Influence of Dietary Copper and Zinc on Rat Lipid Metabolism

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The effects of varying both dietary zinc and copper over wide ranges of concentrations on lipid and nonlipid metabolic parameters in male rats fed a nonatherogenic semipurified diet were investigated. Body weight was directly related to both zinc and copper, and as expected serum zinc and copper levels were directly dependent on dietary zinc and copper, respectively. Liver and kidney copper concentration were directly related to serum copper and ceruloplasmin. The most important findings were those which showed an inverse relationship between serum copper or ceruloplasmin concentrations and levels of serum cholesterol, triglyceride, and phospholipid. There was the expected direct relationship among serum levels of cholesterol, triglycerides, and phospholipids. Other correlations among levels of lipids and metals in serum and tissue were also found, but interestingly no correlation was found between serum or dietary zinc and serum cholesterol.

The role of essential micronutrient metals (trace metals) in lipid metabolism is a subject of recent nutritional investigation. Only manganese, a known cofactor in the biosynthesis of squalene, has been shown to be directly related to serum cholesterol in man and chickens (Doisy, 1972). In addition, Schroeder and co-workers have reported that chromium deficiency is associated in rats with elevated cholesterol levels (1971). In addition, the importance of both manganese and chromium on in vitro synthesis of cholesterol by rat liver preparations has been demonstrated (Curran, 1954).

Reports from our laboratory have shown that dietary and serum copper levels are inversely related to serum cholesterol levels in rats fed a semipurified nonatherogenic diet (Murthy et al., 1972; Murthy and Petering, 1976). In similar studies in which the levels of dietary copper were maintained below the optimal level, it has been suggested that serum cholesterol is related directly to dietary zinc:copper ratios (Klevay, 1971).

We have extended our earlier studies examining relationships in male rats among dietary zinc and copper levels and lipid metabolic parameters, the results of which are presented here.

MATERIALS AND METHODS

Experimental. Seventy-two male weanling Sprague-Dawley rats with a mean body weight of 42.9 g (range 40–46 g) were randomly divided initially into three experimental groups of 24 animals each. They were housed individually in stainless steel cages in environmentally controlled quarters specifically designed for the study of trace metal deficiencies (Klevay et al., 1971). All experimental rats received a semipurified diet low in both zinc and copper (0.51 μg of Zn and 0.43 μg of Cu/g of diet) for the entire experimental period (Table I). During the first 32 days, each of the three experimental groups' dietary regimens was supplemented only with zinc (as zinc acetate) in the distilled demineralized drinking water at a zinc concentration of 2.5, 10.0, or 40.0 μg/mL. Thereafter each of the experimental groups was subdivided and continuously maintained on the previous level of zinc with the further addition of copper (as copper sulfate) to the drinking water: 12 rats from each zinc group were continued on 0.0 μg of Cu/mL and four each were given copper at 0.25, 2.0, and 16.0 μg/mL. Food and water were available ad libitum.

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Table I. Composition of Diet (g/kg)

Corn starch	623.5
Spray dried egg-white	200.0
Fat (corn oil)	90.0
Mineral mixture[a]	40.0
Cellulose powder[b]	30.0
Fat-soluble vitamin mixture[c]	10.0
Water-soluble vitamin mixture[d]	5.0
Choline chloride	1.5

[a] Zinc- and copper-free Wesson modified Osborne-Mendel salt mix (Wesson, 1932). [b] Whatman CF11, (W. & R. Balston, Ltd., London). [c] Corn oil contained (in mg/10 g of mixture) retinyl palmitate, 30 000 IU; ergocalciferol, 4000 IU; and *dl*-α-tocopherol, 100 IU. [d] Corn starch contained (in mg/5 g of mixture): thiamine hydrochloride, 20.0; riboflavin, 20.0; pyridoxine hydrochloride, 10; calcium pantothenate, 60; niacinamide, 100; folic acid, 0.5; menadione, 2.0; biotin, 1.0; inositol, 400; and cyanocobalamine, 0.02.

An additional group of six rats, designated the control group, was maintained throughout the entire experiment on a stock diet of Purina Lab Chow and distilled deionized water. The control animals were housed individually in regular animal quarters.

The experiment lasted 109–119 days. The weight of each rat was recorded weekly. Hematocrit determinations were done 1 day prior to the scheduled kill. Just before the animals were killed they were anesthetized with pentobarbital sodium and blood was drawn from the heart for metal analysis and lipid and ceruloplasmin assays.

