Composition of the Phospholipid Fraction of *Corynebacterium diphtheriae*

By PATRICK J. BRENNAN and DEREK P. LEHANE. (*Department of Biochemistry, Trinity College, Dublin, Irish Republic*)

Corynebacterium diphtheriae gravis previously washed with acetone was extracted with chloroform–methanol–water. Lipids were dried, washed, converted into the sodium form and freeze-dried (Brennan, 1968). This phospholipid fraction was then subjected to the following treatments.

Hydrolysis and chromatography in several solvent systems showed that the lipids were composed of inositol, mannose, glucose, glycerol and five amino acids. Ethanolamine and serine were absent from the ninhydrin-positive products. Deacylation of the lipid fraction and chromatography in two solvents showed the presence of glucose, trehalose, glycerophosphorylinositol, glycerophosphorylinositol dimannoside and a major unidentified product.

About 1·2g. of the phospholipid fraction was applied to a column of DEAE-cellulose and eluted first with chloroform–methanol–water. This removed acylglucoses (Brennan & Lehane, 1969) and acyltrehaloses (Senn, Ioneda, Pudles & Lederer, 1967). Further elution of the column with ammonium acetate removed the partially purified phospholipids. These were subjected to preparative t.l.c. for complete purification.

Phosphatidylinositol was readily identified as one of the major phospholipids. Most of the lipids that yielded glycerophosphorylinositol dimannoside on deacylation were found to be identical with the triacylated dimannophosphoinositide B from mycobacteria (Brennan & Ballou, 1967) with smaller amounts corresponding to the diacylated dimannophosphoinositide C. None of the tetra-acylated dimannophosphoinositide A was evident. A major phospholipid in these fractions has not been fully identified. It contains glycerol and an amino acid and no monosaccharide. Surprisingly, no phosphatidylethanolamine or the higher oligomannophosphoinositides prominent in mycobacteria have been found in *C. diphtheriae*.

The phospholipids of the corynebacteria have been the subject of a number of studies. Chargaff (1931) recognized some of the similarities between the phospholipids of *C. diphtheriae* and those of the mycobacteria. Asselineau (1961) tentatively identified phosphatidylinositol dimannoside in *C. diphtheriae*. However, in *Corynebacterium ovis* the mannophosphoinositides are apparently replaced by arabinophosphoinositides (Lacave, Asselineau & Toubiana, 1967). Brennan (1968) isolated and characterized a phosphoinositide and a dimannophosphoinositide from *Corynebacterium xerosis*. The present work shows that some members of the family of dimannophosphoinositides found in mycobacteria are also present in *C. diphtheriae* and describes other components of the phospholipid fraction.

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The Oxidation of Acetate, Ethanol and Pyruvate by Baker's Yeast

By J. P. GOSLING and P. F. DUGGAN. (*Department of Biochemistry, University College, Dublin, Irish Republic*)

Commercial baker's yeast oxidizes acetate linearly after a short lag period. The duration of this lag can be extended by starvation of the yeast and by the addition of 1mM-ammonium chloride (Gosling & Duggan, 1968).

Cycloheximide (1μg./ml.) completely inhibits adaptation to acetate oxidation such that the initial low rate of oxidation does not increase. Thus cytoplasmic protein synthesis is required for adaptation (Siegel & Sisler, 1965). Chloramphenicol, which inhibits yeast mitochondrial protein synthesis (Roodyn & Wilkie, 1968), has much less effect (at 4mg./ml.) and causes a 5–10% decrease in the final rate.

The activities of a number of enzymes involved in acetate metabolism were measured during adaptation. These were citrate synthase, aconitate hydratase, isocitrate lyase, malate synthase, fumarate hydratase, malate dehydrogenase and fructose diphosphatase. All activities increased at pH4·4 in starved yeast on incubation with 10mM-acetate. At pH7·5 and 20mM-acetate similar though smaller increases were found. Most of these increases occurred at the same time as the increase in oxidation rate.

In contrast, starved baker's yeast oxidizes 10mM-pyruvate at pH4·4 without an adaptation period, though the activities of the above enzymes also increase under these conditions. However, these activities level off or even decrease after 2hr. The rate of pyruvate oxidation is about one-third of the maximal rate of acetate oxidation.