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Diet and cholesterolemia: VII. Effects of methionine, ethionine, and *p*-fluorophenylalanine*

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SUMMARY

The concentration of serum cholesterol in rats fed a diet containing cholesterol, cholic acid, and hydrogenated coconut oil was reduced when ethionine was included in the diet. This effect was not due to depressed intake of food or of cholesterol. The addition of equal amounts of ethionine and methionine to this diet maintained serum cholesterol at levels significantly below those of rats supplemented with ethionine alone. This addition of methionine also very largely prevented the depression of growth caused by ethionine. Administration of *p*-fluorophenylalanine lowered the concentration of cholesterol in the serum but β-2-thienylalanine had little effect.

Ethionine is known to interfere with lipid metabolism in higher animals. In female rats, fatty infiltration of the liver occurs within 12 hr after administration of ethionine (1); in dogs, the concentration of serum lipids is markedly reduced when ethionine is added to the diet (2). Many of the biochemical and pathological effects of ethionine have been described in detail by Fitzgerald and Hellman (3).

Methionine is known to prevent ethionine-induced fatty liver. Effects of methionine on the concentration of serum lipids of animals treated with ethionine, however, have not been reported. The effect of methionine under these conditions should be of interest in view of the known hypocholesteremic effect of methionine in the cholesterol-fed rat (4, 5).

EXPERIMENTAL METHODS

Male rats of the Holtzman strain weighing either 50–55 or 90–100 g were fed the experimental diets for two weeks. There were six rats per group unless otherwise noted. At the end of the experimental period, animals were fasted for 15–18 hr. They were then anesthetized with ether and samples of blood were obtained by cardiac puncture. Serum cholesterol was determined by the method of Henly (6). The total cholesterol and total lipid of the liver were assayed by the method outlined by Fillios and Mann (4). Cholesterol was determined by the Liebermann–Burchardt reaction after isolation as the digitonide.

The basal diet contained: casein, 10%; hydrogenated coconut oil, 25%; salts (7), 5%; cholesterol, 1%; cholic acid, 0.5%; choline, 0.2%; and sucrose to make 100%. All vitamins were supplied in adequate amounts and all additions to the diet were made at the expense of sucrose. The L-isomers of leucine and tyrosine and the DL-forms of other amino acids were used. The methods have been previously described in greater detail (8).

RESULTS

In a preliminary experiment, the concentrations of serum cholesterol of 50–55 g rats fed the basal diet, supplemented with 0.25% or 0.5% of DL-ethionine, for three weeks were found to be 830 and 1280 mg/100 ml, respectively, compared to a concentration of 2110 mg/100 ml for rats fed the unsupplemented basal diet. When both 0.25% of DL-ethionine and 0.25% of DL-methionine were added to the diet, the average concentration of serum cholesterol was only 110 mg/100

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TABLE 1. EFFECTS OF DIFFERENT DIETARY LEVELS OF ETHIONINE AND METHIONINE ON CONCENTRATIONS OF LIPID IN THE SERUM AND LIVER OF 50–55 g RATS

Group	Additions to Basal Diet		Average Wt Change in 2 weeks	Serum Cholesterol	Liver	
	Eth.	Meth.			Total Lipid	Total Cholesterol
	%	%	g	mg/100 ml	% fresh wt	% fresh wt
1	0	0	4 ± 2*	920 ± 103* †	17.2	4.4
2	0.25	0	−9 ± 1	430 ± 92	8.8	1.4
3	0.25	0.25	−1 ± 2	80 ± 11	13.1	1.5
4	0.25	0.5	1 ± 2	130 ± 62	15.0	3.4
5	0.25	1.0	0 ± 1	160 ± 13	16.2	3.3

* Standard error of the mean.

† Statistical significance of differences in serum cholesterol concentration between groups: 1 and 2, $P < 0.01$; 2 and 3, $P < 0.01$; 3 and 5, $P < 0.01$.

ml. Similar results were obtained in an experiment lasting two weeks (Table 1) in which higher levels of methionine, in the presence of 0.25% of ethionine, were found to cause no further reduction in the concentration of serum cholesterol.

Changes in the total lipid or total cholesterol of the liver (Table 1) did not parallel the changes in serum cholesterol. When the diet was supplemented with either ethionine or ethionine plus methionine, however, the concentration of cholesterol in the liver was reduced. Similar values were obtained in several of the other experiments in which the concentration of total cholesterol and total lipid of the liver were determined.