Aliquots of serum were extracted into chloroform–methanol 2:1 (v:v) for determination of the lipid profile (Folch et al., 1954). Lipid extracts were analyzed for cholesterol by a ferric chloride–sulfuric acid method (Zlatkis et al., 1953), for triglycerides by periodate oxidation of glycerol and color development with chromotropic acid (Van Handel and Zilversmit, 1957), for phospholipids by colorimetric determination of inorganic phosphorous in the digested sample (Fiske and Subbarow, 1925), and for fatty acids by colorimetric indicator technique (Mosinger, 1965).

Serum was diluted 1:3 or 1:5 with distilled deionized water and analyzed for zinc and copper by atomic absorption spectrophotometry. Tissue samples were dried and wet-ashed for zinc and copper by the method of Petering et al. (1971). Ceruloplasmin was determined by the method of Houchin (1958) and the results converted to concentration units (Evans and Wiederanders, 1967).

A weighed sample of about 1 g of liver tissue was taken from each rat for lipid analyses. Liver tissue was finely chopped by hand, weighed, and frozen promptly in wide

Table II. Mean Values of Change in Body Weight from Day 32 to Day 109 Related to Dietary Concentrations of Zinc and Copper[a-c]

Dietary zinc, μg/mL of water	Dietary copper (μg/mL of drinking water) 0.0	0.25	2.0	16.0	Lab Chow
2.5	152.5^{a}	172.0	157.0	169.2^{a}	
10.0	$186.5^{a,b}$	179.2	208.5	210.0	214.5
40.0	141.7^{b}_{A}	184.5	186.5	224.7^{a}_{A}	

[a] Analysis of variance showed that dietary zinc and copper significantly affected body weight ($p < 0.02$ in both cases). [b] Means in the same column with same small letter superscripts are significantly different ($p < 0.05$). [c] Means in the same row with same capital letter subscripts are significantly different ($p < 0.05$).

polyethylene test tubes for subsequent analysis. Thawed liver specimens were dehydrated at the time of analysis in 1 mL of 0.9% saline solution and disrupted by sonication before extraction into chloroform–methanol 2:1 (v:v) (Folch et al., 1954). The lipid extracts obtained from this procedure were analyzed for their content of cholesterol triglycerides and phospholipids by colorimetric method described above.

Statistical Analysis. Analysis of variance was performed on each parameter individually to assess the overall effect of dietary zinc and copper and their interaction (Snedecor and Cochran, 1967). In order to stabilize the variances within the dietary groups, it was necessary to transform serum copper, ceruloplasmin, cholesterol, liver copper, and kidney copper values logarithmically before statistical analysis. This transformation succeeded in variance stabilization for all the above variables except cholesterol. The analysis of variance of cholesterol values was carried out excluding the values at 2 and 16 mg of copper and 40 mg of zinc, groups in which very large variability occurred.

In addition to analysis of variance, group means were tested to determine if they were statistically different. All tests (except for cholesterol) were carried out using the error–variance from the analysis of variance table. The F statistic was used to judge significance. In the case of cholesterol, comparisons involving Cu 2/Zn 40 and Cu 16/Zn 40 groups were carried out by the Fisher-Behrens test; the estimates of variation being obtained from the data of the particular cells involved in each comparison. Associations between selected pairs of variables such as serum metals and serum lipids were studied by calculating the Pearson Product Moment correlation coefficients. The correlation coefficients between organ metals and serum parameters were also calculated.

RESULTS

Nonlipid Parameters. Our results showed that the expected response of body growth to dietary zinc was evident at all levels of dietary copper except 0.25 μg of Cu/mL. The maximal response was at 10 μg of Zn/mL of drinking water, and no increase was found when the level of zinc was increased to 40 μg/mL. There was a definite growth response to dietary copper when dietary zinc was given at 10 and 40 μg/mL, but not when it was given at 2.5 μg/mL, the suboptimal level. When dietary levels of copper and zinc were optimal or greater, the final body weight of the rats equaled that found in the control, lab chow, group. The data for change in body weight from day 32 to 109 are shown in Table II.

In Table III are given the nonlipid parameters measured which showed definite responses to dietary zinc and copper changes and which also were correlated with lipid metabolic changes. There were no marked changes in liver and kidney zinc which were readily related to dietary levels of zinc or copper and therefore these values are not presented in detail. The range of values for liver zinc was 74.4 to 96.2 μg/g and for kidney zinc 61.4 to 92.3 μg/g. Thus it appears that liver and kidney zinc levels were remarkably resistant to the lower levels of dietary zinc and copper which were used in this experiment. There were, however, some statistically significant correlations among the other nonlipid parameters themselves and with liver and kidney values for zinc and copper as well.

