Instead of being allowed to feed at will, the animals were trained for 10 days before the experiment to accept a single daily meal, the first group being fed between 9:00a.m. and 12:00 noon and the second between 3:00p.m. and 6:00p.m. The two groups were thus subjected to the same light/dark cycle but fed at different times. On the day of the experiment these feeding times were strictly observed, and liver 3-hydroxy-3-methylglutaryl-CoA reductase activity was measured in animals of each group at 9:00a.m. and at 3h intervals thereafter. The peak of activity was reached 6h later in the second group than in the first, and therefore the timing of maximum activity is related to when the rats feed.

Since rats allowed continuous access to food will feed during the hours of darkness (Suttie, 1968), we suggest that feeding is at least partly responsible for the diurnal variation in liver 3-hydroxy-3-methylglutaryl-CoA reductase activity. Environmental lighting probably establishes the feeding habits of the rat, but it is not itself the cause of the rhythm.

This work was supported by the Scottish Hospital Endowments Research Trust. K. W. G. holds a Science Research Council Studentship.

Hamprecht, B., Nussler, C. & Lynen, F. (1969) *FEBS Lett.* **4**, 117-121

Shapiro, D. J. & Rodwell, V. W. (1971) *J. Biol. Chem.* **246**, 3210-3216

Suttie, J. W. (1968) *J. Nutr.* **96**, 529-536

Some Factors Affecting Liver Microsomal Drug Metabolism in the Chicken

By A. H. DRUMMOND, J. M. MCCALL and W. R. JONDORF (*Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, U.K.*)

It has been established that liver microsomal preparations from chickens can metabolize a variety of substrates under controlled conditions *in vitro* (Brodie & Maickel, 1962; Debackere & Uehleke, 1964; Hitchcock & Murphy, 1971; Gutman & Kidron, 1971). We became interested in studying the effects of various inducers and inhibitors of drug metabolism in the chicken, and in the course of this we also examined other variables such as perinatal development and sex differences in the Hubbard Golden Comet strain of this species.

Livers were obtained from chick embryos (-2 days) and from groups of stunned and decapitated males and females aged 1, 7, 14, 28, 56 and 128 days. They were processed for preparation of microsomal and 105000g supernatant fractions, which were incubated for 30min at 37°C under standard conditions (Jondorf *et al.*, 1966) with aminopyrine or naphthalene as substrates. Incubations were respectively

assayed for metabolic formation of formaldehyde (Cochin & Axelrod, 1959) or 1-naphthol (Booth & Boyland, 1958). In these experiments we found that there was essentially no sex difference in drug-metabolizing activity in preparations from 1-, 7-, 14- and 56-day-old donors. A surprising finding was the marked prenatal drug-metabolizing activity for both substrates, equivalent to 45-60% of the adult rate. Peak drug-metabolizing activity was noted in preparations from 1-day-old chicks, these being more than twice as active as liver microsomal fractions from adult birds or from chicks in the age range 7-56 days. These developmental aspects of drug metabolism differ from corresponding findings in mice and guinea pigs (Jondorf *et al.*, 1958).

When 14-, 28- and 56-day-old female chicks were pretreated for 24h with sodium phenobarbital (80mg intraperitoneally/kg body wt.) or 3-methylcholanthrene (100mg intraperitoneally/kg body wt.), liver microsomal metabolism of aniline, aminopyrine and naphthalene was enhanced by over 100% relative to the controls. We found no evidence of 2-naphthol formation in incubations of induced microsomal preparations with naphthalene. Pretreatment of chicks with 1,1-dichloro-2-*o*-chlorophenyl-2-*p*-chlorophenylethane (100mg intraperitoneally/kg body wt.) for 24h inhibits microsomal metabolism of all three substrates, although at this pretreatment dosage in corresponding experiments with rats drug-metabolizing enzyme activity is induced (Miller *et al.*, 1970).

(-)-Emetine, a known inhibitor of drug-metabolizing enzyme activity in the rat (Miller *et al.*, 1970), can produce comparable inhibitory responses in the chicken, although the drug has to be administered at 40mg intraperitoneally/kg body wt., a dosage that is lethal for the rat (Jondorf & Szapary, 1968). When (-)-emetine at this pretreatment dosage is administered intraperitoneally to chicks in combination with sodium phenobarbital (80mg/kg body wt.) or 3-methylcholanthrene (100mg/kg body wt.) the 24h inductions are blocked.

It remains to be determined whether the time-course of induction and inhibition resembles that seen in previously studied mammalian species, and whether some inducer in the resorbed yolk sac is responsible for the high drug-metabolizing activity of liver microsomal preparations from 1-day-old chicks.

This work was supported in part by the Leverhulme Trust and by the Rankin Fund.

Booth, J. & Boyland, E. (1958) *Biochem. J.* **70**, 681

Brodie, B. B. & Maickel, R. P. (1962) *Proc. Int. Pharmacol. Meet.* **1st**, 1961, **6**, 299

Cochin, J. & Axelrod, J. (1959) *J. Pharmacol. Exp. Ther.* **125**, 105

Debackere, M. & Uehleke, H. (1964) *Proc. Eur. Soc. Study Drug Toxicity* **4**, 40

- 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-