Table 3. Effect of Tween 80 on fatty acid composition of total lipids of *M. phlei* ATCC 354*

Growth medium	Fatty acids (% of total)** 14:0	16:0	16:1	18:0	18:1	Me 18:0
Medium I	4.0 ± 0.11 (3.8 — 4.2)	40.2 ± 0.78 (38.9 — 41.8)	7.6 ± 0.61 (7.0 — 8.3)	4.1 ± 0.33 (3.8 — 4.3)	9.8 ± 0.45 (9.2 — 10.4)	29.3 ± 0.75 (28.2 — 30.4)
Medium II	3.1 ± 0.26 (2.8 — 3.4)	42.0 ± 1.07 (41.1 — 42.8)	9.0 ± 0.60 (8.6 — 9.4)	5.5 ± 0.27*** (5.0 — 6.1)	15.2 ± 0.38**** (14.6 — 15.9)	23.3 ± 0.72**** (22.5 — 24.2)

*Fatty acids were analysed as methyl esters by gas-liquid chromatography. They are: 14:0, myristic; 16:0, palmitic; 16:1, palmitoleic; 18:0, stearic; 18:1, oleic and Me 18:0, tuberculostearic. **The figures in parentheses represent the range of values and the value given is the average ±SE of 3 separate experiments. p-value $\leqslant 0.05$ is considered significant. *** $p < 0.05$; **** $p < 0.005$.

sum of all the individual mannosides and *M. phlei* ATCC 354 loses its unique property. Such a change in the phospholipid pattern is of clinical importance in view of the fact that mannosides are the antigens used for the serological diagnosis of tuberculosis[7], and *M. phlei* ATCC 354 has been shown to be a better source of antigen among various species of mycobacteria[8]. Dubos et al.[9] showed that human and avian type tubercle bacilli, grown in the presence of Tween 80 and injected into rabbits, elicit the production of antibodies directed against this water-soluble ester of oleic acid. That is to say, the sera produced under these conditions contain at the same time antibodies for the bacterial constituents of the injected antigen and other antibodies for the Tween 80 adsorbed on the bacterial surface[9].

There were no significant differences in the content of glycerides in the media with and without Tween 80 (table 2). Among fatty acids, a decrease in tuberculostearic acid with a concomitant increase in oleic acid occurred in cells grown in medium supplemented with Tween 80 (table 3). As the physiological events involved in the breakdown and utilization of Tween 80 are not fully known, it is difficult to explain the mechanism of alterations observed in the present investigation. However, these facts are of importance because they may be a source of confusion in the analysis of the immunological behaviour of mycobacteria grown in presence of Tween 80[9]; and also, in some experiments where Tween 80 is added to the medium, the observed results and changes may not be due to other experimental variations but due to Tween 80 itself.

7 Y. Takahashi, Am. Rev. resp. Dis. *85*, 708 (1962).
8 K. R. Dhariwal, and T. A. Venkitasubramanian, Indian J. Chest Dis. *17*, 147 (1975).
9 R. J. Dubos, B. D. Davis, G. Middlebrook and C. Pierce, Am. Rev. Tuberc. *54*, 204 (1946).

Lecithin-cholesterol acyltransferase activity in carbohydrate-induced hypertriglyceridemia in mice. An immunofluorescent method for identification of isolated thyrotropic cells

M. B. Mattock[1], V. S. Sheorain and D. Subrahmanyam

Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh (India),
25 July 1977

Summary. Feeding to mice of both basal as well as high sucrose diet led to increased levels of plasma triglycerides, which was associated with increased lecithin-cholesterol acyltransferase activity. Although males had significantly higher LCAT activity than females in all the dietary groups, sex difference in the plasma triglycerides was observed in high sucrose group only. Increase in plasma triglycerides in experimental groups was associated with an increase in LCAT activity.

In recent years it has become apparent that at least 2 enzymes, lipoprotein lipase (LPL)[2] and lecithin-cholesterol acyltransferase (LCAT)[3] are involved in the catabolism of the plasma lipoproteins. An increased concentration of plasma triglycerides of the very low density lipoproteins (VLDL) has been supposed to be one of the factors stimulating LCAT activity[4].