Table III. Mean Values of Nonlipid Parameters Related to Dietary Concentration of Zinc and Copper[a-d]

Dietary zinc, μg/mL of water	Dietary copper (μg/mL of drinking water) 0.0	0.25	2.0	16.0	Lab Chow
		Serum zinc (μg/100 mL)[a,c,d]			
2.5	$65.8^{a,b}$	97.5^{a}	92.5^{a}	67.5^{a}	
10.0	$167.3^{a}_{A,B,C}$	112.5^{b}_{A}	125.0^{b}_{B}	127.5^{a}_{C}	150.0
40.0	180.0^{a}	$172.5^{a,b}$	$170.0^{a,b}$	177.5^{a}	
		Serum copper (μg/100 mL)[b-d]			
2.5	$4.4_{A,B}$	$10.6_{A,B}$	84.8_{A}	94.3_{B}	
10.0	$4.3_{A,B}$	$14.3_{A,B}$	136.8_{A}	136.8_{B}	96.7
40.0	$4.1_{A,B}$	$12.9_{A,B}$	96.5_{A}	111.6_{B}	
		Serum ceruloplasmin (mg/100 mL)[b-d]			
2.5	$0.42^{a}_{A,B}$	$1.08_{C,D}$	$24.57_{A,C}$	$29.34_{B,D}$	
10.0	$0.12^{a,b}_{A,B}$	$0.81_{A,B}$	31.12_{A}	29.84_{B}	29.9
40.0	$0.41^{b}_{A,B}$	$1.02_{C,D}$	$26.96_{A,C}$	$33.05_{B,D}$	
		Liver copper (ug/g of dry weight)			
2.5	$3.79_{A,B}$	$5.82_{A,B}$	11.96_{A}	12.88_{B}	
10.0	$3.24_{A,B}$	$6.88_{A,B}$	10.92_{A}	10.92_{B}	13.0
40.0	$3.95_{A,B}$	$5.77_{A,B}$	10.56_{A}	12.59_{B}	
		Kidney copper (ug/g of dry weight)			
2.5	$13.0_{A,B}$	$13.3_{C,D}$	$34.3_{A,C}$	$44.0_{B,D}$	
10.0	$11.4_{A,B}$	$16.2_{A,B}$	35.3_{A}	45.3_{B}	29.7
40.0	10.7_{A}	14.0_{B}	$30.6_{A,B}$	$44.6_{A,B}$	

[a] Analysis of variance showed that dietary zinc significantly affected serum zinc ($p < 0.01$). [b] Analysis of variance showed that dietary copper significantly affected serum copper ($p < 0.01$), ceruloplasmin ($p < 0.01$), liver copper ($p < 0.01$), and kidney copper ($p < 0.01$). [c] Means in the same column with same small letter superscripts are significantly different ($p < 0.05$). [d] Means in the same row with same capital letter subscripts are significantly different.

Table IV. Statistically Significant Correlations of Nonlipid Parameters

	N	r	p
Serum Zn vs. kidney Zn	70	+0.4455	<0.001
Serum Cu vs. liver Zn	71	+0.5863	≪0.001
Serum Cu vs. liver Cu	70	+0.9211	≪0.001
Serum Cu vs. kidney Cu	70	+0.8925	<0.001
Serum Cp vs. liver Zn	69	+0.5334	<0.001
Serum Cp vs. liver Cu	69	+0.8914	≪0.001
Serum Cp vs. kidney Cu	68	+0.8343	≪0.001
Serum Cu vs. Serum Cp	69	+0.913	<0.001
Serum Zn vs. Serum Cu	71	−0.0872	NS
Liver Zn vs. liver Cu	70	+0.6471	≪0.001
Liver Zn vs. kidney Cu	70	+0.4994	<0.001
Liver Cu vs. kidney Cu	69	+0.8291	≪0.001

The data shown in Table IV, which represent the statistically significant interrelationship among the nonlipid parameters studied show a definite metabolic relationship between zinc and copper, especially with respect to liver and serum values. It should also be noted that serum copper or ceruloplasmin values are closely related to liver and kidney copper values, and that serum zinc is associated directly with kidney zinc values.

