The Influence of Dietary Protein and Methionine on Serum Cholesterol Level

KALYAN BAGCHI, B.SC, M.B.B.S., PH.D.,* RANJITA RAY, M.SC.† AND TAPASH DATTA, M.SC.†

Of all the biochemical indices associated with atherosclerosis and the allied coronary disorders, hypercholesterolemia is the most constant. Therefore, the study of nutrition has attracted a large number of investigators. Of the numerous studies carried out in recent years, the most important have been those on the effects of the quantity and quality of fat on the cholesterol level of blood. At the same time, a considerable amount of epidemiologic and biochemical evidence has been collected to support the contention that dietary fat is of great importance in influencing the serum cholesterol level.

Protein and amino acids have received scant attention although epidemiologic, clinical and biochemical evidence is available to demonstrate the important role of these nutrients in cholesterogenesis. Olson et al.[1] have pointed out aptly that the incidence of atherosclerosis in the different areas of the world can be correlated with the amount of protein, especially animal protein, consumed per person. They presented evidence that the dietary intake of protein influences the serum cholesterol level in adult human subjects. When their subjects were put on a diet low in protein, 25 gm. per day, the serum cholesterol value showed a decrease which averaged 44 ± 4 standard error (S.E.) mg. per 100 ml.

It is a well established fact that the serum cholesterol is significantly decreased in protein malnutrition. Ramanathan[2] and others have shown that nutritional edema and kwashiorkor are associated with hypocholesterolemia which tends to return to normal on nutritional rehabilitation. According to some, kwashiorkor and atherosclerosis are, in a sense, reciprocal disorders. The hypocholesterolemia of kwashiorkor can be corrected quickly by the administration of fat free milk powder.

Cholesterol is synthesized mainly in the liver from two carbon fragments, the intermediate products of protein metabolism or of carbohydrate and fat metabolism. If the amount of protein consumed daily is more than the specific need, the excess may be metabolized; the intermediate products of this metabolism can serve as raw material for cholesterol synthesis.

Coenzyme A, which has a functional sulfhydryl group, plays an important part in the synthesis of cholesterol. Enzymes, which have a functional sulfhydryl group, depend to a great extent on the supply of protein and especially on the sulfur-containing amino acids. Bagchi[3] showed that the total sulfhydryl content of the crystalline lens of albino rats depends on the protein content of the diet. Methionine was also found to control the sulfhydryl content. Leaf and Neuberger[4] showed similarly that there was a close correlation between the sulfhydryl content of the liver and the dietary level of protein. It is, therefore, reasonable to speculate that in protein deficiency the concentration of enzymes containing sulfhydryl, including coenzyme A, might be reduced, although evidence to support this contention is not available in the literature.

Olson and Stare[5] reported that pantothenic acid deficiency is associated with a decrease in the tissue content of coenzyme A and reduced capacity for acetylation. Considering the role of coenzyme A in cholesterol biosynthesis, it is thought that the biosynthesis of sterol is

From the All India Institute of Hygiene and Public Health, Calcutta, India.

* Associate Professor of Biochemistry and Nutrition; † Research Assistant.

inhibited in pantothenic acid-deficient animals. Guggenheim and Olson[6] found that the cholesterol content of liver, heart, adrenal glands and blood serum in deficient rats is similar to that in pair-fed control animals. Also there was no indication of a decreased rate of incorporation of carboxyl-labeled acetate in the tissue cholesterol of the deficient animals. Boyd[7] reported that maintenance of cholesterol ester concentration in plasma and liver is dependent upon an adequate dietary intake of pantothenic acid. Coenzyme A contains an organic sulfur, beta mercaptoethylamine, as well as pantothenate, and it has been suggested on the basis of enzymatic studies in rat liver[8] and demonstrations with S^{35}-tagged cystine in the intact rat[9] that cystine is the source of coenzyme A sulfur. Cystine, of course, can be derived from dietary methionine. It has been shown further by Olson and Dinning[10] that sulfur amino acid deficiency in the rat causes a decrease in hepatic coenzyme A levels in the presence of adequate amounts of dietary pantothenate. It seems probable that an abundance of cystine and cystine precursors in the diet would improve the utilization of pantothenic acid for coenzyme A synthesis.

