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MONOOXYGENASE ACTIVITY OF HUMAN LIVER IN MICROSOMAL FRACTIONS OF NEEDLE BIOPSY SPECIMENS

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1 Methods are described for the determination of mixed function oxidase activity in microsomal fractions from percutaneous needle biopsies of human liver.

2 Activities of needle biopsy samples were comparable with those of wedge biopsy samples obtained at laparotomy from different subjects.

3 Although cytochrome P-450 content of liver from rat and man was similar, human AHH activity was only 10% of that in the rat.

4 In biopsies with preserved hepatic architecture, AHH activity and cytochrome P-450 content showed a significant positive correlation.

5 Cigarette smoking significantly increased both AHH activity and turnover number, but not cytochrome P-450 content, of biopsies with normal architecture.

6 The presence of liver disease caused a significant decrease in AHH activity and cytochrome P-450 content.

Introduction

Inter-individual difference in drug response is still a major problem in drug therapy. Many such differences are due to variations in rate of metabolism by hepatic mixed function oxidases (Davies, Thorgeirsson, Breckenridge & Orme, 1973) which may be the result of variations in the amount of enzyme. However, the existence of multiple forms of the haemoprotein cytochrome P-450 (Comai & Gaylor, 1973) may explain such inter-individual differences by variation in the complement of these forms. Animal studies have shown that the different forms of cytochrome P-450 have different substrate specificities (Lu, Levin, West, Jacobson, Ryan, Kuntzman & Conney, 1973) and that they are regio-specific towards the same substrate (Wiebel, Selkirk, Gelboin, Haugen, van der Hoeven & Coon, 1975) although there is considerable overlap in these specificities. The different forms are also selectively affected by inducers (Haugen, Coon & Nebert, 1976) and inhibitors (Ullrich, Weber & Wollenberg, 1975) and show age (Atlas, Boobis, Felton, Thorgeirsson & Nebert, 1977), sex (von Bahr, Hedlund, Karlen, Bäckström & Grasdelen, 1977), tissue (Atlas *et al.*, 1977) and species (Thomas, Lu, Ryan, West, Kawalek & Levin, 1976) differences.

Although the primary function of the hepatic mixed function oxidase system is the detoxication of foreign compounds (Brodie, Axelrod, Cooper, Gaudette, La Du, Mitoma & Udenfriend, 1955) many substrates for this system are activated to more toxic intermediates (Gillette, 1975). Examples of such substrates include paracetamol (Jollow, Mitchell, Potter, Davis, Gillette & Brodie, 1973), isoniazid (Nelson, Mitchell, Timbrell, Snodgrass & Corcoran, 1976), phenytoin (Martz, Failinger & Blake, 1977), α-methyldopa (Dybing, Nelson, Mitchell, Sasame & Gillette, 1976) and polycyclic hydrocarbons (Grover & Sims, 1968). Variations in the complement of these haemoproteins may result in marked differences in toxicological or therapeutic effects of drugs. This has been observed in the toxicity of a variety of compounds such as polycyclic hydrocarbons, aromatic amines and some insecticides in animals (Nebert, Levitt, Orlando & Felton, 1977) but it is not known whether such effects play a significant role in human toxicity of drugs and other foreign compounds.

In order to determine the number and substrate specificity of the different forms of cytochrome P-450 in man and the influence of genetic and environ-

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mental factors on these different forms it will be necessary to find probe substrates which are metabolized to specific products by each form of the enzyme. Such studies must be performed initially with isolated enzyme systems. We now report results of studies on the feasibility of using microsomal fractions from needle biopsies of human liver to determine which substrates may be used *in vivo* as probes for different forms of cytochrome P-450.

Methods

Animal studies

Male Wistar rats (Olac Ltd) weighing between 100 and 150 g were used. Animals received food (Porton Rat Diet, Labsure Animal Foods, Poole, Dorset) and water *ad libitum* until 18 h before study when food was withdrawn. Rats were killed by stunning and exsanguination. Microsomal fractions were prepared from the livers by the method of Atlas *et al.* (1977).

Patients

Percutaneous needle biopsies of human liver were obtained for diagnostic histology in patients with suspected liver disease using the Menghini technique. Tissue surplus to histological requirement was made available to us for studies of microsomal drug metabolism. Wedge biopsies of liver, taken by the Department of Surgery, were obtained for diagnostic purposes during laparotomy in patients with abnormal liver function tests, lymphoma, suspected secondary tumour or macroscopic areas of abnormality of the liver. Part of the biopsy surplus to requirement for histological examination was available for drug metabolism studies. Local Research Ethics Committee permission was obtained to use surplus hepatic tissue in this way.