The effects of injections of ethionine plus methionine on the concentration of serum cholesterol were tested, using 90–100 g rats made hypercholesterolemic by feeding them the basal diet for two weeks. Since fatty livers have been observed within 12 hr in female rats injected with ethionine (1), three injections of 12.5 mg each of ethionine, methionine, or both together were given at 12-hr intervals. Food was available during the injection period but was removed immediately following the final injection. Animals were then fasted for 16 hr before blood samples were taken. The injection of 37.5 mg of ethionine under these conditions (Table 2) resulted in as great a reduction in serum cholesterol as did feeding ethionine for two weeks. There was no difference in food intake of animals injected with saline and animals injected with ethionine during the period of treatment. (In another experiment discussed later (Table 5), the concentrations of serum cholesterol of 50–55 g rats fed the basal diet for 3, 7, or 20 days and then injected with ethionine as described above were well below those of rats fed the basal diet supplemented with ethionine for corresponding periods.) Injection of ethionine and methionine together resulted in no greater reduction in serum cholesterol than did injection of ethionine alone (Table 2). Injection of methionine alone resulted in a reduction comparable to that observed when a diet containing 0.6% of methionine was fed for three weeks (5).

TABLE 2. EFFECTS OF ETHIONINE AND METHIONINE INJECTIONS IN RATS* PREVIOUSLY FED THE BASAL DIET FOR 2 WEEKS

Group	Material Administered	Serum Cholesterol (mg/100 ml)		
		I Subcutaneous Injection	II Intraperitoneal Injection	III Included in Diet (2 weeks)
1	None	670 ± 100†	930 ± 110†	790 ± 110†
2	0.9% Saline	1030 ± 210	660 ± 110	
3	37.5 mg Ethionine	190 ± 40	140 ± 30	
4	0.25% Ethionine			220 ± 30
5	37.5 mg Ethionine			
	37.5 mg Methionine	220 ± 20	170 ± 30	
6	37.5 mg Methionine	460 ± 60	370 ± 60	

* Starting weight of rats, 90–100 g.

† Standard error of the mean. Statistical significance of differences between groups 1 and 3, 1 and 4, 1 and 5, 2 and 3, 2 and 5, was $P < 0.01$ in all columns; 1 and 6, $P > 0.1$ in column I, $P < 0.01$ in column II; 2 and 6, $P < 0.05$ in columns I and II.

Neither choline nor cystine was as effective as methionine in reducing the serum cholesterol of rats fed the diet containing 0.25% of ethionine (Table 3). Also, neither choline nor cystine prevented the depression of growth due to ethionine. The concentration of serum cholesterol of 90–100 g rats was somewhat lower than that of 50–55 g rats, but the combination of ethionine and methionine reduced the serum cholesterol of both groups to within the normal range.

In Table 4 are shown the effects of including ethionine in the complete basal diet and in diets from which cholic acid, or cholesterol and cholic acid, were omitted. In each experiment, the concentration of serum cholesterol of animals fed ethionine was significantly ($p < 0.01$) below that of control animals. However, the concentration of serum cholesterol of rats receiving methionine, in addition to ethionine, did not fall below that of rats receiving ethionine alone, unless cholic acid and cholesterol were present in the diet.

The levels of serum cholesterol of rats fed the basal diet, or the basal diet supplemented with ethionine or ethionine plus methionine, for various lengths of time are shown in Table 5. When ethionine alone was added to the diet, the concentration of serum cholesterol after three days was below the value for rats fed the unsupplemented basal diet (control). At seven days serum cholesterol rose to a value slightly above that of the control group, and, finally, at 14 days, decreased to approximately one-half of the control value. When rats were pre-fed the basal diet for two weeks before receiving the diet containing ethionine, the concentrations of serum cholesterol were below

TABLE 3. EFFECTS OF CHOLINE AND CYSTINE ON GROWTH AND CONCENTRATION OF SERUM CHOLESTEROL OF RATS FED ETHIONINE

Group	Additions to Basal Diet	90–100 g Rats: Average Wt Change in 2 Weeks	90–100 g Rats: Serum Cholesterol	50–55 g Rats: Average Wt Change in 2 Weeks	50–55 g Rats: Serum Cholesterol
		g	*mg/100 ml*	*g*	*mg/100 ml*
1	...	5 + 3*	790 ± 110* †	4 ± 2*	920 ± 100* †
2	0.25% DL-Ethionine	−19 ± 1	220 ± 30	−9 ± 1	430 ± 90
3	0.25% DL-Ethionine 0.25% DL-Methionine	3 ± 4	90 ± 9	−1 ± 2	80 ± 10
4	0.25% DL-Ethionine 0.75% Choline chloride	−16 ± 1	200 ± 60		
5	0.25% DL-Ethionine 0.25% DL-Cystine			−9 ± 1	340 ± 50

* Standard error of the mean.

