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Diet and cholesterolemia: V. effects of sulfur-containing amino acids and protein*

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SUMMARY

Interrelationships among the effects of dietary protein, sulfur-containing amino acids, and choline on the serum cholesterol concentration of the rat have been studied. Hypercholesterolemia was induced by feeding rats a diet containing cholesterol, cholic acid, and saturated fat. When the diet contained choline, additional casein alleviated the hypercholesterolemia. A similar effect was obtained with a supplement of methionine equal to the amount in the casein. Cystine and cysteine, but not taurine, were as effective as methionine. These and other observations suggest that the serum cholesterol-lowering effect of protein supplements is due largely to the sulfur-containing amino acids they provide. The omission of choline from the diet also alleviated the hypercholesterolemia; but when the choline-free diet was supplemented with either choline or methionine, serum cholesterol concentration increased. A supplement of choline alone caused a much greater rise than a supplement of methionine or combined supplementation with choline and methionine. Thus methionine appears to have two opposing effects on serum cholesterol concentration in rats fed a hypercholesterolemic diet lacking choline: (*a*) a cholesterol-elevating effect attributable to its ability to spare choline by providing a supply of preformed methyl groups, and (*b*) a cholesterol-lowering effect common to sulfur-containing amino acids and not dependent upon the provision of methyl groups.

Fillios *et al.* (1) observed that serum cholesterol concentrations of rats fed a hypercholesterolemic diet decreased as the protein content of the diet was increased. A similar study in this laboratory revealed that serum cholesterol concentrations were inversely related not only to the protein content of the diet but also to growth rate and food intake (2). Thus it was not clear whether the lower serum cholesterol concentrations of rats fed a high protein diet were the result of improved nutritional status of the animal (3), higher nitrogen intake, higher intakes of specific amino acids (4), higher protein-to-calorie ratio of the diet (5), or an effect of protein on the utilization of fat (6).

Olson *et al.* (7) noted that rats fed a choline-free diet deficient in methionine exhibit hypocholesterolemia and that the serum cholesterol concentration rises to within the normal range if the methionine content of the diet is increased. In contrast, a high intake of sulfur-containing amino acids causes a reduction in the serum cholesterol concentration of rats fed a hypercholesterolemic diet containing choline (8), so it appears that methionine may have two distinct effects on serum cholesterol concentration, depending upon the nature of the diet. The effects of dietary protein and lipotropic factors on serum cholesterol concentrations have been reviewed recently by Portman and Stare (9).

This paper contains further information about the effects of protein, sulfur-containing amino acids, and choline on serum cholesterol concentration in the rat, and provides evidence that the effect of higher dietary levels of protein is due to the increase in the sulfur-containing amino acid content of the diet.

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EXPERIMENTAL

Male rats of the Holtzman strain weighing 50 to 55 g (six or seven animals per group) were fed the experimental diets for 3 weeks. Then blood was obtained by heart puncture and the animals were killed. Individual serum samples were analyzed for total cholesterol (10) and pooled samples of the livers from each group for total cholesterol and total lipids (8). The basal diet contained 10% casein, 25% hydrogenated coconut oil,[1] 5% salts (11), 1% cholesterol, 0.5% cholic acid, and water-soluble vitamins and sucrose to make 100%. Water-soluble vitamins were included in the diet at the following levels (mg/kg): thiamine HCl, 8.0; riboflavin, 6.0; pyridoxine, 4.0; calcium pantothenate, 40.0; niacin, 50.0; inositol, 200.0; folic acid, 4.0; vitamin B_{12}, 0.04; biotin, 0.20, and choline chloride 2000. Choline was not included in the choline-free diets. Fat-soluble vitamins in ethanol were administered weekly. All changes in the diet were made at the expense of sucrose. The procedures have been discussed previously in greater detail (12).

Throughout this work the serum cholesterol concentrations varied somewhat from experiment to experiment for groups fed a given diet; however, the effects of the various treatments were consistent throughout.

RESULTS

A comparison of the effects on serum cholesterol concentration of dietary supplements of proteins with distinctly different amino acid compositions (Table 1) showed that supplementation of the basal diet with 6% zein and 0.1% tryptophan, a supplement deficient in lysine, caused a growth response and an increase in food intake but did not affect serum cholesterol concentration. Further supplementation of this diet with 0.36% lysine gave a further growth response but caused no significant change in serum cholesterol concentration. Additions of 6% gelatin or casein caused reductions in serum cholesterol concentration, but neither was as effective as 0.5% DL-methionine, even though methionine was less effective in stimulating growth and food intake. This suggested that the quantity of sulfur-containing amino acids in the diet was more important than the over-all protein content in regulating serum cholesterol concentration.

