institute of Nutrition, assembled at Atlantic City, New Jersey, in its Annual Meeting, April 16, 1963, place in its minutes for permanent record this statement of deep regret and sorrow at the passing of its distinguished member, Irvine McQuarrie.

Dr. Irvine McQuarrie, a charter member of the American Institute of Nutrition, devoted much of his research effort to the application of biochemical and nutritional knowledge to the understanding of diseases of children. His accomplishments in this area were outstanding. Of at least equal importance was his ability to select promising young physicians and to stimulate and support their interests in medical research. The accomplishments of his many devoted former students will continue to add to the great esteem in which he was held by all who knew him.

V. *New Members*

The Council received 95 nominations for active membership, of which 77 were unanimously approved by members at the business meetings. All of these have accepted membership in the Institute. They are as follows:

NEW MEMBERS — 1963*

Mildred Adams
Robert Scott Allen
Carl E. Anderson
Zoe Anderson
Dorothy Arata
Billy R. Baumgardt
Lawrence R. Berg
Arthur L. Black
Lynn G. Blaylock
T. H. Blosser
Robert E. Bolinger
Benjamin T. Burton
Harold W. Carroll
Clinton O. Chichester
Donald C. Clanton
Dennis H. Cox
Richard D. Creek
Robert J. Davey
J. Friedrich Diehl
Irwin Allen Dyer
Henry A. Dymsza
Jane C. Ebbs
Royce J. Emerick
Abraham S. Feigenbaum
Coy D. Fitch
Donald F. Flick
Peggy Crooke Fry
Edgar S. Gordon
May Rose Gram
Allen R. Hennes
Donald B. Hudman
Ruth L. Huenemann
Ogden C. Johnson
Richard J. Jones
David Kritchevsky
Jordan G. Lee
Melvin Lee
David A. Libby
Sheldon Margen
John Temple McCall
Donald B. McCormick
Brian H. McCracken
Marion E. McDowell
James H. Meyer
Sanford A. Miller
Nobuko Shimotori Mizuno
Rosemary Shull Morris
Hazel C. Murray
Padmanabhan P. Nair
Marcel E. Nimni
James E. Oldfield
Robert R. Oltjen
Rosemarie Ostwald
Kenneth K. Otagaki
George M. Owen
Ernest R. Peo, Jr.
Anthony V. Pisciotta
John C. Rogler
Magnar Ronning
Harold E. Schendel
John R. Schubert
Alan Jonathan Sheppard
Glen M. Shue
William W. G. Smart, Jr.
David C. Snetsinger
George F. Stewart
Alloys L. Tappel
Milton Toporek
George V. Vahouny
John F. Van Pilsum
William C. Weir
Donald S. Wiggans
W. Lane Williams
Everett L. Wisman
Lloyd A. Witting
George Wolf
Walter Woods

* For institutional affiliations and addresses of new members see the September issue of *Federation Proceedings*.

HONORARY MEMBERS

The election of four new Honorary Members nominated by the Council was unanimously approved by the membership. The new Honorary Members are:

Dr. Kumitaro Arimoto
Dame Harriette Chick
Dr. Herbert M. Evans
Dr. Toshio Oiso

VI. *Treasurer's Report*

A report by the Treasurer, Dr. Douglas V. Frost, from April 9, 1962, to April 11, 1963, was read and approved. The Auditing Committee, Dr. George H. Berryman and Dr. Henry C. Spruth, submitted a report that the Treasurer's accounts were correct and complete. The report was approved.

TREASURER'S REPORT

April 9, 1962 — April 11, 1963

Balance brought forward		
Bank Deposits plus $500 U. S. Series K Bond		$ 8,128.83
Income		
American Institute of Nutrition Dues, 805 members	$1,610.00	
Federation Dues, 811 members	4,866.00	
Journal of Nutrition Subscriptions, 723 members	7,230.00	
Wistar Institute for Editorial Office	9,700.00	
Wistar Institute for Additional Net Subscriptions	1,001.25	
American Society for Clinical Nutrition	1,679.00	
American Society for Clinical Nutrition, Refund overpayment	106.00	
Federation Rebate from 1962 Annual Meeting	2,816.01	
U. S. Series K Bond Interest	6.90	
Bank Deposit Interest	87.50	
Overpayment from Members	37.64	
Nutrition Foundation and Borden Company for Award Winner Reception	552.26	29,692.56
Total receipts and balance brought forward		$37,821.39
Expenses		
Federation Office:		
Invoice for 829 active, 10 honorary, 2 retired members	5,046.00	
Addressing Dues Notices	37.52	
Fall Mailing	117.21	
Wistar Institute, *Journal of Nutrition* subscriptions	6,911.50*	
Wistar Institute, *Journal of Nutrition* for 1961–62	10.00	
Wistar Printing of Dues Cards and Stamped Envelopes	87.06	
American Society for Clinical Nutrition	1,793.50	
Secretary's Office Expenses	650.00	
Reception for Award Winners at Annual Meeting	552.26	
Cornell University, for Editorial Office	9,600.00	
Operating Deficit for Year Ending June 30, 1962	689.64	
Delegate to Canadian Nutrition Society, June 7–8, 1962	166.30	
Travel Expenses, Council Meeting, October 31, 1962, Washington	640.41	
American Institute of Biological Sciences Affiliation	100.00	
Bank Charges	15.53	
Refunds to Overpaid Members	40.04	
Total expenditures		$26,456.97
* Estimate of payment still due Wistar for *Journal of Nutrition*		320.00
TOTAL		$26.776.97
Over-cash balance		— 58.87
		$26,718.10
Balance on hand, April 11, 1963		
Cash	10,923.29	
U. S. Series K Bond	500.00	
		$11,423.29
Estimated payment due Wistar		320.00
Expected balance		$11,103.29

DOUGLAS V. FROST, *Treasurer*

VII. *Dues*

The president, Dr. Norris, reported on the recommendation of the Council that the AIN dues be increased $3.00. Dr. Norris discussed the needs for an increase in dues. The current increase is to assist in defraying the additional costs of the offices of the Secretary and the Treasurer. He explained the desire of the Council to establish a permanent Secretariat at the Federation headquarters.

