hydrate content and the ammonia released into the supernatant during starvation of cells grown under these conditions, has been studied. In agreement with Dawes & Ribbons (1965) batch cells grown on glucose contain appreciable quantities of reserve carbohydrate which are completely utilized during the first 2hr. of starvation, the release of NH_3 into the supernatant being suppressed during this time. Cells grown batchwise on acetate do not contain reserve carbohydrate and ammonia is immediately released into the supernatant.

Cells grown in the chemostat on glucose contain only very small amounts of reserve carbohydrate and consequently NH_3 is released immediately they are starved. Comparisons will be made with cells grown in the chemostat on acetate.

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Nicotinamide–Adenine Dinucleotide-Specific Isocitrate Dehydrogenase from Pea Mitochondria

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NAD-Specific isocitrate dehydrogenase has been extracted from etiolated pea shoot mitochondria and purified 50-fold. (Cox & Davies, 1967). The enzyme is exceedingly unstable but considerable stabilization can be achieved by including 5M-glycerol in all solutions used.

Both the crude and the partially purified enzyme give a sigmoid plot of initial rate against substrate concentration, and both preparations are activated by low concentrations of citrate. In the presence of 1mM-citrate in the assay system a hyperbolic plot of initial rate against substrate concentration is obtained, although at high isocitrate concentrations the citrate behaves as a competitive inhibitor.

The enzyme is inhibited by monovalent anions and their presence in the assay system results in an increase in the sigmoid nature of the plot of rate against substrate concentration. For this reason tris-chloride has also been found to inhibit and, therefore, all assays have been carried out in *N*-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (Hepes) (Good, *et al.* 1966) adjusted to pH 7·6 with sodium hydroxide.

NAD-specific isocitrate dehydrogenase is not activated by AMP as in yeast (Hathaway & Atkinson, 1963) and *Neurospora crassa* (Sanwal, Zink & Stachow, 1964) or ADP as in heart (Chen & Plaut, 1963). However, control of the enzyme may be exerted through the ratio of reduced and oxidized coenzyme. Using a crude extract which has been passed through a column of Biogel P-10 to remove small molecules it can be shown that although the response of initial rate to change in NAD^+ concentration is hyperbolic, in the presence of 0·073mM-NADH the plot becomes sigmoid. The NADH also acts as a competitive inhibitor. The partially purified enzyme does not respond in this way to NADH but the NADH still acts as a competitive inhibitor. This suggests that the NADH acts at two sites, one the active site and the other an allosteric site, the latter being sensitive to purification.

Density gradient centrifugation has been carried out on glycerol gradients using the crude enzyme. A comparison of the enzyme on a gradient containing 0·1M-citrate throughout with one containing no citrate has shown that citrate considerably reduces the distance of movement from the origin.

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The Allosteric Nature of 3-Phosphoglycerate Dehydrogenase

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3-Phosphoglycerate dehydrogenase is the first enzyme unique to the 'phosphorylated pathway' for the formation of serine from 3-phosphoglycerate. Pizer (1963) has reported that this enzyme from *Escherichia coli* is inhibited by serine whereas Walsh & Sallach (1965) using enzyme from chicken liver and Bridgers (1965) using mouse brain extracts, found no effect of serine on the enzyme. 3-Phosphoglycerate dehydrogenase is present in crude extracts of etiolated pea epicotyls and is specifically inhibited by low concentrations of L-serine. Many other related substances were without effect at 1·0mM-concentration.

The allosteric effect has been found to be unstable under all the conditions investigated. Stability is increased in the presence of glycerol or L-serine or by carrying out extraction at room temperature. Allosteric activity can be regenerated in crude extracts prepared in potassium phosphate buffer which have been aged for 90min. at 2°, by

either making the extract 2·5M to glycerol or by raising the temperature to 25°.