Analytical procedures

The hepatic tissue was placed in ice cold 0.25M potassium phosphate, 0.15M potassium chloride buffer pH 7.25 containing 1.0 mM EDTA (phosphate buffer) immediately upon removal from the patient. Samples were kept at 4°C during all subsequent procedures. Microsomal fractions were isolated by a modification of the method of Atlas *et al.* (1977), after tissue samples were blotted and weighed. Up to 100 volumes of phosphate buffer were added to needle biopsy samples and 10-20 volumes of buffer added to wedge biopsy samples. The tissue was homogenized using eight strokes of a Teflon-glass homogenizer (A. H. Thomas) with a motor-driven pestle. This was on average within 45 min of the biopsy procedure (maximum interval 85 min). The resultant homogenates were centrifuged at $14000 \times g_{av}$ for 15 min on an MSE Superspeed 50 ultracentrifuge. The post-mitochondrial supernatant was recentrifuged at $105{,}000 \times g_{av}$ for 60 min. The microsomal pellet was resuspended in a volume of 0.25M potassium phosphate buffer, pH 7.25, containing 30% (v/v) glycerol (phosphate – glycerol buffer), equivalent to the wet weight of tissue used, using a glass-glass microhomogenizer (Jobling Ltd) with a volume of 0.1 ml for needle biopsies and a Teflon-glass homogenizer (A. H. Thomas) size 0 for wedge biopsies. Samples were either assayed fresh or frozen immediately at −80°C in aliquots with a protein concentration of 8–16 mg/ml. No significant loss of activity occurred during 12 months storage (data not shown).

Concentration of microsomal protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystalline bovine serum albumin as standard. The assay volumes were reduced five-fold enabling the measurement to be performed on 2 µl aliquots of microsomal fraction with less than 2 mg protein/ml.

Cytochrome P-450 content was determined by a modification of the method of Omura & Sato (1964) enabling the assay to be performed on as little as 400 µg microsomal protein. An aliquot of the microsomal suspension was diluted with phosphate-glycerol buffer to a final concentration of approximately 0.5 mg protein/ml. The sample was reduced with sodium dithionite and divided between two 1.5 ml cuvettes. The difference spectrum was recorded between 400 and 500 nm on a Pye Unicam SP 800 split beam recording spectrophotometer fitted with an SP 850 scale expansion accessory with full scale deflection set to 0.05 or 0.10 O.D. units. The sample cuvette was bubbled with carbon monoxide for 30 s and the difference spectrum between 400 and 500 nm again recorded. The concentration of cytochrome P-450 was calculated assuming an extinction coefficient of 91 mM cm^{-1} between 450 and 490 nm for reduced cytochrome P-450 (Omura & Sato, 1964).

NADPH-cytochrome c reductase activity was assayed by a modification of the method of Mazel (1971). Sodium-potassium phosphate buffer (0.04 M, pH 7.6 1 ml) containing 0.8 mM EDTA, 1.2 mM potassium cyanide and 0.06 mM cytochrome c was equilibrated at 37°C in a 1.5 ml cuvette for 4 min. Two µl of microsomal suspension were added to give a final protein concentration of approximately 20 µg/ml, and the base-line absorbance recorded for 2 min at 550 nm on a Pye Unicam SP 500 spectrophotometer at 37°C fitted with an external Devices chart recorder set to a full scale deflection of 0.1 O.D. units. Five µl of 12.5 mM NADPH solution were then added, the solution mixed rapidly and the

Table 1 Microsomal mixed function oxidase activity in needle and wedge samples of rat liver

Sample	*Weight (mg)*	*Microsomal protein (mg/g)*	*Cyt P-450 (nmol/mg)*	*Cyt c reductase (nmol reduced mg^{-1} min^{-1})*	*AHH (pmol mg^{-1} min^{-1})*
Needles	137.3 ± 13.4	18.8 ± 0.6	0.67 ± 0.04	122.5 ± 8.2	278.4 ± 46.8
Wedges	2520 ± 150	21.6 ± 0.8	0.59 ± 0.06	121.6 ± 3.6	305.8 ± 58.1
P		< 0.05	> 0.30	> 0.90	> 0.70

Values are means ± s.e. mean from three animals.

absorption recorded over the next few minutes. The amount of cytochrome c reduced was calculated assuming a net extinction coefficient for reduced cytochrome c at 550 nm of 19.1 mM cm^{-1}.

Aryl hydrocarbon hydroxylase (AHH) activity was determined with benzo (a) pyrene as substrate by the micro method of Atlas, Vesell & Nebert (1976) using synthetic 3-hydroxybenzo(a)pyrene as standard (a gift from Dr D.M. Jerina, NIH).

Comparison of values was performed using two-tailed Student's *t*-test for samples of unequal size.

Results

The technique used in the preparation of microsomal fraction from small samples of liver obtained by needle biopsy involving dilution with up to 100 volumes buffer was compared, using rat liver, with more conventional techniques of microsomal preparation. Multiple needle biopsies were taken to give approximately 100 mg from each liver and an intact lobe taken for comparison. The needle biopsy material was treated as would be the human needle biopsy samples and the larger liver samples were treated by standard techniques (Atlas *et al.*, 1977). There were no significant differences in the parameters of mono-oxygenase activity between the two microsomal preparations, although the wedge samples yielded slightly more microsomal protein (Table 1). Each of the scaled down assays of monooxygenase activity was checked against the original 'macro' assay. No significant effects of the modifications adopted on any of these activities were observed (results not shown).

