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Human liver microsomal drug metabolism

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RELATIVELY few studies have been made of drug metabolism *in vitro* by subcellular fractions of human liver. Coccia and Westerfeld[1] investigated chlorpromazine metabolism by supernatant fractions obtained from 12–18 hr post-mortem human livers and Kuntzman *et al.*[2] studied pentobarbital, acetophenetidin and 3,4-benzpyrene metabolism by whole homogenates of biopsy samples. Creaven and Williams[3] demonstrated aromatic hydroxylating activity in 4–28 hr post-mortem livers. No investigations appear to have been made using microsomal preparations,* and this communication describes experiments which we have performed with microsomal fractions prepared from liver samples obtained 2–5½ hr post-mortem. We have found that such preparations can metabolize codeine, hexobarbital and aniline, and we have also been able to measure $NADPH_2$-cytochrome *c* reductase (EC 1.6.2.3.) activities and cytochrome P-450 levels.

Experimental

Human liver tissue was obtained as soon as possible after death had occurred. The samples were packed on ice and taken to the laboratory where ice-cold 1·15% KCl containing 0·005 M potassium phosphate buffer pH 7·4 was added (3 ml per g liver) and they were macerated for 30 sec at 0° with an MSE top-drive blender. A Potter–Elvehjem P.T.F.E. glass homogenizer was then used to make a homogenate suitable for differential centrifugation. It proved impossible with any of the liver samples to make an adequate homogenate using only the Potter–Elvehjem homogenizer. The final homogenate was centrifuged for 60 min at 105,000 *g*. The red, gelatinous pellet was resuspended in ice-cold buffered 1·15% KCl so that 2 ml contained 1 g liver equivalent. This suspension is referred to as "the microsomal preparation".

Incubation mixtures contained 2·0 ml microsomal preparation, 2·0 ml 0·1 M potassium phosphate buffer pH 7·4, 0·05 ml 0·2 M $MgSO_4$, 0·5 ml 0·2 M nicotinamide, 0·25 ml of $NADPH_2$ generation mixture (glucose-6-phosphate, 12 μmoles; $NADP^+$, 0·5 μmole; glucose-6-phosphate dehydrogenase, 3 units); and the appropriate drug. The final volume was made up to 5·0 ml with H_2O. Codeine phosphate (5 μmoles) and redistilled aniline (6 μmoles) were added as aqueous solutions while hexobarbital-Na (2·4 μmoles) (gift from May & Baker Limited) was added in 20 μl ethanol. Incubations were carried out in duplicate for 30 min at 37° with shaking. Appropriate control flasks were run simultaneously to check the $NADPH_2$-dependence of the reactions and to determine blank and recovery values.

The amount of morphine formed from codeine was measured by the method of Snell and Snell[4] as described by Johannesson *et al.*[5] Hexobarbital remaining was assayed as described by Cooper and Brodie.[6] The formation of *p*-aminophenol from aniline was determined by the method of Brodie and Axelrod.[7]

$NADPH_2$-cytochrome *c* reductase activity was measured at 22° by the method of Williams and Kamin.[8] Cytochrome P-450 was assayed following Omura and Sato[9] using an Optica double-beam recording spectrophotometer. The protein content of the microsomal preparation was determined using the procedure of Lowry *et al.*[10]

Results and discussion

Table 1 shows the rates of drug metabolism obtained with seven separate liver samples, and also includes data concerning their origin. A dash (—) indicates that no metabolism at all was detectable

* After the draft article describing this work was submitted for publication, a paper by Alvares, Schilling, Levin, Kuntzman, Brand and Mark was published [*Clin. Pharmac. Ther.* **10**, 655 (1969)] describing the properties of cytochrome P-450 and 3,4-benzpyrene hydroxylase in human liver microsomes.

TABLE 1. *IN VITRO* METABOLISM OF DRUGS BY POST-MORTEM HUMAN LIVER MICROSOMAL PREPARATIONS

Experiment no.	Cause of death	Age at death	Sex	Known drug treatment before death	Time elapsed between death and removal of liver sample (hr)	Rate of drug metabolism (nmoles/100 mg protein/30 min)			$NADPH_2$-Cytochrome *c* reductase activity: (Units/100 mg protein)	Cytochrome P-450 (nmoles/100 mg protein)
						Codeine	Hexobarbital	Aniline		
1	*Myocardial infarction	55	Female	—	2	350	1930	240	62·3	—
2	†Alcohol/Barbiturate Poisoning	72	Female	Amobarbital, Chlordiazepoxide	5·5	—	—	—	20·6	38·6
3	‡Haemorrhage following aortic graft	66	Male	Meperidine Codeine Chlorpromazine	5	200	930	500	26·0	—
4	*Myocardial infarction	67	Male	Pentobarbital Digoxin	2·5	320	2680	480	111·0	62·3
5	*Myocardial infarction	68	Male	—	2·5	270	—	—	30·5	29·8
6	‡Heart valve failure	47	Male	Meperidine Phenoperidine Warfarin	4	240	—	300	41·0	8·5
7	§Carcinoma of oesophagus	55	Female	Thiopental	3·75	—	—	—	3·0	—

