Br. J. clin. Pharmac. (1983), **15**, 667–672

THE EFFECT OF ALCOHOLIC CIRRHOSIS ON THE ACTIVITIES OF MICROSOMAL ALDRIN EPOXIDASE, 7-ETHOXYCOUMARIN *O*-DE-ETHYLASE AND EPOXIDE HYDROLASE, AND ON THE CONCENTRATIONS OF REDUCED GLUTATHIONE IN HUMAN LIVER

K.W. WOODHOUSE, FAITH M. WILLIAMS, ELAINE MUTCH, P. WRIGHT[1], O.F.W. JAMES[2] & M.D. RAWLINS

Wolfson Unit of Clinical Pharmacology, [1]Department of Surgery and [2]Department of Medicine (Geriatrics), The University of Newcastle-upon-Tyne, Newcastle-upon-Tyne

1 Activities of the microsomal mono-oxygenases 7-ethoxycoumarin *O*-de-ethylase (EOC) and aldrin epoxidase (AE), together with microsomal epoxide hydrolase (EH) activity and concentrations of reduced glutathione (GSH) have been measured in liver from patients with alcoholic cirrhosis and in normals.

2 Activities of both mono-oxygenases were significantly reduced in alcoholic cirrhosis. EOC activity (pmol 7-OH coumarin formed/mg microsomal protein/min) was 108.0 ± 10.6 (n = 8) in normals and 60.9 ± 11.6 (n = 8) in alcoholic cirrhosis ($P < 0.01$). AE activity (pmol dieldrin formed/mg microsomal protein/min) was 58.9 ± 9.5 (n = 11) in normal liver biopsies and 29.9 ± 8.6 (n = 9) in alcoholic cirrhosis ($P < 0.05$).

3 Microsomal EH activity (nmol styrene glycol formed/mg microsomal protein/min) was similar in normals (39.2 ± 4.4, n = 11) and alcoholic cirrhosis (40.5 ± 9.1, n = 6).

4 GSH concentrations (μg GSH/g liver tissue) were lower ($P < 0.01$) in alcoholic cirrhosis (792 ± 73, n = 10) compared to normals (1182 ± 76, n = 6).

Introduction

The hepatic microsomal monooxygenases are responsible for the biotransformation of a wide variety of exogenous and endogenous substances. Although their primary role may be the conversion of lipotrophic agents to more polar compounds, and thus to facilitate their renal excretion, many substances may be 'activated' to highly reactive intermediates (frequently epoxides) which may be responsible for cytotoxicity or carcinogenicity (Miller & Miller, 1966, 1974, 1977; Jollow & Smith 1977; Sims 1980). Inactivation of reactive intermediates may proceed through several pathways, but particularly important are hydration of epoxides by epoxide hydrolase, and conjugation (enzymatic or non-enzymatic) with glutathione (Arias & Jakoby 1976; Oesch *et al.*, 1977).

Patients with alcoholic cirrhosis are at particular risk from adverse drug reactions (Naranjo *et al.*, 1978) and in addition, suffer a high incidence of primary liver cancer (Johnson *et al.*, 1978), a disease in which environmental carcinogens may play an important role. We have therefore examined the activities of two mono-oxygenases (7-ethoxycoumarin *O*-de-ethylase (EOC), and aldrin epoxidase, (AE)), epoxide hydrolase (EH), and the concentrations of reduced glutathione (GSH), in liver from patients with alcoholic cirrhosis and in normals.

Methods

Patients

Liver was obtained from patients undergoing diagnostic percutaneous Menghini needle biopsy, using tissue surplus to histological requirement. Normal liver was obtained either from patients who underwent liver biopsy during diagnostic workup and were subsequently shown to have normal histology, or needle biopsy taken at cholecystectomy. Most patients in the cirrhotic groups were receiving multivitamin supplements ('Multivite') and all but five (two in the EOC study and one each in the AE, EH and GSH studies) admitted to consuming greater than an estimated 60 g of alcohol per day. None of the 'normal' patients consumed over 20 g of alcohol per day. Other drug therapy and smoking habits are de-

