The above observations could have vast implications for the thiamine status of foods and feeds kept or prepared in water treated with chlorine. Chlorination of water is widely practised in many parts of the world where the thiamine intake in humans may only be marginal[13,14].

1 We thank the International Foundation for Science, Sweden, and the Thai University Development Council for financial support.
2 N. Yagi and Y. Itokawa, J. nutr. Sci. Vitaminol. *25*, 281 (1979).
3 A.I. Vogel, in: A Textbook of Quantitative Inorganic Analysis, 3rd edn, p.364. Longmans, London 1961.
4 M. Fujiwara and K. Matsui, Analyt. Chem. *25*, 810 (1953).
5 B. Panijpan and P. Detkriangkraikun, Am. J. clin. Nutr. *32*, 723 (1979).
6 P.P. Waring, W.C. Goad and Z.Z. Ziporin, Analyt. Biochem. *24*, 185 (1968).
7 J.J. Doherty, N. Cane and F. Wokes, J. Pharm. Pharmac. *7*, 1053 (1955).
8 A.A. Gallo, I.L. Hansen, H.Z. Sable and T.J. Swift, J. biol. Chem. *247*, 5913 (1972).
9 A.A. Gallo and H.Z. Sable, J. biol. Chem. *249*, 1382 (1974).
10 A.M. Chauvet-Monges, C. Rogeret, C. Brand and A. Crevat, Biochim. biophys. Acta *304*, 748 (1973).
11 S.K. Chakrabartty and H.O. Kretschmer, J. chem. Soc. Perkin *1*, 222 (1974).
12 Y. Ushijima and M. Nakano, Biochem. biophys. Res. Commun. *93*, 1232 (1980).
13 S.L. Vimokesant, S. Nakornchai, S. Dhanamitta and D.M. Hilker, Am. J. clin. Nutr. *28*, 1458 (1975).
14 C.S. Farkas, Can. med. Ass. J. *122*, 1356 (1980).

Plasma lecithin: cholesterol acyltransferase activity in high- and low-responding rhesus monkeys

A.K. Bhattacharyya

Departments of Pathology and Physiology, Louisiana State University Medical Center, New Orleans (LA 70112, USA), 3 February 1981

Summary. The initial rate of esterification of plasma cholesterol by lecithin: cholesterol acyltransferase (LCAT) was measured in high- and low-responding rhesus monkeys fed a moderately high cholesterol (0.15 mg/kcal) diet. The results show that the rate of esterification of cholesterol in the plasma of the high-responders was significantly ($p < 0.025$) higher than that of the low-responding animals. In view of known relationships between LCAT activity and plasma lipoprotein metabolism, it is suggested that the lipoprotein metabolism in the high-responders would differ from that in the low-responders.

Among rhesus monkeys fed cholesterol some develop severe hypercholesterolemia (high-responders), while some show only mild elevation in serum cholesterol level, (low-responders)[2]. Previously, we reported that high-responders absorb significantly higher percentage of intestinal luminal cholesterol than low-responders[2-4]. This difference in the intestinal absorption of cholesterol has been the only difference observed so far in respect to cholesterol metabolism between high- and low-responders that explains the differential response in plasma cholesterol concentration when fed cholesterol[2-4]. Based on free and esterified cholesterol content and specific radioactivity of free and esterified cholesterol in plasma chylomicrons 4 h after feeding a test meal by gavage containing radiolabeled cholesterol, we have suggested that the high absorption of cholesterol in high-responders is probably due to increased uptake and esterification of cholesterol by the intestinal mucosa[3]. Cholesterol, besides being esterified within the intestinal mucosa by the esterifying enzymes, cholesterol esterase[5] and acylcoenzyme A cholesterol acyltransferase (ACAT)[6-7], is also esterified in the systemic circulation by the enzyme present in the plasma, lecithin cholesterol acyltransferase (LCAT)[8]. It was of interest, therefore, to study the rate of esterification of cholesterol by LCAT in the plasma of the 2 highly selected groups of rhesus monkeys.

Materials and methods. 10 adult, male rhesus monkeys, weighing between 8 and 14 kg, were used in the study. The monkeys were selected previously as high- or low-responders from a group of 36 young adult male monkeys on the basis of the response of plasma cholesterol to an atherogenic diet[2]. Of the 10 animals, 6 were high-responders and 4 were low-responders.

The monkeys were fed for more than a year a semisynthetic moderately high cholesterol diet providing fat at 38% of calories and proteins at 15% calories. The cholesterol content of the diet was 0.15 mg/kcal[4]. The animals were fed once daily about 150 g diet or 600 calories a day which was sufficient to maintain body weight.

