On the Mannophosphoinositides of *Mycobacterium 607*

Evidence has accumulated, in recent years[1-5], on the presence of antibodies to phospholipids of tubercle bacilli in tuberculous patients. Studies on the nature of the phospholipid-antigens involved in the serological reactions revealed that the inositomannosides are the active lipids[3,4]. The elegant work of BALLOU et al.[6] established the presence of several mannose-containing phospholipids in mycobacteria. However, recent studies of PANGBORN and MCKINNEY[4] and BRENNAN and BALLOU[7] suggest that these lipids are more complex than they were presumed to be as several of them were found to contain higher fatty acid-phosphorus ratios. This report pertains to the nature of the mannose-containing phospholipids of *Mycobacterium 607*.

Materials and methods. *M. 607* were grown for 48–56 h and methods used in the cultivation, harvesting the cells, extraction and purification of the lipids were as described elsewhere[8,9]. The lipids were separated by thin-layer chromatography (TLC) using silica gel G coated on glass plates at 0.3–0.5 mm thickness. The phospholipids were separated on the plates with chloroform-methanol-water 65:25:4, 60:35:8 and 80:35:7 respectively. The lipids were identified by exposing the plates to iodine vapour and phospholipids by molybdate spray[10]. Glycolipids were detected by phenol-phosphoric acid spray[7]. In preparative TLC, marker plates were run simultaneously to locate the position of the phospholipids. The phospholipids were quantitatively eluted from the silica gel by extracting twice with suitable aliquots of ethanol-chloroform-water, 5:2:2; chloroform-methanol, 1:1; 1:4 and methanol.

Phosphorus in the spots was estimated by direct digestion. Mannose was determined both by the cysteine-sulphuric acid method of DIEDRICH and ANDERSON[11] and by the anthrone method[12]. Fatty acids were quantitated according to the procedure of STERN and SHAPIRO[13]. Lipid samples were hydrolyzed with 50% HCl for 6 h at 110 °C in sealed tubes and inositol in the hydrolyzate was determined as detailed elsewhere[3].

Results and discussion. Phospholipids were found to be separated into 8 spots on TLC with chloroform-methanol-water, 65:25:4 (Figure 1). Of these phospholipids, the first 3 from the origin (M, VI and VII) were found to be

[1] Y. TAKAHASHI, K. MOCHIZUKI and Y. NAGAYAMA, J. exp. Med. *114*, 555 (1966).
[2] G. FAVEZ, S. JEQUIER and P. VULLIEMOZ, Am. Rev. resp. Dis. *94*, 905 (1966).
[3] D. SUBRAHMANYAM and D. R. SINGHVI, Proc. Soc. exp. Biol. Med. *120*, 102 (1965).
[4] M. C. PANGBORN and J. A. MCKINNEY, J. Lipid Res. *7*, 627 (1966).
[5] D. SUBRAHMANYAM, in *Conference on Tuberculosis and Leprosy* (United States – Japan Cooperative Medical Science Program, Tokyo 1966).
[6] Y. C. LEE and C. E. BALLOU, Biochemistry *4*, 1395 (1965).
[7] P. BRENNAN and C. E. BALLOU, J. biol. Chem. *242*, 3046 (1967).
[8] D. SUBRAHMANYAM, Can. J. Biochem. *42*, 1195 (1964).
[9] D. SUBRAHMANYAM, Ind. J. Biochem. *1*, 34 (1964).
[10] J. C. DITTMER and R. L. LESTER, J. Lipid Res. *5*, 126 (1964).
[11] D. F. DIEDRICH and L. ANDERSON, Analyt. Biochem. *2*, 70 (1961).
[12] R. JOHANSON, Analyt. Chem. *26*, 1331 (1954).
[13] I. STERN and B. SHAPIRO, J. clin. Path. *6*, 158 (1953).

