content in whole blood, it increased by 24% after 3 h of treatment. This could conceivably be due to a release of glutathione from tissues such as liver[2,3] and kidney[3], which have been shown to exhibit a moderate diminution in cellular glutathione (10–15% and 22% respectively) at this experimental time[2,3].

Following 3–6 h of the administration of ethanol the levels of GSH_T in the erythrocytes increased, reaching basal values at 6 h. This was due to a marked increase in the concentrations of GSH within the red blood cell, which reached a level above control values. GSSG levels were also enhanced, but they remained lower than levels found at time zero. The recovery of GSH levels in the erythrocyte could be related to an active resynthesis of the tripeptide, since γ-glutamyl-cysteine synthetase, the first enzyme involved in this process, is markedly inhibited by physiological levels of GSH[11]. Furthermore, GSSG is also able to inhibit hepatic γ-glutamyl-cysteine synthetase[11]. Thus, the decrease in the erythrocyte levels of GSH and GSSG observed after 3 h of ethanol intoxication could activate this enzyme by de-inhibition. The basal values of GSH_T observed in red blood cells after 6 h of ethanol intoxication were found concomitantly with an increased content (209%) of GSH_T in plasma. These findings indicate either that the erythrocyte is still releasing glutathione to the plasma and/or that another tissue is contributing to this effect. Although previous studies have indicated that the liver is maximally depleted of GSH after 6 h of ethanol ingestion[2,5], pointing to this organ as the main source of plasma GSH_T, further studies are needed to clarify this.

It is concluded that acute ethanol ingestion diminishes the glutathione levels of the erythrocyte, as is found in the liver tissue. However, this effect of ethanol appears earlier in the erythrocyte than in the liver and seems to be due to a translocation of glutathione from the red blood cell into the plasma, mainly as GSSG. Both an enhanced release of GSSG and an increased GSH synthesis in the erythrocyte could conceivably explain the change in the redox state of the glutathione couple previously observed after 6 h of ethanol ingestion, represented by an increase in the GSH/GSSG ratio[5].

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Subcellular distribution of mannophosphoinositides in *Mycobacterium smegmatis* during growth

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Summary. Subcellular distribution of total phospholipids and mannophosphoinositides (mannosides) was examined in *Mycobacterium smegmatis* ATCC 607 during its transition from the early exponential to the stationary phase of growth. There was relatively more of these substances in the cell membrane than in the cell wall, and the total amount increased with the age of the culture. Among individual mannosides, dimannophosphoinositides (dimannosides) were distributed equally in the cell wall and membrane. However, hexamannophosphoinositides (hexamannosides) were more predominant in the cell membrane, and the level increased with the age of the culture.

A number of bacterial genera belonging to the class Actinomycetales have the unique characteristic of possessing mannose-containing phospholipids called mannophosphoinositides[3]. These phospholipids have been known to possess antigenic characteristics in mycobacteria[4] and nocardia[5]. The cellular distribution of these components in mycobacteria is unclear[6, 7]. The present investigation was undertaken in an attempt to ascertain the cellular distribution of these phospholipids in mycobacteria. Changes, if any, in localization of these mannosides within mycobacterial organelles were also studied with respect to age of the culture.

Materials and methods. Mycobacterium smegmatis ATCC 607 was obtained from the American Type Culture Collection, and maintained as described earlier[8]. Cells in early exponential (2 days), mid-exponential (4 days) and early stationary (6 days) phases were harvested by filtration, washed thoroughly with normal saline (0.85% sodium chloride) and dried at 60 °C. Extraction of lipids and phosphorus estimation were carried out according to standard procedures[9]. Mannosides were resolved on silica gel H plates using the solvent system chloroform-methanol-water, 10:5:1 (v/v/v)[10]. Areas from the TLC plate corresponding to particular phosphatides were scraped off into Packard vials containing scintillation fluid and radioactivity was monitored in a Packard Liquid Scintillation counter[8].

Isolation of cell wall and cell membrane fractions. Mycobacterium smegmatis ATCC 607 cells, grown in Youman's medium containing ^{32}P-orthophosphate (500 µCi/100 ml of medium), were harvested after 2, 4 and 6 days of growth. A 25% suspension of the cells of a particular age was prepared in 0.01 M phosphate buffer (pH 7.0). Cells were sonicated

for 10 min at 4 °C and the sonicate was subjected to differential centrifugation[11]. Mycobacterial cell wall was obtained at 25,000 × g and the cell membrane at 105,000 × g. The purity of the subcellular fractions was assessed by electron microscopy and by measuring the activity of ATPase according to the method of Grover et al.[12]

Chemicals. Carrier-free ^{32}P-orthophosphate was purchased from Bhabha Atomic Research Centre, India. Silica gel H was obtained from Chemical Division, Glaxo Laboratories Ltd, India. All other reagents were of analytical grade.

