- Gutman, Y. & Kidron, M. (1971) *Biochem. Pharmacol.* **20**, 3547
- Hitchcock, M. & Murphy, S. D. (1971) *Toxicol. Appl. Pharmacol.* **19**, 37
- Jondorf, W. R., Maickel, R. P. & Brodie, B. B. (1958) *Biochem. Pharmacol.* **1**, 352
- Jondorf, W. R., Simon, D. C. & Avnimelech, M. (1966) *Mol. Pharmacol.* **2**, 506
- Jondorf, W. R. & Szapary, D. (1968) *Arch. Biochem. Biophys.* **126**, 892
- Miller, H. H., Johnson, R. K., Donahue, J. D. & Jondorf, W. R. (1970) *Biochem. J.* **117**, 491

Stimulation of Tryptophan Metabolism in the Rat by 4-Dimethylamino-3'-methylazobenzene and 2-Acetamidofluorene

By JAMES G. CHALMERS (*Beatson Institute for Cancer Research, 132 Hill Street, Glasgow G3 6UD, U.K.*)

Tryptophan 2,3-dioxygenase may be induced in rat liver by cortisol or tryptophan, and the activity of the enzyme is measured by the conversion of tryptophan into kynurenine under suitable conditions. Altman & Greengard (1966) found that the administration of cortisol to patients caused increases in the activity of tryptophan 2,3-dioxygenase and a corresponding increase in urinary kynurenine. In the rat Tenconi & Pasquariello (1967) described increases in urinary kynurenine and other tryptophan metabolites after treatment with cortisol. It has since been reported that triamterene (2,4,7-triamino-6-phenylpteridine), a synthetic pteridine, increased the cortisol induction of tryptophan 2,3-dioxygenase and also the excretion of kynurenine in the rat (Duncan & Ghosh, 1969; Ghosh & Green, 1970).

A number of studies have been made of the induction of liver enzymes during hepatocarcinogenesis. In a feeding experiment in which rats were given 4-dimethylamino-3'-methylazobenzene for 5 weeks Poirier & Pitot (1969b) found no significant change in the dietary induction of tryptophan 2,3-dioxygenase. Poirier & Pitot (1969a) reported an inhibition of enzyme induction after the administration of 2-acetamidofluorene. Kizer *et al.* (1969) also recorded an inhibition of the cortisol induction of tryptophan 2,3-dioxygenase after the administration of 4-dimethylamino-3'-methylazobenzene.

In the present experiments male rats were fed on a diet containing a supplement of 4-dimethylamino-3'-methylazobenzene (0.06%) or 2-acetamidofluorene (0.03%). At intervals during the experiment the rats were injected with a combination of cortisol and tryptophan, and the urine was collected individually for 5h thereafter. Compared with controls it was found that there was an increased elimination of

kynurenine in the rats receiving prior treatment with the carcinogens.

In view of the findings by other workers that in feeding experiments with liver carcinogens the induction of tryptophan 2,3-dioxygenase is unchanged or inhibited, it was expected that the kynurenine elimination would also be unchanged or decreased. There were differences, however, in the experimental conditions, chiefly in the method of enzyme induction with cortisol and tryptophan but also in the duration of feeding.

- Altman, K. & Greengard, O. (1966) *J. Clin. Invest.* **45**, 1527
- Duncan, J. E. & Ghosh, D. (1969) *Biochem. Biophys. Res. Commun.* **37**, 404
- Ghosh, D. & Green, R. (1970) *Biochem. Biophys. Res. Commun.* **41**, 1421
- Kizer, D. E., Cox, B., Howell, B. A. & Shirley, B. C. (1969) *Cancer Res.* **29**, 2039
- Poirier, L. A. & Pitot, H. C. (1969a) *Cancer Res.* **29**, 464
- Poirier, L. A. & Pitot, H. C. (1969b) *Cancer Res.* **29**, 475
- Tenconi, L. T. & Pasquariello, G. (1967) *Acta Vitaminol. Enzymol.* **21**, 17

Lysine Biosynthesis in Mammals

By FLORENCE C. I. WANG and MAXWELL H. R. LEWIS (*Departments of Child Health and Biochemistry, Queen's University of Belfast, Belfast BT9 7BL, U.K.*)

The synthesis of saccharopine [ϵ -N-(L-glutaryl-2)-L-lysine] from L-lysine and α -oxoglutarate is catalysed by the enzyme 'lysine-oxoglutarate reductase' [L-lysine-2-oxoglutarate reductase (saccharopine-synthesizing)], which has been partially purified from human liver (Hutzler & Dancis, 1968). Our studies on ox and human liver mitochondria have shown the presence of an enzyme capable of hydrolysing saccharopine to lysine and α -oxoglutarate. This enzyme, 'saccharopine oxidoreductase' [saccharopine-NADP⁺ oxidoreductase (hydrolysing)], has an absolute requirement for NADP⁺ and has a pH optimum about 9.0. 'Saccharopine oxidoreductase' preparations from human or ox liver are stimulated by NH₄Cl, which was found to inhibit 'lysine-oxoglutarate reductase'. The two enzymes exhibit different stabilities on incubation, 'saccharopine oxidoreductase' losing its stability after 90min incubation at 30°C and 'lysine-oxoglutarate reductase' being stable for a period of 4h. The two enzymes have been partially resolved by fractionation of mitochondrial extracts from human and ox livers.