Lipid Parameters. The relationships of cholesterol, triglyceride, phospholipid, and free fatty acid levels in serum and liver are shown in Table V. Although liver phospholipids were determined they did not vary markedly and thus are not given in Table V. The values for liver phospholipids ranged between 20.20 mg/g (wet weight) and 23.62 mg/g, the control value being 25.18 mg/g.

The values given in Table V show significant changes with respect to dietary zinc and copper and have been further related among themselves and with metabolic values for zinc and copper. These results are given in Table VI, in which statistically significant correlations of lipid parameters and nonlipid parameters are shown.

There was a significant interaction between serum cholesterol and dietary zinc and copper ($F = 3.5$, $p = 0.01$) which was evidenced by a sharp decrease in serum cholesterol at Cu 2/Zn 40 and Cu 16/Zn 40. Because of the large variability shown in these two groups and their small number, there was no significant difference in their values. The serum cholesterol results of Table V are interpreted according to the statistical methods outlined above. Serum triglyceride values were also inversely related to dietary copper ($p < 0.05$) and to zinc ($p < 0.01$). Serum free fatty acid values (FFA) and serum phospholipids were directly related only to dietary zinc ($p = 0.05$ and $p < 0.01$, respectively).

The data given in Table VI show a marked direct interrelationship among the levels of serum cholesterol, triglyceride (TG) and phospholipid (PL), but do not include any similar relationship with FFA levels. They also indicate that serum cholesterol and serum triglyceride values are primarily related in an inverse fashion to serum copper and ceruloplasmin and not to serum zinc.

Since there was no relationship between serum zinc and serum cholesterol, it is obvious that the relationship between serum Zn/Cu and serum cholesterol ($p < 0.01$) must be a direct one, since the important variable, serum copper, is in the denominator.

The serum lipid parameters also showed an inverse (or negative) correlation with the liver zinc and copper values, indicating that serum lipid values are related to the metabolic activities of zinc and copper.

When we examine the possible relationship between liver lipids and liver zinc or copper levels we do not find any evidence that the metal concentrations are in any way associated with liver lipid levels.

It is of interest to point out that the control values for liver and serum lipid concentrations were variable in relation to the experimental groups. Thus, the control

Table V. Mean Values of Lipid Parameters Related to Dietary Concentration of Zinc and Copper[a-d]

Dietary zinc, μg/mL of water	Dietary copper (μg/mL of drinking water) 0.0	0.25	2.0	16.0	Lab Chow
		Serum cholesterol (mg/100 mL)			
2.5	94.0_{A}	73.0	66.6^{a}_{A}	79.1	
10.0	101.0_{A}	98.8	93.7^{a}	72.7_{A}	58.2
40.0	105.7_{A}	99.4	58.9	46.7_{A}	
		Serum triglyceride (mg/100 mL)			
2.5	72.2^{a}	49.3	52.0	66.7	
10.0	98.1^{b}_{A}	84.5^{a}	73.8^{a}	53.3_{A}	99.4
40.0	$52.2^{a,b}$	47.5^{a}	28.8^{a}	32.3	
		Serum phospholipid (mg/100 mL)			
2.5	114.7^{a}	93.0^{a}	112.8^{a}	119.0	
10.0	144.0^{a}_{A}	143.2^{a}_{B}	$146.8^{a,b}_{C}$	$113.5_{A,B,C}$	100.0
40.0	127.5_{A}	115.5	91.5^{b}_{A}	103.8	
		Serum free fatty acid (mg/100 mL)			
2.5	0.97	1.24	0.74	0.84^{a}	
10.0	0.94^{a}	0.85	0.83	0.97	1.01
40.0	1.34^{a}	1.32	1.19	1.62^{a}	
		Liver cholesterol (mg/g of wet weight)			
2.5	3.86	3.95	3.97	4.46^{a}	
10.0	4.13_{A}	3.90	3.65	3.32^{a}_{A}	3.86
40.0	3.90	4.44	3.95	3.90	
		Liver triglyceride (mg/g of wet weight)			
2.5	1.88^{a}	1.93	1.50^{a}	1.51^{a}	
10.0	2.24^{b}	2.24	1.84	2.17^{b}	1.91
40.0	$3.04^{a,b}_{A}$	2.90_{B}	2.80^{a}_{C}	$4.21^{a,b}_{A,B,C}$	

[a] Analysis of variance showed that dietary zinc significantly affected serum triglycerides ($p < 0.01$), serum phospholipids ($p < 0.05$), serum free fatty acids ($p < 0.05$), and liver triglycerides ($p < 0.05$). [b] Analysis of variance showed that dietary copper significantly affected serum cholesterol ($p < 0.01$), serum triglycerides ($p < 0.05$), and serum phospholipids ($p < 0.01$). [c] Means in the same column with same small letter superscripts are significantly different ($p < 0.05$). [d] Means in the same row with same capital letter subscripts are significant different ($p < 0.05$).