Results and discussion. Electron microscopic examination of the cell wall and the membrane fractions of mycobacteria revealed them to be of the desired purity. Comparison of ATPase activity in the 2 fractions showed the membranes to have much higher activity (1.27 µmoles Pi liberated/mg protein/30 min) as compared to the cell walls (0.11 µmoles Pi liberated/mg protein/30 min). The mechanism of membrane lipid synthesis can be ascertained by measuring uptake of radioactive membrane precursors, since such precursors have been observed to be incorporated into functionally distinct components of the cell at differing rates. Thus, by measuring the incorporation of ^{32}P-orthophosphate, our observations (table 1) revealed a higher content of total phospholipids in the cell membrane fraction as compared to the wall in the early exponential phase. This is in agreement with the reports on several gram positive bacteria including *Mycobacterium phlei*[13] and atypical mycobacteria *Mycobacterium* P_6^{14}. As phospholipids are the primary constituents of the cytoplasmic membrane, it was thought that these lipids may play a role in the uptake of phosphate from the surrounding medium[15]. In agreement with earlier work carried out on whole cells of mycobacteria[16], corynebacteria[17], streptomyces[18] and nocardia[19], it was observed that the total phospholipid content in the subcellular fractions of *Mycobacterium smegmatis* 607 (table 1) increased with the age of the culture. By mid-exponential phase phospholipids increased in both the fractions, with the membrane showing a 10% higher content of phospholipids than the wall. There was a further increase in the content of phospholipids synthesized in both fractions by the early stationary phase, the membrane being richer in phospholipids than the wall by 7%.

An analysis of the total mannoside content (expressed as percent of total phospholipids) as shown in table 2, revealed a higher content of mannosides in the cell wall as compared to the cell membrane in early exponential phase. The percentage of mannosides in the cell wall of *Mycobacterium smegmatis* 607 maintained a steady level while in the cell membrane, it increased continuously during growth. In the early stationary phase, there was a 32% rise in the mannoside content in the cell membrane resulting in a higher content of mannosides in the membrane as compared to wall. These variations in the relative proportion of mannosides in cell wall and membrane with age cannot be explained at present. However, in *Streptomyces griseus*[9] and *Mycobacterium tuberculosis* $H_{37}Rv$[6] in late exponential phase, they have been shown to be mainly present in the cell wall.

Among individual mannosides (table 3) tri- and tetraacylated dimannosides contributed towards the bulk of mannosides (approximately 80–90%) in the cell wall as well as membrane fractions. These mannosides were distributed equally in both fractions and did not vary with age of the culture. Since the enzyme responsible for synthesis of mannosides is present not only in the cell membrane but also in the wall[7], an asymmetric distribution of mannosides, especially dimannosides, can be explained. Hexamannoside content increased with growth in the membrane fraction and at the end of the stationary phase, they were 30% higher in the membrane than in the wall. Diacylated dimannoside and triacylated trimannoside levels decreased with age of the culture in the wall fraction with a concomitant increase in membranes.

Homogeneous layers are an exception in bacterial organization, as there are intergradations and intrusions from one layer into another[20]. Thus, if mannosides are extended continuously from their points of origin in the wall to the membrane, it is reasonable to expect higher homologues, viz. hexamannosides, in the membrane. Analogous to these

Table 1. Distribution of total phospholipids in cell wall and cell membrane fractions of *Mycobacterium smegmatis* ATCC 607 at different growth phases

Age	Cell wall (cpm/µg lipid P)*	Cell membrane (cpm/µg lipid P)*
Early exponential	1673	2206
Mid-exponential	3151	3480
Early stationary	3527	3809

*Results are the average of 2 different batches.

Table 2. Distribution of total mannophosphoinositides in cell wall and cell membrane fractions of *Mycobacterium smegmatis* ATCC 607 at different growth phases

Age	Cell wall*	Cell membrane*
Early exponential	48.2	39.7
Mid-exponential	51.4	51.5
Early stationary	51.2	58.4

*Results are the average of 2 different batches. Values are expressed as percentage of cpm incorporated in mannosides.