The motion was made, seconded and unanimously passed by the membership to increase the dues by $3.00.

The Council also recommended that foreign subscribers to either of the official journals be assessed the additional mailing charge.

Dues for the coming year will be as follows:

AIN ($5.00) and Federation ($6.00) dues	$11.00
Clinical Division dues	2.00
The Journal of Nutrition (Optional to Clinical Division members)	10.00
American Journal of Clinical Nutrition (Required of Clinical Division members — optional to AIN members)	8.50

VIII. *Editor's Report*

The editor of *The Journal of Nutrition*, Dr. Richard H. Barnes, submitted his report for the period July 1, 1962 to June 30, 1963. It was approved and is summarized below:

Summary of Finances in the Operation of the Editor's Offices — *The Journal of Nutrition*

Balance brought forward	$(−689.64)
Receipts — AIN	10,289.64
Total receipts and balance available	9,600.00
Expenditures, partially estimated, per schedule	11,905.64
Estimated balance July 1, 1963 (loss)	($−2,305.64)

The AIN Council approved payment from the AIN treasury to cover the deficit of the Editor's office for the current year ending June 30, 1963. Of the above deficit, $1,428.88 is due to the cost of preparing the 11-year Cumulative Index. It is estimated that an additional $1,100 will be required to complete the Cumulative Index during the coming year. The Council recommended that the expenses of the editorial office for preparation of the 11-year Cumulative Index be recovered by negotiating the sale price of the index with The Wistar Institute.

Editing and Publication Operations

(January 1, 1962 — December 31, 1962)

	1960	1961	1962
Volumes published	70, 71, 72	73, 74, 75	76, 77, 78
Pages published (including papers, biographies, announcements, and proceedings)	1486	1399	1447
(Scientific papers only)		(1357)	(1403)
Papers published (including 3 biographies)	208	203	209
Papers submitted	231	287	321
Papers rejected	29	59	85
Rejection rate (based on % of papers submitted)	13%	20%	26%
Supplements published	—	—	1

Biographies: Cyrus Edwin French, Edward Bright Vedder, Wilbur O. Atwater

Operating Schedule	1960	1961	1962
Average time lapse from receipt of manuscript to publication date:			
Avg no. days with reviewer	17.3	20.6	20
Avg no. days out for revision	17.3	18.3	17.9
Avg no. days in office, in mail, or in unavoidable delay	27.1	21.8	32.6
Total days	61.7	60.7	70.5
Avg no. months being processed by Editorial Office	2.1	2.0	2.4
Avg no. months with Wistar Press	3.0	3.2	3.4
Total, months	5.1	5.2	5.8

IX. *Reports of Committees and Representatives*

A. *Committee on Publication Management:* Dr. W. J. Darby, chairman.

The Publication Management Committee continues to consider means of improving the publication program and financial support of the Society's journal(s). Our present agreement with The Wistar Institute Press for the publication of the *Journal of Nutrition* continues through next year. Wistar pays $9,700 to the Editor's office and an arrangement has been completed whereby the American Institute of Nutrition participates in the portion of the income obtained from nonmember subscriptions to the Journal that are in excess of the number of member subscriptions. Also, the Institute receives the income from page charges. (Wistar will charge AIN for publication of pages in excess of 1,500 per year.) This income is for the twofold purpose of support of the editorial and Journal expenses by the AIN and of providing funds for the payment by AIN to Wistar Press of costs of publication of pages in excess of the agreed number. The experience with this arrangement is now accumulating but it is too early to assess the income accurately since only with this April issue of the Journal were manuscripts published on which page charges were levied.

The costs of the editorial office continue to rise at about 6 to 8% per year as may be anticipated. This is due to normal salary adjustments and the expense of handling a larger volume of manuscripts.

This year the cost will approximate a total of $11,905 — a sum $2,200 in excess of the lump payment by Wistar. This cost next year may be expected to increase slightly.

In addition, the Editor's office is completing an 11-year Cumulative Index through 1960. This will have cost some $2,500 for its preparation. Your Committee will enter into negotiations with Wistar to recover this cost from the income on sales of this index.

The Management Committee continues to explore a long-range plan of publication and some editorial device which will provide adequate services to relieve the editorial office of as much of the time-consuming mechanical work as possible.

Helpful discussions have been held with the editors of both of the Institute's journals and consideration will be given to the needs and problems of both in arriving at any proposal in the future.

The Council has instructed this Committee to make recommendations to it at the fall, 1963, meeting concerning the future publishing arrangements for *The Journal of Nutrition.*

B. *Public Information Committee:* Dr. Philip L. White, chairman.

The pamphlet, "Career Opportunities in Nutrition," continues to be popular. More than 4,000 copies were mailed from the Chairman's office. A number of career opportunity registers have included an abstract of the pamphlet. The Committee and the Secretary responded to numerous communications from the public.

The size and scope of the Public Information Committee was expanded during the year in preparation for increased activities if and when a permanent Secretariat is established for the American Institute of Nutrition.