Table VI. Statistically Significant Correlations among Lipid and Nonlipid Parameters

	N	r	p
Serum cholesterol			
vs. serum TG	67	+0.570	<0.001
vs. serum PL	68	+0.666	≪0.001
vs. serum Cu	68	−0.470	<0.001
vs. serum Cp	66	−0.501	<0.001
vs. liver Cu	67	−0.474	<0.001
vs. liver Zn	68	−0.337	<0.01
vs. kidney Cu	67	−0.492	<0.001
vs. liver PL	65	−0.259	0.05
Serum triglycerides			
vs. serum PL	67	+0.598	<0.001
vs. serum Cu	67	−0.331	<0.01
vs. serum Cp	67	−0.391	0.001
vs. liver Zn	67	−0.353	<0.01
vs. liver Cu	67	−0.380	<0.01
vs. kidney Cu	66	−0.286	0.02
Serum phospholipids			
vs. serum Cu	68	−0.187	NS
vs. serum Cp	66	−0.311	0.02
vs. liver PL	65	−0.316	0.02
vs. liver Zn	68	−0.333	<0.01
Serum free fatty acids			
Liver triglycerides	62	+0.359	<0.01
Liver cholesterol			
Liver phospholipids	68	+0.322	<0.01
Liver triglycerides			
vs. serum Zn	67	+0.422	<0.001
Serum Zn/Cu			
vs. serum cholesterol	(12[a])	+0.716	<0.01
vs. serum (cholesterol + triglycerides)	12	+0.576	=0.05
Dietary Zn/Cu			
vs. serum cholesterol	12	+0.632	<0.05

[a] Group values used instead of individual values.

serum cholesterol and PL levels were similar to that of the lowest values from the experimental groups, while the control serum triglyceride level was similar to the values from the highest experimental groups.

DISCUSSION

The possibility of an important relationship between dietary zinc and copper on the one hand and lipid metabolism in the rat was first suggested by Murthy et al. in a report to the Ninth International Congress of Nutrition (1972). Since then, Murthy and Petering (1976) have reported that serum cholesterol is inversely correlated with serum copper in rats fed a nonatherogenic diet similar to the one used here. Previously, Guthrie et al. (1974) and Amine and Hegstead (1971) found that dietary iron was inversely related to serum lipid, and recently Iacano (1974) found that dietary calcium could alter cholesterol and lipid metabolism in the rabbit. Thus, we see that there is increasing evidence that the mineral contents of the diets of experimental animals may have an important bearing on lipid metabolism.

Since lipid metabolism and dietary lipid quality and concentration are considered to be factors in the etiology of atherosclerosis in man, the possibility of dietary minerals having a role in lipid metabolism must be viewed as important. The data presented here and the reports of Elwood et al. (1970), Klevay (1975), and others mentioned above indicate that more attention needs to be paid to the mineral contents of the diets of man and experimental animals in order to understand the relation between nutrition and cardiovascular diseases.

Some differences in the design of the experiment reported here and those described by Murthy and Petering (1976) and Klevay (1975) need to be emphasized The salt mixture used here was a zinc- and copper-free Wesson (1932) modification of Osborn-Mendel formula which contained about 3 ppm of manganese, while Klevay used the Jones-Foster salt mix (1942) which contains about 52 ppm of manganese.

We also fed our diets devoid of added copper for 32 days before administering the various levels of dietary copper. This allowed all animals to begin the experiment with low stores of copper, which may be a vital part of our experimental design. Neither Murthy and Petering (1976) nor Klevay (1975) followed this experimental pattern.

As shown in the data presented here and in our previous report (Murthy and Petering, 1976), the relationship between either dietary zinc/copper or serum zinc/copper and serum lipids is an artifact and results from the fact that serum and dietary copper have the major and definitive role in determining serum cholesterol as well as serum triglyceride levels, and zinc has none or a minor one. Under these conditions, when the neutral or minor role of dietary or serum zinc is divided by the major negative role of copper a positive relationship of zinc/copper vs. cholesterol or triglyceride results as shown in Table VI.