A supplement of threonine increased the growth rate of rats fed the diet containing methionine but had little effect on serum cholesterol concentration (Table 1). Since blood could be taken more easily from larger rats, the next experiment was designed to ascertain whether threonine could be included routinely in the diets used in studying the effect of methionine. The results in Figure 1 show that, regardless of the methi-

TABLE 1. Comparison of Growth, Food Intake, and Serum Cholesterol Concentration of Rats Fed a 10% Casein Diet Supplemented with Protein or Methionine

Diet	Weight Gain	Food Intake	Serum Cholesterol
	g/rat/3 wks	*g/rat/day*	*mg/100 ml*
10% casein	28 ± 2 *	5.6	1670 ± 320 *
10% casein + 6% zein + 0.1% DL-tryptophan	41 ± 4	6.4	1670 ± 350
10% casein + 6% zein + 0.1% DL-tryptophan + 0.36% L-lysine·HCl	47 ± 4	6.9	1500 ± 230
10% casein + 6% gelatin	52 ± 4	8.4	1080 ± 110
10% casein + 6% gelatin + 0.1% DL-tryptophan	46 ± 1	7.1	1200 ± 210
16% casein	82 ± 4	9.0	970 ± 120
10% casein + 0.5% DL-methionine	42 ± 5	6.1	590 ± 70
10% casein + 0.5% DL-methionine + 0.5% DL-threonine	65 ± 4	8.7	520 ± 70

* Standard error of the mean.

[1] Hydrol, Durkee Famous Foods, Chicago, Ill.

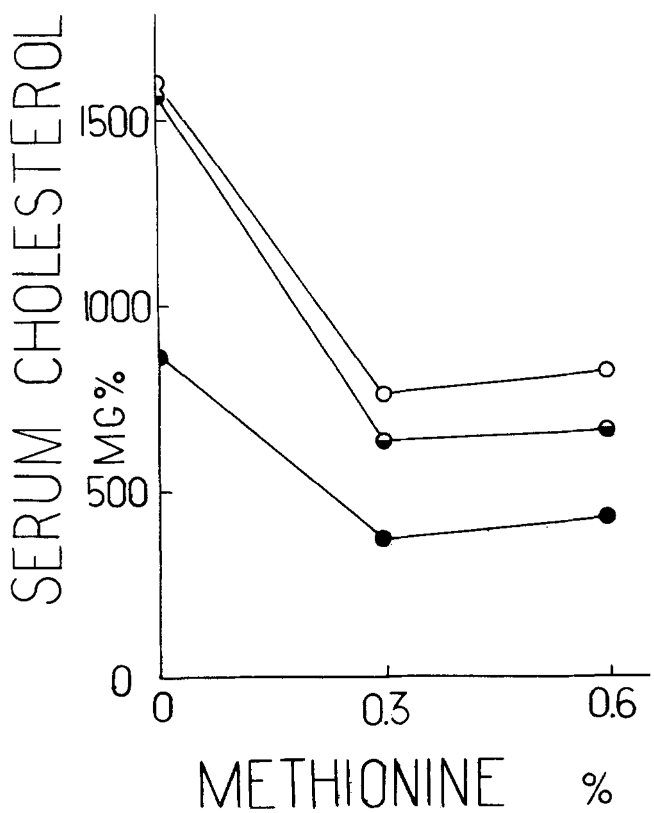

Fig. 1. Effect of supplements of threonine and methionine on serum cholesterol concentrations of rats fed a hypercholesterolemic diet containing 10% casein. (o——o) = no threonine; (◒——◒) = 0.5% DL-threonine; (•——•) = 2.0% DL-threonine.

onine content of the diet, addition of 0.5% DL-threonine caused no significant change in serum cholesterol concentration. However, when the threonine supplement was increased to 2%, serum cholesterol concentrations decreased.

Further evidence that the dietary content of sulfur-containing amino acids is more important in the regulation of serum cholesterol concentration than the protein content per se was obtained by feeding diets differing in protein content but equal in their content of sulfur-containing amino acids (Table 2). The serum cholesterol concentrations of rats fed on diets containing either 10% casein supplemented with 0.6% DL-methionine or 28% casein were similar. Both diets contained 1% sulfur-containing amino acids according to values given by Block and Weiss (13).