The main function of the Committee to date has been the evaluation of abstracts for their newsworthiness. This has been done in cooperation with the office of Dr. Sidney Negus, Director of Public Information for the Federation. The Committee is grateful for the cooperation extended by the moderators in helping to select topics thought to be newsworthy.

C. *U. S. National Committee — International Union of Nutritional Sciences:* Dr. W. H. Sebrell, chairman.

1. *Travel Grants to the Sixth International Congress of Nutrition.* The U. S. National Committee — IUNS established a Travel Grant Award Committee composed of Dr. Floyd S. Daft, chairman, Dr. A. E. Schaefer, secretary, and Drs. W. H. Sebrell, C. G. King and R. W. Engel. The total funds available for travel grant awards was approximately $50,000, of which a $25,000 grant was obtained by the American Institute of Nutrition from the National Institutes of Health, $17,500 from the National Science Foundation, and $8,000 plus interest from the fund designated "U. S. — IUNS," in accordance with the action of the American Institute of Nutrition at the 1962 annual meeting. Arrangements were completed to have the Federation of American Societies for Experimental Biology handle the accounting and disbursement of funds. A total of 96 grants has been awarded to date. Grants cover economy jet fares round trip from the recipient's home station to Edinburgh.

2. *Group Travel.* Dr. Schaefer reported on the arrangements for group air travel which were worked out with the Ambassador Travel Service and Pan American Airways. Approximately 240 individuals who will attend the Sixth International Congress of Nutrition have taken advantage of this reduced group fare.

3. *U. S. National Committee — IUNS Delegates to Sixth International Congress of Nutrition.* The U. S. National Committee — IUNS designated two official delegates to the Sixth International Congress of Nutrition, Dr. W. H. Sebrell and Dr. Grace Goldsmith, whose appointments have been approved by the State Department. The State Department has graciously approved paying the expenses of one of the delegates. One of the objectives of the U. S. — IUNS will be to work towards the admission of IUNS to the International Council of Scientific Unions (ICSU).

D. *Representatives to the AAAS Council:* Dr. J. H. Roe and Dr. Paul B. Pearson.

Delegates attended the 129th meeting of the American Association for the Advancement of Science, December 26–30, 1962.

The AAAS is a super-federation consisting of 304 affiliated and associated societies and academies of science, the membership of which probably exceeds seven million. It has a direct active membership of 81,000 persons.

1. *Report of the Committee on Council Affairs.*

a. The Council Study Committee on International Scientific Communications considered the desirability and feasibility of preparing an International Science Register. The Committee decided that it would be clearly desirable to have an international scientific directory, but the costs and problems involved in producing such a directory are great and there seems no feasible way of handling them at present.

b. The Study Committee on International Scientific Communication reported that plans are going forward, in cooperation with the Japanese Science Council, for a Symposium on Japanese science, to be held at the AAAS meeting in Cleveland in 1963.

c. The decision of the Board of Directors, that the AAAS should neither oppose nor support a proposed amendment to the National Science Foundation Act to alter the qualifications for receipt of an NSF fellowship, was approved by the Council.

2. *Report of Committee on Affiliation.* The following eight societies, on recommendation of this committee, were elected to membership:

The American Academy of Arts and Sciences
American Society of Clinical Hypnosis
American Vacuum Society
Biophysical Society
Conference Board of the Mathematical Sciences
Medical Library Association, Inc.
Society for General Systems Research
Society of Technical Writers and Publishers, Inc.

3. *Report of Committee on Science in the Promotion of Human Welfare.* This committee concerned itself with the following:

a. Organization of a Commission on Air Pollution.

b. Planning conferences to facilitate communication between scientists and other members of the community.

c. Organization for the Philadelphia meeting of a symposium on "The Integrity of Science."

4. *Report of Committee on Public Understanding of Science.* The following achievements were noted:

a. Supported by a grant from the National Science Foundation, the AAAS is presenting a once-a-week program for a scientific audience over the educational television station of New York City.

b. In cooperation with the Brookings Institute, the AAAS is presenting a third series of seminars on scientific research topics for members of Congress.

c. The Holiday Science Lecture Series, supported by a grant from the National Science Foundation, is being well received. This consists of a lecture series given in New York, with Rockefeller Institute as co-sponsor; in Boston, with the American Academy of Arts and Sciences as co-sponsor; in Chicago, with the Chicago Museum of Natural History as co-sponsor; and in Seattle, with the University of Washington as co-sponsor.

5. *Report of the Executive Officer and Presentation of 1963 Budget.* The Executive Officer, Dr. Dael Wolfle, reported the following:

a. During the year 1962 the largest number of new members ever to join the AAAS, 18,358, was reported. This number is 50 per cent greater than the number of new members admitted during previous high admission years.

b. A budget of $2,420,700 was presented which would yield a surplus of receipts over expenses estimated to be $133,000.

c. Grants received during 1962, amounting to $586,000 were reported.

E. *Representative to the Food and Agriculture Organization:* Dr. B. S. Schweigert.

The Food and Agriculture Organization's Nutrition Division has shown a general expansion of activities in the current year. They have initiated an expanded program in January of 1963 in conjunction with the United Nations Special Fund, the World Food Programme and the Expanded Program for Technological Assistance to governments. Approximately 90 million dollars in foods, cash or services have been pledged in support of this program. Specific activities will include emergency aid, establishment of food reserves, and economic and social development. FAO highlighted the midpoint of their Freedom from Hunger campaign by featuring Freedom From Hunger Week in February of 1963. A World Food Congress will be held in the United States in June 1963 under their sponsorship. Joint assistance with the United Nations Children's Fund now extends to over 40 developing countries with applied nutrition projects. FAO, in collaboration with other international agencies, is also active in the production and distribution of low cost protein preparations to developing countries.

