incubation mixture, except that 0·2ml. of 0·01% dichlorophenolindophenol (DCPI) substituted for NAD^+ and CoA. The activity was also determined either at increasing substrate concentration, or in the presence of oxaloacetate and pyruvate.

The main results showed that 0·16mM-glyoxylate inhibited oxoglutarate dehydrogenase approximately 4 times more than oxalomalate and hydroxy-oxoglutarate. The inhibition by glyoxylate was still evident at low concentrations (0·05mM) whereas oxalomalate and hydroxyoxoglutarate were without effect. The inhibition produced by 0·2mM-glyoxylate was removed by equimolar concentrations of either the substrate or the oxaloacetate, only when these substances were added together with glyoxylate, but not when the addition occurred 10min. later. Pyruvate and TPP also removed the inhibition, but, respectively, at concentrations 5- and 300-fold that of glyoxylate. All these results suggested that glyoxylate inhibited by binding to the TPP-site of the enzyme. This possibility is under investigation with labelled glyoxylate.

Ruffo, A., Testa, E., Adinolfi, A., Pelizza, G. & Moratti, R. (1967). *Biochem. J.* **103**, 19.
Sanadi, D. R., Littlefield, J. W. & Bock, R. M. (1952). *J. biol. Chem.* **197**, 851.

Evidence for the Existence of a Pyruvate Permease in Rat-Heart Muscle

By D. J. WATTS and P. J. RANDLE. (*Department of Biochemistry, University of Bristol*)

Lactate production and pyruvate uptake by rat hearts perfused with concentrations of pyruvate from 0·5 to 10mM are maximal at 2mM-pyruvate. The $V_{max.}$ of lactate production (0·6 μmoles/min./g.) is lower than the $V_{max.}$ for lactate dehydrogenase (greater than 100units/g.). In hearts perfused with 5 or 10mM-pyruvate the distribution volume is no greater than that of extracellular water (pyruvate space 47ml./100g.; sorbitol space 58ml./100g.). At lower pyruvate concentrations (0·5mM) the distribution volume may be lower than at 5mM.

Pyruvate uptake by rat heart muscle was reduced by perfusion of the normal heart with short or long chain fatty acids, ketone bodies, by alloxan-diabetes (Garland, Newsholme & Randle, 1964), by anoxia or by inhibition of cardiac contraction with media containing 33mM-K^+. Lactate production is not diminished by these factors. The diminution in pyruvate uptake is not associated with any change in the distribution of pyruvate with 5mM-pyruvate which remains extracellular.

In hearts perfused with 5·5mM-glucose and insulin (0·05units/ml.) the concentration ratio of intracellular pyruvate/extracellular pyruvate was 0·74:1. The ratio was the same in hearts in which ATP was depleted by 2:4-dinitrophenol. If it is assumed that intramitochondrial pyruvate concentration is near zero the intracellular/extracellular ratio approximates to unity.

These observations suggest that the entry and exit of pyruvate in cardiac muscle involves a carrier system which is not energy linked. Attempts to make kinetic measurements were frustrated by the incomplete extracellular distribution at low pyruvate concentrations.

Garland, P. B., Newsholme, E. A. & Randle, P. J. (1964). *Biochem. J.* **93**, 665.

The Rates of Penetration of Oxaloacetate and L-Malate into Mitochondria

By J. M. HASLAM and H. A. KREBS. (*Department of Biochemistry, University of Oxford*)

L-Malate has been considered as a carrier for reducing equivalents between mitochondria and cytoplasm (see Krebs, 1967; Krebs, Gascoyne & Notton, 1967). A flow of reducing equivalents mediated by the L-malate–oxaloacetate system requires that the mitochondrial membrane be readily permeable to both L-malate and oxaloacetate. Lardy, Paetkau & Walter (1965) have presented evidence that the rate of diffusion of oxaloacetate out of mitochondria is slow and requires special transport mechanisms, but no direct measurements of the rates of penetration of oxaloacetate and L-malate into mitochondria have been reported.

We have investigated the penetration of oxaloacetate and L-malate into isolated, washed mitochondria from rat liver, kidney and heart by measuring the effect of oxaloacetate or L-malate on the concentration of intra-mitochondrial NADH using the Aminco-Chance dual-wavelength spectrophotometer at (340–374)mμ. The reaction mixture in these studies contained 0·25M-sucrose; 20mM-tris-HCl, pH 7·6 or 20mM-tricine-KOH, pH 7·6, and 1–3mg. of mitochondrial protein. As the activity of the mitochondrial malate dehydrogenase under the test conditions was very high in relation to the rates of penetration of oxaloacetate and L-malate, the initial rate of oxidation of NADH was a measure of the rate of penetration of oxaloacetate, and the initial rate of reduction of NAD^+ was a measure of the penetration of L-malate.

In experiments with oxaloacetate, a high initial concentration of NADH was generated from endogenous substrate by inhibiting NADH oxidase with rotenone plus antimycin A. The continual