0306–5251 83 0600–6670 $02.00

Table 1 Patient details

Parameter measured	*Histology (Number of biopsies)*	*Male : Female*	*Smokers*	*Liver function tests*			
				Serum albumin (g/l)	*Serum bilirubin (μmol/l)*	*Serum AST (iu/l)*	*Serum alkaline phosphatase (iu/l)*
Aldrin epoxidase	Normal ($n = 11$)	3 : 8	5	42.6 ± 0.9	10.2 ± 0.9	22.4 ± 2.0	99.1 ± 6.9
	Cirrhosis ($n = 9$)	7 : 2	6	33.4 ± 1.8	106.7 ± 71.2	65.1 ± 7.4	164.9 ± 21.6
7-ethoxycoumarin *O*-de-ethylase	Normal ($n = 8$)	3 : 5	4	42.5 ± 0.9	14.3 ± 4.0	28.0 ± 1.9	104.5 ± 17.4
	Cirrhosis ($n = 8$)	6 : 2	5	34.0 ± 1.7	45.0 ± 18.6	88.9 ± 29.1	230.0 ± 53.3
Epoxide hydrolase	Normal ($n = 11$)	6 : 5	4	42.5 ± 1.1	12.3 ± 1.5	26.0 ± 2.9	115.4 ± 12.6
	Cirrhosis ($n = 6$)	5 : 1	4	32.8 ± 2.2	36.0 ± 9.3	92.7 ± 22.4	135.7 ± 21.9
Reduced glutathione	Normal ($n = 6$)	4 : 2	4	43.5 ± 1.5	12.2 ± 2.0	41.1 ± 12.0	140.8 ± 34.3
	Cirrhosis ($n = 10$)	7 : 3	7	32.8 ± 1.7	53.9 ± 21.4	99.9 ± 22.4	229.3 ± 50.0
Microsomal protein recovery	Normal ($n = 10$	3 : 7	5	42.6 ± 1.0	10.3 ± 0.9	23.0 ± 2.1	98.6 ± 7.6
	Cirrhosis ($n = 8$)	6 : 2	5	33.3 ± 2.0	116.4 ± 80.0	66.8 ± 8.2	161.3 ± 24.1

AST = aspartate aminotransferase

tailed below. All patients in the disease group had fully established, histologically proven cirrhosis. Sex ratios, smoking habits and liver function tests are shown in Table 1.

Methodology

Storage Biopsy specimens for enzyme studies were either placed in ice cold buffer and assayed immediately or frozen in liquid nitrogen and stored at −80°C until analysis. All biopsies for GSH studies were immediately frozen and stored in liquid nitrogen until analysis. Only one parameter was usually measured in each biopsy due to availability of tissue (< 60 mg).

Aldrin epoxidase assay Biopsies were homogenised in ice-cold buffer (0.25 M potassium phosphate, 0.15 M potassium chloride, 1.0 mM EDTA, pH 7.25) using a glass-glass homogeniser. The homogenate was centrifuged at 10,000 *g* for 10 min and the supernatant removed and centrifuged at 100,000 *g* for 60 min. The microsomal pellet was resuspended in 0.25 M potassium phosphate buffer pH, 7.5, containing 30% v/v glycerol. AE activity was measured by determination of the rate of formation of the stable epoxide, dieldrin, from the aldrin substrate (100 μM) as previously described; dieldrin was measured by gas chromatography with electron capture detection (Wolff *et al.*, 1979; Williams *et al.*, 1981, 1982). Activity was expressed as pmol dieldrin formed/mg microsomal protein/min (pmol/mg/min).

7-Ethoxycoumarin O-de-ethylase assay Microsomes were prepared as above. The microsomal pellet was resuspended in 0.25 M potassium phosphate, 6.4 mM magnesium chloride buffer, pH 7.5, containing 30% v/v glycerol. EOC activity was determined using 7-ethoxycoumarin as substrate (1 mM), and measuring the rate of formation of the fluorescent metabolite, 7-hydroxycoumarin (Greenlee & Poland, 1978). Activity was expressed as pmol 7-hydroxycoumarin formed/mg microsomal protein/min (pmol/mg/min).

Epoxide hydrolase assay Biopsies were homogenised in ice-cold 0.15 M potassium chloride, 10 mM potassium phosphate buffer, (pH 8.0) and microsomes prepared as described above. The microsomal pellet was resuspended in this buffer. EH activity was measured using a modification of the method of Oesch, measuring the rate of formation of (3H) styrene glycol from (3H) styrene oxide and separating the reaction products by high pressure liquid chromatography (Oesch, 1974; O'Neill *et al.*, 1981). Activity was expressed as nmol styrene glycol formed/mg microsomal protein/min (nmol/mg/min).

