Effect of Tween 80 on lipids of *Mycobacterium phlei* ATCC 354

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Summary. Addition of Tween 80 to the growth medium brings about qualitative and quantitative changes in the lipids of *Mycobacterium phlei* ATCC 354. The results suggest that Tween 80 itself may be a variant in the system.

Tween 80 (polyoxyethylene derivative of sorbitan monooleate) is generally used as a detergent when bacteria are grown as shake culture. Submerged and dispersed growth is facilitated by the addition of Tween 80 to the suspending medium[2]. Its effect in enhancing growth and bringing about morphological changes in mycobacteria is well known[3,4]. Stinson and Solotorovsky[5] observed that addition of Tween 80 to the medium doubles the quantity of lipid produced by *Mycobacterium avium*. This communication reports the effect of tween 80 on the production of various lipid components by *Mycobacterium phlei* ATCC 354. It was hoped that Tween 80, by interfering with the integrity of the cell membrane, might change the content of individual lipids, especially of phospholipids associated with the cytoplasmic membrane of the cell.

Table 2. Effect of Tween 80 on glycerides of *M. phlei* ATCC 354*

Growth medium	mg/100 mg dry weight of bacteria** Total	MG	DG	TG
Medium I	1.09	0.09 ± 0.02 (0.08 – 0.11)	0.11 ± 0.01 (0.09 – 0.13)	0.89 ± 0.04 (0.82 – 0.96)
Medium II	1.12	0.10 ± 0.02 (0.09 – 0.12)	0.11 ± 0.02 (0.09 – 0.12)	0.91 ± 0.05 (0.87 – 0.95)

*The glycerides were separated from each other by TLC using solvent systems of 2 different compositions i.e. petroleum hydrocarbon-diethyl ether-acetic acid (60:40:1, v/v/v) (solvent I) and (90:10:1, v/v/v) (solvent II). The chromatoplates were developed in solvent I for 7.5 cm and then were taken out, air dried and developed in solvent II for 15 cm. The bands were scraped into test tubes and eluted with n-hexane-ether (1:1, v/v). Glyceride glycerol was estimated using mono-, di- and tripalmitin as standards. Abbreviations are: MG, monoglycerides; DG, diglycerides and TG, triglycerides. **The figures in parentheses represent the range of values and the value given is the average ± SE of 3 separate experiments.

Materials and methods. *Mycobacterium phlei* ATCC 354 (American Type Culture Collection, Rockville, Maryland, USA) was grown as shake culture at 37 °C in a medium containing the following in g: asparagine, 5; potassium dihydrogen phosphate, 5.9; potassium sulfate, 0.5; citric acid, 1.5; magnesium carbonate, 0.6 and glycerol, 20. The pH of the medium was adjusted to 7.0 and volume made to 1 l. This medium was designated as medium I. 2 g of Tween 80 were added per litre of the medium in addition to the above ingredients and this constituted medium II. Cells were harvested at the late logarithmic phase of growth. Extraction of lipids, separation, identification and estimations of various lipid components were as described earlier[6].

Results and discussion. A significant increase in the content of total phospholipids was observed when the cells were grown in presence of Tween 80 (table 1). *M. phlei* ATCC 354 has been shown to be a unique strain in that phosphatidylinositol mannosides, phosphatidylinositol dimannoside A in particular, are the major phospholipids, whereas in all other species of mycobacteria, cardiolipin has been shown to be present at a concentration higher than the sum total of all the individual mannosides[6]. In a medium supplemented with Tween 80, a decrease in the content of mannosides was observed with a simultaneous increase in cardiolipin (table 1). This increase in cardiolipin content is so sharp that it overcomes the

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Table 1. Effect of Tween 80 on phospholipids of *M. phlei* ATCC 354*

Growth Medium	mg/100 mg dry weight of bacteria** Total lipids	Total phospholipids	CL	PE	PIMx	PIM_2A	PIM_2B	PIM_5
Medium I	11.60 ± 0.32 (11.00 – 12.10)	3.20 ± 0.07 (3.08 – 3.33)	0.93 ± 0.08 (0.83 – 1.03)	0.43 ± 0.02 (0.38 – 0.48)	1.52	1.00 ± 0.08 (0.95 – 1.07)	0.39 ± 0.05 (0.34 – 0.44)	0.13 ± 0.01 (0.11 – 0.15)
Medium II	12.80 ± 0.36 (12.30 – 13.50)	4.00 ± 0.23*** (3.60 – 4.40)	1.50 ± 0.10**** (1.39 – 1.62)	0.47 ± 0.04 (0.41 – 0.54)	1.33	0.91 ± 0.02 (0.85 – 0.98)	0.32 ± 0.02 (0.29 – 0.35)	0.10 ± 0.02 (0.08 – 0.11)

*Phospholipids were separated from each other by TLC using chloroform-methanol- 7 M ammonia (115:45:7.5, v/v/v). Abbreviations are: CL, cardiolipin; PE, phosphatidyl ethanolamine; PIMx, phosphatidylinositol mannosides; PIM_2A, phosphatidylinositol dimannoside A tetra acylated; PIM_2B, phosphatidylinositol dimannoside B triacylated and PIM_5, phosphatidylinositol pentamannoside tetra acylated. The value for PIMx is calculated by the sum of PIM_2A, PIM_2B and PIM_5. **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. *** $p < 0.05$; **** $p < 0.01$.

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.

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

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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