The present investigation was carried out on albino rats and children to get further evidence on the effects of dietary protein and methionine on cholesterogenesis.

EXPERIMENTAL

Albino rats of the Glaxo strain were used as the experimental animals. Both healthy and malnourished children attending the Nutrition Clinic of the Urban Health Center of this Institute were the human subjects.

Weanling albino rats were kept on diets with protein levels at 4, 10 and 18 per cent; the amount of calories and other nutrients was the same. Adjustment was made in calorie content for the different levels of protein by changes in the amount of starch. The requirements for water-soluble vitamins were supplied through yeast, and the fat-soluble vitamins were administered orally twice a week in arachis oil medium. The composition of the diets is given in Table I.

Total serum cholesterol was determined by the colorimetric method of Henley[11] and the serum protein and albumin by Kingsley's method modified by Weichselbaum.[12] The total sulfhydryl content of the tissues was determined by the spectrophotometric method of Flesch and Kun[13] using Bennett's reagent (1:4 chloromercuriphenylazo-β-naphthol). The thymol turbidity test[14] was carried out to assess liver function, which plays an important role in cholesterogenesis, and because of its close link with protein malnutrition

TABLE I
Composition of Diets

Nutrient	Protein Level		
	18%	10%	4%
Starch (%)	73	81	87
Casein (%)	16	8	2
Groundnut oil (%)	5	5	5
Yeast (%)	4	4	4
Mineral mixture (%)	2	2	2

During the investigation it was decided to determine the effect of methionine supplementation on rats on low protein diets in order to assess whether or not the addition of this amino acid produces any change in the serum cholesterol level. For this purpose a group of albino rats on 4 and 10 per cent protein diets were given supplements of 50 mg. of methionine orally each day for fifteen days. At the end of this period the serum cholesterol level was determined as well as the total sulfhydryl content of the liver.

Since methionine sulfoximine was found to be an antimetabolite of methionine in earlier investigations,[15] it was decided to administer this compound and observe its effect on cholesterogenesis: Methionine sulfoximine was synthesized in the laboratory according to the method of Bentley et al.[16] DL-Methionine was oxidized to methionine sulfoxide by the action of hydrogen peroxide and then converted into sulfoximine by the action of sodium azide. The compound was administered intraperitoneally at 20 mg. per kg. body weight in aqueous solution. The injections were given on five alternate days; subsequently the animals were killed and the usual investigations carried out. A separate group of albino rats were given methonine sulfoximine and simultaneously a daily supplement of 50 mg. methionine orally to determine whether or not methionine supplementation protects the animals from the deleterious effects of the antimetabolite.

The investigations on the children were carried out in two stages. The first phase consisted of

TABLE II

Serum Cholesterol Values and Total Sulfhydryl Content of the Liver in Albino Rats on Diets with Different Levels of Protein

Protein Level (%)	No. Animals	Total Sulfhydryl (mg./100 gm. liver weight)		Serum Cholesterol (mg./100 cc.)	
		Mean	Range	Mean	Range
4	10	207	188–216	71	68–81
10	12	289	265–320	88	82–94
18	10	386	361–406	121	118–131

determining serum cholesterol, protein and albumin levels and assessing liver function in two groups of children four to five years old. In one group children were included who exhibited clinical signs of a mild degree of protein malnutrition, i.e., enlarged tender liver, hair changes, relative lack of growth with inadequate muscle mass and hypotonia. Eight children with frank cases of kwashiorkor were also included in this group. The other group included children of the same age group who, on clinical examination, were found to be healthy.

