- Smith, J. N., Smithies, R. H. & Williams, R. T. (1954b)
Biochem. J. **57**, 74
 Sundvik, E. (1886) *Jahresber. Thierchem.* **16**, 76

Liver L-Serine-Pyruvate Aminotransferase Activity in Different Animal Species

By E. V. ROWSELL, A. H. AL-TAI and J. A. CARNIE
 (Department of Biochemistry, University of Manchester Institute of Science and Technology, Manchester M60 1QD, U.K.) and KATHLEEN V. ROWSELL
 (Department of Medical Biochemistry, University of Manchester, Manchester M13 9PL, U.K.)

In a comparative survey for liver serine dehydratase, covering the pig, sheep, rabbit, guinea pig, rat, mouse and common shrew, activity was found to increase consistently with decreasing body size—with increasing basal metabolism (Rowsell *et al.*, 1965). From recent assays we can report that the body size relationship still holds when we include values for cat and dog liver. With amphibian liver (from common frog, green tree frog, natterjack toad), however, we find no measurable activity.

The distribution pattern for L-serine-pyruvate aminotransferase activity is altogether different. Assays were made on homogenates of fresh liver by measuring hydroxypyruvate formation with D-glycerate dehydrogenase (Rowsell *et al.*, 1969). The results obtained, expressed as μmol of hydroxypyruvate/h per g of tissue, as means $\pm$ S.D. (nos. of animals), were as follows: mouse, 4.66 ± 0.86 (5); blackhooded rat, 1.59 ± 0.27 (10); Sprague-Dawley rat, 4.45 ± 0.77 (9); Wistar rat, 3.30 ± 0.47 (7); guinea pig, 0.53 ± 0.19 (3); rabbit, 24.8 ± 4.5 (12); sheep, 1.07 ± 0.20 (4); pig, 1.42 ± 0.44 (4); cat, 94 ± 32 (6); dog, 126 ± 49 (14); common frog, 54 ± 10 (5); common toad, 51.5 and 46.3. The most notable feature is the outstandingly high activity in the carnivorous species.

The reverse reaction, with hydroxypyruvate and L-alanine as substrates, was assayed, by measuring serine formation with ninhydrin after separation by paper chromatography (Kay *et al.*, 1956). Dog and frog liver activities were 2–3-fold greater than with L-serine and pyruvate as substrates; and we note that Sallach's (1956) original studies on serine formation by transamination employed a dog liver enzyme. Even so, it is difficult to accept that the function of this enzyme is to form L-serine: the flesh eater, presumably, derives an abundant supply of amino acids from its food; it seems unlikely it would be especially well equipped for their synthesis. We assume, rather, that the enzyme operates, at least in the carnivore, to convert L-serine into hydroxypyruvate as the first step towards glucose formation via D-glycerate.

Moreover for rat liver there is evidence that L-serine is converted into glucose without being liberated as pyruvate, from perfusion studies; testing the effects of quinolinate, a potent inhibitor of phosphoenolpyruvate carboxykinase (Lardy *et al.*, 1969). The authors conclude that L-serine-pyruvate aminotransferase, rather than L-serine dehydratase, is the enzyme initiating gluconeogenesis from L-serine. Our comparative findings are consistent with that suggestion.

- Kay, R. E., Harris, D. C. & Entenman, C. (1956) *Arch. Biochem. Biophys.* **63**, 14
 Lardy, H. A., Veneziale, C. M. & Gabrielli, F. (1969) *FEBS Symp.* **19**, 55
 Rowsell, E. V., Carnie, J. A. & Wahbi, S. D. (1965) *Biochem. J.* **96**, 13P
 Rowsell, E. V., Snell, K., Carnie, J. A. & Al-Tai, A. H. (1969) *Biochem. J.* **115**, 1071
 Sallach, H. J. (1956) *J. Biol. Chem.* **223**, 1101

Activities of Phosphopyruvate Carboxylase and Citrate Synthase in Rat and Cow Liver after Glucocorticoid Administration

By R. J. HEITZMAN, I. D. HERRIMAN and C. B. MALLINSON (Agricultural Research Council Institute for Research on Animal Diseases, Compton, Newbury, Berks., U.K.)

The administration of a glucocorticoid to rats increases hepatic gluconeogenesis and ketogenesis. However, Baird & Heitzman (1971) have shown that glucocorticoids are antiketogenic and possibly do not increase gluconeogenesis in bovine liver.

In investigations of the reasons for this difference, the metabolic role of oxaloacetate has been an important consideration. Oxaloacetate is at a key branching point: it can be converted into phosphoenolpyruvate via the phosphopyruvate carboxylase reaction or into citrate through the citrate synthase reaction. The activities of these enzymes are important, since they could affect the fate of oxaloacetate and thus gluconeogenesis and ketogenesis. The sub-cellular distribution of these enzymes is also important; in rat liver phosphopyruvate carboxylase is almost exclusively located in the cytoplasm whereas citrate synthase is a mitochondrial enzyme. The enzymes distribute much more evenly between cytoplasm and mitochondria in bovine liver cells.