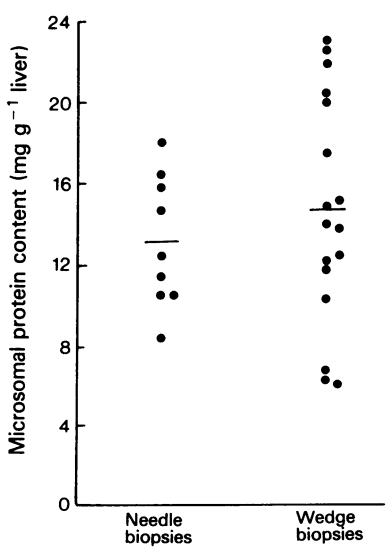

Figure 1 Microsomal protein content of needle and wedge biopsies of human liver with normal architecture. Horizontal bars indicate means.

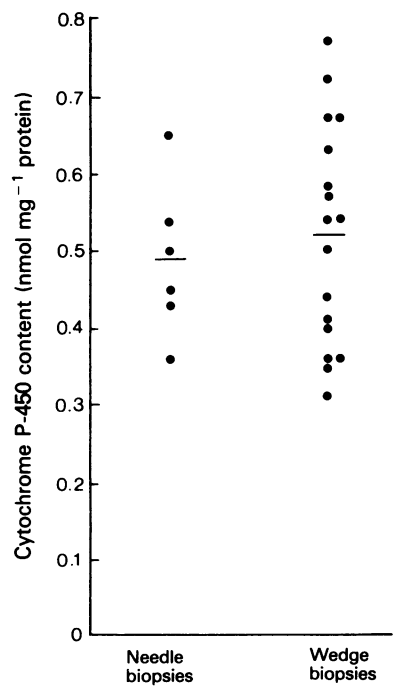

Figure 2 Microsomal cytochrome P-450 content of needle and wedge biopsies of human liver with normal architecture. Horizontal bars indicate means.

Table 2 Activities (mean ± s.e. mean) of components of microsomal mixed function oxidase system in needle and wedge biopsies of human liver with normal architecture expressed per g wet weight of tissue

Sample	*n*	*Cyt P-450* (*nmol/g*)	*NADPH-Cyt c reductase* (*nmol reduced* g^{-1} min^{-1})	*AHH* (*pmol* g^{-1} min^{-1})
Needle biopsies	9	7.2 ± 0.6	1929 ± 189	398 ± 64
Wedge biopsies	17	7.4 ± 0.8	2149 ± 168	443 ± 61
P		> 0.80	> 0.40	> 0.60

The yield of microsomal protein from needle and wedge biopsies of human liver with normal architecture is shown in Figure 1. Protein content of microsomal fraction from needle biopsies ($n = 9$) was 13.2 ± 1.1 mg/g liver (mean ± s.e. mean) and from wedge biopsies ($n = 17$) was 14.7 ± 1.4, a difference that was not significant ($P > 0.4$). Cytochrome P-450 content for the two groups of biopsy samples is shown in Figure 2. In needle biopsies ($n = 6$) cytochrome P-450 content was 0.49 ± 0.04 nmol/mg protein which was not significantly different ($P > 0.5$) from the cytochrome P-450 content of wedge biopsies ($n = 17$) which was 0.52 ± 0.03 nmol/mg protein. Activities expressed per g wet weight of liver are shown in Table 2. No significant differences were found when comparisons were made between activities expressed in this way or per mg microsomal protein.

NADPH-cytochrome c reductase activity (Figure 3) in needle biopsies ($n = 9$) was 146.2 ± 8.4 nmol cytochrome c reduced mg^{-1} protein min^{-1} and was not significantly different from the activity in wedge biopsies ($P > 0.80$) which was 149.2 ± 10.5 nmol cytochrome c reduced mg^{-1} min^{-1}. Comparison of aryl hydrocarbon hydroxylase activities in the two biopsy groups (Figure 4) revealed no significant difference between them ($P > 0.9$). AHH activity in the needle biopsy samples was 30.4 ± 4.2 pmol product produced mg^{-1} protein min^{-1} and in wedge biopsy samples it was 29.9 ± 2.6 pmol mg^{-1} min^{-1}.

Comparison of monooxygenase activity in microsomal fractions from wedge biopsy samples of human liver and from whole rat liver is shown in Table 3. There were no significant differences between man and rat in protein yield or cytochrome P-450 content. NADPH-cytochrome c reductase activity was slightly higher ($P < 0.05$) in the human samples. Despite

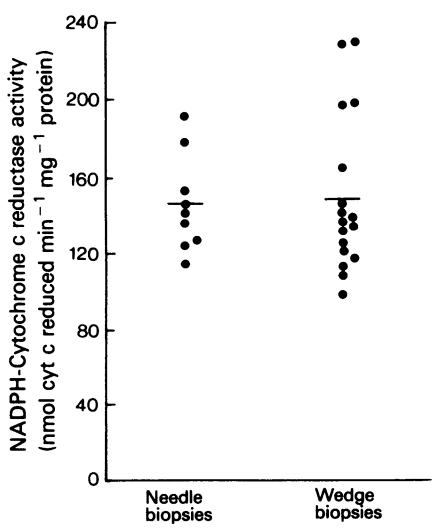

Figure 3 Microsomal NADPH-cytochrome c reductase activity of needle and wedge biopsies of human liver with normal architecture. Horizontal bars indicate means.