The statistical analysis of the data show the extensive and pronounced negative correlation between serum cholesterol and triglycerides and parameters which measure copper nutriture and metabolism. Since, as was shown in Table IV, there is a very close association between values of serum copper, liver copper, liver zinc, and kidney copper, it is readily understood that serum cholesterol and serum triglyceride values, being dependent on copper nutriture and copper metabolism, are also highly correlated with these tissue parameters of copper metabolism.

Serum triglyceride levels were inversely related to liver zinc values while liver triglyceride values were positively related to serum zinc. Therefore, it seems that as serum zinc levels increase there may be a tendency to move triglyceride from serum to the liver, or to decrease the release of triglyceride (TG) from the liver into the plasma.

There is less of a relationship between serum-free fatty acid levels (FFA) and parameters of either zinc or copper nutriture and metabolism than there is with the other blood lipids. There is, however, an indirect relationship of FFA levels with serum zinc in that liver TG is positively associated with serum zinc and serum FFA is also associated with liver TG. On the other hand, serum FFA levels have an inverse relationship with serum triglycerides of a low order of significance ($p < 0.05$) and none with serum cholesterol and phospholipid values.

The data presented here give a detailed picture of the complex relationships between copper and zinc nutriture and metabolism on the one hand and parameters of lipid metabolism in the rat. All of the data point to a highly significant inverse relationship between parameters of copper metabolism and those of lipid metabolism, with zinc nutriture modifying these relationships but not being determinative. These results indicate a need to identify more carefully the dietary minerals in both experimental animals and man when relationships between diet and lipid metabolism are being studied.

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Effect of Ammoniated Casein in the Diet on the Growth of Weanling Rats

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Effect of ammoniated casein in the diet on the growth of weanling rats was studied using feeds containing 11.4, 20, 30, and 40% casein which had been subjected to treatment in the dry state with ammonia gas. No significant differences in growth were found at any protein level between controls fed untreated casein and those fed ammoniated casein containing 66.4 mmol of ammonia/100 g of casein. Of a total of 44, one rat fed ammoniated casein (30%) died after 10 days, presumably of unrelated causes. No other adverse nutritional effects were observed over the time period studied. The kidney weight/body weight ratios after 35 days on the diets were correlated to the protein level (r = +0.701) but were not affected by the ammonia. There was no significant difference between the observed protein efficiency ratio of the ammoniated casein and that of the control; values were 2.88 ± 0.14 and 2.80 ± 0.02, respectively.

The absorption of ammonia gas by dry isoelectric casein has been shown to be an effective method for converting this protein into a water-soluble form similar to ammonium caseinate (Girdhar and Hansen, 1974). Such treatment markedly improves the physical and chemical properties of the casein but results in the retention of 1.0–1.8% nitrogen as ammonia in the product. Although treatment with ammonia gas has previously been proposed for fumigating citrus fruit and corn (Bothast et al., 1973) and for decontaminating aflatoxins from peanut and cottonseed meal (Gardner et al., 1971), little is known regarding the mechanism of action of ammonia on the protein or the nutritive value and possible toxic effects of the treated material.

According to a review of the status of the safety of ammonium ions (National Technical Information Service, 1973), ammonia salts are generally recognized as safe (GRAS) and should not be expected to produce adverse effects if ingested in moderate amounts. However, large amounts of ammonia salts are toxic and may cause acidosis and liver and kidney damage.

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The purpose of this study was to gain information about the nutritive value of ammoniated casein and to determine if the feeding of diets containing elevated levels of this product as the sole source of protein had any detrimental effects on the growth of weanling rats.

MATERIALS AND METHODS

Ammoniated casein was prepared by placing 3000-g batches of vitamin-free casein under vacuum in a chamber (31.25 cm × 27.5 cm) connected to a water aspirator. After 10–15 min, the pump was closed off and dry ammonia gas was admitted such that a slightly reduced pressure was maintained over a period of approximately 50 min. During the process, the temperature of the casein increased from 25 to 50 °C. The treated casein was then heated to 60 °C and degassed under vacuum for 60 min in order to remove excess ammonia from the casein particles. After degassing, the powder contained 0.93% additional nitrogen due to residual adsorbed ammonia. A 5% aqueous solution had a pH of 7.4.

Nitrogen was determined on the original and ammoniated caseins and on all the mixed diets by using the macroKjeldahl procedure (AOAC, 1970). Total nitrogen in the control samples was converted to protein by use of the factor 6.38. Nitrogen due to residual ammonia was determined by difference and was not included in the calculation of the protein content.

Male, 35 to 46 g, weanling albino rats of the Sprague-Dawley strain were obtained commercially. The rats were