The second stage consisted of dividing the malnourished children into three comparable groups; two served as experimental groups and the third as the control group. Supplements of vegetable protein in the form of Chick pea or Bengal gram (Cicer arietinum) were given to one experimental group daily, and animal protein in the form of skimmed milk was given to the other experimental group daily; the protein and calorie content of the two supplements was the same (21 gm. of protein and 480 cal.). The children in the control group were given no dietary supplement. The supplementation continued for about two years after which the serum cholesterol, plasma protein and albumin and liver functions were determined.

RESULTS

In Table II, the serum cholesterol values for albino rats kept on diets with different protein levels are presented. In addition, the total sulfhydryl content of the liver is given to indicate the correlation between serum cholesterol and dietary protein on the one hand and liver sulfhydryl content on the other.

The effect of methionine supplementation on the liver sulfhydryl content and the serum cholesterol level of albino rats on diets low in protein (4 and 10 per cent level) is presented in Table III. Supplementation with 50 mg. of methionine per day for fifteen days not only increased the content of sulfhydryl in the liver but also simultaneously increased the serum cholesterol level. This increase was more marked in rats on the 10 per cent protein diet than those on the 4 per cent protein diet. In the former group, the serum cholesterol showed an increase of 33 per cent; in the latter the increase was about 18 per cent.

To determine the effect of an antimetabolite of methionine on liver sulfhydryl content and serum cholesterol level, methionine sulfoximine was administered to albino rats on 18 per cent protein diets and the total liver sulfhydryl content and serum cholesterol values determined; the findings are presented in Table IV. The antimetabolite produces a marked fall in the sulfhydryl content of the liver and a simultaneous reduction of serum cholesterol to the extent of about 30 per cent. Supplementation of 50 mg. daily of methionine to rats having the antimetabolite prevented to a large extent these changes in the liver and in the serum.

Children who were clinically healthy, those with manifestations of mild degrees of protein malnutrition and those with frank cases of kwashiorkor were then taken as subjects for investigation. Serum protein, serum albumin and serum cholesterol were determined, and

TABLE III

Effect of Methionine Supplementation on Liver Sulfhydryl Content and Serum Cholesterol Levels in Rats

No. of Animals	Methionine Supplementation (mg./day for 15 days)	Total Liver Sulfhydryl (mg./100 gm. wet liver)		Serum Cholesterol (mg./100 cc. serum)	
		Mean	Range	Mean	Range
4% Protein Level					
8	0	212	181–262	67	59–82
8	50	326	298–396	84	78–96
10% Protein Level					
8	0	279	226–324	81	70–96
8	50	374	305–428	118	106–129

TABLE IV

Effect of Methionine Sulfoximine on Liver Sulfhydryl Content and Serum Cholesterol Levels in Rats

Regimen	No. of Animals	Total Liver Sulfhydryl (mg./100 gm. wet liver)		Serum Cholesterol (mg./100 cc. serum)	
		Mean	Range	Mean	Range
18% protein	6	364	316–401	124	112–148
18% protein and methionine sulfoximine	6	216	182–261	68	58–79
18% protein, methionine sulfoximine and 50 mg. methionine daily for 15 days	6	342	301–396	112	104–140

TABLE V

Serum Protein, Liver Function and Total Serum Cholesterol in Healthy and Malnourished Children

Description of Subjects	Age (yr.)	No. of Children	Serum Albumin (gm. %)		Albumin: Globulin Ratio (mean)	Liver Function Thymol Turbidity (units)		Serum Cholesterol (mg. %)	
			Mean	Range		Mean	Range	Mean	Range
Healthy	5–6	15	4.5	4.8–3.9	2.3	7.6	5.6–8.1	142	156–136
Signs of mild degree of protein malnutrition	5–6	16	2.6	3.0–1.9	1.1	15.8	17.6–13.2	126	132–119
Healthy	3–4	10	3.4	3.8–2.9	1.5	9.8	11.1– 8.6	131	142–128
Kwashiorkor	3–4	8	2.4	2.7–1.3	1.0	17.6	19.9–15.2	112	121–109