Table 3 shows the effect on serum cholesterol concentration of adding increasing increments of methionine to a diet containing 10% casein. Maximum lowering of serum cholesterol concentration occurred with the addition of 1.2% DL-methionine, i.e., with a total of 1.5% methionine in the diet. However, the difference between the value for the group receiving an additional 0.6% DL-methionine and that for the group receiving an additional 1.2% was not statistically significant. When the DL-methionine supplement

TABLE 2. Effects of Diets Differing in Protein Content but Not in Methionine Content on Serum Cholesterol Concentration

Diet	Number of Rats	Weight Gain	Food Intake	Serum Cholesterol
		g/rat/3 wks	*g/rat/day*	*mg/100 ml*
10% casein	11	34 ± 2 *	6.2	1140 ± 20 *
10% casein + 0.6% DL-methionine + 0.5% DL-threonine	12	62 ± 3	7.9	580 ± 30
25% casein	6	113 ± 4	10.3	760 ± 50 †
28% casein	6	111 ± 3	9.4	580 ± 80 †

* Standard error of the mean.
† Values obtained in two separate experiments. The control groups for the two experiments have been averaged.

TABLE 3. Effect of Dietary Methionine Content on Serum and Liver Cholesterol Concentration

Diet	Weight Gain	Food Intake	Serum Cholesterol	Liver	
				Total Lipid	Total Cholesterol
	g/rat/3 wks	*g/rat/day*	*mg/100 ml*	*% fresh wt*	*% fresh wt*
Experiment I					
10% casein	31 ± 3 *	5.9	1880 ± 260 *	15.5	6.2
10% casein + 0.3% DL-methionine †	65 ± 7	8.5	730 ± 40	21.1	5.8
10% casein + 0.6% DL-methionine †	58 ± 4	7.0	450 ± 50	18.3	5.8
10% casein + 0.9% DL-methionine †	64 ± 1	7.4	420 ± 40	19.3	5.4
10% casein + 1.2% DL-methionine †	61 ± 5	7.0	410 ± 30	19.9	5.4
10% casein + 2.5% DL-methionine †	19 ± 1	4.1	430 ± 60	13.8	3.5
Experiment II					
10% casein	34 ± 2	5.9	1310 ± 150		
10% casein + 6% zein ‡	53 ± 1	8.4	1310 ± 100		
10% casein + 6% zein + 0.3% DL-methionine ‡	69 ± 3	8.1	980 ± 70		
10% casein + 6% zein + 0.6% DL-methionine ‡	69 ± 4	9.0	690 ± 50		
15% wheat gluten	5 ± 1	5.3	760 ± 90		
15% wheat gluten + 0.5% DL-methionine	6 ± 1	5.5	480 ± 70		
15% wheat gluten + 0.5% L-lysine·HCl	30 ± 4	7.5	1160 ± 180		
15% wheat gluten + 0.5% L-lysine·HCl + 0.5% DL-methionine	38 ± 4	7.7	870 ± 140		

* Standard error of the mean.
† 0.5% DL-threonine also added.
‡ 0.1% DL-tryptophan and 0.3% L-lysine·HCl also added.

was increased to 2.5%, a marked retardation in growth was observed; but serum cholesterol concentration was unaffected. Addition of 0.3% DL-methionine to the diet containing 10% casein and 6% zein (supplemented with tryptophan and lysine) caused a decrease in serum cholesterol concentration and an improvement in growth, but increasing the methionine supplement from 0.3% to 0.6% further depressed serum cholesterol concentration without affecting growth.

The sulfur-containing amino acids are limiting for the growth of rats fed on diets containing casein, so the effect of methionine was determined under conditions in which it would give no growth response. When a diet containing 15% wheat gluten,[2] which is not limiting in sulfur-containing amino acids, was fed, a supplement of methionine caused a depression in serum cholesterol but no significant increase in growth (Table 3).

Subsequently the effects of some other sulfur-containing amino acids were determined. Cystine and cysteine were as effective as methionine in lowering serum cholesterol concentration (Table 4). When both cysteine and betaine were added, the effect was no greater than that obtained with cysteine alone. Growth and food intake of the group fed the diet supplemented with methionine were greater than those of groups fed on diets supplemented with cystine or cysteine, but the serum cholesterol concentrations of groups receiving cystine or cysteine were lower.