† Statistical significance of differences in serum cholesterol concentrations: (a) 90–100 g rats, 1 and 2, $P < 0.01$; 2 and 3, $P < 0.01$; 3 and 4, $P < 0.01$; (b) 50–55 g rats, 1 and 2, $P < 0.01$; 2 and 3, $P < 0.01$; 3 and 5, $P < 0.01$.

TABLE 4. EFFECTS OF ETHIONINE AND METHIONINE ON THE CONCENTRATION OF SERUM CHOLESTEROL IN THE PRESENCE AND ABSENCE OF CHOLIC ACID AND CHOLESTEROL*

Group	Additions to Basal Diet: Eth.	Additions to Basal Diet: Meth.	Serum Cholesterol (mg/100 ml): Expt 1 Complete Basal Diet	Expt 2 Cholic Acid Omitted	Expt 3 Cholic Acid and Cholesterol Omitted
	%	%			
1	0	0	1190 ± 190†	210 ± 21†	90 ± 4†
2	0.25	0	150 ± 32	80 ± 8	70 ± 3
3	0.25	0.25	90 ± 16	80 ± 8	70 ± 6

* Starting weight of rats, 90–100 g.

† Standard error of the mean. Statistical significance of differences between groups 1 and 2 was $P < 0.01$ in all experiments; 2 and 3, $P > 0.1$ in experiment 1.

control values after seven days of ethionine supplementation (see Table 6, Experiment 2).

The concentration of serum cholesterol of rats fed both ethionine and methionine (Table 5) was the same as that of rats fed ethionine alone after three days but, in contrast, decreased at seven days and remained below 100 mg/100 ml at 14 and 20 days.

The serum cholesterol of three groups of rats given injections of ethionine over a 24-hr period after either 3, 7, or 20 days on the basal diet was in all cases lower than that of rats fed the diet containing ethionine for the corresponding number of days.

The specificity of ethionine in reducing the concentration of serum cholesterol was tested (1) by pair-feeding a group receiving the basal diet and a group receiving the basal diet plus 0.25% of ethionine, and (2) by feeding other amino acid antagonists as shown in Table 6. The serum cholesterol of the pair-fed control group was lower than that of the control group fed *ad libitum* (Experiment 1, Table 6) but significantly ($p < 0.01$) above that of the group receiving 0.25% of ethionine.

In Experiment 2 (Table 6), rats were fed the basal diet for two weeks, then the test diets for seven days. The addition of either 0.5% of DL-*p*-fluorophenylalanine or 1.0% of β-2-thienylalanine to the diet caused marked depressions of growth. Only *p*-fluorophenylalanine, however, produced a reduction in serum cholesterol comparable to that produced by ethionine. A

TABLE 5. EFFECTS OF THE ADMINISTRATION OF ETHIONINE AND METHIONINE FOR VARIOUS TIMES ON SERUM CHOLESTEROL*

Additions to Basal Diet	Days on Diet: 3	7	14	20
	Serum Cholesterol (*mg/100 ml*)			
None	460 ± 66†	730 ± 62†	920 ± 100†	2090 ± 240 †
0.25% Ethionine	200 ± 26	800 ± 105	430 ± 90	1010 ± 104
0.25% Ethionine 0.25% Methionine	230 ± 23	120 ± 11	80 ± 11	60 ± 6
37 mg Ethionine‡ injected on final day	90 ± 14	100 ± 20		110§

* Starting weight of rats, 50–55 g.

† Standard error of the mean.

‡ Rats were fed the basal diet for the number of days indicated and given 3 injections of ethionine in the following 24-hr period.

§ 75 mg of ethionine injected and samples pooled.

