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 $^{32}P_i$ 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-Analyser 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 phosphoglucomutase 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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