TABLE 4. Effect of Sulfur-Containing Amino Acids on Serum Cholesterol Concentration of Rats Fed a Diet Containing 10% Casein and 0.5% DL-threonine

Additions to Diet	Weight Gain	Food Intake	Cholesterol
	g/rat/3 wks	*g/rat/day*	*mg/100 ml*
—	34 ± 2 *	5.9	1310 ± 150 *
0.6% DL-methionine	68 ± 2	7.9	690 ± 30
0.9% L-cystine	63 ± 2	7.5	560 ± 50
0.9% L-cysteine	52 ± 2	6.9	620 ± 50
0.9% L-cysteine +0.9% betaine	46 ± 3	6.8	590 ± 50

* Standard error of the mean.

Since the sulfur-containing amino acids are precursors of taurine, which is conjugated with cholic acid to form taurocholic acid in the rat, the effects of feeding taurine and sodium taurocholate were determined (Table 5). Supplementation of the basal diet with taurine resulted in lower serum cholesterol concentrations, but the effect of taurine was less than that of methionine. Also, the replacement of the cholic acid in the diet with an equivalent amount of sodium taurocholate gave approximately the same decrease in serum cholesterol concentration as did the addition of taurine.

TABLE 5. Effect of Taurine and Sodium Taurocholate on Serum Cholesterol Concentration of Rats Fed a Diet Containing 10% Casein

Additions to Diet	Weight Gain	Food Intake	Serum Cholesterol
	g/rat/3 wks	*g/rat/day*	*mg/100 ml*
—	24 ± 1 *	7.1	1690 ± 130 *
0.5% DL-methionine	42 ± 3	8.3	750 ± 70
0.4% taurine	29 ± 2	6.9	1390 ± 40
1.0% taurine	29 ± 2	7.2	1200 ± 210
2.0% taurine	30 ± 2	7.2	1480 ± 180
0.625% sodium taurocholate †	27 ± 2	6.5	1110 ± 170

* Standard error of the mean.
† Equivalent to 0.5% cholic acid. Cholic acid omitted from this diet.

In an earlier experiment, addition of 1% DL-methionine to a diet containing 25% casein, but no cholesterol or cholic acid, caused no change in serum cholesterol concentration. However, 25% casein supplies 0.9% methionine, and serum cholesterol concentrations of rats fed on diets containing cholesterol and cholic acid were not decreased by dietary supplements of methionine if the methionine content of the diet was above 0.9%. Therefore the effect of supplementation with methionine or cystine in the absence of dietary cholesterol and cholic acid was investigated, using rats fed on diets containing either 10% casein or 10% soybean protein[3] (Table 6). Although a supplement of methionine reduced the serum cholesterol concentration of rats fed the diet containing casein and cholesterol but no cholic acid, neither methionine nor cystine affected serum cholesterol concentration when cholesterol and cholic acid were both omitted. Neither methionine nor cystine affected serum cholesterol concentrations of rats fed a diet containing soybean protein but no cholesterol or cholic acid.

Although the determinations on liver were done on pooled samples and no statistical measure of variability can be given, certain trends are apparent from

[2] Wheat gluten containing less than 1% lipid kindly supplied by Dr. John Andrews, General Mills, Minneapolis, Minn.

[3] C-1 Assay Protein, Drackett Product Company, Cincinnati, Ohio.

TABLE 6. Effect of Sulfur-Containing Amino Acids on Serum Cholesterol Concentrations of Rats Fed on Diets Containing No Cholic Acid or Cholesterol

Additions to Diet	Weight Gain	Food Intake	Serum Cholesterol
	g/rat/3 wks	*g/rat/day*	*mg/100 ml*
Experiment I. 10% Casein			
1% cholesterol	42 ± 3 *	6.8	281 ± 18 *
1% cholesterol † + 0.6% methionine	61 ± 2	8.1	182 ± 11
No addition	36 ± 3	6.2	121 ± 10
0.6% methionine †	55 ± 2	7.9	115 ± 4
0.9% cystine †	65 ± 4	8.5	105 ± 4
Experiment II. Soybean Protein			
No addition	24 ± 2	7.3	144 ± 7
0.6% methionine	44 ± 3	8.7	140 ± 5
1.2% methionine	34 ± 3	7.5	144 ± 1
0.48% cystine	18 ± 1	5.7	139 ± 2
0.96% cystine	20 ± 1	6.0	151 ± 8
1.5% cystine	19 ± 1	5.9	136 ± 9

The diet contained no cholesterol or cholic acid unless otherwise noted. DL-Methionine and L-cystine used as dietary supplements.