We can look forward to continuing attention to the crucial area of improved nutrition by FAO and interrelated problems of food preservation and distribution as a part of their total program.

F. *Representative to the Division of Biology and Agriculture, National Research Council:* Mr. N. R. Ellis.

Your representative to the Division of Biology and Agriculture of the National Research Council attended the several meetings of the constituent boards, committees and other units held in Washington during the past 12 months.

During 1962, Dr. M. B. Russell succeeded Dr. W. E. Krauss as Chairman of the Agricultural Board. Also, Dr. Paul F. Sharp assumed his duties as Executive Secretary of this organization. Publication 990, "Nutrient Requirements of Laboratory Animals," was issued recently. This is a

96-page booklet that covers the nutrient requirements of the cat, guinea pig, monkey, hamster, mouse and rat. There is now a coverage of 16 animal species in this series of publications.

During the past year a revision was issued on "Nutrient Requirements of Dogs" (Pub. 989). Revisions of publications on beef cattle, dairy cattle, foxes and minks, rabbits, sheep and swine are currently underway. Dr. L. C. Norris, longtime Chairman of the Committee on Animal Nutrition, asked to be relieved of this office and in his place Dr. W. M. Beeson was appointed to head this committee.

The Agricultural Research Institute held its Eleventh Annual Meeting on October 15–16, 1962. There was a varied program on general topics such as "Research needed to solve industry's 1970 problems," "Research in USDA pioneering laboratories," biological and related methods of insect control and cooperative agricultural research programs. Consideration has been given in the past two years to the merging of ARI and the Agricultural Board. It was decided not to do this since much of the original needs for merger has been achieved through the integration of representation in the two organizations.

The Food and Nutrition Board held two general meetings, one on November 2, 1962, and the other on April 5, 1963, at which the committees serving under the Board reported on progress of studies. The Board responded to several inquiries from government agencies for advisory services. One from the Department of Interior concerned fish protein concentrates. A committee has considered the problem and has concluded that a "wholesome, safe and nutritious product can be made from whole fish." Experimental and small scale plant studies have shown that such a product can be prepared. However, thus far successful large scale production still presents problems of quality control, including freedom from solvent extraction residues.

In March, 1962, the Secretary of Agriculture requested a statement on dairy products as concerned with consumer questions of fat in relation to cardiovascular disease, fallout contamination, pesticide residues, research needs and others. A five-page statement entitled "The nutritional significance and safety of milk and milk products in the National diet" was issued on May 25, 1962.

Consideration has been given to minimal food and water requirements for survival in connection with the Navy's fallout shelter tests.

As has been true for a number of years, much attention is given to international food problems. A prominent current question concerns the adequacy and nutritional balance of overseas shipments of foods in the Food for Peace program from the standpoint of possible malnutrition developing in people in the receiving country. Some recommendations on policy principles have been made.

On April 9–10, 1963, there was held the annual meeting of the National Research Council along with the constituent divisions, including the Division of Biology and Agriculture. There are now 41 scientific societies represented with membership along with 23 additional individuals either classed "at large" or "liaison." Besides the presentation of reports of activities of the Food and Nutrition Board, the Agricultural Board and other committees, there was discussion of the recreation of the Biology Council. As outlined by the Chairman of the Division, Dr. T. C. Byerly, it is the plan to have such a council consider and outline the emerging problems in biology and to identify new concepts that are on the horizon.

International activities received attention also. Consideration is being given to a proposal for development of an international biological program. Work continues on assessment of factors involved in plant and animal pests. A resumé was presented on bills introduced in Congress on the humane care and treatment of vertebrate animals used in research. At the time of the report, seven bills had been introduced in the present session of the 88th Congress. All are similar in intent but differ somewhat in detail of application and manner of operation. While the general sentiment of biologists is that legislation is not needed and would restrict and curtail research, the opinion was expressed that enactment of legislation on the subject is a likely possibility and that support of the most constructive bill may be the best course. It is suggested that such a bill may be S1041, the Randolph Bill.

G. *Committee for Joint AIN — Nutrition Society of Canada Meeting in 1964:* Dr. H. H. Williams.

The joint meeting will be held at the Royal York Hotel, Toronto, Ontario, September 14–15, 1964. Two half-day sessions will be devoted to symposia and two half days will include two sessions of scientific papers. It is anticipated that the announcement regarding the program, abstracts, registration fees, etc. will be included in the fall AIN mailing. Deadline for abstracts will be approximately June 30, 1964.

H. *Federation.*

Dr. Grace A. Goldsmith reported on Federation activities. She described proposals that are being considered regarding a long-range program for the Federation. The Federation Board approved raising the non-member registration fee for the annual meetings from $15.00 to $20.00, and having a registration fee of $10.00 for graduate students and retired members.

The Council proposed a resolution to the membership which was passed unanimously expressing the AIN's gratitude to the Federation staff, Dr. Milton Lee and his assistants, for their dedicated, efficient operation and foresight in future planning to continue to serve the member societies and the individual members.

The dates of the 1964 Federation Meetings, to be held in Chicago, are April 12–18.

X. *Report of the Clinical Division:* Dr. Robert E. Hodges, Secretary-Treasurer of ASCN.

The membership of the American Society for Clinical Nutrition is 141 and there is a balance of $3,204.06 in its treasury. A constitutional amendment has been approved which states that in the event of termination of ASCN any assets remaining should be turned over to the AIN. Dues and journal assessments will remain the same for the Clinical Division. The Third Annual Meeting was to be held Saturday, April 27, 1963, in the Terrace Room of the Colton Manor Hotel in Atlantic City. The 1964 meeting will be held in the same hotel on May 2. Newly elected officers are listed on the last page of these minutes.