Reduced glutathione assay Biopsies were homogenised in 4 ml buffer (0.1 M sodium phosphate, 0.005 M EDTA, pH 8.0) and 1 ml 25% metaphosphoric acid as a protein precipitant. The homogenate was centrifuged at 10,000 *g* for 30 min, and the supernatant used for GSH estimation using the fluorometric method of Hissin & Hilf (1976). This method, however, proved unsuitable for the measurement of oxidised glutathione. Concentrations are expressed as μg GSH/g wet weight liver (μg GHS/g).

Protein assay Microsomal protein concentrations were measured by the method of Lowry *et al.* (1951).

Statistical analysis Results are expressed as mean ± s.e. mean. The significance of differences between means was assessed by Student's *t*-test for unpaired values.

Results

Aldrin epoxidase

Microsomal AE activity (Figure 1) was measured in 20 liver biopsies from 11 normals, and 9 alcoholic cirrhotics. Two patients in the cirrhotic group were taking carbamazepine and one of these was also taking phenytoin. AE activity was significantly lower ($P < 0.05$) in alcoholic cirrhosis (29.9 ± 8.6 pmol/mg/min) than in normals (58.9 ± 9.5 pmol/mg/min).

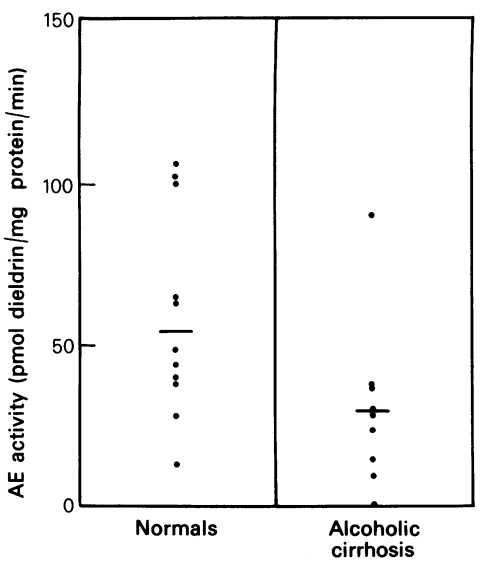

Figure 1 Microsomal aldrin epoxidase (AE) activity in normal and cirrhotic human liver ($P < 0.05$).

7-ethoxycoumarin O-de-ethylase

Microsomal EOC activity (Figure 2) was measured in 16 liver biopsies from eight normals and eight alcoholic cirrhotics. Two patients in the cirrhotic group were taking spironolactone. As with AE, EOC activity was significantly lower ($P < 0.01$) in biopsies from patients with alcoholic cirrhosis (60.9 ± 11.6 pmol/mg/min) than in normals (108.0 ± 10.6 pmol/mg/min).

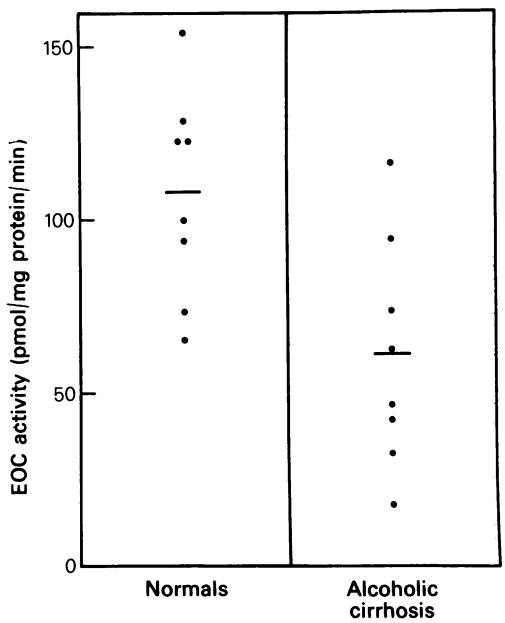

Figure 2 Microsomal 7-ethoxycoumarin-*O*-de-ethylase (EOC) activity in normal and cirrhotic human liver ($P < 0.01$).