* Standard error of the mean.

† 0.5% DL-threonine also added.

the results. In four separate experiments (Tables 3, 7, 8) the total lipid content of the liver increased by 4% to 6% when methionine was included in the basal diet containing choline. This effect of methionine was less evident when the diet contained threonine (Table 7). Methionine supplements did not cause a consistent rise in liver total cholesterol under these conditions, but perhaps of more importance is the fact that additions of methionine up to 1.2% of the diet, levels that caused maximum lowering of serum cholesterol concentration, did not in any case cause a lowering of liver total cholesterol. The effect of 2.5% DL-methionine is an exception, but with this level of methionine in the diet, growth and food intake were severely depressed.

TABLE 7. Effect of Methionine and Threonine on Liver Cholesterol and Total Lipids

Additions to Diet		Liver	
DL-Methionine	DL-Threonine	Total Lipids	Total Cholesterol
		% of fresh wt	*% of fresh wt*
—	—	11.1	3.9
0.3	—	16.0	5.7
0.6	—	19.0	5.1
—	2.0	12.0	4.5
0.3	2.0	13.0	3.5
0.6	2.0	14.5	4.4

Finally, the effect of methionine was studied in rats fed a choline-free diet containing cholic acid and cholesterol. The omission of choline from the diet caused a large drop in serum cholesterol concentration (Table 8). The addition of methionine or betaine to the choline-deficient diet resulted in an increase in serum cholesterol concentration, but the addition of taurine had little effect. Higher levels of methionine caused no greater increase under these conditions than 0.3% DL-methionine. In contrast to its effect in choline-deficient diets, a supplement of methionine caused serum cholesterol concentrations of rats receiving adequate amounts of choline to fall, but not to as low a value as when choline was omitted from the diet. Although again the use of pooled samples does not permit a measure of individual variation, the liver cholesterol values for each of the three groups receiving neither choline nor additional methionine were 2% to 3% below the values for the other groups (Table 8). The total amount of cholesterol per liver was 356 mg for rats fed the basal diet containing choline and 310 mg when a choline-free diet was fed. On a fat-free dry weight basis the values were 409 and 277 mg per g, respectively.

DISCUSSION

The observation that the serum cholesterol concentrations of rats fed on diets containing equal amounts of sulfur-containing amino acids but different amounts of casein were the same suggests that the greater sulfur-containing amino acid content of high casein diets is responsible, in part if not entirely, for their serum cholesterol-lowering effect. That this may be true of other proteins as well is suggested by the observation that the relative effects of casein and gelatin supplements were proportional to the amounts of sulfur-containing amino acids they provided. The lack of effect of zein on serum cholesterol concentration may well be a consequence of the unavailability of many of the amino acids of this protein (14). Apparently the quantity of the sulfur-containing amino acids that becomes available from zein is sufficient to stimulate growth, but not to affect serum cholesterol concentration (Table 1).

The reduction in serum cholesterol concentration that occurs when the protein or methionine content of the diet is increased is not a result of improved nutritional status (increased growth and food intake) or

TABLE 8. Effects of Methionine and Choline on the Cholesterol Content of Serum and Liver

Additions to 10% Casein Choline-free Diet	Weight Gain	Food Intake	Serum Cholesterol	Liver	
				Total Cholesterol	Total Lipids
	g/rat/3 wks	*g/rat/day*	*mg/100 ml*	*% fresh wt*	*% fresh wt*
Experiment I					
0.2% choline chloride	27 ± 3 *	5.4	2100 ± 380 *	7.1	16.9
0.2% choline chloride + 0.6% methionine †	60 ± 3	7.1	960 ± 100	7.8	21.2
No addition	12 ± 3	4.2	380 ± 60	5.3	32.1
0.3% methionine †	43 ± 4	5.9	710 ± 40	7.8	22.3
0.6% methionine †	50 ± 2	6.5	630 ± 30	6.6	22.4
1.2% methionine †	45 ± 3	5.5	650 ± 50	7.5	21.5
2.0% methionine †	25 ± 3	4.4	700 ± 90	6.1	19.6
Experiment II					
0.2% choline chloride	30 ± 2	7.0	1500 ± 310	7.6	18.2
0.2% choline chloride + 0.5% methionine	43 ± 3	8.0	800 ± 140	7.6	24.6
0.2% choline chloride + 0.4% taurine	27 ± 2	6.0	1330 ± 340	6.9	17.2
No addition	27 ± 3	6.3	160 ± 10	4.2	38.2
0.5% methionine	47 ± 4	8.5	530 ± 50	7.5	29.6
0.4% taurine	20 ± 1	6.0	220 ± 20	4.7	36.3
0.52% betaine hydrochloride	26 ± 4	5.9	840 ± 90	7.3	21.0