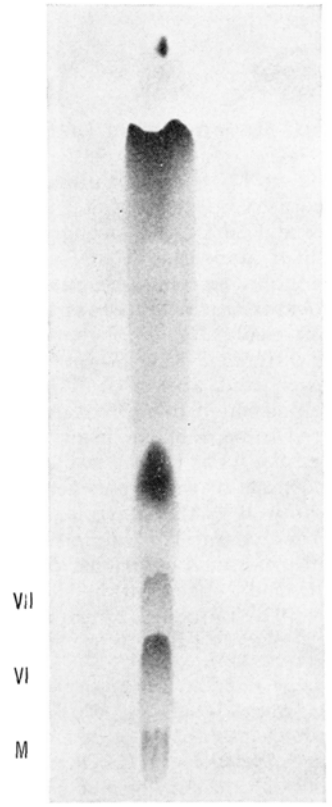

Fig. 1. Phospholipids of *M. 607* separated by TLC with chloroform-methanol-water, 65:25:4 as developing solvent. The spots were located by molybdate spray. Spots M, VI and VII were glycolipids.

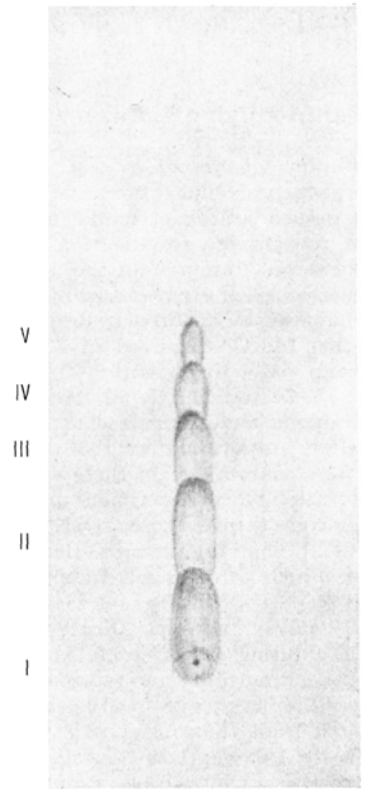

Fig. 2. Further separation of spot M of Figure 1 by TLC with chloroform-methanol-water, 80:35:7 as developing solvent. The spots were identified by phenol-phosphoric acid spray.

glycolipids. These glycolipids were purified further by TLC with chloroform-methanol-water, 60:35:8. The purified lipids were then examined by continuous development for 3 h with chloroform-methanol-water (80:35:7) on TLC plates. The first spot (M) further separated into 5 spots (I–V, Figure 2) in this solvent and these lipids were isolated in amounts adequate for analysis. Analytical data on the glycolipids, as presented in the Table, suggest that all the 7 lipids were mannophosphoinositides containing varying amounts of mannose (1–6) and fatty acids (2–4)/g-atom of phosphorus. Monomannoside was the only phospholipid among the isolated lipids with a fatty acid-phosphorus ratio of 2. The mannose-containing lipids accounted for 30% of the total phospholipids of *M. 607* of which the monomannoside was the major glycolipid.

Mannophosphoinositides of *M. 607*

Phospho-lipid	No. of moles/g-atom P			Inference
	Mannose	Carboxyl ester	Inositol	
I	5.7–6.2 (4)	4.0–4.5 (3)	1.0	Hexamannoside
II	4.9–5.3 (4)	4.0–4.2 (3)	1.0	Pentamannoside
III	3.4–3.5 (4)	3.1–3.3 (3)	1.1	Tetramannoside
IV	2.8–3.0 (3)	3.5–3.9 (2)	1.2	Trimannoside
V	1.6–1.8 (2)	3.5–3.7 (2)	0.8	Dimannoside
VI	1.1–1.3 (4)	2.3 (4)	1.3	Monomannoside
VII	2.2–2.4 (4)	4.2–4.4 (4)	1.0	Dimannoside

No. of determinations are given in parentheses.