Michaelis-Menten plots in the presence and absence of L-serine using fresh crude extract as enzyme are all hyperbolic and indicate that L-serine acts as a non-competitive inhibitor. Under certain circumstances velocity/serine concentration curves of sigmoid form can be obtained which depend on the concentration of substrate in agreement with the allosteric V system of Monod, Wyman & Changeux (1965).

Sigmoid inhibition curves develop with time and have not been shown with fresh extracts. A simple model of step-wise denaturation of the enzyme on extraction can account for the development of sigmoid form with age. The enzyme is assumed to pass through two intermediate states before losing activity completely. Only the native form can be inhibited by L-serine and the sigmoid inhibition curve results when sufficient of the first denaturation product has accumulated to form an artificial allosteric V system.

The effects of glycerol, L-serine and temperature on the stability of the catalytic site of 3-phosphoglycerate dehydrogenase are consistent with the proposed mechanism of denaturation.

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Glyoxylate as a Substrate for Pyruvic Decarboxylase

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Purified preparations of wheat germ pyruvic decarboxylase decarboxylate pyruvate and glyoxylate. Glyoxylate inhibits pyruvate decarboxylation, whereas pyruvate and acetaldehyde stimulate glyoxylate decarboxylation. The product formed from glyoxylate and pyruvate has been tentatively identified as lactaldehyde and the same product is formed from glyoxylate and acetaldehyde (Corbett & Davies, unpublished results). Glycolaldehyde also stimulates glyoxylate decarboxylation and the reaction product has been identified as glyceraldehyde.

The kinetics of pyruvate and glyoxylate decarboxylation have been studied at low substrate concentrations and the plot $V/[S]$ shown to be sigmoid in both cases. Hill plots of the data give $N = 2$. The physical meaning of N is in this case taken to mean that the decarboxylation of pyruvate and glyoxylate follows second order kinetics, without implicating allosteric properties.

The purified preparations also catalyse the synergistic decarboxylation of 2-oxoglutarate and glyoxylate. The products of this reaction have been isolated as 2,4-dinitrophenylhydrazones but not yet identified. The enzymic decarboxylation of 2-oxoglutarate is also stimulated by formaldehyde and acetaldehyde.

Preparations of pyruvic oxidase obtained from pea seedling mitochondria catalyse similar reactions though the pH optimum of the reaction is higher.

The mechanism of these decarboxylations will be discussed and compared with the synergistic decarboxylation of glyoxylate and 2-oxoglutarate observed in animal mitochondria (Koch & Stokstad 1966, Stewart & Quayle, 1967).

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Inhibition of Oxoglutarate Dehydrogenase by Glyoxylate and its Condensation Compounds

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In order to investigate the mechanism of inhibition produced by oxalomalate on oxoglutarate dehydrogenase (Ruffo, Testa, Adinolfi, Pelizza & Moratti, 1967), we used unpurified samples of oxalomalate, prepared by enzymic condensation of glyoxylate plus oxaloacetate, for obtaining the optically active isomer. Since the samples were contaminated by glyoxylate, we observed that also glyoxylate alone inhibited the activity of oxoglutarate dehydrogenase. The present communication deals with the effects produced by glyoxylate, hydroxyoxoglutarate and oxalomalate on this enzyme.

Oxoglutarate dehydrogenase (EC 1.2.4.2) purified according to Sanadi, Littlefield & Bock (1952) was preincubated in the presence or in the absence of oxalomalate, hydroxyoxoglutarate and respectively glyoxylate, and the activity determined as reported by Ruffo *et al.* (1967). In further experiments the preincubation was shortened to 10min. and the enzyme concentration increased 25-fold. In these mixtures glyoxylate was added either alone, or together with oxoglutarate, oxaloacetate, pyruvate and respectively thiamine pyrophosphate (TPP). Portions of either 0·02ml. were taken for determining the activity as previously reported (Ruffo *et al.* 1967), or 0·1ml. were added to 2·9ml. of the same