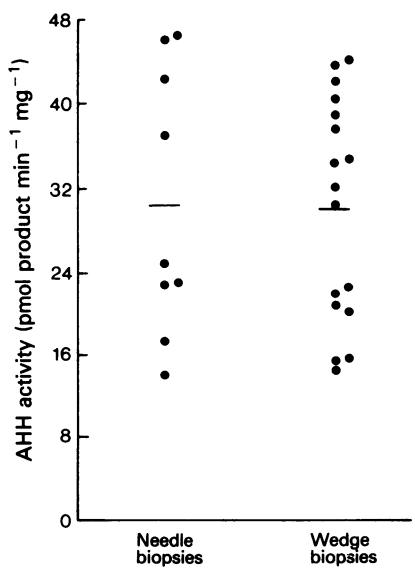

Figure 4 Microsomal aryl hydrocarbon hydroxylase (AHH) activity of needle and wedge biopsies of human liver with normal architecture. Horizontal bars indicate means.

Table 3 Mixed function oxidase activity (mean ± s.e. mean) in human and rat liver microsomes

Sample	n	*Protein (mg/g liver)*	*Cyt P-450 (nmol/mg)*	*Cyt c reductase (nmol mg^{-1} min^{-1})*	*AHH (pmol mg^{-1} min^{-1})*
Human liver wedge biopsies with normal architecture	17	14.7 ± 1.4	0.52 ± 0.03	149.2 ± 10.5	29.9 ± 2.6
Whole rat liver	6	17.5 ± 1.1	0.63 ± 0.04	110.3 ± 3.4	270.0 ± 19.4
P		> 0.20	> 0.05	< 0.05	< 0.001

these similarities AHH activity in human liver was only 10% of that in rat liver ($P < 0.001$).

Figure 5 shows the relationship between cytochrome P-450 content and AHH activity in needle and wedge biopsies of human liver with normal architecture. There were significant correlations between cytochrome P-450 content and AHH within both biopsy groups and the slopes of the regression lines did not differ significantly ($P > 0.60$). Needle and wedge biopsies with normal architecture were therefore treated as a single population in further comparisons.

Values of the various parameters of monooxygenase activity were compared between smokers and non-smokers and are shown in Table 4. There was no significant difference between the two groups in protein yield, NADPH-cytochrome c reductase activity or cytochrome P-450 content. AHH activity was significantly increased ($P < 0.01$) by cigarette smoking. Comparison of the turnover number for AHH, expressed in pmol product formed nmol^{-1} cytochrome P-450 min^{-1} (Figure 6), revealed a significant difference between the two groups ($P < 0.01$). Mean activity in non-smokers was 52.9 ± 4.0 pmol nmol^{-1} cytochrome P-450 min^{-1} and in smokers 72.8 ± 5.5 pmol nmol^{-1} cytochrome P-450 min^{-1}.

Activity was also compared between subjects with and without liver disease, diagnosed histologically from the biopsy material. Biopsies classified as showing liver disease comprised those with fatty change, cirrhosis and hepatitis. The results are shown in Table 5. No significant difference in protein yield or NADPH-cytochrome c reductase activity was found between the two groups. However, there was a significant decrease in cytochrome P-450 content of over 50% ($P < 0.001$) in the liver disease group and in AHH activity of 30% ($P < 0.005$). Despite these changes AHH turnover number was significantly increased ($P < 0.005$) by 52% (from 59.8 ± 3.5 pmol nmol^{-1} min^{-1} to 90.7 ± 9.5 pmol nmol^{-1} min^{-1}) in this group.

Discussion

The procedures used to prepare microsomal fractions from needle biopsy samples of liver and the modifications of the assays adopted were assessed using microsomal fraction from rat liver. No significant differences in mixed function oxidase activity were found using these techniques compared with standard procedures.

Several groups have reported values for mixed function oxidase activity of human liver, using either microsomal fractions of wedge biopsies (Davies & Thorgeirsson, 1971; Black, Perrett & Carter, 1973), or whole homogenate (Schoene, Fleischmann, Remmer & van Olderhausen, 1972) or post-mitochondrial supernatant (Fraser, Williams, Davies, Draffan & Davies, 1976) of needle biopsies. We now report results of studies on cytochrome P-450

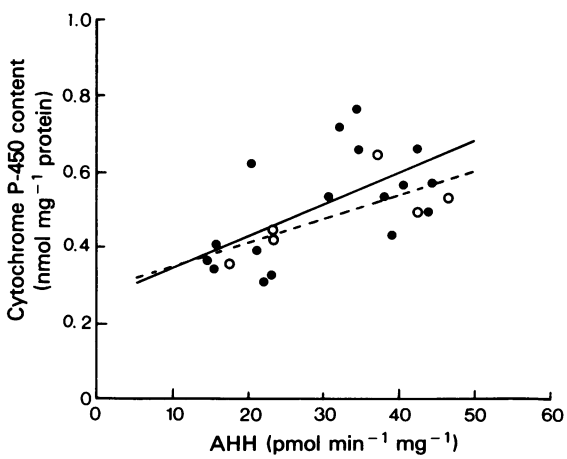

Figure 5 Relationship between microsomal cytochrome P-450 content and aryl hydrocarbon hydroxylase (AHH) activity in needle and wedge biopsies of human liver with normal architecture.
----○----Needle biopsies ($r = 0.81$; $P < 0.05$),
——●——Wedge biopsies ($r = 0.58$, $P < 0.02$).

