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Daily Rhythmic Variations in Hamster Liver Drug Metabolism and Associated Parameters

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A number of drug-metabolizing enzymes have been shown to exhibit diurnal rhythms in activity in the rat and the mouse (Jori *et al.*, 1971). The relationships between microsomal metabolism and binding of drugs and steroids and the concentrations of cytochromes *P*-450 and b_5 have not previously been determined, so that the nature of the changes remains uncertain. We have investigated diurnal rhythmic parameters in hepatic microsomal preparations from adult male hamsters. These were caged as litter-mate groups and living under a regime comprising a fixed 12h light–12h dark cycle (06:30–18:30h light phase) and constant temperature and dietary conditions. The animals were regularly handled during the month before the experiment to minimize pre-death stressing.

The microsomal parameters examined included the metabolism and spectrally apparent 'binding' of biphenyl, aniline and corticosterone and the concentrations of cytochromes *P*-450 and b_5. The spectral 'binding' magnitudes of biphenyl and aniline, type I and type II substrates respectively, were greatest between 06:00 and 08:00h ($10^{-3} \times \Delta E$/mg of protein: biphenyl, 54; aniline, 36) and least between 18:00 and 20:00h ($10^{-3} \times \Delta E$/mg of protein: biphenyl, 41; aniline, 27). Biphenyl and aniline 4-hydroxylation activities followed a similar rhythm, achieving their highest rates between 06:00 and 08:00h (nmol of product formed/min per mg of protein: biphenyl, 9.0; aniline, 1.9) and their lowest rates between 18:00 and 20:00h (nmol of product/min per mg of protein: biphenyl, 1.3; aniline, 1.3). Differences, in all cases, between the rhythmic extremes were significant ($P < 0.05$). These patterns were similar but considerably more pronounced than that observed for cytochrome *P*-450, whereas, in contrast, no diurnal rhythm was found in biphenyl 2-hydroxylating activity. Corticosterone (type I) showed a slightly different diurnal 'binding' rhythm from biphenyl and aniline, with a maximum between 06:00 and 08:00h but a highly variable minimum stretching between 12:00 and 18:00h. No diurnal rhythms were detectable in the largely cytochrome *P*-450-independent metabolism of corticosterone and in plasma 11-hydroxy corticosteroid concentrations.

In those microsomal parameters exhibiting diurnal rhythms, the occurence of maximum substrate binding and metabolism correlated well with the exposure of animals to light and that of minimum binding and metabolism with the onset of darkness. The results may reflect greater extents of microsomal binding of endogenous materials, e.g. steroids, in the 'evening', when hamsters are more active and hence sustaining high concentrations of these compounds.

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Evidence that the Diurnal Variation in Rat Liver 3-Hydroxy-3-methylglutaryl-Coenzyme A Reductase Activity is Initiated by Feeding

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In rats allowed free access to food and subjected to alternate 12h light and dark periods, liver 3-hydroxy-3-methylglutaryl-CoA reductase (EC 1.1.1.34) activity varies between a maximum at 12:00 midnight and a minimum during the day, midnight coinciding with the middle of the dark period (Hamprecht *et al.*, 1969; Shapiro & Rodwell, 1971). Our experiments were designed to identify the cause of this rhythm.

Rats were allowed to feed at will and subjected to consecutive 12h light and dark periods. The first group was in darkness from 6:00a.m. to 6:00p.m. and the second from 6:00p.m. to 6:00a.m. After 1 week under these conditions liver 3-hydroxy-3-methylglutaryl-CoA reductase activity, measured at 12:00 noon on the same day, was sevenfold higher in the first group than in the second. Enzyme activity thus depends on the environmental lighting rather than on the time of day.

During further studies rats were kept in a room illuminated between 9:00p.m. and 9:00a.m. and in darkness between 9:00a.m. and 9:00p.m.

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.

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Some Factors Affecting Liver Microsomal Drug Metabolism in the Chicken

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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 105000*g* 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.

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