of the enzyme with phosphate at acid pH can be simplified, if glutamate is also added, by gel-filtering the enzyme through the reaction mixture.

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Regeneration of Aspartate Aminotransferase from its Apoenzyme

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Churchich & Farrelly (1968) found that the apoenzyme of pig heart aspartate aminotransferase (EC 2.6.1.1) recovered activity only slowly on incubation with 1 mM-pyridoxal 5-phosphate (PLP) in 0.1 M-phosphate buffer, pH 6.8, at 30°, although the aldimine bond with a specific lysine residue was rapidly formed. We found, however, that reactivation of the apoenzyme was almost complete in 10 min. at 20° in 2.0 ml. of 40 μ M-PLP-100 mM-tris-62.5 mM-HCl, which was made into the assay solution by addition of 0.5 ml. of 50 mM-aspartic acid-20 mM- α -oxoglutaric acid-0.1 M-tris. Comparison of these conditions, together with the known facilitation of the dissociation of pyridoxamine 5-phosphate (PMP) in acid solution by phosphate (Scardi, Scotto, Iaccarino & Scarano, 1963), suggested that phosphate might slow the recombination of PLP, and so this was investigated.

The apoenzyme was treated at 20° with PLP or PMP (10 μ M) in either 50 mM-tris-37.5 mM-HCl-100 mM - Na₂HPO₄ or 50 mM - tris - 25 mM - HCl - 287.5 mM-NaCl. These two solutions have the same pH and ionic strength. Samples were taken at various times and introduced into the assay mixture. Phosphate greatly slowed the rate of recovery of activity.

When phosphate accelerates the dissociation of PMP in acid solution (Scardi *et al.* 1963), it does not act merely by inhibiting recombination, since the rate of dissociation increases with phosphate concentration even under conditions in which the position of equilibrium overwhelmingly favours dissociation. It is therefore likely that there is an intermediate stage in which the phosphate ion has displaced the phosphate group of PMP, but in which the rest of the molecule is still bound. Com-

parison of the present results with those of Churchich & Farrelly (1968) points to the formation of a somewhat analogous intermediate during the reassociation of PLP. The aldimine bond is formed in this intermediate, but a phosphate ion prevents the prosthetic group from adopting the position in which it can function.

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Adaptation of Baker's Yeast to the Oxidation of Acetate

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After a short lag baker's yeast oxidizes acetate linearly (Weinhouse & Millington, 1947; Stoppani, Conches, de Flavelukes & Sacerdote, 1958; Duggan, 1964). The final rate attained is pH-dependent, being greater at lower pH values. However, when the concentration is sufficiently high the readily diffusible undissociated acetic acid interferes with its own oxidation (Duggan, 1964).

The lag in O₂-uptake rate decreases and disappears on storage at 4°. The lag may be extended by starvation of the yeast and this extension is proportional to oxygenation time whether the yeast is shaken in air or bubbled with O₂.

When 1 mM-ammonium chloride is added with 9 mM-acetate (final concentrations), it delays further the attainment of a linear O₂-uptake rate by starved yeast. However, if acetate or acetate and ammonium are added to yeast that is already oxidizing acetate linearly, there is no divergence in the subsequent O₂-uptake rates. Ammonium therefore does not affect the oxidation of acetate by adapted yeast. Ammonium has a stimulatory effect on the oxidation of glucose, an effect also found by Holzer & Witt (1964).

O₂-uptake rates and the specific activities of isocitrate lyase and malate dehydrogenase were measured in yeast oxidizing acetate. Starved and unstarved yeast was used at pH values 7.5 and 4.3. Glucose 6-phosphate dehydrogenase activity was also measured, as this enzyme is neither induced nor repressed in *Saccharomyces cerevisiae* under these conditions (Witt, Kronau & Holzer, 1966). In all cases there was close correlation between increases in O₂-uptake rate and increases in the specific activities of isocitrate lyase and malate dehydrogenase.

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