Table 4 Mixed function oxidase activity (mean ± s.e. mean) in liver biopsies with normal architecture in smokers and non-smokers

Sample	n	*Protein (mg/g liver)*	*Cytochrome P-450 (nmol/mg)*	*Cyt c reductase (nmol mg^{-1} min^{-1})*	*AHH (pmol mg^{-1} min^{-1})*
Non-smokers	16	12.8 ± 1.1	0.49 ± 0.05	154.1 ± 10.6	26.7 ± 2.6
Smokers	10	16.4 ± 1.6	0.55 ± 0.03	139.2 ± 8.6	38.4 ± 2.1
P		> 0.05	> 0.30	> 0.30	< 0.01

content, together with other parameters of mixed function oxidase activity, for human liver using microsomal fractions of needle biopsy samples. The values obtained in the present study are in close agreement with those reported by other groups using a variety of other preparations of liver (Table 6). In the present study no differences in any of the activities measured were found between microsomal fractions prepared from needle or wedge biopsy samples with normal hepatic architecture from separate groups of subjects. The techniques described have thus enabled optium yield of the microsomal fraction to be achieved from samples of human liver obtained by percutaneous needle biopsy and make it possible to perform at least four assays of drug metabolizing activity on as little as 50 mg liver.

Comparison of activities in rat and human liver reveal that cytochrome P-450 content of the microsomal fraction is very similar as has been reported by others (Pelkonen, Kaltiala, Larmi & Kärki, 1974). However, despite this and the greater NADPH-cytochrome c reductase activity of human liver, AHH activity is only 10% of that of rat liver. The difference between rat and human hepatic monooxygenase activities varies considerably, depending upon the substrate used. Amylobarbitone hydroxylation by human liver is only 8% that of rat liver (Fraser *et al.*, 1976), aminopyrine demethylation, 15% (Kamataki, Kitada & Kitagawa, 1973), ethylmorphine demethylation, 48% (Davies & Thorgeirsson, 1971), aniline hydroxylation, 65% (Deckert & Remmer, 1972), hexobarbitone hydroxylation 70% (Kamataki *et al.*, 1973) and phenacetin deethylation almost the same (unpublished observations) as rat liver. This would suggest that the differences between the type of cytochrome P-450 in man and rat are very marked and further studies on the characterization of the cytochromes P-450 present in human liver are currently in progress.

The association between cytochrome P-450 content and AHH activity in liver biopsies with normal architecture is of some interest. It has recently been shown that changes in induced AHH activity correlate most closely with specific forms of cytochrome P-450 in the rabbit (Atlas *et al.*, 1977) and in the mouse and rat (Boobis, Nebert & Felton, 1977). Whether such a relationship explains the correlation reported here for human liver remains to be established. The almost identical regression lines for needle and wedge biopsy samples support the conclusion that needle biopsy samples with normal architecture are representative of the activity of the whole liver. This confirms the work of others using whole homogenate of needle biopsies, comparing the results with *in vivo* drug metabolism (Sontaniemi, Pelkonen, Ahokas, Pirttiaho & Ahlqvist, 1978).

Comparison of activities of the different components of the hepatic monooxygenase system in liver biopsies with normal architecture from smokers and non-smokers revealed no significant differences in protein yield, cytochrome P-450 content or NADPH-cytochrome c reductase activity between the two groups. However, there were significant increases in hepatic AHH activity in biopsies from cigarette smokers confirming the work of others (Pelkonen, Jouppila, Kaltiala & Kärki, 1975).

When the turnover numbers for AHH, expressed per nmol cytochrome P-450, were compared between smokers and non-smokers a marked elevation was found in the smokers. Thus hepatic AHH activity is

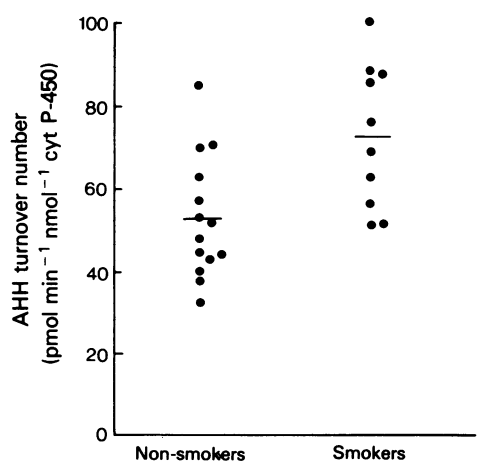

Figure 6 Microsomal aryl hydrocarbon hydroxylase (AHH) turnover number in biopsies of human liver with normal architecture from non-smokers and smokers. Horizontal bars indicate means.