Table 3. Distribution of individual mannophosphoinositide components in cell wall and cell membrane fractions of *Mycobacterium smegmatis* ATCC 607 at different growth phases

Age	Fraction	PIM_2-3F	PIM_2-4F	PIM_2-2F + PIM_3-3F	PIM_6-3F + PIM_6-4F
Early exponential	Wall	62.1	20.2	12.5	5.2
	Membrane	65.2	24.4	6.2	4.2
Mid-exponential	Wall	57.5	29.3	7.1	6.1
	Membrane	61.2	26.0	5.2	7.6
Early stationary	Wall	62.6	25.8	6.0	5.5
	Membrane	53.9	29.4	8.9	7.9

Results are the average of 2 different batches. Values are expressed as percent of total mannosides. Abbreviations: PIM_2-3F, triacylated dimannoside; PIM_2-4F, tetraacylated dimannoside; PIM_2-2F, diacylated dimannoside; Pim_3-3F, triacylated trimannoside; PIM_6-3F, triacylated hexamannoside; PIM_6-4F, tetraacylated hexamannoside.

observations is the 'dynamic membrane flow hypothesis'[21,22] which suggests the interconversion of phospholipids between different cellular membrane systems and organelles. This can be further emphasized by the postulated role of phospholipids as carriers of cations and other substances across the cell membrane[23].

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The effect of diet on esterase band pattern in *Myzus persicae* (Sulzer) – a disclaimer

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Summary. Aphids cultured on artificial diet did not exhibit extra esterase band patterns, nor did their patterns differ from those in aphids cultured on plants.

Bunting and van Emden[2] reported that polyacrylamide gel electrophoretograms of aphids from the insecticide-susceptible Y5 strain of *Myzus persicae* cultured for over 2 years on Dadd and Mittler's[3] artificial diet exhibited a number of hitherto unreported esterase band patterns; and these bands were not present in gels made from aphids taken from this clone and reared for several generations on potato plants. While discussing a number of possible explanations for this phenomenon and concluding that the final answer must await further work, they stated that their work '... demonstrates beyond doubt that diet can have a profound effect on esterase band pattern'.[2] (My emphasis).

Materials and methods. As a prelude to investigating further this reported influence of diet on changes in band patterns of esterases of *M. persicae,* I set out to provide the conventional repetition of original results by running several preliminary gels of bulk homogenates of aphids taken from this same Y5 clone. I used the same materials and methods as those used by Bunting and van Emden[2], conducted the work in the same laboratory, using the same equipment, and, as a control, ran, on the same gels, homogenates of aphids from brussel sprout plants growing in the Department greenhouse. The only known difference was that the Y5 clone had now been maintained on the artificial diet for more than 5 years without interruption.

Results. These first runs produced gels which showed no difference in esterase band patterns between the aphids grown on the artificial diet and those from the brussel sprout plants. And neither population produced gels showing any sign of the extra esterase bands reported by Bunting and van Emden[2]. I then ran further gels contrasting alate and apterous adults, nymphs and adults, populations from different glasshouses and different host plants; and using concentrations of homogenate from 60 µg aphid/µl to 120 µg aphid/µl. I varied the voltage and times for running the gels and the times for staining the gels.

I also ran gels of aphids taken from every sachet of artificial diet on which the Y5 clone was being maintained, from Y5 individuals parasitized in the laboratory by *Aphidius matricariae,* from Y5 populations transferred to plants and back to artificial diet, from sachets of Y5 populations kept for 4 weeks in a refrigerator at approximately 5 °C, and using homogenates frozen for up to 72 h prior to use.

None of these gels produced any sign of the extra esterase bands. I then contacted Bunting and discussed his techniques in some detail. As a result I made several minor adjustments to the method of preparing solutions and of preparing and running the gels. But further runs still failed to produce gels showing any sign of extra bands.

Conclusions and discussions. It can be assumed with a fair degree of confidence that the present Y5 clone of *M. persicae* no longer includes aphids which produce polyacrylamide gels showing extra esterase bands, or patterns of bands which differ in any significant way from those of aphids feeding on plants.

A number of explanations could be proposed to account for this failure to repeat Bunting and van Emden's[2] findings. But Bunting (personal communication) states that gels illustrating these bands in the original Y5 population were never made prior to establishing the sub-population from that clone onto potato plants. Nor were the 2 sub-populations from which his definitive gels were finally made