XI. *Report of AIN Council Actions:* Dr. Leo C. Norris.

A. The Council recommended that a special committee be established to investigate the possibilities for establishing a secretariat with an Executive Secretary at the Federation office, Beaumont House, and to determine possible ways and means for defraying the cost of such an office.

B. The Council approved reimbursement to the AIN Treasurer of the Secretary's costs that have been involved in notification of awards and arrangements for the Sixth International Congress of Nutrition. Reimbursement would be made from funds specified for this purpose from funds of the U. S. — IUNS.

C. Council approved re-establishment of the Mead Johnson Award, expressing gratitude and thanks to the Mead Johnson Company. Criteria for the award will be as follows:

1. The award shall be entitled "Mead Johnson Award for Research in Nutrtiion."

2. The award shall be made annually, consisting of $1,000 and a certificate.

3. The recipient(s) of the award shall be restricted to investigators who have not reached their 46th birthday during the calendar year in which the award is given.

4. The award shall be based on a single outstanding piece of research in nutrition, published in the year preceding the annual meeting, or on a series of papers on the same subject published within not more than the three years preceding the annual meeting.

5. Membership in the American Institute of Nutrition shall not be a condition for receiving the award.

6. The American Institute of Nutrition shall proceed as the Council determines is best for the selection of the recipient.

D. Dr. Norris discussed the establishment of three new committees, one an ad hoc Committee on Nutrition Training and Fellowships. This committee will be charged with the responsibility of developing a report giving guidelines for the development of curricula for nutrition training in various fields of nutrition — animal, plant and human, and of developing a background paper to encourage fellowship awards by the National Academy of Sciences in the various fields of nutrition.

Council has created a committee in charge of symposia for the annual meetings. In the past, the responsibility for organizing symposia has been that of the outgoing Council member. At the 1963 meeting, Dr. Engel reported on the success of an ad hoc committee that was developed to assist him in organizing the five symposia. The chairman of this committee will be the outgoing Council member.

The third committee formed is an ad hoc Committee on International Nutrition, a primary function of which would be to keep members of the AIN informed about international nutrition activities.

E. A tentative resolution on policies of administering federal grants approved by the Council was presented to the membership. With a revision suggested by Dr. M. O. Schultze, the following resolution was adopted by the membership:

> The recent criticisms made by the Intergovernmental Relations Subcommittee of the House Committee on Government Operations concerning the management of the research grant programs of the National Institutes of Health, Department of Health, Education and Welfare, has made it highly desirable to have a clearer definition of the policies underlying the administration of federal programs in support of research at our universities and research laboratories. A critical study is, therefore, needed of the relationships which should properly exist between the Federal government and our research institutions and their scientific investigators. Accordingly the members of the American Institute of Nutrition in assembly at Atlantic City, New Jersey, April 18, 1963, urgently request that the National Academy of Sciences — National Research Council, undertake a critical appraisal of the policies of the Federal government and of recipient research and educational institutions pertaining to the research grant programs not only of the National Institutes of Health, but also to those of all other federal agencies. We request, furthermore, that following this critical appraisal the NAS — NRC enunciate principles of a basic policy which will serve as a guide in the future conduct and administration of Federal programs in support of scientific investigations.

ANNUAL DINNER AND PRESENTATION OF FELLOWS AND AWARDS

The annual banquet was held on Friday, April 19, at the Shelburne Hotel, with approximately 300 members and quests attending. Dr. Norris, as toastmaster, introduced the special guests and awardees.

Dr. David Hand presented Certificates of Fellow to the following persons selected for their distinguished careers in nutrition:

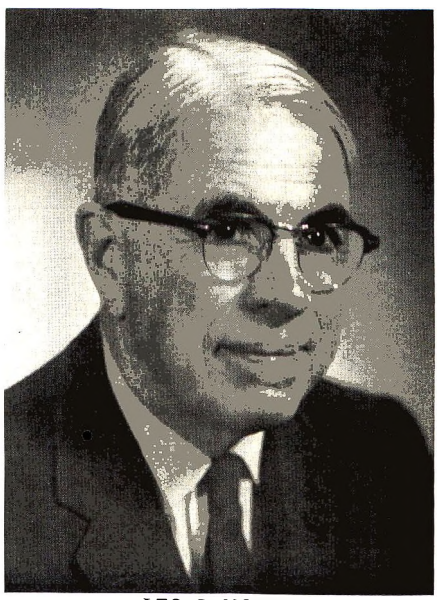
WENDELL H. GRIFFITH — "For a distinguished career in experimental nutrition in research areas of choline metabolism and amino acid interrelationships — for his service in the field of international nutrition both as Chief of Nutrition Branch, Office of the Chief Surgeon, European Theater of Operations in World War II, where he received the Legion of Merit Decoration and the Bronze Star Award, and as Nutrition Advisor to India for UNICEF — for his service and contribution as a member of a number of nutrition boards, including the Food and Nutrition Board of the National Academy of Sciences — National Research Council, the Council on Foods and Nutrition of the American Medical Association, Advisory Panel of the Interdepartmental Committee on Nutrition for National Defense, and for his service to the American Institute of Nutrition as its Past President and Council member."

WENDELL H. GRIFFITH

C. GLEN KING — "A charter member of the American Institute of Nutrition, for a distinguished career as President of the Nutrition Foundation since it was organized in 1942 — for his pioneer research work in the isolation and identification of vitamin C — for his many services in activities as a member of the Food and Nutrition Board of the National Academy of Sciences — National Research Council, Past President of the American Institute of Nutrition, and as a member of the Expert Advisory Committee on Nutrition of the World Health Organization."