Experiments with mammals have shown that lysine does not participate in a reversible deamination-reamination reaction (Schoenheimer & Rittenberg, 1940; Weissman & Schoenheimer, 1941) and is directly incorporated into proteins (Clark & Ritten-

berg, 1951). However, in micro-organisms two distinct pathways of lysine biosynthesis are known (Meister, 1965; Rodwell, 1969). One is based on the formation and subsequent decarboxylation of α -diaminopimelate (Gilvarg, 1960), the other involves α -aminoadipate as the key intermediary metabolite (Broquist, 1966).

From studies of patients with genetic defects in lysine metabolism it appears that lysine degradation in humans proceeds by a reversal of the α -aminoadipate pathway established for its biosynthesis in yeast and *Neurospora* (Kao *et al.*, 1964; Saunders & Broquist, 1967; Trupin & Broquist, 1965). 'Saccharopine dehydrogenase' [saccharopine-NAD⁺ oxidoreductase (hydrolysing)] has been isolated, purified and characterized (Hutzler & Dancis, 1970) from human liver. This enzyme reduces NAD⁺ in the presence of saccharopine, with the formation of glutamate and L-2-aminoadipic δ -semialdehyde. Our evidence shows 'saccharopine oxidoreductase' to be distinct from 'saccharopine dehydrogenase'.

We have also studied the activity of 'saccharopine oxidoreductase' in the livers of different mammals. Our results show that herbivorous animals have the highest activity of the enzyme and that this activity is considerably less in omnivores and carnivores. Excessive amounts of saccharopine and lysine are observed in the plasma and urine of patients suffering from saccharopinuria (Carson, 1969; Carson *et al.*, 1968; Simell *et al.*, 1972). The high concentrations of lysine in the body fluids of these patients may result from repression or inhibition by saccharopine of 'lysine-oxoglutarate reductase'. Alternatively there may be an induction of 'saccharopine oxidoreductase', as indicated by our current findings.

- Broquist, H. P. (1966) *Annu. Rev. Biochem.* **35**, 231-236
Carson, N. A. J. (1969) *Proc. Symp. Soc. Study Inborn Errors Metab.* 6th. 163-172
Carson, N. A. J., Scally, B. G., Neill, D. W. & Carre, I. J. (1968) *Nature (London)* **218**, 679
Clark, I. & Rittenberg, D. (1951) *J. Biol. Chem.* **189**, 521-528
Gilvarg, C. (1960) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **19**, 948-952
Hutzler, J. & Dancis, J. (1968) *Biochim. Biophys. Acta* **158**, 62-69
Hutzler, J. & Dancis, J. (1970) *Biochim. Biophys. Acta* **206**, 205-214
Kao, M. H., Saunders, P. O. & Broquist, H. P. (1964) *J. Biol. Chem.* **241**, 508-515
Meister, A. (1965) *Biochemistry of the Amino Acids*, vol. 2, pp. 928-951, Academic Press, London and New York
Rodwell, V. W. (1969) *Metab. Pathways* **3**, 334-350
Saunders, P. O. & Broquist, H. P. (1967) *J. Biol. Chem.* **241**, 3435-3440
Schoenheimer, R. & Rittenberg, D. (1940) *Physiol. Rev.* **20**, 218-248
Simell, O., Visakorpi, J. K. & Donner, M. (1972) *Arch. Dis. Childhood* **47**, 52-55

- Trupin, J. S. & Broquist, H. P. (1965) *J. Biol. Chem.* **240**, 2524-2530
Weissman, N. & Schoenheimer, R. (1941) *J. Biol. Chem.* **140**, 779-795

Regulation of L-Serine Dehydratase in Developing Rat Liver

By KEITH SNELL and DERYCK G. WALKER (*Department of Biochemistry, University of Birmingham, Birmingham B15 2TT, U.K.*)

Previous workers have reported a peak of L-serine dehydratase activity in developing rat liver either at 2 (Yeung & Oliver, 1971) or at 20-26 days *post partum* (Vernon *et al.*, 1968; Evered & Roffe, 1971; Goswami *et al.*, 1972). In the present work L-serine dehydratase activity was assayed in unfractionated liver homogenates (5%, w/v) under the incubation conditions of Greengard & Dewey (1967) but by using a discontinuous procedure. Two peaks of activity, at 2 and 18 days *post partum*, both having essentially similar isoenzyme compositions (Inoue *et al.*, 1971), have thus been observed, and this developmental profile is similar to that of some other enzymes of amino acid metabolism (Snell & Walker, 1972).

L-Serine dehydratase activity was precociously induced 2 days before term by injection *in utero* of glucagon or dibutyryl cyclic AMP (6-*N*,2'-*O*-dibutyryladenosine 3':5'-cyclic monophosphate), but not by cortisol or thyroxine, and this induction, as with the natural postnatal increase, was blocked by cycloheximide or actinomycin D. Glucose administration to caesarian-delivered newborn rats partially blocked the postnatal increase in activity, but had no effect on glucagon-mediated induction at birth (see also Yeung & Oliver, 1971; Greengard & Dewey, 1967). Glucose may act indirectly to prevent postnatal hypoglycaemia and subsequent endogenous glucagon release.

The second postnatal rise in enzyme activity could be prematurely induced at 11 days by cortisol; tryptophan and an intragastrically-administered amino acid mixture were less effective, and induction by these agents was not additive to that by cortisol. Glucagon and dibutyryl cyclic AMP were completely ineffective at this age. Induction by cortisol or tryptophan was blocked by actinomycin D. Glucose also prevented the cortisol-stimulated increase in activity, and lowered the naturally elevated enzyme activity at 18 days.

At 21 days, after the second postnatal peak, L-serine dehydratase was stimulated equally by cortisol or tryptophan injection or by intubation of an amino acid mixture, and again the inductive effects were