Epoxide hydrolase

Microsomal EH activity (Figure 3) was measured in tissue from 17 patients, comprising 11 normals, and six alcoholic cirrhotics. No patient was on drugs known to interfere with microsomal enzyme activity. EH activity was 39.2 ± 4.4 nmol/mg/min in normals and 40.5 ± 9.1 nmol/mg/min in alcoholic cirrhosis ($P > 0.8$).

Reduced glutathione

GSH concentrations (Figure 4) were measured in 16 specimens (all obtained under local anaesthesia), comprising six normals and 10 with alcoholic cirrhosis. One patient in the 'normal' group was taking frusemide and spironolactone. GSH concentrations were again

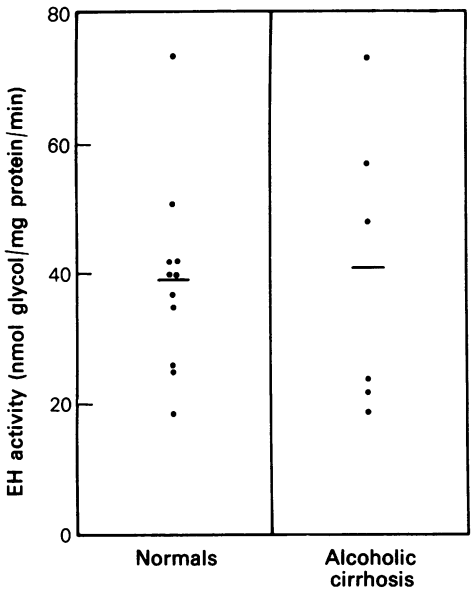

Figure 3 Microsomal epoxide hydrolase (EH) activity in normal and cirrhotic human liver ($P > 0.8$).

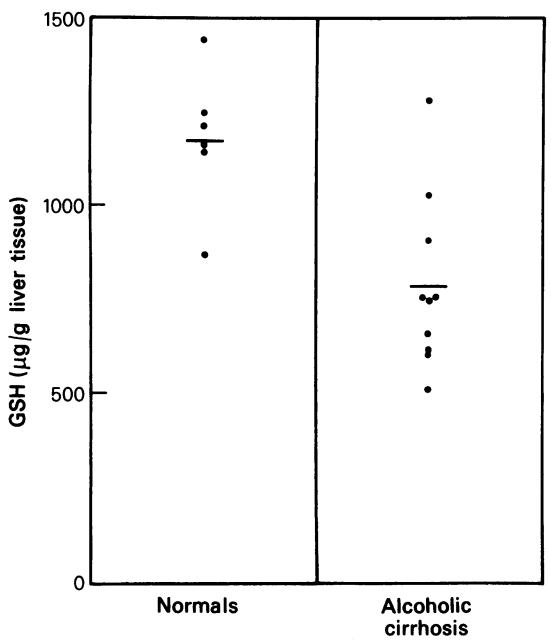

Figure 4 Reduced glutathione concentrations in normal and cirrhotic human liver ($P < 0.01$).

lower in the cirrhotic group (792 ± 73 μg GSH/g) than in normals (1182 ± 76 μg GSH/g), ($P < 0.01$).

Microsomal protein content

Microsomal protein content, expressed as mg microsomal protein/g wet weight liver (mg protein/g) was estimated in 18 specimens (used in the AE study). Two patients in the cirrhotic group were taking carbamazepine and one of them was also taking phenytoin. Recovery of microsomal protein was similar in biopsies from patients with alcoholic cirrhosis (10.6 ± 2.6 mg protein/g, $n = 8$) and normals (11.5 ± 2.0 mg protein/g, $n = 10$).

Discussion

In this study several pathways involved in the hepatic metabolism of drugs and environmental agents have been examined *in vitro* in tissue from patients with alcoholic cirrhosis and from normals. We have demonstrated that in alcoholic cirrhosis there is a significant decrease in the activities of the microsomal monooxygenases aldrin epoxidase and 7-ethoxycoumarin *O*-de-ethylase when compared with normals. In addition, we have shown that hepatic reduced glutathione content is also diminished in these patients. By contrast, microsomal epoxide hydrolase activity appears to be unaltered.

