agent was injected alone. The block in glycogenolysis at birth may be partly due to a relative corticosteroid insufficiency.

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Increased Phospholipid Depletion Alters the Apparent Specificity of Phospholipid-Dependence of Glucose 6-Phosphatase Activity

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A biphasic inhibition of glucose 6-phosphatase (glucose 6-phosphohydrolase) occurs when rat liver microsomal fraction is treated with *Clostridium welchii* and/or *Bacillus cereus* phospholipase C. This results in partial (50–75%) loss of activity at 5°C. Subsequent post-incubation at 20° or 37°C in the absence of substrate causes complete and irreversible loss of the remaining activity without further detectable phospholipid hydrolysis (Cater *et al.*, 1970). With *Cl. welchii* phospholipase C the glucose 6-phosphatase activity lost at 5°C is completely restored by the addition of sonicated phosphatidylcholine (lecithin) or a 1:1 mixture of phosphatidylethanolamine+phosphatidylserine. Further, if bovine serum albumin is present throughout the phospholipase treatment, extensive protection against the loss of glucose 6-phosphatase activity at 5°C is apparent. This latter observation raised doubt as to the specific and obligatory dependence of glucose 6-phosphatase activity on phospholipids.

Marked differences occur if phospholipid depletion is carried out by using both the phospholipase C preparations together. The loss of activity at 5°C is only 0–15% greater than that observed when treatment was with *Cl. welchii* phospholipase C alone, but the glucose 6-phosphatase activity is now no longer restored by the addition of sonicated phosphatidylcholine, nor is any protection conferred at 5°C by bovine serum albumin. However, 80–100% of the glucose 6-phosphatase activity is still restored by the addition of a sonicated mixture of phosphatidylethanolamine+phosphatidylserine (1:1). Therefore

it seems that the greater and qualitatively different phospholipid removal due to the wider substrate specificity of *B. cereus* phospholipase C exerts a critical effect on the apparent phospholipid specificity for glucose 6-phosphatase reactivation. Although both phospholipases hydrolyse phosphatidylcholine completely, the *B. cereus* enzyme, unlike that from *Cl. welchii*, also hydrolyses virtually all the phosphatidylethanolamine and phosphatidylserine and partially hydrolyses phosphatidylinositides, but does not attack sphingomyelin.

We interpret these results as indicating that phosphatidylcholine alone is not able to restore glucose 6-phosphatase activity, but that the acidic phospholipids remaining after the action of *Cl. welchii* phospholipase C in some way allow it to restore glucose 6-phosphatase activity. The lack of protection by bovine serum albumin in the system extensively depleted of phospholipid would also appear to indicate a specific and obligatory phospholipid requirement for glucose 6-phosphatase activity. It would thus appear that almost complete phospholipid depletion of an enzyme system may be required before unequivocal conclusions about the specificity of its phospholipid-dependence can be drawn.

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Phosphoenzyme Formation Involving Serine with Phosphoglucomutase from *Micrococcus lysodeikticus*

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Hanabusa *et al.* (1966) isolated a phosphoglucomutase from *Micrococcus lysodeikticus* that required glucose 1,6-diphosphate as a cofactor, but that could not be obtained in a phosphorylated form, and for which the initial-velocity patterns suggested that glucose 1,6-diphosphate and the substrates had to be present simultaneously on the enzyme molecule. Thus its mechanism appeared to be different from the phosphoenzyme mechanism of the rabbit muscle phosphoglucomutase (Ray & Roscelli, 1964; Britton & Clarke, 1968), and the active form of the enzyme appeared to be an enzyme–glucose 1,6-diphosphate complex. However, despite these findings, recent induced-transport studies have shown that it has a phosphoenzyme mechanism (Britton & Clarke, 1972). Nevertheless it was still possible that the active form of the mutase could be an enzyme–glucose

1,6-diphosphate complex performing the role of a phosphoenzyme. Attempts have therefore been made to isolate the active form of the mutase.

