We have shown that with hamster liver microsomal preparations Tween 80 produces a typical type I binding spectrum (cf. Schenkman *et al.*, 1967) at low concentrations, but that at high concentrations a novel spectrum is observed. This interaction of Tween 80 with microsomal preparations might be expected to interfere with both the binding and metabolism of 'cytochrome *P*-450 substrates'. Tween 80 modifies the typical type I binding spectrum of biphenyl (Bridges & Burke, 1971), but does not affect the binding characteristics of aniline, a type II substrate. At low concentrations of Tween 80 (less than 0.5%) this inhibition of biphenyl binding is apparently competitive, but at higher concentrations (up to 1.0%) it appears to be of mixed mechanism. Tween 80 over the concentration range 0.25–2.5% significantly inhibits the hydroxylation of biphenyl to 2-hydroxybiphenyl and 4-hydroxybiphenyl and also the further hydroxylations of 2-hydroxybiphenyl and 4-hydroxybiphenyl; it stimulates the 4-hydroxylation of aniline. Acetone and ethanol also interfere with biphenyl metabolism both in the hamster and in the rat.

Tween 80 also causes difficulties in the solvent extraction and fluorimetric assays of hydroxylated products both for biphenyl and for several other compounds. It appears likely that Tween 80 produces changes in the binding and metabolism of other 'cytochrome *P*-450 substrates', and its use or the use of other solvents should therefore be approached with great caution.

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The Influence of Storage Conditions on Microsomal Drug-Metabolizing Activity

By M. DANNY BURKE and JAMES W. BRIDGES
(Department of Biochemistry, University of Surrey,
Guildford, Surrey, U.K.)

Microsomal drug-metabolizing enzymes quickly lose their activity during storage at room temperature, a phenomenon partially ascribable to the conversion of cytochrome *P*-450 into cytochrome *P*-420 (Hewick & Fouts, 1970). Preservation of valuable liver samples, e.g. post-mortem specimens, and the purification of cytochrome *P*-450 oxygenase systems necessitate knowledge of the nature of the changes

occurring during storage and means of minimizing them.

We have investigated changes incurred, during storage of hamster liver microsomal preparations for 72h at 2° and 20°C, in the metabolism and spectrally apparent 'binding' of biphenyl and aniline and in contents of cytochromes *b*₅ and *P*-450, phospholipid, triglyceride, cholesterol and free fatty acids. Three storage preparations were compared: microsomal suspension in 0.1M-phosphate buffer, pH7.6 (preparation A), suspension in 0.1M-phosphate–20% glycerol buffer, pH7.6 (preparation B), and microsomal pellet overlaid with phosphate buffer (preparation C). In all the fresh preparations biphenyl initiated type I spectrally apparent interactions (Bridges & Burke, 1971) with kinetically dissimilar affinities (cf. Walker, 1963), a high-affinity K_s 1.5×10^{-4} M and a persistently lower-affinity K_s 4×10^{-4} M. Aniline underwent a type II interaction with a kinetically unique affinity (K_s 8.7×10^{-3} M), which was almost doubled by the presence of glycerol. During storage of preparation A at 2°C there was a progressive decrease in the low-affinity biphenyl K_s over 72h, whereas the high-affinity interaction was stable with respect to its K_s for 24h but was completely lost by 48h. $\Delta E_{\max}$ values for the high-affinity and low-affinity interactions decreased to zero after 24 and 48h respectively. Comparable changes in aniline parameters showed its type II interaction to be more stable than the biphenyl type I 'binding'. Storage of preparations B or C preserved unchanged the biphenyl type I affinities, but $\Delta E_{\max}$ changes indicated progressive loss of considerable quantities of microsomal 'binding' components. In contrast, preparation B achieved partial protection of the aniline type II $\Delta E_{\max}$ without any preservation of the interaction affinity.

Changes in $\Delta E_{\max}$ at 20°C roughly correlated directly with loss of biphenyl 2- and 4-hydroxylating activities and inversely with that for further hydroxylation of 2-hydroxybiphenyl, but there was no correlation for the further hydroxylation of 4-hydroxybiphenyl. The results suggest that it is inappropriate to ascribe specific roles in 2- or 4-hydroxylation to the low-affinity and high-affinity 'binding' sites.

Changes in cytochrome *P*-450 concentrations in the various preparations did not correlate with changes in the extents of substrate 'binding' and hydroxylation. Cytochrome *P*-450 was very stable, even at 20°C, and formation of cytochrome *P*-420 was slight. Glycerol preserved cytochrome *P*-450, as expected (Ichikawa & Yamano, 1967), without exerting comparable conservation of microsomal binding and biphenyl metabolism activity.

Cytochrome *P*-450 stability was reflected in the stability of microsomal phospholipid, but the decreases in biphenyl binding and metabolism accompanied the liberation of free fatty acids on storage.

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Daily Rhythmic Variations in Hamster Liver Drug Metabolism and Associated Parameters

By M. DANNY BURKE, BRIAN G. LAKE, J. MICHAEL TREDGER, JAGADISH CHAKRABORTY and JAMES W. BRIDGES (*Department of Biochemistry, University of Surrey, Guildford, Surrey, U.K.*)

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

By ROGER BOOTH, KEITH W. GREGORY and CARMEN ZENAROSA SMITH (*Department of Biochemistry, University of Dundee, Dundee DD1 4HN, U.K.*)

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.