A NOTE ON PERIODIC VARIATIONS IN SERUM CHOLESTEROL, SERUM LECITHIN-CHOLESTEROL ACYL TRANSFERASE ACTIVITY AND FAECAL STEROL AND BILE ACID EXCRETION IN THE RAT FED DIFFERENT LEVELS OF DIETARY PROTEIN

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Effects of three different levels of dietary protein (12,20 and 42%) on periodic variations in the serum cholesterol concentration, serum LCAT activity and faecal excretion of bile acids in rats fed hypercholesteremic diet were investigated. Serum free cholesterol concentration and serum LCAT activity showed minor variations with time in rats ingesting 12% protein diet, but progressively increased in rats ingesting 42% protein diets upto 32 days. Serum cholesterol concentration increased progressively but serum LCAT activity showed increase only at the end of 32 days in rats ingesting 20% protein diets. Average daily faecal excretion of bile acids per rat during different periods was lower in animals consuming 12% protein than those consuming higher levels of dietary protein.

Previous studies from this Laboratory[1] have emphasised the importance of dietary level of protein in the regulation of serum cholesterol, serum lecithin-cholesterolacyl transferase (LCAT) activity and faecal excretion of bile acids in the rat. The dependence of these responses on the duration of the experiment led us to investigate the time-course of changes in these parameters. Such studies will be helpful in understanding blood cholesterol regulation and thus may throw some light on processes involved in atherogenesis and coronary heart disease, the incidence of which is increasing in the armed forces and particularly among the officers.

Male albino rats raised from the stock originally procured from the Zoological House, Amritsar, were used in this investigation. The animals were divided into three groups (7-8 animals per group) and were fed *ad libitum* diets containing 12, 20 and 42% protein for 32 days. The initial weights of the animals averaged 119±11, 109±12 and 109±14 grams in the respective groups. Hypercholesteremia was produced by feeding 0.5% of cholesterol and 0.25% of cholic acid in the diets. Blood was drawn from each animal after 8-day periods, serum separated and analysed for free and total cholesterol[2] and for LCAT activity[3]. The data were considered by analysis of variance and students' 't' test was used to determine which means were different from each other and at what level of significance. Faecal analysis was done on pooled samples collected over 8-day periods. Faecal bile acids were estimated by the procedure of Lewis[4]. Futher details about the material and methods used, diet and other experimental conditions have been described earlier.

Hypercholesteremia observed in rats in various groups is caused by increased absorption of exogenous cholesterol as a result of feeding cholic acid alongwith cholesterol[5,6]. Lower degree of hypercholesteremia was observed in animals ingesting higher levels (20 or 42 %) of dietary protein, an effect which was statistically significant. Serum concentrations of free, esterified and total cholesterol of rats ingesting 12% protein diets were markedly higher even 8 days after feeding of experimental diets. Serum esterified and total cholesterol concentrations increased progressively for 24 days in animals ingesting three different levels of dietary protein. The increase at the end of 24 days compared to the respective values at the end of 8 days was statistically significant. Compared to the values at the end of 24th day, the animals in respective groups registered a substantial and significant reduction in serum esterified cholesterol fraction and serum total cholesterol concentration at the end of 32 days. This decrease at the end of 32 days in animals in all the groups suggests that the rats get adapted to this hypercholesteremic regimen within 24 days and this adaptation then inhibits subsequent hypercholesteremia. However, it must be noted that at all stages the animals ingesting higher levels of dietry protein had lower serum esterified and serum total cholesterol concentration, compared to the animals ingesting 12% protein diets. Increasing protein level from 20% to 42%, however, had small effect on these parameters.

At the end of 8-day period serum free cholesterol concentration of rats ingesting 12% protein diets were significantly higher than those of animals fed either 20% or 42% protein diets. The subsequent changes in free cholesterol concentration in rats on 12% protein diet were minor and insignificant. In contrast, the serum free cholesterol concentration progressively increased upto 32 days in rats ingesting either 20% or 42% protein diets; the increase was significant at the end of 32 days. The value of free cholesterol at the end of 32 days in animals on higher protein tended to approach the values observed in animals on 12% protein diets. Moreover, these changes in serum cholesterol concentration are not related to the differences in food intake or weight gain, a finding which confirms our earlier observations[1]. Further these differences in different cholesterol fractions in rats ingesting different levels of protein are reflected in the changes in serum esterified cholesterol/free cholesterol ratio.