[^{14}C]Glucose 1,6-di[^{32}P]phosphate was incubated with the enzyme at pH 8.3, and the enzyme was then separated from the substrates by gel filtration (on Sephadex G-25). ^{32}P (approx. 1 mol/mol of enzyme) but no ^{14}C was associated with the enzyme, and more than 90% of the ^{32}P could be exchanged with the substrates. On incubation at 30°C (at pH 8.3) the amount of exchangeable ^{32}P fell, indicating a half-life for the phosphoenzyme of approx. 9h. HClO_4 precipitated all of the ^{32}P with the protein, and no $^{32}\text{P}_i$ was released. Electrophoresis in sodium dodecyl sulphate showed that ^{32}P was associated with a protein of molecular weight 70000. After acid hydrolysis and Dowex chromatography on a Technicon Auto-Analyzer the radioactivity was eluted with phosphoserine. Phosphate migration may occur with acid hydrolysis, but the half-life of the phosphoprotein in alkali (0.25M-NaOH at 37°C) was similar to that of phosphoserine (Kennedy & Koshland, 1957).

It is concluded that the phosphoglucumutase from *M. lysodeikticus* reacts with glucose 1,6-diphosphate to give a phosphoenzyme with the phosphate bound to serine, and that, in contrast with the conclusions of Hanabusa *et al.* (1966), the mechanism appears to be essentially that of the rabbit muscle enzyme.

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Lipoamide Dehydrogenase Mutants of *Escherichia coli* K12

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The pyruvate dehydrogenase and α -oxoglutarate dehydrogenase complexes of *Escherichia coli* contain specific dehydrogenase (E1) and transacylase (E2) components, but the lipoamide dehydrogenase (E3) components are functionally interchangeable and immunologically and electrophoretically indistinguishable (Pettit & Reed, 1967). Studies with mutants requiring acetate or succinate for aerobic growth on glucose minimal medium have established the existence of two pairs of closely linked structural

genes, *aceE* and *F* (near *leu*) for the E1 and E2 components of the pyruvate dehydrogenase complex and *sucA* and *B* (near *gal*) for the corresponding components of the α -oxoglutarate dehydrogenase complex (Henning *et al.*, 1966; Herbert & Guest, 1969).

Mutants requiring both acetate and succinate and lacking activity for both complexes have been obtained. Some are blocked in lipoic acid biosynthesis (Herbert & Guest, 1968), but others, which do not respond to lipoic acid, have now been isolated and designated lipoamide dehydrogenase mutants (*lpd*⁻). Ultrasonic extracts of 10 such mutants had between 1 and 10% of the lipoamide dehydrogenase activity of parental strains. They also lacked the overall activities for both dehydrogenase complexes, but these could be restored by adding either purified lipoamide dehydrogenase or extract of an *ace*⁻, *suc*⁻ double-*amber* mutant (which lacks both types of E1 and E2 component but has 30% of parental lipoamide dehydrogenase activity).

Studies with one mutant showed that spontaneous revertants selected for either acetate- or succinate-independence were independent of both supplements and regained activity for both dehydrogenase complexes as well as lipoamide dehydrogenase. These three activities could also be restored by P1-transduction with a wild-type donor strain and selection on unsupplemented glucose or acetate minimal medium. Further, the mutant phenotype could be introduced into suitable recipients by co-transduction of *lpd*⁻ with linked donor markers.

Genetic studies with the same mutant showed that *lpd* is linked to *aceE* (93%), *aceF* (97%), *nadC* (56%), *leu* (8–41%), *pan* (21%) and *tonA* (17%) but not to *thr*, *nadA* or *gal* (<1% co-transduction with each); the following order was indicated: *thr-leu-nadC-aceE-aceF-lpd-pan-tonA*.

These results indicate that there is a lipoate dehydrogenase gene in or near the *ace* region and, unless the mutants prove to be complex, they suggest that a single lipoamide dehydrogenase gene specifies the E3 components of both dehydrogenase complexes.

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