Table 5 Microsomal mixed function oxidase activity (mean ± s.e. mean) in biopsy samples of patients with and without liver disease

Sample	n	*Protein (mg/g liver)*	*Cyt P-450 (nmol/mg)*	*Cyt c Reductase (nmol mg^{-1} min^{-1})*	*AHH (pmol mg^{-1} min^{-1})*
Biopsies with normal architecture	26	14.2 ± 1.0	0.51 ± 0.03	148.1 ± 7.2	30.1 ± 2.2
Biopsies with abnormal architecture	20	13.3 ± 0.6	0.25 ± 0.02	132.4 ± 7.7	20.8 ± 1.9
P		> 0.40	< 0.001	> 0.10	< 0.005

Table 6 Comparison of mixed function oxidase activity in human liver microsomes with values reported by other workers

Investigation	*Sample*	*Cyt. P-450 (nmol/mg)*	*NADPH-cytochrome c reductase (nmol reduced mg^{-1} min^{-1})*	*AHH (pmol mg^{-1} min^{-1})*
Present study	Needles and wedges	0.51 ± 0.14	148.1 ± 36.0 (37°C)	30.1 ± 11.2
Alvares *et al.* (1969)	Wedges	0.25	—	—
Ackermann & Heinrich (1970)	Wedges	0.29	124 (37°C)	—
Raj *et al.* (1971)	Autopsy	0.27	—	—
Nelson *et al.* (1971)	Autopsy	0.28	24 (25°C)	—
Davies & Thorgeirsson (1971)	Wedges	0.85	200 (37°C)	—
Schoene *et al.* (1972)	Needles	0.42 ± 0.10	69 ± 19 (25°C)	—
Ackermann (1972)	Wedges	0.20	—	—
Black *et al.* (1973)	Wedges	0.47 ± 0.22	199 ± 42 (?)	—
Darby & Grundy (1973)	Wedges	0.54 ± 0.14	—	—
Pelkonen *et al.* (1973)	Wedges	0.53	78 (22°C)	5.4 ± 2.5
Kamataki *et al.* (1973)	Autopsy	0.14, 0.78	71, 178 (25°C)	—
Pelkonen *et al.* (1974)	Wedges	0.59 ± 0.28	115 ± 30 (22°C)	2.73 ± 1.58
Pelkonen *et al.* (1975b)	Wedges	—	—	3.6 ± 1.6
Kapitulnik *et al.* (1977)	Autopsy	—	—	41.2 ± 37.7
Sato *et al.* (1977)	Needles	0.52 ± 0.17	—	—
Sontaniemi *et al.* (1978)	Needles	0.41 ± 0.07	—	—

Where indicated values are mean ± s.d.

inducible in man by cigarette smoking, presumably by polycyclic hydrocarbon constituents.

The presence of liver disease has a significant effect on both cytochrome P-450 content and AHH activity. In studies using whole homogenate of needle biopsies, liver disease was found to cause a marked reduction in cytochrome P-450 content (Hartoma, Sontaniemi, Pelkonen & Ahlqvist, 1977; Sato, Kamada, Abe, Suamatsu, Kawano, Hayashi, Matsumara & Hagihara, 1977). This has also been found in the present study, using microsomal fractions of needle biopsies. In addition AHH activity was significantly reduced in these biopsies. However, AHH turnover number was significantly increased in this group. The possible differential effect of liver disease on the different components of the monooxygenase system and on different forms of cytochrome P-450 has yet to be resolved. The influence of liver disease on hepatic monooxygenase activity will be considered in detail in a separate paper.

Studies with microsomal fractions are necessary for accurate comparison of specific activities of drug metabolizing enzymes and for the determination of such activities in the absence of soluble enzymes. The present study shows the feasibility of using microsomal fractions from small samples of human liver obtained by percutaneous needle biopsy in studies on substrate specificity of the monooxygenase system.

We wish to thank Drs T.J. Peters and V.S. Chadwick for making human liver samples available to us for use in these studies.

ARB is an MRC Research Fellow. Reprint requests should be addressed to Dr A.R. Boobis, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 0HS.

References

ACKERMANN, E. (1972). Entalkylierung von Athylmorphin und p-c-hydroxylierung von Anilin in Lebermikrosomen von Menschen und von mannlichen und weiblicken Ratten. *Biochem. Pharmac.*, **21**, 2168–2180.

ACKERMANN, E. & HEINRICH, I. (1970). Die Aktivitat der N- und O-demethylase in der leber des menschen. *Biochem. Pharmac.*, **19**, 327–342.

ALVARES, A.P., SCHILLING, G., LEVIN, W., KUNTZMAN, R., BRAND, L. & MARK, L.C. (1969). Cytochromes P-450 and b_5 in human liver microsomes. *Clin. Pharmac. Ther.*, **10**, 655–659.

ATLAS, S.A., BOOBIS, A.R., FELTON, J.S., THORGEIRSSON, S.S. & NEBERT, D.W. (1977). Ontogenic expression of polycyclic aromatic compound-inducible monooxygenase activities and forms of cytochrome P-450 in rabbit. Evidence for temporal control and organ specificity of two genetic regulatory systems. *J. biol. Chem.*, **252**, 4712–4721.