Reduced degree of hypercholesteremia observed in rats ingesting higher level of dietary protein may have been caused by the effect of high protein intake on absorption of exogenous cholesterol and its distribution to other tissues, on excretion of end-products of cholesterol metabolism, or on synthesis of cholesterol

in various tissues and its mobilisation to blood. One or more of these factors may be involved in the regulation of blood cholesterol by dietary protein. Serum LCAT is primarily involved in serum cholesterol esterification and may also modify cholesterol transport and distribution[7] and changes in the activity of this enzyme may influence blood cholesterol and particularly its fractions. The LCAT activity of animals ingesting 12% protein diets showed minor and insignificant change with time. LCAT activity of animals on 20% protein diets followed similar trend for 24 days but increased significantly at the end of 32 days. Animals on 42% protein diets, however, recorded a marked increase in LCAT activity at the end of 24 days and highly significant increase at the end of 32 days and this value was significantly higher than that of animals on 12% protein diets. Paradoxically increased LCAT activity *in vitro* is not associated with higher esterified cholesterol fraction and such observations have been reported earlier also[1,8,9]. However, suggested relation of increased LCAT activity *in vitro* to increased turnover rate of cholesterol *in vivo*[8] may partly account for reduced hypercholesteremia observed in rats ingesting higher levels of dietary protein. The periodic variations in LCAT activity observed in this investigation may have contributed to the periodic variations in serum cholesterol fractions in rats fed on different levels of dietary protein.

There was no regular trend in the time-course of changes in bile acid excretion, however, the animals on high protein diets tended to excrete greater amounts of faecal bile acids per day. Daily faecal excretion of bile acids averaged highest in animals on 42% protein diets. Reduced hypercholesteremia observed in rats on high protein diets is partly explained by increased faecal excretion of bile acids in these rats. This may also partly explain periodic variations in serum cholesterol concentration in different groups of animals. Published reports[10,11] also emphasised the role of faecal bile acid excretion in the regulation of blood cholesterol. Inability to eliminate the absorbed sterol has been suggested to be the cause of accumulation of exogenous cholesterol in hypophysectomised rats [12,13]. However, our data suggest that reduced hypercholesteremia in rats ingesting higher levels of dietary protein is not caused by differences in mean daily faecal excretion of sterols and this finding was observed earlier[1,14] also. A different approach is required to evaluate the role of some other fractors in the regulation of blood cholesterol.

TABLE 1

PERIODIC VARIATIONS IN SERUM CHOLESTEROL, SERUM LCAT ACTIVITY AND FAECAL BILE ACID EXCRETION IN RATS FED HYPERCHOLESTEREMIC DIETS CONTAINING DIFFERENT LEVELS OF DIETARY PROTEIN

Dietary level of protein	Values of constituents after	8 days	16 days	24 days	32 days
12%	Free chol.*	33·7±3·1**	30·5±4·6	30·3±4·8	30·3±1·6
	Total chol.*	111·1±6·5	123·3±5·3	161·5±14·3	129·7±8·6
	Esterified Chol*	77·4±6·8	92·8±5·6	131·2±13·5	99·4±10·5
	LCAT activity†	6·45±1·65	5·40±1·33	6·26±1·79	6·77±0·89
	Faecal bile acids excretion§	5·6	8·3	5·8	5·3
20%	Free Chol*	21·6±2·7	23·4±1·2	27·0±2·7	29·1±2·3
	Total Chol*	89·8±5·2	103·2±3·9	117·8±8·2	88·0±5·5
	Esterified Chol*	68·2±6·2	79·8±2·3	90·8±7·7	58·9±5·2
	LCAT activity†	5·70±0·92	3·94±0·75	5·88±1·41	8·74±1·46
	Faecal bile acids excretion§	10·2	11·1	8·0	10·2
42%	Free Chol*	20·7±1·5	21·8±1·9	28·2±2·0	27·5±3·6
	Total Chol*	80·9±5·0	78·9±5·4	111·1±6·4	90·1±5·2
	Esterified Chol*	60·2±7·5	57·1±5·4	82·9±6·0	62·6±6·8
	LCAT activity†	5·46±0·97	5·48±1·16	7·24±1·35	11·19±1·37
	Faecal bile acids excretion§	9·3	12·2	14·6	11·1

*Cholesterol mg. per 100 ml. serum. **Standard error of the mean. †LCAT activity as mg. of cholesterol esterified per 100 ml. of serum during 24 hours incubation. §as mg. of cholic acid/rat/day.

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