Blood samples were obtained after an overnight fast in tubes containing disodium EDTA (1 mg/ml) and were immediately cooled in crushed ice. The plasma was separated by centrifugation at 4 °C and was used immediately for determination of LCAT activity.

Plasma cholesterol esterification or LCAT activity was measured by the method of Marcel and Vezina[9] by measuring the decrease in the concentration of plasma free cholesterol before and after incubation. Aliquots of the plasma in triplicate were incubated in stoppered erlenmyer flasks in a shaking incubator at 37 °C for 30 min. The reaction was stopped by addition of 5 ml methanol and lipids were extracted by the method of Folch et al.[10]. The lipid extract was evaporated to dryness under N_2 and the residue dissolved in small volume of hexane was subjected to TLC for free and esterified cholesterol separation using silica gel G plate with light petroleum/diethyl ether/glacial acetic acid (80:20:1, v/v). The free and ester bands were scraped and eluted with diethyl ether. The free sterol was subjected to as trimethylsilyl ether derivative with 5 α-cholestane used as the internal standard as described below. The sterol esters were saponified with alcoholic KOH, extracted with hexane and processed as the free sterol.

GLC was equipped with a hydrogen flame ionization detector and an automatic digital integrator. The glass column (183 cm × 4 mm inner diameter) was packed with 3% SE-30 on 100–120 mesh Gas-Chrom Q (Applied Science Labs, State College, PA). Temperatures of column, detector and flash heater were 235,220 and 250 °C, respectively. Helium was used as carrier gas at 30 ml/min; the inlet pressure was 40 psi.

Results. The plasma total cholesterol in the high-responding rhesus monkeys was significantly higher than the low-responding animals (230 vs 142 mg/dl, $p < 0.025$) (table). Similarly plasma free and esterified cholesterol concentrations in the high-responders were also significantly higher than the low-responding group. However, plasma esterified cholesterol concentration when expressed as percent of

plasma total cholesterol, was similar in both groups, about 70%.

The plasma LCAT activity was significantly higher by about 39% in the high-responders than in the low-responders (79 vs 57 n moles/hr/ml plasma, $p < 0.025$) (table).

Discussion. The present study showed that the initial rate of esterification of cholesterol in the plasma by LCAT was significantly higher by about 39% in the high-responding rhesus monkeys than in the low-responding animals (table). Although absolute levels of LCAT enzyme were not determined and since the substrate and enzyme concentrations are limiting factors in the in vitro method used for measuring the enzyme activity, the high LCAT activity in high-responders is probably due to the high concentration of free cholesterol in the plasma. In in vivo situation this would imply that due to high cholesterol absorption in the high-responders[2-4] resulting in increased input of free cholesterol into the plasma produced the increased esterification of cholesterol in the high-responders. The high input of free cholesterol into the plasma during absorption is indicated by the fact that the free cholesterol content of the plasma chylomicrons after feeding a test meal containing cholesterol was significantly higher in the high-responders than in the low-responders[3].

The significance of the high LCAT activity in the high-responding rhesus monkeys is not clear. In several animal species and in humans, elevated rates of cholesterol esterification (LCAT activity) with high plasma unesterified cholesterol content has been demonstrated[11,12]. It is well known that the enzyme LCAT circulates in the plasma associated with high density lipoproteins HDL[13] and catalyzes the transfer of fatty acid from the 2-position of lecithin to hydroxyl group of cholesterol producing esterified cholesterol and lysolecithin[8]. This reaction is of great significance in the metabolism of plasma lipoproteins because it has been suggested that from chylomicrons and very low density lipoprotein remnants (VLDL remnants) LCAT removes the unesterified cholesterol and lecithin by converting them to esterified cholesterol and lysolecithin[14,15]. The chylomicrons and VLDL remnants are thus converted to smaller lipoprotein particles which can then undergo further metabolism. Further, it has been suggested that LCAT also plays a role in the conversion of nascent HDL secreted by the liver to normal plasma HDL[16]. The normal plasma HDL, particularly the smaller HDL subfraction (HDL_3, $d = 1.125$–1.210 g/ml), has been shown to be the preferred substrate for LCAT[8,17]. Also there is increasing evidence that the indicence of atherosclerotic disease is inversely related to the concentration of HDL-cholesterol[18]. It has been suggested that HDL protects against the development of atherosclerosis[19] by promoting the transport of peripheral tissue cholesterol to liver for its metabolism and excretion[20]. In view of these relationships between LCAT and the metabolism of plasma lipoproteins, the observed higher activity of LCAT in the high-responders than in the low-responders suggests that there would be differences in the rates of lipoprotein metabolism between the 2 groups. In fact, drastic changes in the concentration and composition of the plasma lipoproteins have been observed in the high-responders than in the low-responders upon feeding cholesterol[21], although cholesterol turnover and metabolism in these 2 groups of highly selected rhesus monkeys have been shown to be similar[2-4]. The only observation that so far has provided a reasonable explanation for the differential hypercholesterolemia produced upon cholesterol feeding in these 2 groups of monkeys is that the high-responders have significantly higher percentage of absorption of cholesterol than the low-responders[2-4].