ATLAS, S.A., VESELL, E.S. & NEBERT, D.W. (1976). Genetic control of inter-individual variations in the inducibility of aryl hydrocarbon hydroxylase in cultured human lymphocytes. *Cancer Res.*, **36**, 4619–4630.

von BAHR, C., HEDLUND, I., KARLEN, B., BÄCKSTRÖM, D. & GRASDALEN, H. (1977). Evidence for two catalytically different binding sites of liver microsomal cytochrome P-450: importance for species and sex differences in oxidation pattern of lidocaine. *Acta pharmac. tox.*, **41**, 39–48.

BLACK, M., PERRETT, R.D. & CARTER, A.E. (1973). Hepatic bilirubin UDP-glucuronyl transferase activity and cytochrome P-450 content in a surgical population, and the effects of preoperative drug therapy. *J. lab. clin. Med.*, **81**, 704–712.

BOOBIS, A.R., NEBERT, D.W. & FELTON, J.S. (1977). Comparison of β-naphthoflavone and 3-methylcholanthrene as inducers of hepatic cytochrome(s) P-448 and aryl hydrocarbon (benzo(a)pyrene) hydroxylase activity. *Molec. Pharmac.*, **13**, 259–268.

BRODIE, B.B., AXELROD, J., COOPER, J.R., GAUDETTE, L., LA DU, B.N., MITOMA, C. & UDENFRIEND, S. (1955). Detoxication of drugs and other foreign compounds by liver microsomes. *Science, N.Y.*, **121**, 603–604.

COMAI, K. & GAYLOR, J.L. (1973). Existence and separation of three forms of cytochrome P-450 from rat liver microsomes. *J. biol. Chem.*, **248**, 4947–4955.

DARBY, F.J. & GRUNDY, R.K. (1973). The metabolism of (ureyl-^{14}C) tolbutamide *in vitro* and *in vivo* in man. *Life Sci.*, **13**, 97–105.

DAVIES, D.S. & THORGEIRSSON, S.S. (1971). Mechanism of hepatic drug oxidation and its relationship to individual differences in rates of drug oxidation in man. *Ann. N.Y. Acad. Sci.*, **179**, 411–420.

DAVIES, D.S., THORGEIRSSON, S.S., BRECKENRIDGE, A. & ORME, M. (1973). Interindividual differences in rates of drug oxidation in man. *Drug Metab. Disposition*, **1**, 411–417.

DECKERT, F.W. & REMMER, H.K. (1972). *In vitro* inhibition of rat and human liver microsomal enzymes by 4-hydroxycoumarin anticoagulants and related compounds. *Chem.-biol. Interact.*, **5**, 255–263.

DYBING, E., NELSON, S.D., MITCHELL, J.R., SASAME, H. & GILLETTE, J.R. (1976). Oxidation of α-methyldopa and other catechols by cytochrome P-450-generated superoxide anion: possible mechanism of methyldopa hepatitis. *Molec. Pharmac.*, **2**, 911–920.

FRASER, H.S., WILLIAMS, F.M., DAVIES, D.L., DRAFFAN, G.H. & DAVIES, D.S. (1976). Amylobarbitone hydroxylation kinetics in small samples of rat and human liver. *Xenobiotica*, **6**, 465–472.

GILLETTE, J.R. (1975). Formation of reactive drug metabolites as a basis of drug action and toxicity. *Israel J. Chem.*, **14**, 193–204.

GROVER, P.L. & SIMS, P. (1968). Enzyme-catalysed reactions of polycyclic hydrocarbons with deoxyribonucleic acid and protein *in vitro*. *Biochem. J.*, **110**, 159–160.

HARTOMA, T.R., SONTANIEMI, E.A., PELKONEN, O. & AHLQVIST, J. (1977). Serum zinc and serum copper and indices of drug metabolism in alcoholics. *Eur. J. clin. Pharmac.*, **12**, 147–151.

HAUGEN, D.A., COON, M.J. & NEBERT, D.W. (1976). Induction of multiple forms of mouse liver cyotchrome P-450. Evidence for genetically controlled *de novo* protein synthesis in response to treatment with β-naphthoflavone or phenobarbital. *J. biol. Chem.*, **251**, 1817–1827.

JOLLOW, D.J., MITCHELL, J.R., POTTER, W.Z., DAVIS, D.C., GILLETTE, J.R. & BRODIE, B.B. (1973). Acetaminophen-induced hepatic necrosis II. Role of covalent binding *in vivo*. *J. Pharmac. exp. Ther.*, **189**, 195–202.

KAMATAKI, T., KITADA, M. & KITAGAWA, H. (1973). Some observations on the microsomal electron transport system and activities of drug oxidizing enzymes in human liver. *Chem. pharm. Bull.*, **21**, 8–11.

KAPITULNIK, J., POPPERS, P.J. & CONNEY, A.H. (1977). Comparative metabolism of benzo(a)pyrene and drugs in human liver. *Clin. Pharmac. Ther.*, **21**, 166–176.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265–275.

LU, A.Y.H., LEVIN, W., WEST, S.B., JACOBSON, M., RYAN, D., KUNTZMAN, R. & CONNEY, A.H. (1973). Reconstituted liver microsomal enzyme system that hydroxylates drugs, other foreign compounds, and endogenous substrates VI. Different substrate specificities of the cytochrome P-450 fractions from control and phenobarbital-treated rats. *J. biol. Chem.*, **248**, 456–460.

