but evidence from the survey perhaps gives some clues as to what these might be. Thus potatoes contained 0.001 mg/kg, and carrots, brussels sprouts and cabbage contained 0.003 mg/kg. The samples were obtained direct from producers and none had been treated with mercury pesticides. On this point there was no correlation between the apparent mercury content of fruit and vegetables and pesticide treatment. Flour used in the U.K. has been shown to contain 0.002-0.007 mg/kg (Hart & Willis, 1972). These may not reflect absolute background concentrations, since most seed grain is treated with mercury compounds, although it has been reported that this has little effect on mercury concentrations in the crop (Smart, 1968). Meat such as beef, chicken, ham and bacon contained less than 0.005 mg/kg, but values of 0.04 and 0.02 mg/kg for pig kidney and pig liver were similar to results obtained in Sweden (Westöö, 1968). An approximate estimate of the mercury content of the meat component of the diet may be obtained from the analyses of the composite meat–fish and fish samples. This is estimated to be in the region of 0.003 mg/kg, indicating that the mercury concentration in the meat component is no greater and may even be less than that in the diet as a whole. For food other than fish it is not clear how much of the total mercury present is in the form of organomercury compounds. Swedish studies on three whole diets not containing fish indicated that the concentrations of methylmercury compounds averaged about 50% of the total mercury content, although Japanese data for individual foods are much lower and in food as a whole including fish amount to less than 10% (M. Kondo, paper presented at the 3rd. Technical Meeting of the O.E.C.D. Sector Group on Unintended Occurrence of Chemicals in the Environment at West Berlin in 1972).

It would appear that fish is the major contributor to what little mercury there is in the diet of the average person. For most people in the U.K. this will largely be from ‘fresh’ fish, since canned fish contained less mercury and much less canned fish is consumed.

Fish consumption in this country is about one-quarter of that in Japan and less than one-half of that in Sweden. Additionally, in these countries a large proportion of the fish supply is taken from inland and coastal waters, where fish have been found to contain higher-than-average mercury concentrations. Some 65% of the fish landed in this country is caught in distant waters, where mercury concentrations averaged about 0.06 mg/kg. Fish from coastal waters of England and Wales contained 0.21 mg/kg, and these fish account for some 7% of the total fish supplies of the U.K. The average mercury concentration in the total fish supply was estimated to be about 0.08 mg/kg. More than 90% of the total mercury in fish was present as methylmercury compounds, this value being similar to Swedish values (Johansson et al., 1970). Values produced in Japan are lower, generally less than 50%. There is no detailed information about the variation in fish consumption between individuals in this country, but a hypothetical range of mercury intake may be obtained by making certain assumptions about frequency of fish consumption, size of meal and mercury concentrations in fish consumed by individuals.

Hart, H. V. & Willis, K. H. (1972) Bull. Flour Mills Bakers Res. Ass. no. 1
Ministry of Agriculture, Fisheries and Food (1971b) Press Notice no. 348
Smart, N. Å. (1968) Residue Rev. 23, 21

COMMUNICATIONS

The Influence of Tween 80 on the Metabolism of Biphenyl Microsomal Oxygenases

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

The study of the interaction of highly lipophilic compounds with enzymes necessitates the use of solvents or sonication. Sonication, acetone, ethanol and dimethyl sulphoxide have all been shown to interfere with hydroxylations mediated by cytochrome P-450 (Anders, 1971; Stock et al., 1970).

Tween 80, a non-ionic detergent, is extensively employed as a vehicle for the addition of water-insoluble compounds such as biphenyl, imipramine [10,11-dihydro-5-(3-dimethylaminopropyl) dibenz- [b,f]azepine] and amitryptaline [3-(3-dimethylaminopropylide)-1,2,4,5-dibenzocyclohepta-1,4-diene] to microsomal systems, yet the possibility of its interaction with these enzymes has not properly been investigated.
We have shown that with hamster liver microsomal preparations Tween 80 produces a typical type I binding spectrum (cf. Schenken 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 10%) 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.

We are grateful to the Medical Research Council for financial support of this work through a Studentship for M. D. B.


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°C and 20°C, in the metabolism and spectrally apparent 'binding' of biphenyl and aniline and in contents of cytochromes b5 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 Ks 1.5 x 10^-4 M and a persistently lower-affinity Ks 4 x 10^-5 M. Aniline underwent a type II interaction with a kinetically unique affinity (Ks 8.7 x 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 Ks over 72h, whereas the high-affinity interaction was stable with respect to its Ks for 24h but was completely lost by 48h. ΔE_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 ΔE_max changes indicated progressive loss of considerable quantities of microsomal 'binding' components. In contrast, preparation B achieved partial protection of the aniline type II ΔE_max without any preservation of the interaction affinity.

Changes in ΔE_max at 20°C roughly correlated directly with loss of biphenyl 2- and 4-hydroxylation 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.