The identification of only 2 dimannosides and 1 pentamannoside in *M. 607* is in contrast with the higher number, some of which with different fatty acid-phosphorus ratios, found in BCG and *M. tuberculosis* by PANGBORN[4] and in *M. phlei* by BALLOU[7]. The dimannosides in *M. 607* differed in their mobility on TLC plates probably due to differences in the position of the fatty acids on the molecules. The extent of variation in the glycolipids due to differences between strains, and to age and conditions of growth within a particular strain, is unknown and such a study might throw some light on this aspect[14].

Zusammenfassung. Aus *Mycobacterium 607* wurden 7 Mannophosphoinositiden isoliert und durch präparative Dünnschichtchromatographie getrennt. Diese Lipide machen ca. 30% der gesamten Phosphatide des Stammes aus und setzen sich aus einem Mono-, zwei Di- und je einem Tri-, Tetra-, Penta- und Hexamannosiden zusammen. Mit Ausnahme der Monomannoside (2) haben sämtliche Mannoside mehr als 3 Fettsäuren pro Phosphor-g-Atom.

G. K. KHULLER and D. SUBRAHMANYAM

Biochemistry Division, National Institute of Communicable Diseases, Delhi (India), 11 March 1967.

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Inhibitory and Modifying Influence of Excess of Vitamin A on Tail Regeneration in *Bufo* Tadpoles

Excess of vitamin A is known to exert a profound influence on morphogenesis and differentiation on embryonic and adult tissues both in vivo and in vitro[1–5]. This communication reports the results of a study on the influence of excessive vitamin A in the medium on tail regeneration in tadpoles of *Bufo andersonii* Bouleng.

Appropriate quantities of Arrovit drops (Vitamin A palmitate, Roche, India), dissolved in a little ethanol, were added to tap water to prepare vitamin A solutions of 1, 2.5, 5, 10, 15, 20 and 30 I.U./ml concentration. The experimental tadpoles were immersed in these solutions immediately after amputation of the tail across the middle. They were maintained in these solutions either for the entire duration of the experiment or for 1 day only after which they were transferred to conditions similar to those of controls. A small quantity of ethanol was added to the water in which controls were kept. The animals were fed boiled spinach regularly and their medium was changed every 2 days. The experiments were made at room temperature during July–September.

Apart from greater mortality and retardation in general body growth in vitamin A treated tadpoles their regenerated tails differed from those in controls in several respects (Figure a–d). The length of the tail regenerated in experimental tadpoles was much less than in controls; it decreased progressively with rise in vitamin A concentration in the medium. Thus, while the regenerated tail in controls attained a length of 2.95–4.7 mm in 9 days the animals immersed in 1 I.U./ml vitamin solution for all this period regenerated tail lengths of 1.8–2.95 mm only. In solutions of 20 and 30 I.U./ml strength, no regenerate attained a length of more than 1 mm even in 12 days. Generally, the tadpoles treated with vitamin A solutions for 1 day only showed greater regeneration than their fellows kept in these solutions for the entire period of the experiment. The difference was, however, small in concentrations below 5 and above 10 I.U./ml. The small regenerates in tadpoles kept in high vitamin A concentrations also appeared to be deficient in pigmentation.

Regeneration of the axial tissues was affected particularly severely and none of the experimental tadpoles regenerated them as well as the controls. There was some regeneration of these tissues in animals treated with 1, 2.5 and 5 I.U./ml vitamin A solutions, those maintained in the last mentioned concentration showing the least amount of growth. The tadpoles kept in these concentrations of vitamin A for only 1 day showed relatively better

[1] M. B. AYDELLOTE, J. Embryol. exp. Morph. *11*, 621 (1963).
[2] H. B. FELL, J. Embryol. exp. Morph. *10*, 379 (1962).
[3] D. M. KOCHHAR and P. M. JOHNSON, J. Embryol. exp. Morph. *14*, 223 (1965).
[4] M. MARIN-PADILLA, J. Embryol. exp. Morph. *15*, 262 (1966).
[5] G. WEISEMANN, Nature *192*, 235 (1961).