The administration of 10mg of dexamethasone 21-isonicotinate to healthy cows resulted, 48h later, in a significant decrease of the hepatic phosphopyruvate carboxylase activity in the cytoplasm to 36% of the control value with no effect on the activity of the particulate enzyme. The citrate synthase activity in bovine liver was increased to 180% of the control value in the particulate fraction and to 187% in the

cytoplasmic fraction. These changes in enzyme activity would favour an anti-ketogenic action of a glucocorticoid in the bovine liver.

Administration of 1 mg of dexamethasone 21-isonicotinate or 1 mg of betamethasone to healthy female rats fed on an adequate ration resulted in effects in liver, 24 and 48 h later, quite the opposite from those observed in bovine liver. Hepatic phosphopyruvate carboxylase activity was increased in the cytoplasmic fraction and hepatic citrate synthase activity was depressed by 30% in the particulate fraction. These enzyme changes would favour the diversion of oxaloacetate to phosphoenolpyruvate in rat liver.

A noteworthy observation was that in bovine liver there was a significant correlation between the citrate concentration and the citrate synthase activity. This correlation was not observed in rat liver experiments.

C. B. M. is a Placement Student from Huddersfield Polytechnic, Queensgate, Huddersfield HD1 3DH, Yorks., U.K.

Baird, G. D. & Heitzman, R. J. (1971) *Biochim. Biophys. Acta* 252, 184

Single-Step Purification of Dihydrofolate Reductases on Pteridine Matrices

By J. G. DANN, N. G. L. HARDING, P. C. H. NEWBOLD and J. M. WHITELEY (*The Postgraduate Medical School, Hills Road, Cambridge CB2 2QH, U.K.*)

Intracellular dihydrofolate reductase maintains the tetrahydrofolate pool at the expense of NADPH. The enzyme uses folate or 7,8-dihydrofolate as substrate, and it is powerfully inhibited by their 2,4-diamino analogues, e.g. methotrexate. The basal dihydrofolate reductase activity of wild-type cells is often increased after therapy with methotrexate, following oncogenic transformation, or with the emergence of mutants resistant to methotrexate. In the latter case two forms of the intracellular enzyme were observed, and showed disparate increase in activity as resistance developed (Harding *et al.*, 1970).

Attempts to define the mechanism underlying these increases in reductase activity have been unrewarding, since poor yields of active protein are obtained by conventional chromatography. Since the 2,4-diamino substituents are largely responsible for binding of the inhibitors to the enzyme, a distal carboxyl group of these derivatives is coupled to aminoalkyl-agarose to produce insoluble matrices that will retard or retain the mammalian and bacterial enzyme according to the K_m or K_i of the compound (Newbold & Harding, 1971). Similar affinity matrices will also bind the enzyme from T4 bacteriophage (Erickson & Mathews, 1971) and chicken liver (Kaufman & Pierce, 1971), and will retain reductase-independent

protein (Newbold & Harding, 1971; Kaufman & Pierce, 1971).

We have sought to answer whether an increase in the intracellular lifetime or rate of synthesis of each form of the reductase would account for the previous observations (Harding *et al.*, 1970). Trial of the affinity columns with the enzyme from *Lactobacillus casei* showed that one of the two forms was lost during purification. This was due to substrate-dependent interconversion by loss of bound NADPH. Whether this occurs by a catalytic function of the enzyme or by elution of the bound NADPH is not clear.

Apart from the enzyme from *Plasmodium* (Ferone *et al.*, 1969), dihydrofolate reductases are relatively small proteins (mol.wt. approx. 20000). Although the pteridine moiety of the folate molecule primarily determines interaction with the active site, the *p*-aminobenzoylglutamate side chain contributes to the binding (Baker, 1967). For maximal interaction between the reductase and the matrix, there must be an extended linking agent between the agarose and the inhibitor. With compounds of K_i 10^{-8} – 10^{-9} M a spacer length of C_4 – C_6 is required for good recovery (>50%) of catalytic activity. If the γ -carboxyl group of the folate derivative is linked to the spacer, addition of one methylene group to a C_3 spacer changes the pteridine-agarose distance by about 6%. Since the enzyme structure is not resolved, we are unable to explain how this small change considerably alters retention and elution of the enzyme.

For high yields of active enzyme 5-formyltetrahydrofolate, 7,8-dihydrofolate and folate are particularly effective counter-substrates. Buffer composition is important, both for determining stability of the enzyme and for elution of reductase-independent proteins. Dissociation of the enzyme from the matrix can lead to inactivation, whereas binding to it does not. Active and inactive species of the eluted enzyme migrate identically on sodium dodecyl sulphate-polyacrylamide gel. Hence loss of activity is apparently due to irreversible deformation of the secondary or tertiary structure of the enzyme. Inactivation occurs particularly when folate and NADPH are both in the eluent, although two forms are eluted when NADPH is present. In contrast, NADPH stabilizes the reductase against intracellular degradation (N. G. L. Harding and G. Court, unpublished work).

This work is supported by grants from the Medical Research Council, the Leukaemia Research Fund and the British Medical Association. P. C. H. N. is in receipt of the Elmore Studentship, University of Cambridge.

Baker, B. R. (1967) *Design of Active-Site-Directed Irreversible Enzyme Inhibitors*, pp. 192–266, John Wiley and Sons, New York

Erickson, J. S. & Mathews, C. K. (1971) *Biochem. Biophys. Res. Commun.* 43, 1164