This was later on confirmed by Marcel and Vezina[5] that in in vitro experiments when increasing concentrations of 2 triglyceride-rich lipoprotein fractions (chylomicrons and VLDL) were added, there was a proportional increase in plasma LCAT activity. This was found to be true in severe hypertriglyceridemic patients only when exogenous substrate was used, ad not in cases where autologous substrate was used for enzyme assay[6]. In the mouse an increased VLDL-triglyceride concentration was observed on feeding high sucrose diet for 12 days[7]. It was then of interest to see whether this hypertriglyceridemic state was accompanied by an increased LCAT activity.

Materials and methods. Male and female Swiss mice of Institute colony (Virus Research Centre, Poona strain) weighing approximately 20 g and 18 g respectively were kept on commercial pellets (Hind Lever, Bombay) and water ad libitum. Animals were then given basal diet for 2 weeks, the composition of which was as follows: butter fat, 20%; casein, 20%; salt mixture, 4%; vitamin mixture, 1%; cellulose powder, 5%; and sucrose, 50%. Thereafter one group of animals was continued on the basal diet for 12 days, whereas the other group was kept on high sucrose diet (70% sucrose and not fat) for the same period. At the end of experimental period, the

Plasma triglycerides and LCAT activity of mice fed on various diets

Dietary groups	Sex	Triglyceride levels mg/100 ml (mean ± SEM)	p-value**	LCAT activity* μmoles cholesterol/L/h
Pellet	M	60.0 ± 2.5	–	97.7
control	F	57.5 ± 2.0	–	51.8
Basal	M	75.0 ± 2.9	< 0.01	130.9
	F	68.9 ± 3.3	< 0.02	102.1
High	M	128.0 ± 4.5***	< 0.001	154.2
sucrose	F	110.0 ± 5.0	< 0.001	117.3

*Assayed from pooled plasma; each value was mean of 4 readings; **When compared with control; ***Male vs female, $p < 0.05$.

animals were sacrificed under light ether anaesthesia and blood was collected in ice-cold heparinized tubes by cardiac puncture. Plasma was separated and used for various investigations. Cholesterol was estimated by colorimetry[8], and LCAT was assayed essentially by the method of Stokke and Norum[9]. The enzyme was also assayed by the method of Glomset[10]. Though the results of this method were not included in this paper. The enzyme activity in mouse was almost 2 fold to that in humans, and therefore the enzyme concentration and the incubation period were reduced by one-half of that in the original method. Triglycerides were estimated by the method of Van Handel and Zilversmit[11].

Results and discussion. The data presented in the table clearly demonstrate increased triglyceride levels in the basal and high sucrose-fed animals when compared with those fed on control diet. Among the 2 experimental dietary groups, high sucrose-fed animals had higher levels. There was a marked sex difference in plasma triglyceride levels of high sucrose-fed animals. LCAT activity was found to be increased in both basal as well as high sucrose group but more so in case of latter. Males had higher enzyme activity in plasma than female in all the animals including controls. There appeared some association between increase in plasma triglycerides and LCAT activity during this study. Our observations supported the earlier findings[4,5]. The results in the present study were unlike those observed by Goren and Simons[6] because they found a decrease in LCAT activity (when assayed by the method of Stokke and Norum[9]) in hypertriglyceridemic patients, whereas LCAT activity increased (when assayed according to the method of Glomset[10]) in the same patients. We also used both methods and in both cases the activity followed the same pattern. These experiments, therefore, further confirm the view that LCAT activity is influenced in part by the triglyceride concentration of the plasma.