TABLE 6. EFFECT OF FOOD RESTRICTION AND AMINO ACID ANTAGONISTS ON GROWTH AND SERUM CHOLESTEROL

Diet	Expt 1*		Expt 2*	
	Average Wt Change	Serum Cholesterol	Average Wt Change	Serum Cholesterol
	g	*mg/100 ml*	*g*	*mg/100 ml*
Basal	8 ± 2†	1350 ± 260	14 ± 2	830 ± 50
Basal pair-fed	−7 ± 1	730 ± 140		
0.25% DL-Ethionine	−21 ± 2	100 ± 30	−5 ± 2	180 ± 30
0.25% DL-Ethionine				
0.25% DL-Methionine	14 ± 2	40 ± 6	12 ± 2	230 ± 40
0.5% DL-*p*-Fluorophenylalanine			−17 ± 1	240 ± 70
0.5% DL-*p*-Fluorophenylalanine‡			−15 ± 1	260 ± 40
1.0% β-2-Thienylalanine			−13 ± 1	610 ± 50
1.0% β-2-Thienylalanine§			−10 ± 1	1000 ± 270
5.0% L-Leucine			9 ± 1	980 ± 130

* In Experiment 1, 90–100 g rats were fed the experimental diets for 11 days. In Experiment 2, 90–100 g rats were fed the basal diet for 2 weeks, followed by the experimental diets for 7 days.

† Standard error of the mean.

‡ 0.375% DL-phenylalanine, 0.125% L-tyrosine, and 0.25% DL-tryptophan added with analogue.

§ 0.6% DL-phenylalanine, 0.3% L-tyrosine, and 0.5% DL-tryptophan added with analogue.

slight reduction in serum cholesterol resulted from feeding β-2-thienylalanine. The addition of 5% of L-leucine to the diet caused little depression of growth, and an increase in serum cholesterol.

The marked depression of growth observed when rats were fed the diet containing 0.25% of ethionine (Tables 1 and 3) was partially prevented by the addition of an equal amount of methionine.

Rats transferred directly from a natural diet to the ethionine-supplemented diet lost approximately 20% of their original body weight. This entire weight loss occurred within the first seven days of the experiments and body weight was then essentially constant up to 20 days (Fig. 1). However, 90–100 g rats that had been fed the basal diet for two weeks and then transferred to the diet supplemented with ethionine lost only 5 g in seven days (Table 6, Experiment 2), while the same size rats that had not been pre-fed the basal diet lost 19 g in seven days.

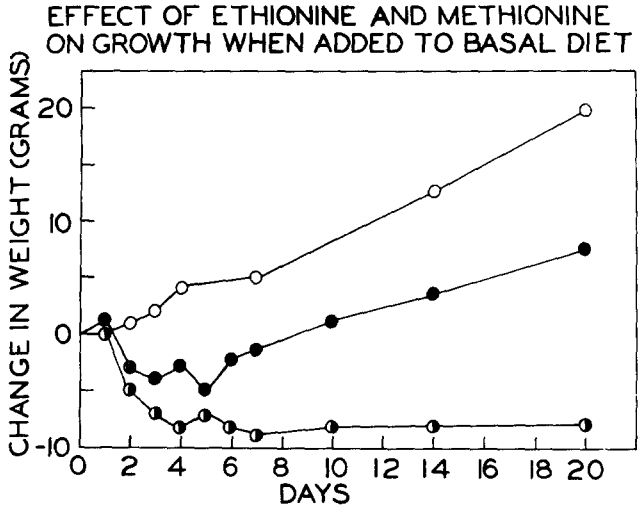

FIG. 1. Effects of ethionine and methionine on growth of rats transferred from stock diet to purified diet producing hypercholesteremia. (O——O) = basal diet; (◑——◑) = basal diet + 0.25% DL-ethionine; (●——●) = basal diet + 0.25% DL-ethionine and 0.25% DL-methionine.

DISCUSSION

Administration of ethionine, either by injection or by addition to the diet, lowered the concentration of serum cholesterol of rats on a hypercholesteremic regimen. This is in agreement with the observation of Feinberg et al. (2) and others (9–11) that the concentration of serum lipids falls in ethionine-treated animals. Since a lowering of serum cholesterol was observed in our experiments within 40 hr after the initial injection of ethionine, during a period in which food intake and growth were not affected, this action of ethionine cannot be attributed to reduced intake of food or cholesterol. In addition, restriction of food and retardation of growth due to paired-feeding or the administration of β-2-thienylalanine were much less effective than ethionine in lowering the concentration of serum cholesterol.