MARTZ, F., FAILINGER, C. & BLAKE, D.A. (1977). Phenytoin teratogenesis: correlation between embryopathic effect and covalent binding of putative arene oxide metabolite to gestational tissue. *J. Pharmac. exp. Ther.*, **203**, 231–239.

MAZEL, P. (1971). Experiments illustrating drug metabolism *in vitro*. In *Fundamentals of Drug Metabolism and Drug Disposition*, eds. La Du, B.N., Mandel, H.G. & Way, E.L., pp. 546–582. Baltimore: The Williams and Wilkins Company.

NEBERT, D.W., LEVITT, R.C., ORLANDO, M.M. & FELTON, J.S. (1977). Effects of environmental chemicals on the genetic regulation of microsomal enzyme systems. *Clin. Pharmac. Ther.*, **22**, 640–658.

NELSON, E.B., RAJ, P.P., BELFI, K.J. & MASTERS, B.S.S. (1971). Oxidative drug metabolism in human liver microsomes. *J. Pharmac. exp. Ther.*, **178**, 580–588.

NELSON, S.D., MITCHELL, J.R., TIMBRELL, J.A., SNODGRASS, W.R. & CORCORAN, G.B. (1976). Isoniazid and iproniazid: activation of metabolites to toxic intermediates in man and rat. *Science, N.Y.*, **193**, 901–903.

OMURA, T. & SATO, R. (1964). The carbon monoxide-binding pigment of liver microsomes I. Evidence for its hemoprotein nature. *J. biol. Chem.*, **239**, 2370–2378.

PELKONEN, O. JOUPPILA, P., KALTIALA, E.H. & KÄRKI, n.t. (1975). Aryl hydrocarbon hydroxylase and cytochrome P-450 in the Human liver: fetal development and cigarette smoking. In *Proc. Eur. Soc. Tox.* Vol. **XVI**, *Developmental and genetic aspects of drug and environmental toxicity*, eds. Duncan, W.A.M., Julou, D. & Kramer, M. pp. 181–185. Amsterdam: Excerpta Medica.

PELKONEN, O., KALTIALA, E.H., KÄRKI, N.T., JALONEN, K. & PYÖRÄLÄ, K. (1975). Properties of benzpyrene hydroxylase from human liver and comparison with the rat, rabbit and guinea pig enzymes *Xenobiotica*, **8**, 501–509.

PELKONEN, O., KALTIALA, E.H., LARMI, T.K.I. & KÄRKI, N.T. (1973). Comparison of activities of drug-metabolizing enzymes in human fetal liver and adult livers. *Clin. Pharmac. Ther.*, **14**, 840–846.

PELKONEN, O., KALTIALA, E.H., LARMI, T.K.I. & KÄRKI, N.T. (1974). Cytochrome P-450-linked monooxygenase system and drug-induced spectral interactions in human liver microsomes. *Chem.-biol. Interact.*, **9**, 205–216.

RAJ, P., NELSON, E.B. & ESTABROOK, R.W. (1971). Studies on drug metabolism in human liver. *Chem.-biol. Interact.*, **3**, 303–304.

SATO, N., KAMADA, T., ABE, H., SUEMATSU, T., KAWANO, S., HAYASHI, N., MATSUMARA, T. & HAGIHARA, B. (1977). Simultaneous measurement of mitochondrial and microsomal cytochrome levels in human liver biopsy. *Clinica chim. Acta*, **80**, 243–251.

SCHOENE, B, FLEISCHMANN, R.A., REMMER, H. & VAN OLDERHAUSEN, H.F. (1972). Determination of drug metabolising enzymes in needle biopsies of human liver. *Eur. J. clin. Pharmac.*, **4**, 65–73.

SONTANIEMI, E.A., PELKONEN, O., AHOKAS, J., PIRTTIAHO, H.I. & AHLQVIST, J. (1978). Drug metabolism in epileptics: *in vivo* and *in vitro* correlations. *Br. J. clin. Pharmac.*, **5**, 71–76.

THOMAS, P.E., LU, A.Y.H., RYAN, D., WEST, S.B., KAWALEK, J. & LEVIN, W. (1976). Immunochemical evidence for six forms of rat liver cytochrome P-450 obtained using antibodies against purified rat liver cytochrome P-450 and P-448. *Molec. Pharmac.*, **12**, 746–758.

ULLRICH, V., WEBER, P. & WOLLENBERG, P. (1975). Tetrahydrofuran – an inhibitor for ethanol-induced liver microsomal cytochrome P-450. *Biochem. biophys. Res. Commun.*, **64**, 808–813.

WIEBEL, F.J., SELKIRK, J.K., GELBOIN, H.V., HAUGEN, D.A., VAN DER HOEVEN, T.A. & COON, M.J. (1975). Position-specific oxygenation of benzo(a)pyrene by different forms of purified cytochrome P-450 from rabbit liver. *Proc. Nat. Acad. Sci. USA*, **72**, 3917–3920.

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