1 Present address: Unit for Metabolic Medicine, Dept. of Medicine, Guy's Hospital Medical School, London Bridge, SE1 9RT, England.
2 A. V. Nichols, E. H. Strisower, F. T. Lindgren, G. L. Adamson and E. L. Coggiola, Clin. chim. Acta *20*, 277 (1968).
3 J. A. Glomset and K. R. Norum, Adv. Lipid Res. *11*, 1 (1973).
4 P. J. Nestel, Clin. Sci. *38*, 593 (1970).
5 Y. L. Marcel and C. Vezina, J. biol. Chem. *248*, 8254 (1973).
6 R.. Goren and L. Simons, Clin. chim. Acta *74*, 289 (1977).
7 V. S. Sheorain, Indian J. Biochem. Biophys. *13*, 23 (1976), abstract.
8 B. Zak, Am. J. clin. Path. *27*, 583 (1957).
9 K. T. Stokke and K. R. Norum, Scand. J. clin. Lab. Invest. *27*, 21 (1971).
10 J. A. Glomset, J. Lipid Res. *9*, 155 (1968).
11 E. Van Handel and D. B. Zilversmit, J. Lab. clin. Med. *50*, 152 (1957).

Dopamine-β-hydroxylase activity in stroke-prone spontaneously hypertensive rats

T. Nagatsu, T. Kato, Y. Hashimoto, Y. Numata (Sudo), Y. Yamori and K. Okamoto[1]

Laboratory of Cell Physiology, Department of Life Chemistry, Graduate School at Nagatsuta, Tokyo Institute of Technology, Yokohama 227 (Japan); Department of Biochemistry, School of Dentistry, Aichi-Gakuin University, Nagoya 464 (Japan); Department of Pathology, National Shimane Medical School, Izumo 693 (Japan); and Department of Pathology, Faculty of Medicine, Kinki University, Osaka 589 (Japan), 5 August 1977

Summary. Dopamine-β-hydroxylase (DBH) activity was higher in the serum, the mesenteric artery and the cerebral cortex of 4-week-old stroke-prone spontaneously hypertensive rats (SHRSP), and lower in the nucleus tractus solitarii than it was in spontaneously hypertensive rats (SHR).

Dopamine-β-hydroxylase (DBH) activity was found to be higher in the serum, the mesenteric vessels, of 3-week-old spontaneously hypertensive rats (SHR)[2,3] and lower in the locus coeruleus than it was in the control Wistar-Kyoto rats[4,5]. At 16 weeks of age, when hypertension of SHR was fixed, DBH activity in the serum, the mesenteric vessels, and the locus coeruleus, there were no significant differences between SHR and normotensive Wistar-Kyoto rats. In contrast, DBH and tyrosine hydroxylase (TH) activities in the adrenal glands and the vas deferens were significantly higher in SHR than in Wistar-Kyoto rats[6,7]. These changes suggest that the nervous system is an important regulator of blood pressure, especially in an early phase in the development of hypertension of SHR. Stroke-prone spontaneously hypertensive rats (SHRSP)[8] were isolated as a mutant of SHR by

1 The authors wish to thank Prof. Masao Sano (Nagoya University) for his aid in dissecting the nucleus tractus solitarii and the locus coeruleus.

2 K. Okamoto, Folia endocr. jap. *38*, 782 (1962).
3 K. Okamoto and K. Aoki, Jap. Circul. J. *27*, 282 (1963).
4 T. Nagatsu, T. Kato, Y. Numata (Sudo), K. Ikuta, H. Umezawa, M. Matsuzaki and T. Takeuchi, Nature *251*, 630 (1974).
5 T. Nagatsu, K. Ikuta, Y. Numata (Sudo), T. Kato, M. Sano, I. Nagatsu, H. Umezawa, M. Matsuzaki and T. Takeuchi, Science *191*, 290 (1976).
6 T. Nagatsu, T. Kato, Y. Numata (Sudo), K. Ikuta, H. Kuzuya, H. Umezawa, M. Matsuzaki and T. Takeuchi, Experientia *31*, 767 (1975).
7 T. Nagatsu, T. Kato, Y. Numata (Sudo), K. Ikuta, M. Sano, I. Nagatsu, H. Umezawa, M. Matsuzaki and T. Takeuchi, Jap. J. Pharmac. *27*, 531 (1977).
8 K. Okamoto, Y. Yamori and A. Nagaoka, Circ. Res. *34/35*, 143 (1974).