C. GLEN KING

LEO C. NORRIS — "For a distinguished career in experimental nutrition of animals, especially his contributions to studies of the B complex vitamins and trace minerals — for his leadership in the development of standards of nutrient requirements for farm and laboratory animals — for his many contributions as a member of the Agricultural Board of the National Academy of Sciences — National Research Council from 1949 to 1961, and as Chairman of the Board's Committee on Animal Nutrition, 1945 to 1962, as a recipient of the Borden Award from the Poultry Science Association in 1938 and the Teaching Award in 1957, and as President of the American Institute of Nutrition."



LEO C. NORRIS

Osborne and Mendel Award

The 1963 Osborne and Mendel Award of $1,000 and a scroll was presented to Dr. James B. Allison, director of the Rutgers University Bureau of Biological Research and head of the Rutgers Research Council, for his studies on protein quality, protein depletion, protein reserves, and protein metabolism — for his success in training workers in nutrition — and for his services to national and international bodies concerned with world-wide problems of malnutrition.

Borden Award

The 1963 Borden Award of $1,000 and a gold medal, was presented to Dr. Arthur L. Black of the Department of Physiological Sciences, University of California, Davis, for his studies on the origin of the amino acids of casein, on the role of the citric acid and pentose cycles in lactation, and on the utilization of C^{14}-labeled, short-chain fatty acids which have elucidated metabolic pathways involved in milk formation in the intact cow.

JAMES B. ALLISON

ARTHUR L. BLACK

OFFICERS AND COMMITTEES

AMERICAN INSTITUTE OF NUTRITION

July 1, 1963 – June 30, 1964

Council

President: Grace A. Goldsmith, Tulane University School of Medicine, New Orleans, Louisiana

President-Elect: R. W. Engel, Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg, Virginia

Past-President: Leo C. Norris, College of Agriculture, Department of Poultry Husbandry, University of California, Davis, California

Secretary: Olaf Mickelsen, Department of Foods and Nutrition, College of Home Economics, Michigan State University, East Lansing, Michigan (1966)

Treasurer: Douglas V. Frost, Research Division, Abbott Laboratories, North Chicago, Illinois (1965)

Councilors: W. A. Krehl (1964), H. H. Williams (1965), D. M. Hegsted (1966)

Committees

Nominating Committee: George K. Davis, chairman; Herbert Bird, George M. Briggs, Vernon Cheldelin, Robert Hodges

Joint Committee on Biochemical Nomenclature: F. W. Quackenbush, chairman (1964); Vernon Cheldelin (1965)

Committee on Publication Management: W. J. Darby, chairman (1964); P. L. Harris (1964), J. K. Loosli (1964), J. M. Hundley (1965), George Berryman (1965), Olaf Mickelsen (ex officio), John F. Mueller (ex officio)

Nominating Committee for Borden Award: Robert Shank, chairman (1964); Boyd O'Dell (1965), L. D. Wright (1966)

Nominating Committee for Osborne-Mendel Award: Max K. Horwitt, chairman (1964); F. W. Hill (1965), Nevin Scrimshaw (1966)

Nominating Committee for Mead Johnson Award: Robert Olson, chairman (1964); Carl Baumann (1965), B. Connor Johnson (1966)

Fellows Committee: Icie Macy Hoobler, chairman (1964); H. E. Robinson (1964), James Waddell (1965), Charlotte M. Young (1966), Paul E. Howe (1966)

Public Information Committee: P. L. White, chairman (1964); M. S. Read (1964), Allen D. Tillman (1964), L. J. Teply (1964), Martha F. Trulson (1965), Samuel B. Tove (1965), Allan L. Forbes (1965), J. M. R. Beveridge (1965), Olaf Mickelsen (ex officio) (1966)

Auditing Committee: Paul E. Johnson (1964), Edwin L. Hove (1964)

Tellers' Committee: Louis L. Rusoff (1964), Robert L. Squibb (1964)

Committee on Honorary Memberships: C. G. King, chairman; Helen Burch, W. D. Salmon

Symposium Committee: Willard A. Krehl, chairman; George K. Davis, B. S. Schweigert, A. E. Harper, Robert Olson

Ad hoc Committee on Establishment of an Executive Secretariat: Floyd S. Daft, chairman; George M. Briggs, Willard A. Krehl, Elmer L. Severinghaus, Philip L. White

Ad hoc Committee on International Nutrition: William Pearson, chairman; Guillermo Arroyave, K. W. King, Arnold E. Schaefer, Walter C. Unglaub, Calvin Woodruff, C. F. Asenjo

Ad hoc Committee on Careers in Nutrition: Theodore B. Van Itallie, chairman; Myron Brin, William G. Huekstra, George V. Mann, William J. McGanity, H. E. Sauberlich, E. L. R. Stokstad, J. J. Vitale, Leslie T. Webster

Ad hoc Committee on Nutrition Training and Fellowships: L. C. Norris, chairman; James McGinnis, co-chairman; C. O. Chichester, James Dinning, Wendell Griffith, Robert Hodges, O. Neal Miller, Clara Storvick

Ad hoc Committee to Outline Differentiation in AIN Awards: Paul György, chairman; W. H. Sebrell, O. L. Kline

Committee on Recommended Constitutional Changes: A. E. Axelrod, chairman (1964); L. M. Henderson, Marian E. Swendseid, U. D. Register, W. J. Visek

Organizing Committee for Joint Meeting with Nutrition Society of Canada: H. H. Williams, chairman; M. O. Lee, D. A. Richert