These findings are compatible with the work of Boobis *et al.* (1980) and Brodie *et al.* (1981) who reported that the activity of the hepatic microsomal monooxygenase aryl hydrocarbon hydroxylase (AHH) is decreased in cirrhotic liver of mixed aetiology. Since a greater proportion of our cirrhotic patients were smokers, their lower monooxygenase activities cannot be attributed to lack of induction by tobacco consumption. Similarly, the only patients receiving drugs which induce microsomal monooxygenases were in the cirrhotic group. Most of our cirrhotic patients continued to have a significant alcohol intake, and as chronic ethanol exposure has been shown in man to cause a reduction in monooxygenase (AHH) activity which may be unrelated to the degree of liver damage (Brodie *et al.*, 1981), both factors may have contributed to the reduction in EOC and AE activities in our cirrhotic patients.

The demonstration of a parallel reduction in the activities of AE and EOC is particularly interesting. Studies using human liver have shown that AE activity is inhibited by metyrapone, but to a lesser extent by α-naphthoflavone (Williams *et al.*, 1982). EOC activity (at high substrate concentrations) is also inhibited by metyrapone but is induced by α-naphthoflavone (Boobis *et al.*, 1981). These observations suggest that two populations of cytochome P-450 are involved in the two reactions. Our results, together with those of others (Brodie *et al.*, 1981) therefore indicate that

there is a broad impairment of hepatic microsomal monooxygenase activity in alcoholic cirrhosis.

Microsomal epoxide hydrolase activity was similar in alcoholic cirrhotics and normals, thus suggesting that whilst the cytochrome P-450 dependent monooxygenases decrease in parallel in alcoholic cirrhosis, there is a differential loss of some microsomal enzymes, with EH remaining intact. Similarly, Boobis *et al.* (1980) have shown that activity of microsomal NADPH cytochrome c-reductase is unaltered by the presence of severe liver disease.

Concentrations of reduced glutathione were, however, lower in cirrhotic liver compared to normals. In view of the similar microsomal protein yield from cirrhotic and normal liver, we feel that this is probably a true reduction, rather than simply a reflection of a smaller number of functional hepatocytes in biopsies from cirrhotic liver. Both acute and chronic exposure to alcohol have been shown to cause a reduction in hepatic GSH content in rats (Fernandez & Videla, 1981), and from our results, it is not possible to say if the observed reduction is due simply to the presence of severe liver damage or a consequence of continued heavy alcohol intake.

These results have several implications. First, we confirm that there is diminished hepatic monooxygenase activity, *in vitro*, in tissue from patients with alcoholic cirrhosis, and of a magnitude which can explain the impaired intrinsic clearance of drugs metabolised by this system *in vivo*. Moreover, a reduced intrinsic clearance will account for the increased incidence of type A (dose-dependent) adverse drug effects in cirrhotics. Second, the unaltered EH activity in alcoholic cirrhosis suggests that these patients would not be especially susceptible to hepatotoxicity from agents whose toxic intermediates are inactivated by enzymatic hydration. By contrast, hepatotoxic xenobiotic intermediates which are predominantly inactivated by conjugation with glutathione, may be more likely to be cytotoxic or carcinogenic in patients with this disorder.

Finally, our finding that two mono-oxygenase reactions dependent on different cytochrome P-450 populations are reduced in parallel in alcoholic cirrhosis, emphasises the need to investigate the effects of liver disease on different cytochrome P-450 species in man.

This work was supported by the North of England Cancer Research Campaign.

K.W. Woodhouse is an MRC Training Fellow.

References

ARIAS, I.M. & JAKOBY, W.B. (1976). *Glutathione, metabolism and function.* New York: Raven Press.

BOOBIS, A.R., BRODIE, M.J., KAHN, G.C., FLETCHER, D.R., SAUNDERS, J.H. & DAVIES, D.S. (1980). Monooxygenase activity of human liver in microsomal fractions of needle biopsy specimens. *Br. J. clin. Pharmac.*, **9**, 11–19.

BOOBIS, A.R., KAHN, G.C., WHYTE, C., BRODIE, M.J. & DAVIES, D.S. (1981). Biphasic 0-deethylation of phenacetin and 7-ethoxycoumarin by human and rat liver microsomal fractions. *Biochem. Pharmac.*, **30**, 2451–2456.

BRODIE, M.J., BOOBIS, A.R., BULPITT, C.J. & DAVIES, D.S. (1981). Influence of liver disease and environmental factors on hepatic mono-oxygenase activity *in vitro*. *Eur. J. clin. Pharmac.*, **20**, 39–46.