TABLE VI

Effect of Protein Supplementation on Serum Protein and Cholesterol Levels

Description of Subjects*	No. of Children	Serum Albumin (gm. %)		Liver Function Thymol Turbidity (units)		Serum Cholesterol (mg. %)	
		Mean	Range	Mean	Range	Mean	Range
Apparently normal, on deficient diet	32	2.1	1.8–2.6	16.2	14.6–17.8	118	106–126
Daily supplementation of 21 gm. milk protein for 1 year	28	4.2	3.6–4.8	7.8	5.8– 9.6	138	131–152
vegetable protein for 1 year	18	3.9	3.1–4.6	8.4	7.6–10.1	140	129–162

* All subjects were four to five years old.

liver function was assessed. These values are presented in Table v.

It is evident that the serum cholesterol level has an inverse relation to liver function, which in turn has a relationship to the serum protein and albumin level and also to the albumin: globulin ratio. The same trend was also observed when children four to five years old were divided into three groups at random. There was a more significant rise in the serum protein level of the group given the supplement than in the control groups; simultaneously there was also a rise in the serum cholesterol level (Table vi).

COMMENTS

It is an established fact that fat influences serum cholesterol both quantitatively and qualitatively in experimental animals as well as in human beings. The present investigation was planned to determine the role of dietary protein and methionine in cholesterogenesis when the other nutrients in the diet remained at the same level.

The investigations carried out on albino rats on diets with different levels of protein showed that the serum cholesterol level is strongly influenced by dietary protein. Functional activity of the liver is probably one of the factors responsible since there are simultaneous fluctuations of the total sulfhydryl content of the liver. In this respect the effect of protein on the liver is possibly due to either its protective action on the liver, maintained by its structural integrity and, therefore, its functions, promoting the synthesis of cholesterol or to a larger amount of protein supplying the raw material for cholesterol synthesis or by both. Since methionine has a role in the maintenance of sulfhydryl groups and methionine sulphoximine drastically reduces the sulfhydryl groups in some tissues[15,17] the effect of this amino acid and its antimetabolite was determined as far as the level of serum cholesterol was concerned. Since methionine supplements given to rats on 4 and 10 per cent protein diets produced a significant elevation of serum cholesterol level and the administration of the antimetabolite to rats on 18 per cent protein diet decreased the cholesterol level, it is evident that this amino acid plays an important part in maintaining the serum cholesterol level in albino rats. The role of methionine as a lipotropic factor maintaining the functional integrity of the liver for cholesterol synthesis might explain this action. The effect of methionine which, among many other enzymes, is also the functional unit of coenzyme A, an enzyme essential for the synthesis of cholesterol, in maintaining the sulfhydryl groups in tissues is also relevant in this context.

The serum cholesterol levels in children, broadly falling into the following three groups: (1) clinically healthy; (2) showing mild signs of protein malnutrition; and (3) showing frank manifestations of protein malnutrition in the form of kwashiorkor, also show the same trend. The serum cholesterol level showed an inverse relation to the protein nutritional status. The functional integrity of the liver was found to be an important determining factor in this respect. The serum albumin level, the albumin globulin ratio and the thymol turbidity test results indicate the same relationship. The effect of protein and methionine in influencing the serum cholesterol level is possibly mediated through the liver. It is reasonable to speculate that hepatic dysfunction and hypercholesterolemia cannot be observed in the same person. In a healthy functioning liver cholesterol will be synthesized from two carbon fragments, derived from dietary protein in excess of the specific need.

The effect of protein supplementation, in the form of vegetable or animal protein, on children having a protein-deficient diet results not only in improved growth (height and weight) but also in a significant elevation of the serum cholesterol level, a finding which is similar to the results obtained in experiments with albino rats maintained on different levels of dietary protein.

