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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 ($10^{-3} \times \Delta E/\text{mg}$ of protein: biphenyl, 54; aniline, 36) and least between 18:00 and 20:00h ($10^{-3} \times \Delta E/\text{mg}$ 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 occurrence 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.