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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The increase in isocitrate lyase activity also closely corresponded with the increase in O_2 -uptake rate where this had been delayed by the presence of ammonium ion. Oxidation rate therefore is closely related to the increase in isocitrate lyase activity in unstarved, starved and ammonium-inhibited yeast.

From these results it appears that the glyoxylate cycle may be induced at the same time as the increase in O_2 -uptake rate in baker's yeast adapting to acetate oxidation. Starvation depletes the endogenous stores of the yeast and so delays enzyme induction. In the presence of ammonium ion adaptation is delayed, possibly because of a stimulation of glycolytic activity with resulting elevated levels of phosphoenolpyruvate, a repressor of isocitrate lyase (Kornberg, 1965).

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Ultramicrochemical Studies on the Site of Formation of Dehydroepiandrosterone Sulphate in the Adrenal Cortex

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Biochemical studies have shown that cortisol and 11β -hydroxyandrostenedione are synthesized by the fascicular and reticular zones of the human adrenal cortex (Griffiths, Grant & Symington, 1963). Little is known, however, about the site of formation of dehydroepiandrosterone sulphate (DHAS). The work now reported shows that the reticular zone of the cortex is mainly responsible for the sulphation of dehydroepiandrosterone (DHA) both in the guinea pig and in the human.

Fresh adrenal tissue was frozen in solid carbon dioxide, transferred to a cryostat where cylinders of tissue were bored out and fresh frozen sections (2.5 mm. diameter, 16μ thick) prepared (Grunbaum, Geary & Glick, 1956). One section was placed on a microscope slide for histological studies and the next three were placed in a reaction tube for enzyme assay. This sequence was followed through the entire cortex into the medulla. The tissue in the tubes was incubated with $0.1\mu C$ of $[^3H]DHA$ ($48m\mu g.$) in $25\mu l.$ of a medium containing phosphate buffer, pH 7.0, ATP, $MgSO_4$ and K_2SO_4 .

After incubation, $100\mu l.$ of ethyl acetate containing $100\mu g.$ of DHAS was added, followed by $50\mu l.$ of 25% (w/v) NaCl. After mixing, the organic phase was removed and the aqueous phase was extracted twice more with ethyl acetate ($100\mu l.$). The residues from each extraction were chromatographed on silica gel in the solvent system 2-methylpropan-2-ol-ethyl acetate-aq. 5N- NH_4OH (41:50:20, by vol.) to obtain the sulphate fractions. Solvolysis of the sulphates yielded free extracts of steroid; these were acetylated and their specific radioactivities measured. Specific radioactivity measurements were also carried out on the free steroid obtained by hydrolysis of the acetates. The percentage conversion of DHA into DHAS in each incubation was then calculated from the mean value of the specific radioactivities.

The DHA-sulphating activity was based on the protein-nitrogen content of the tissue remaining in the reaction tube, which was determined by the bromsulphalein method of Nayyer & Glick (1954).

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The Absorption of Vitamin E in Man

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Low plasma vitamin E concentrations have been reported in patients with steatorrhoea (Losowsky & Leonard, 1967) and this has been confirmed in the present study. The cause of this deficiency is presumed to be diminished absorption of the vitamin, but little is known about the absorption in man. In the few reported studies, large unphysiological doses have been used and absorption indirectly assessed from plasma concentrations, which depend also on other factors. Rosenkrantz, Milhorat & Farber (1953) and Klatskin & Molander (1952) measured faecal quantities after large doses, but the methods used were non-specific.

In the present study fasting subjects were given oral doses of DL- α -[5-methyl- 3H]tocopherol in oily solution containing approximately 0.2 mg. of total α -tocopherol. Radioactivity in urine, faeces, plasma and erythrocytes was followed for the next 14 days. A total of 39 subjects were studied, 20 without steatorrhoea (group A) and 19 with steatorrhoea (group B). Three deficient subjects in group B were also studied after repletion with