U. S. National Committee — IUNS

A. E. Schaefer (1964), F. S. Daft (1964), W. J. Darby (1964), W. H. Sebrell, Jr. (1964), Paul György (1965), W. M. Beeson (1965), G. F. Combs (1966), Charlotte M. Young (1966), O. L. Kline (1966)

Also ex officio (voting) member is Grace A. Goldsmith (1964) and ex officio (non-voting) members: T. C. Byerly, R. K. Cannan, Harrison Brown, E. C. Rowan

Representatives to Other Organizations

Federation Board: L. C. Norris (1964), Grace A. Goldsmith (1965), R. W. Engel (1966)

Federation Advisory Committee: Grace A. Goldsmith (1965)

National Research Council Boards and Divisions: Gerald F. Combs (1965)

American Association for the Advancement of Science: Paul B. Pearson, Delegate (1964); James M. Orten, Alternate (1965)

Food and Agriculture Organization: B. S. Schweigert (1964)

Editorial Board

The Journal of Nutrition

R. H. Barnes, editor (1964); Harold H. Williams, associate editor (1964); G. F. Combs (1964), R. M. Leverton (1964), G. V. Mann (1964), M. O. Schultze (1964), G. K. Davis (1965), A. E. Axelrod (1965), R. H. Follis, Jr. (1965), E. L. R. Stokstad (1965), J. G. Bieri (1966), Harry P. Broquist (1966), R. G. Hansen (1966), R. T. Holman (1966), G. M. Briggs (1967), R. M. Forbes (1967), Jules Hirsch (1967), E. E. Howe (1967)

Officers, American Society for Clinical Nutrition

(A division of the American Institute of Nutrition)

President, W. Henry Sebrell, Jr.; Vice-President/President-Elect, Willard A. Krehl; Past-President, William B. Bean; Secretary-Treasurer, John F. Mueller, Department of Medicine, Veterans Administration Hospital, Denver 20, Colorado; Councilors: George V. Mann, Robert E. Shank, Theodore Van Itallie

Editorial Board

American Journal of Clinical Nutrition

W. A. Krehl, editor (1967); Robert E. Hodges, associate editor; Laurance Kinsell, Charles S. Davidson, M. M. Wintrobe, William D. Robinson, Robert E. Olson, D. Mark Hegsted, Donald Watkin, Maurice E. Shils, Margaret Albrink, George Gabuzda.

Respectfully submitted,

ARNOLD E. SCHAEFER, *Secretary*

Invitation for Nominations for 1964 American Institute of Nutrition Awards

Nominations are requested for the 1964 annual awards administered by the American Institute of Nutrition to be presented at the next annual meeting. Nominations may be made by anyone, including members of the Nominating Committees and non-members of the Institute.

The following information must be submitted: (1) Name of the award for which the candidate is proposed. (2) A brief convincing statement setting forth the basis for the nomination. A bibliography and supporting letters are not to be submitted. (3) Five copies of the nominating letter must be sent to the chairman of the appropriate nominating committee *before October 1, 1963,* to be considered for the 1964 awards.

General regulations for A.I.N. awards. Membership in the American Institute of Nutrition is not a requirement for eligibility for an award and there is no limitation as to age except as specified for the Mead Johnson Award. An individual who has received one Institute award is ineligible to receive another Institute award unless it is for outstanding research subsequent to or not covered by the first award. A Jury of Award composed of A.I.N. members, which makes final selection and remains anonymous, may recommend that an award be omitted in any given year if in its opinion the work of the candidates nominated does not warrant the award. An award is usually given to one person, but, if circumstances and justice so dictate, a Jury of Award may recommend that any particular award be divided between two or more collaborators in a given research.

Presentation of awards will be made at the annual dinner at the annual meeting.

1964 Borden Award in Nutrition

The Borden Award in Nutrition, consisting of $1000 and a gold medal, is made available by the Borden Company Foundation, Inc. The award is given in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of milk or its components. The award will be made primarily for the publication of specific papers during the previous calendar year, but the Jury of Award may recommend that it be given for important contributions made over a more extended period of time not necessarily including the previous calendar year. Employees of the Borden Company are not eligible for this award nor are individuals who have received a Borden Award from another administering association unless the new award be for outstanding research on a different subject or for specific accomplishment subsequent to the first award.

Former recipients of this award are:

1944 – E. V. McCollum
1945 – H. H. Mitchell
1946 – P. C. Jeans and Genevieve Stearns
1947 – L. A. Maynard
1948 – C. A. Cary
1949 – H. J. Deuel, Jr.
1950 – H. C. Sherman
1951 – P. György
1952 – M. Kleiber
1953 – H. H. Williams
1954 – A. F. Morgan and A. H. Smith
1955 – A. G. Hogan
1956 – F. M. Strong
1957 – no award
1958 – L. D. Wright
1959 – H. Steenbock
1960 – R. G. Hansen
1961 – K. Schwarz
1962 – H. A. Barker
1963 – Arthur L. Black

NOMINATING COMMITTEE:
ROBERT E. SHANK, *Chairman*
BOYD L. O'DELL
L. D. WRIGHT

Send nominations to:

DR. ROBERT E. SHANK
Department of Preventive Medicine and Public Health
School of Medicine
Washington University
St. Louis 10, Missouri

1964 Osborne and Mendel Award

The Osborne and Mendel Award of $1000 and an inscribed scroll has been established by the Nutrition Foundation, Inc., for the recognition of outstanding recent basic research accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the

most significant published contribution in approximately the calendar year preceding the annual meeting of the Institute, or who has published recently a series of papers of outstanding significance. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration.