DL-Methionine and L-cystine used as dietary supplements.
* Standard error of the mean.
† 0.5% threonine also included.

higher nitrogen intake per se, because the addition of zein to the basal diet stimulated growth and food intake without affecting serum cholesterol concentration. Also, a gelatin supplement stimulated growth more than a supplement of methionine but had only a slight effect on serum cholesterol concentration (Table 1), and the addition of methionine to diets in which the sulfur-containing amino acids were not limiting caused a reduction in serum cholesterol concentration without affecting growth (Table 3). In fact, no evidence of a correlation between growth and serum cholesterol concentration was seen in these experiments. These observations also make it unlikely that the protein-to-calorie ratio (5) or the effect of protein on the utilization of fat (6) influence serum cholesterol concentration to any great extent under the conditions of this study.

Herrmann (15) has suggested that the effect of sulfur-containing amino acids on serum cholesterol concentration in the cholesterol-fed rat may be due to increased formation of taurine and its conjugation with cholic acid. However, our results show that dietary supplements of methionine were more effective than equivalent amounts of taurine in reducing serum cholesterol concentration; therefore it appears that the effect of sulfur-containing amino acids cannot be entirely accounted for by their conversion to taurine.

Shapiro and Freedman (16) have presented evidence that the effect of methionine on serum cholesterol concentration is influenced by the type of dietary fat. Work done in this laboratory suggests that polyunsaturated fatty acids and methionine have supplementary effects in lowering serum cholesterol concentration.[4]

Olson *et al.* (7) found that the hypocholesterolemia observed in rats fed a diet deficient in both choline and methionine did not occur if the diet was supplemented with an adequate quantity of either methionine or choline. They suggested that the effect of methionine in preventing hypocholesterolemia in choline-deficient rats was the result of an increase in endogenous choline synthesis upon increasing the methionine intake. Our findings that the methyl donors methionine and betaine

[4] N. Nath, J. C. Seidel, and A. E. Harper. In preparation.

partially prevented the lower serum cholesterol concentrations observed in choline deficiency, while taurine was ineffective, lend further support to this conclusion.

However, methionine appears to have two effects when added to a diet deficient in both choline and sulfur-containing amino acids. It causes an increase in serum cholesterol concentration, an effect of the labile methyl groups of methionine in promoting choline synthesis; at the same time it exerts a cholesterol-lowering effect in the serum, owing to the greater intake of the nonmethyl portion of the molecule. Thus the final serum cholesterol concentration represents the resultant of the "hypercholesterolemic" effect of the labile methyl groups and the "hypocholesterolemic" effect of the sulfur-containing amino acids. Part of the difference between our results and those of Olson *et al.* can be explained by the fact that the sulfur-containing amino acid content of all diets used by Olson *et al.* was kept constant by varying the cystine content. Since either cystine or methionine is effective in reducing serum cholesterol concentration when the supply of methyl groups is adequate, no serum cholesterol-lowering effect of methionine could be expected under these conditions.

It also seems probable that under our dietary conditions the addition of 0.6% methionine to a choline-deficient diet is not sufficient to completely replace choline in its effect on serum cholesterol concentration, for serum cholesterol concentrations of rats fed on a choline-deficient diet supplemented with 0.6% methionine were lower than those of rats fed a similar diet containing an adequate quantity of choline.

Nishida *et al.* (17) have reported that methionine supplementation also lowers serum cholesterol concentration in chicks fed a hypercholesterolemic diet containing choline; but in contrast to the observations in the rat, they did not find that the serum cholesterol concentration of the choline-deficient chick was low. Hegsted *et al.* (18) could demonstrate no lipotropic effect of choline in the chick such as is readily demonstrated in the rat, and it would appear that the effect of choline deficiency on serum cholesterol concentration is also distinctly different in these two species.