FARRELL, G.C., COOKSLEY, W.G.E. & ROWELL, L.W. (1979). Drug metabolism in liver disease: Activity of hepatic microsomal metabolising enzymes. *Clin. Pharmac. Ther.*, **26**, 483–492.

FERNANDEZ, V. & VIDELA, L.A. (1981). Effect of acute and chronic ethanol ingestion of the content of reduced glutathione of various tissues of the rat. *Experientia*, **37**, 392–394.

GREENLEE, W.F. & POLAND, A. (1978). An improved assay of 7-ethoxycoumarin 0-deethylase activity: Induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-doxin. *J. Pharmac. exp. Ther.*, **205**, 596–656.

HISSIN, P.J. & HILF, R. (1976). A fluorimetric method for determination of oxidised and reduced glutathione in tissue. *Analyt. Biochem.*, **74**, 214–226.

JOHNSON, P.J., KRASER, B., PORTMAN, B., EDDLESTON, A.L.W.P. & WILLIAMS, R. (1978). Hepatocellular carcinoma in Great Britain: Influence of age, sex, HBsAg status and aetiology of underlying cirrhosis. *Gut*, **19**, 1022–1026.

JOLLOW, D.J. & SMIGH, C. (1977). Biochemical aspects of toxic metabolites: Formation, detoxication and covalent binding. In *Biological reactive intermediates*, eds Jollow, D.J., Kocsis, J.J., Snyder, R. & Vainio, H., pp. 42–59. New York: Plenum Press.

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

MILLER, E.C. & MILLER, J.A. (1966). Mechanisms of chemical carcinogens: Nature of proximate carcinogens and interactions with macromolecules. *Pharmac. Rev.*, **18**, 805–838.

MILLER, E.C. & MILLER, J.A. (1974). Biochemical mechanisms of chemical carcinogenesis. In *Molecular Biology of Cancer*, ed Busch, H., pp. 377–402. New York: Academic Press.

MILLER, J.A. & MILLER, E.C. (1977). The concept of reactive electrophilic metabolites in chemical carcinogenesis: Recent results with aromatic amines, Safrole and Aflatoxin B. In *Biological reactive intermediates*, eds Jollow, D.J., Kocsis, J.J., Snyder, R. & Vainio, H., pp. 6–24 New York: Plenum Press.

NARANJO, C.A., BUSTO, U. & MARDONES, R. (1978). Adverse drug reactions in liver cirrhosis. *Eur. J. clin. Pharmac.*, **13**, 429–434.

OESCH, F. (1974). Purification and specificity of a human microsomal epoxide hydrolase. *Biochem. J.*, **139**, 77–78.

OESCH, F., BENTLEY, P. & GIATT, H.R. (1977). Epoxide hydrolase: Purification to apparent homogeneity as a specific probe for the relative importance of epoxides among other reactive metabolites. In *Biological reactive intermediates*, eds Jollow, D.J., Kocsis, J.J., Snyder, R. & Vainio, H., pp. 181–206. New York: Plenum Press.

O'NEILL, V.A., RAWLINS, M.D. & CHAPMAN, P.H. (1981). Epoxide hydrolase activity in human skin. *Br. J. clin. Pharmac.*, **12**, 517–522.

SIMS, P. (1980). The metabolic activation of chemical carcinogens. *Br. med. Bull.*, **36**, 11–18.

WILLIAMS, FAITH M., WOODHOUSE, K.W., JAMES O.F.W. & RAWLINS, M.D. (1981). Aldrin epoxidation by human liver microsomes. *Br. J. clin. Pharmac.*, **13**, 595P.

WILLIAMS, FAITH M., WOODHOUSE, K.W., MIDDLETON, D., WRIGHT, P., JAMES, O. & RAWLINS, M.D. (1982). Aldrin epoxidation in small samples of human liver. *Biochem. Pharmac.*, **31**, 3701–3703.

WOLFF, T., DEML, E. & WANDERS, H. (1979). Aldrin epoxidation, a highly sensitive indicator specific for cytochrome P-450-dependent mono-oxygenase activities. *Drug Metab. Dispos.*, **7**, 301–305.

(Received October 29, 1982, accepted February 8, 1983)