SUMMARY

Serum cholesterol levels in albino rats correlate closely with the level of dietary protein and the total sulfhydryl content of the liver. Methionine supplementation of the low protein diet increased the serum cholesterol level. Intraperitoneal administration of methionine sulfoximine, an antimetabolite of methionine, to albino rats on adequate protein diet significantly reduced the total sulfhydryl content of the liver as well as the serum cholesterol level. In children aged four to five years there is a close correlation between the serum cholesterol level and the protein nutritional status. The serum cholesterol level and the serum albumin, albumin:globulin ratio and the liver functions are correlated. Supplementation of the diet of children with animal or vegetable protein produced a significant increase in the serum albumin and cholesterol levels.

ACKNOWLEDGMENT

We are indebted to the director of the Indian Council of Medical Research for making a grant available for conducting this investigation. Thanks are due to Miss Krishna Munshi for her technical assistance during the course of this investigation.

REFERENCES

1. Olson, R. E., Jablonski, J. B. and Taylor, E. The effect of dietary protein, fat and choline upon the serum lipids and lipoprotein of the rat. *Am. J. Clin. Nutrition*, 6: 111, 1958.
2. Ramanathan, M. K. Biochemical changes in the serum in the nutritional oedema syndrome (kwashiorkor). *Indian J. M. Res.*, 43: 517, 1955.
3. Bagchi, K. Experimental cataract in protein deficiency. *J. Indian. M. A.*, 31: 271, 1958.
4. Leaf, P. and Neuberger, G. The effect of diet on the glutathione content of the liver. *Biochem. J.*, 41: 280, 1947.
5. Olson, R. E. and Stare, F. J. The metabolism in vitro of cardiac muscle in pantothenic acid deficiency. *J. Biol. Chem.*, 190: 149, 1951.
6. Guggenheim, K. and Olson, R. E. Studies of lipogenesis in certain B-vitamin deficiencies. *J. Nutrition*, 48: 345, 1952.
7. Boyd, G. S. The possible role of coenzyme A in the biosynthesis of cholesterol in the rat. *Biochem. J.*, 55: 892, 1953.
8. Hoagland, M. B. and Novelli, G. D. Biosynthesis of coenzyme A from phosphopantetheine and of pantetheine from pantothenate. *J. Biol. Chem.*, 207: 767, 1954.
9. Yang, C. S. and Olson, R. E. Effect of dietary hepatic necrosis in rats upon liver coenzymes. *Fed. Proc.*, 13: 483, 1954.
10. Olson, R. E. and Dinning, J. S. Enzyme abnormalities associated with dietary necrotic liver degeneration in rats. *Ann. New York Acad. Sc.*, 57: 889, 1954.
11. Henley, A. A. The determination of serum cholesterol. *Analyst*, 82: 286, 1957.
12. Weichselbaum, T. E. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am. J. Clin. Path.*, 7: 40, 1946.
13. Flesch, P. and Kun, E. A colorimetric method for the determination of SH group in tissue homogenate by RSR. *Proc. Soc. Exper. Biol. & Med.*, 74: 249, 1950.
14. Shank, R. F. and Hoagland, C. L. A modified method for the quantitative determination of the thymol turbidity reaction of serum. *J. Biol. Chem.*, 162: 133, 1946.
15. Bagchi, K. The effect of dietary protein on the sulfhydryl content of crystalline lens. *Indian J. M. Res.*, 47: 184, 1959.
16. Bentley, H. R., McDermott, E. G. and Whitehead, J. K. Methionine sulphoximine synthesis. *Proc. Roy. Soc. London s.B*, 138: 265, 1951.
17. Bagchi, K. The effect of methionine sulfoximine induced methionine deficiency on the crystalline lens of the albino rats. *Indian J. M. Res.*, 47: 437, 1959.