The effect of choline deficiency in decreasing the cholesterol content of the liver is in agreement with the observation of Cuthbertson *et al.* (19), who fed 2.0% cholesterol and 0.5% cholic acid, but does not agree with the earlier report of Ridout *et al.* (20), that the liver cholesterol-ester content of choline-deficient animals is high.

A marked decrease in the cholesterol content of the liver was observed in the present study when the results were expressed as per cent of fresh liver weight. Much of this effect was the result of infiltration of lipid material into the livers of choline-deficient rats, with a subsequent increase in liver weight. However, even when the results were expressed as the total amount of cholesterol per liver, or as per cent of fat-free dry matter, the cholesterol content of the livers of choline-deficient rats was lower. The divergence between our results and those of Ridout *et al.* may be due to differences in the diets used. The cholesterol content of the liver in our experiments was considerably higher than that reported by Ridout *et al.*, probably because of the inclusion of cholic acid in our diets.

The difference between the effects of choline deficiency on the cholesterol and total lipid content of the liver suggests that under our dietary conditions choline may exert an effect on liver cholesterol concentration that is independent of its lipotropic activity. It is also of interest that methionine is as effective as choline or betaine in preventing a decrease in liver cholesterol concentration in choline-deficient rats, but is less effective than either choline or betaine in preventing the accumulation of liver fat and the decrease of serum cholesterol concentration.

It is of passing interest that rats fed on diets containing 9% casein supplemented with methionine develop fatty livers, which can be prevented by a supplement of threonine, an effect attributed to an amino acid imbalance (21). The parallel results observed in the present work involving both the total lipid and cholesterol contents of the liver suggest that the effects of this amino acid imbalance can also be demonstrated in rats on a hypercholesterolemic regimen.

REFERENCES

1. Fillios, L. C., S. B. Andrus, G. V. Mann, and F. J. Stare. *J. Exptl. Med.* **104**: 539, 1956.
2. Nath, N., A. E. Harper and C. A. Elvehjem. *Can. J. Biochem. and Physiol.* **37**: 1375, 1959.
3. Goldner, M. G., L. Loewe, R. Lasser, and I. Stern. *Proc. Soc. Exptl. Biol. Med.* **87**: 105, 1954.
4. Johnson, D., Jr., G. A. Leveille and H. Fisher. *J. Nutrition* **66**: 367, 1958.
5. Kokatnur, M., N. T. Rand and F. A. Kummerow. *Circulation Research* **6**: 424, 1958.
6. March, B. E., and J. Biely. *J. Nutrition* **69**: 105, 1959.
7. Olson, R. E., J. R. Jablonski and E. Taylor. *Am. J. Clin. Nutrition* **6**: 111, 1958.
8. Fillios, L. C., and G. V. Mann. *Metabolism, Clin. and Exptl.* **3**: 16, 1954.
9. Portman, O. W., and F. J. Stare. *Physiol. Revs.* **39**: 407, 1959.
10. Henly, A. A. *Analyst* **82**: 286, 1957.

11. Harper, A. E. *J. Nutrition* **68**: 405, 1959.
12. Nath, N., R. Wiener, A. E. Harper, and C. A. Elvehjem. *J. Nutrition* **67**: 289, 1959.
13. Block, R. J., and K. W. Weiss. *Amino Acid Handbook: Methods and Results of Protein Analysis.* Charles C Thomas, Springfield, Ill., 1956, p. 342.
14. Geiger, E., G. W. Courtney and L. E. Geiger. *Arch. Biochem. Biophys.* **41**: 74, 1952.
15. Herrmann, R. G. *Circulation Research* **7**: 224, 1959.
16. Shapiro, S. L., and L. Freedman. *Am. J. Physiol.* **181**: 441, 1955.
17. Nishida, T., F. Takenaka and F. A. Kummerow. *Circulation Research* **6**: 194, 1958.
18. Hegsted, D. M., R. C. Mills, C. A. Elvehjem, and E. B. Hart. *J. Biol. Chem.* **138**: 459, 1941.
19. Cuthbertson, W. F. J., P. V. Elcoate, D. M. Ireland, D. C. B. Mills, and P. Shearley. *Brit. J. Nutrition* **13**: 227, 1959.
20. Ridout, J. H., C. C. Lucas, J. M. Patterson, and C. H. Best. *Biochem. J.* **58**: 301, 1954.
21. Harper, A. E., D. A. Benton, M. E. Winje, and C. A. Elvehjem. *J. Biol. Chem.* **209**: 159, 1954.