possible that these different forms of carbonic anhydrase are artifacts of the preparation procedure, extracts from more than 12 plants in diverse Families all showed multiple forms of carbonic anhydrase. In a number of dicotyledons the size of both the major isoenzymes is close to that of the enzyme from parsley, which has a molecular weight of 180 000 (Tobin, 1970). The isoenzymes from the monocotyledons examined had molecular weights of less than 40 000. Like erythrocyte and parsley carbonic anhydrases, the enzymes from *T. albiflora* contained zinc.

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Histochemical Localization of Enzymes in Heart Muscle

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Histochemical localization by formazan deposition, in an agarose gel-film medium, by using phenazine methosulphate to reduce interference from tetrazolium reductases, has been used for extensive studies on the localization of mitochondrial and cytoplasmic enzymes in rat liver (Pette & Brandau, 1966), rabbit skeletal muscle (Sigel & Pette, 1969) and insect flight muscle (Brandau & Pette, 1966). This variant of the tetrazolium localization method has not been used to study the distribution of soluble enzymes in heart muscle.

We now report the results of studies on the distribution of succinate dehydrogenase (EC 1.3.99.1), glutamate dehydrogenase (EC 1.4.1.2), glutamate-oxaloacetate transaminase (EC 2.6.1.1) and lactate dehydrogenase (EC 1.1.1.27) in rat heart muscle. The muscle fibres were caused to relax by soaking the organ in iso-osmotic potassium chloride solution before freezing and sectioning. This pretreatment made it possible to detect the striations of the myofibrils, normally invisible in freshly excised heart tissue. All the enzymes showed a banded distribution, but that for the purely mitochondrial enzymes succinate dehydrogenase and glutamate

dehydrogenase was coarser and more irregular, in keeping with the expected localization of these enzymes in mitochondria, which are concentrated, but not exclusively located, opposite the I-bands of the fibrils (Klingenberg, 1964). The picture for glutamate-oxaloacetate transaminase, which is known to occur in both cytoplasm and mitochondria in this tissue, was similar to that for the purely cytoplasmic enzyme lactate dehydrogenase.

It cannot at present be proved conclusively that the 'soluble' enzymes are located within the myofibrils, but the localization pattern appears to be in accord with the hypothesis of a concentrated distribution of many glycolytic and related enzymes around the isotropic zone of skeletal-muscle fibres, which has been put forward by Brandau & Pette (1966) and Sigel & Pette (1969).

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Isoenzymes of Glutamine Synthetase in Rat Tissues

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It was recently shown (Iqbal & Ottaway, 1970) that glutamine synthetase from rat muscle has different kinetic properties from that from kidney. In particular, product inhibition by P_i is competitive with respect to ATP. This is incompatible with the reaction mechanism proposed by Meister and co-workers (see Meister, 1968), but is compatible with a mechanism involving binding of P_i to the enzyme and random release of P_i and ADP as products. This latter mechanism predicts that P_i will be an uncompetitive inhibitor of the muscle enzyme with respect to hydroxylamine. It has been possible to show that this is indeed the case, and a semi-random mechanism is now regarded as established for rat muscle glutamine synthetase.

The inhibition of kidney glutamine synthetase by P_i was also studied. P_i was found to inhibit uncompetitively with both hydroxylamine and glutamate as variable substrates; the K_i is about 5 mM in each case. These observations are not in accord with the Meister mechanism, which predicts

no inhibition with respect to hydroxylamine and non-competitive inhibition with respect to glutamate.

It was also found that AMP is a powerful competitive inhibitor of the kidney enzyme with respect to hydroxylamine. The K_i is approx. $10\mu\text{M}$. AMP does not affect the activity of muscle glutamine synthetase.

Although it is difficult to interpret several of these observations, they underline further the marked difference in properties between the enzymes from the two tissues.

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The Development of Hypoxanthine/Guanine Phosphoribosyltransferase Activity in Man

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Hypoxanthine/guanine phosphoribosyltransferase (EC 2.4.2.8) is important to man. Its absence is associated with the childhood syndrome of mental deficiency, self-mutilating behaviour and hyperuricaemia (Nyhan, 1968). The enzyme catalyses the conversion of 5-phosphoribosyl 1-pyrophosphate and hypoxanthine or guanine into the corresponding 5'-mononucleotides.

The measurement of hypoxanthine/guanine phosphoribosyltransferase activity in tissues other than erythrocytes is complicated by destruction of the product by 5'-nucleotidase (EC 3.1.3.5). However, 5'-nucleotidase can be inhibited by TTP (Murray & Friedrichs, 1969). Assays based on the method of Cartier & Hamet (1968) were performed on the 25000g supernatant from homogenates. When the hydrolysis of GMP by brain homogenates was decreased to 15% of control values by 1.5mM-TTP there was a fourfold increase in measured hypoxanthine/guanine phosphoribosyltransferase activity. Other tissues showed less marked changes, and activity in erythrocytes was unaffected. A survey in foetal, childhood and adult tissues showed that activity was present in all the tissues examined; in most, including liver and kidney, little change occurred with growth and development. In contrast, activity in regions of brain rose during the postnatal period to reach values that, in the adult were markedly higher than those in other tissues.

Activity in the testis was also somewhat higher than those in other organs.

Hypoxanthine/guanine phosphoribosyltransferase activity can be separated into at least two components by electrophoresis on cellulose acetate or starch gel, there being no major alteration in the pattern from brain during development. Reaction products from both components were destroyed by 5'-nucleotidase, and the reaction was inhibited partially by 0.7mM-6-mercaptopurine and totally by 40mM-EDTA. No marked alteration in electrophoretic pattern was observed after treatment with 5mM-sodium dodecyl sulphate. Electrophoretic separation of hypoxanthine/guanine phosphoribosyltransferase components on polyacrylamide gel has also been reported by Bakay & Nyhan (1971).

No differences in thermal stability were detected in different tissues.

The distribution and activity of hypoxanthine/guanine phosphoribosyltransferase seem consistent with its postulated 'salvage' role and possibly with its involvement in the use of exogenous purines for cellular nucleotide biosynthesis (see Raivio & Seegmiller, 1970).

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The Effect of Starvation on the Cholinesterase Activity of the Liver and Serum of Young Male Rats

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Harrison & Brown (1951) showed that adult male rats starved for periods up to 6 days produced a decrease in liver cholinesterase (EC 3.1.1.8; formerly pseudocholinesterase) activity but no change in the activity in plasma. We now report the effects of starvation on cholinesterase activity in young male rats. This study was made to establish base-line values of enzyme activity as a preliminary to examining the effect of feeding on hepatic cholinesterase synthesis.