Former recipients of this award are:

1949 – W. C. Rose
1950 – C. A. Elvehjem
1951 – E. E. Snell
1952 – Icie Macy Hoobler
1953 – V. du Vigneaud
1954 – L. A. Maynard
1955 – E. V. McCollum
1956 – A. G. Hogan
1957 – G. R. Cowgill
1958 – P. György
1959 – Grace A. Goldsmith
1960 – N. S. Scrimshaw
1961 – Max K. Horwitt
1962 – William J. Darby
1963 – James B. Allison

NOMINATING COMMITTEE:

MAX K. HORWITT, *Chairman*
F. W. HILL
NEVIN SCRIMSHAW

Send nominations to:

DR. M. K. HORWITT
L. B. Mendel Research Laboratory
Elgin State Hospital
Elgin, Illinois

1964 Mead Johnson Award for Research in Nutrition

The Mead Johnson Award for Research in Nutrition has been re-established by the Mead Johnson Company. This award of $1000 and a certificate will be presented each year to an investigator who has not reached his 46th birthday during the calendar year in which the award is given and will be based on a single outstanding piece of research in nutrition published in the year preceding the annual meeting, or on a series of papers on the same subject published within not more than the three years preceding the annual meeting.

Former recipients of this award are:

1939 – C. A. Elvehjem
1940 – W. H. Sebrell, Jr.
J. C. Keresztesy
J. R. Stevens
S. A. Harris
E. T. Stiller
K. Folkers
1941 – R. J. Williams
1942 – G. R. Cowgill
1943 – V. du Vigneaud
1944 – A. G. Hogan
1945 – D. W. Woolley
1946 – E. E. Snell
1947 – W. J. Darby
P. L. Day
E. L. R. Stokstad
1948 – F. Lipmann
1949 – Mary S. Shorb
K. Folkers
1950 – W. B. Castle
1951 – no award
1952 – H. E. Sauberlich

NOMINATING COMMITTEE:

ROBERT OLSON, *Chairman*
CARL BAUMANN
B. CONNOR JOHNSON

Send nominations to:

DR. ROBERT OLSON
Department of Biochemistry and Nutrition
Graduate School of Public Health
University of Pittsburgh
Pittsburgh 13, Pennsylvania

Invitation for Nominations for 1964 American Institute of Nutrition Fellows

The Fellows Committee of the American Institute of Nutrition invites nominations for Fellows in the Society. Eligible candidates are active or retired members of the Society who have passed their sixty-fifth birthday (by the time of the annual meeting) and who have had distinguished careers in nutrition. Up to three Fellows will be chosen each year.

Nominations may be made to the Chairman of the Fellows Committee by any member of the Society, including members of the Committee.

Nominations (in 5 copies) are due by October 1. A supporting statement giving the reason for the nomination is desirable but not necessary.

Final selection will be made by the Fellows Committee and a suitable citation will be presented at the Annual Dinner in April.

Fellows Committee:

ICIE MACY HOOBLER, *Chairman*
H. E. ROBINSON
JAMES WADDELL
CHARLOTTE M. YOUNG
PAUL E. HOWE

Send nominations to:

DR. ICIE MACY HOOBLER
502 Burson Place
Ann Arbor, Michigan

The following persons have been elected previously as Fellows of the Society:

Thorne M. Carpenter (1958)
George R.Cowgill (1958)
Eugene F. DuBois (1958)
R. Adams Dutcher (1961)
Ernest B. Forbes (1958)
Casimir Funk (1958)
Wendell H. Griffith (1963)
Albert G. Hogan (1959)
Icie Macy Hoobler (1960)
Paul E. Howe (1960)
J. S. Hughes (1962)
C. Glen King (1963)
Leonard A. Maynard (1960)
Elmer V. McCollum (1958)
Harold H. Mitchell (1958)
Agnes Fay Morgan (1959)
John R. Murlin (1958)
Leo C. Norris (1963)
Helen T. Parsons (1961)
Lydia J. Roberts (1962)
William C. Rose (1959)
W. D. Salmon (1962)
Arthur H. Smith (1961)
Harry Steenbock (1958)
Robert R. Williams (1958)

Invitation for Nominations for Honorary Membership in the American Institute of Nutrition

The Committee on Honorary Memberships of the American Institute of Nutrition invites nominations for Honorary Members.

Distinguished individuals of any country who are not members of the American Institute of Nutrition and who have contributed to the advance of the science of nutrition shall be eligible for proposal as Honorary Members of the Society.

Nominations may be made to the Chairman of the Committee on Honorary Memberships by two members of the Society.

Nominations (in three copies) are due by October 1. A supporting statement giving the reason for the nomination is desirable but not necessary.

Final selection of nominees will be made by the Council of the American Institute of Nutrition and such nominations submitted to the Society at the spring meeting. Election requires a two-thirds majority of the ballots cast.

Honorary members pay no membership fees but are eligible to subscribe to the official journal(s) at member's rates.

Committee on Honorary Memberships:

C. GLEN KING, *Chairman*
HELEN BURCH
W. D. SALMON

Send nominations to:

DR. C. GLEN KING
Nutrition Foundation
99 Park Avenue
New York 16, N. Y.

The following persons have been elected previously as Honorary Members of the Society:

Kunitaro Arimoto	David P. Cuthbertson
W. R. Aykroyd	Herbert M. Evans
Frank B. Berry	Toshio Oiso
Frank G. Boudreau	Lord John Boyd Orr
Robert C. Burgess	V. N. Patwardhan
Harriette Chick	Emile F. Terroine
F. W. A. Clements	Artturi I. Virtanen

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