The poor weight gain of rats fed the basal diet was due to the cholic acid and the high level of saturated fat in the diet. Omission of cholic acid from the diet resulted in a weight gain of 2 g/day. Rats fed a diet

containing 10% casein, without the high level of saturated fat, will gain 3–4 g/day.

Although the concentration of serum cholesterol of rats fed the ethionine-supplemented diet rose with time, it remained well below the value for rats fed the basal diet after two to three weeks. When methionine was included with ethionine in the basal diet, however, the serum cholesterol gradually decreased with time. Addition of methionine alone to this basal diet caused a marked reduction in serum cholesterol, and a similar effect was obtained when methionine was replaced by cystine or cysteine (4, 5). However, when 0.25% of ethionine was present in the diet, cystine had very little effect. Also, methionine alone did not reduce the concentration of serum cholesterol below 400 mg/100 ml; in the presence of ethionine, however, values as low as 60 mg/100 ml were observed. Thus, the general hypocholesteremic effect of the sulfur-containing amino acids cannot account for the effect of methionine over and above that of ethionine. Also, serum cholesterol of rats fed the diet supplemented with ethionine and methionine fell from the third to the twentieth day, while those of rats fed the diet supplemented with ethionine alone rose during this period. Therefore, the additional effect of methionine described in this paper appears to be related in some way to the specific ability of methionine to counteract ethionine toxicity.

Adaptation to ethionine has been observed by Spolter and Harper (12) after four or five days in rats fed diets containing 9% of casein and as much as 1% of ethionine. In the present study, rats fed the diet supplemented with 0.25% of ethionine did not gain weight but any loss of weight occurred within the first seven days. The particularly high values for serum cholesterol in rats receiving ethionine for seven days, and the lack of difference between those receiving ethionine and those receiving ethionine and methionine for three days (Table 5), occurred at or near the time of adaptation and may be related to the ability of rats to adapt to the ethionine-containing diet used in this work. Rats pre-fed the basal diet for two weeks adapted slightly better to the ethionine containing diet and the concentrations of serum cholesterol after seven days were below values for rats fed the basal diet. It should be noted that rats pre-fed the basal diet were two weeks older than those not pre-fed the basal diet when supplementation with ethionine was begun; thus, the different responses to ethionine may be partly an effect of age.

Para-fluorophenylalanine is an antagonist of phenylalanine in microorganisms (13–16) and inhibits the formation of several enzymes in microorganisms and pigeon liver slices (17, 18). It is shown here to inhibit the growth and markedly reduce the concentration of serum cholesterol of rats fed a diet containing cholesterol, cholic acid, and hydrogenated coconut oil.

It has been suggested that ethionine might act as an antagonist of choline as well as of methionine, possibly through a mechanism involving transethylation leading to the formation of an ethylated derivative of choline (19). The significance of this hypothesis is questionable in relation to the effects of ethionine on serum and liver lipids. Choline prevents neither the ethionine-induced fatty liver (1) nor the inhibition of fatty acid oxidation (20). In our experiments, ethionine did not act as an antagonist of choline; an excess of choline in the diet neither reversed the growth depression nor had any significant effect on the concentration of serum cholesterol of ethionine-fed rats.

Considerable evidence suggests that ethionine acts as an inhibitor of protein synthesis. Ethionine has been shown to inhibit the incorporation of C^{14}-labeled glycine and methionine into liver proteins in the rat (21) and to inhibit competitively the incorporation of leucine and methionine into protein in the Ehrlich-Ascites tumor (22). Ethionine itself is incorporated into liver proteins in the rat (23). Adaptive increases in several enzymes of rat liver are inhibited by ethionine. These include tryptophan peroxidase (24, 25), xanthine oxidase (26, 27), threonine dehydrase (28), and glucose-6-phosphatase (29). It is generally accepted that the serum lipoproteins are obligatory vehicles for the transport of cholesterol, phospholipids, and triglycerides in the blood (30). Since ethionine might inhibit protein synthesis in rat liver, and since the synthesis of serum lipoproteins apparently occurs in the liver (31, 32), it is possible that an inhibition of the synthesis of the protein moiety of the lipoproteins might account for the reduction of serum cholesterol concentrations by ethionine. A recent report on this subject indicates that the synthesis of serum lipoproteins is indeed depressed in rats receiving ethionine (33).

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