* Sudden death. † Long-term poisoning. ‡ Died during operation. § Extensive post-operative resuscitation attempted.

with our assay methods. The corresponding cytochrome P-450 concentrations and $NADPH_2$-cytochrome *c* reductase activities are also shown. It is of interest that the presence of cytochrome P-450 did not apparently ensure that drug metabolism could occur *in vitro*, while drug metabolism could apparently occur in the absence of detectable cytochrome P-450. $NADPH_2$-cytochrome *c* reductase activity was always detected.

The values in Table 1 are similar to those which we have found for rat liver microsomes; codeine, 790 nmoles/100 mg/30 min: hexobarbital, 1600; aniline, 200; $NADPH_2$-cytochrome *c* reductase activity, 72·6 units/100 mg protein; cytochrome P-450, 91·3 nmoles/100 mg protein. These values are themselves in good agreement with those reported by others; for example Klinger *et al.*;[11] Gram and Fouts;[12] and Kato and Gillette [13] although higher rates of metabolism have been reported (Kato and Takanaka;[14] Gram Rogers and Fouts).[15]

As probably all seven livers came from drug-treated patients, we expect induction of drug-metabolizing activities to have occurred. In view of the time-factors involved, the fact that active microsomal preparations can be made indicates a high degree of stability of the enzyme system concerned. However, owing perhaps to the harsh method used for homogenisation, the human liver microsomal preparations go off quite rapidly even at 0°. Washing the microsomal preparations by a second

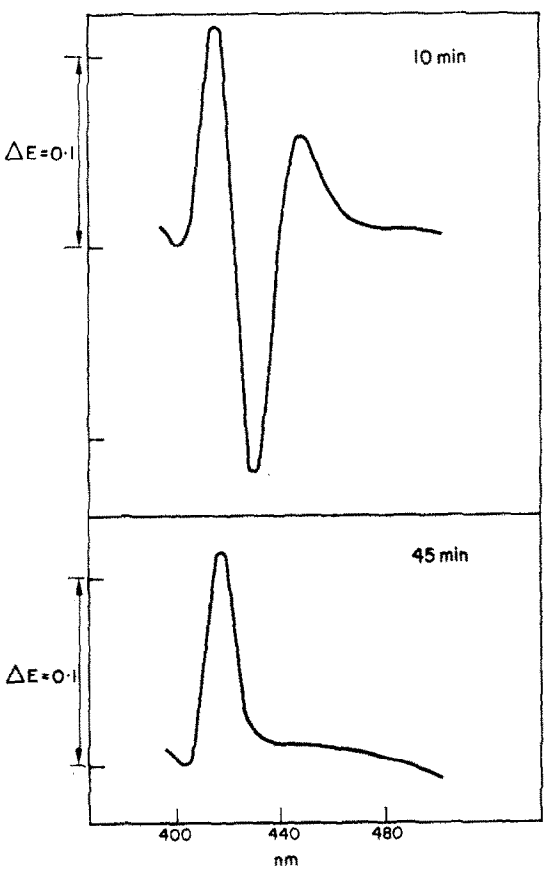

FIG. 1. Difference spectra [reduced (CO)—reduced (N_2)] obtained after standing a humanliver microsomal preparation at 0° for 10 and 45 min. In each case, cuvettes contained 2·5 ml 0·1 M potassium phosphate buffer pH 7·4; 4 mg sodium dithionite and 0·5 ml microsomal preparation (10 min or 45 min old). After bubbling with CO or N_2 for 1 min, difference spectra were recorded.

centrifugation and resuspension destroys all drug-metabolizing activities and eliminates cytochrome P-450. $NADPH_2$-cytochrome *c* reductase activities are reduced to very low levels. Figure 1 illustrates the effect of standing the microsomal preparations at 0° for 45 min on the difference spectra [reduced (CO)–reduced (N_2)] obtained in the course of cytochrome P-450 determination. Even after 45 min at 0° the peak at 450 nm has flattened out. With rat liver microsomes, the spectrum after 45 min standing is identical with the one obtained after 10 min standing.

The large peak at 418 nm is probably a result of contamination of our human liver preparations by haemoglobin. Our failure to detect cytochrome P-450 and yet still be able to demonstrate drug-metabolizing and $NADPH_2$-cytochrome *c* reductase activities *in vitro* may be an expression of the differing labilities of the varying components at present thought to make up the drug-metabolizing enzyme system. It is premature to speculate on the basis of the data presented here that more than one system may be involved, the separate systems handling different types of compound. Neither can we point to obvious sex and age differences. One clear conclusion may be drawn, however. This is that extensive liver damage resulting from, for example, long-term anoxia destroys all capacity to metabolize drugs.

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