The initial rate of reaction of plasma lecithin: cholesterol acyltransferase and plasma cholesterol levels in high-responding and low-responding rhesus monkeys

Group and animal No.	Plasma cholesterol (mg/dl) Total	Free	Esterified	LCAT activity nmoles/h/ml plasma
High-responders				
1	231	66	165 (71.4)*	81
2	317	76	241 (76.0)	91
3	179	53	126 (70.4)	75
4	266	102	164 (61.6)	95
5	188	55	133 (70.7)	65
6	201	51	150 (74.6)	68
Mean ± SEM	230 ± 22**	67 ± 8***	163 ± 17*** (70.8 ± 2.0)	79 ± 5**
Low-responders				
1	115	37	78 (67.8)	61
2	172	54	118 (68.6)	52
3	119	39	80 (67.2)	63
4	164	39	125 (76.2)	53
Mean ± SEM	142 ± 15	42 ± 4	100 ± 12 (70.0 ± 2.0)	57 ± 3

* Figures in parentheses are percent of total. ** Significantly higher than values from low-responders at $p < 0.025$ level. *** Significantly higher than values from low-responders at $p < 0.05$ level.

1 Acknowledgments. Financed by USPHS research grant HL-08974 from the National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Md.

2 D.A. Eggen, J. Lipid Res. *17*, 663 (1973).
3 A.K. Bhattacharyya and D.A. Eggen, in Atherosclerosis IV. Proc. 4th int. Symposium, Tokyo 1976, p. 293. Ed. G. Schettler, Y. Goto, Y. Hata and G. Klose. Springer, New York 1977.
4 A.K. Bhattacharyya and D.A. Eggen, J. Lipid Res. *21*, 518 (1980).
5 C.R. Treadwell and G.V. Vahouny, in: Handbook of Physiology, sect. 6, vol. III, chapt. 72, p. 1407. Am. Physiol. Soc., Washington, DC 1968.
6 R. Haugen and K.R. Norum, Scand. J. Gastroenterol. *11*, 615 (1976).
7 K.R. Norum, A.-C. Lilljeqvist and C.A. Drevon, Scand. J. Gastroenterol. *12*, 281 (1977).
8 J.A. Glomset and K.R. Norum, Adv. Lipid Res. *11*, 1 (1973).
9 Y.L. Marcel and C. Vezina, Biochim. biophys. Acta *306*, 497 (1973).
10 J. Folch, M. Lees and G.H. Sloane-Stanley, J. biol. Chem. *226*, 497 (1957).
11 K.T. Stokke, Atherosclerosis *19*, 393 (1974).
12 A.G. Lacko, K.G. Varma, H.L. Rutenberg and L.A. Soloff, Scand. J. clin. Lab. Invest. *33*, suppl. 137, 29 (1974).
13 J.A. Glomset, in: Blood lipids and Lipoproteins: Quantitation, composition and metabolism, p. 745. Ed. G.J. Nelson. Wiley-Interscience, New York 1972.
14 J.A. Glomset, K.R. Norum, A.V. Nichols, W.C. King, C.D. Mitchell, K.R. Applegate, E.L. Gong and E. Gjone, Scand. J. clin. Lab. Invest. *35*, suppl. 142, 3 (1975).
15 V.N. Schumaker and G.H. Adams, J. theor. Biol. *26*, 89 (1970).
16 R.L. Hamilton, M.C. Williams, C.J. Fielding and R.J. Havel, J. clin. Invest. *58*, 667 (1976).
17 C.J. Fielding and P.E. Fielding, FEBS Lett. *15*, 355 (1971).
18 G.J. Miller and N.E. Miller, Lancet *1*, 16 (1975).
19 N.E. Miller, P.J. Nestel and P. Clifton-Bligh, Atherosclerosis *23*, 535 (1975).
20 R.L. Hamilton, in: Disturbances in Lipid and lipoprotein Metabolism, p. 155. Ed. J.M. Dietschy, A.M. Gotto, Jr, and J.A. Ontko. Am. Physiol. Soc., Bethesda, MD 1978.
21 L.H. Myers, A.K. Bhattacharyya and D.A. Eggen, Clin. Chem. *25*, 1114 (1979); abstract.