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GLUCURONIC ACID CONJUGATION BY HEPATIC MICROSOMAL FRACTIONS ISOLATED FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Abstract—The hepatic glucuronidation of 1-naphthol and 4-nitrophenol (3-methylcholanthrene inducible substrates of glucuronyltransferase, GT 1) was found to be deficient in freshly prepared untreated "native" microsomes from streptozotocin-induced diabetic male rats. The defect was not observed in female rats. Moreover, the glucuronidation of 1-naphthol and 4-nitrophenol was higher in "native" microsomes from male control rats than in those from female controls. This sex difference in the glucuronidation of the GT 1 substrates was abolished by detergent activation of the transferase enzyme *in vitro*. Streptozotocin treatment did not alter the glucuronidation of paracetamol or phenolphthalein (phenobarbitone inducible substrates for GT 2). This diabetes-induced defect in the glucuronidation of GT 1 substrates was abolished by insulin treatment of the animals and was diminished or completely abolished by detergent activation of the transferase enzyme *in vitro*. Increased membrane constraint is proposed as the mechanism responsible for the transferase defect. 3-Methylcholanthrene induction abolished the streptozotocin-induced defect in 4-nitrophenol glucuronidation, whereas phenobarbitone did not. This is attributed to the differential effect of these inducers on the microsomal membrane.

There are many reports of altered oxidative metabolism of xenobiotics in experimentally-induced diabetes [1–6]. However, only a few investigations have concentrated on the effect of experimentally-induced diabetes on drug conjugation reactions and those have produced conflicting results [7–10]. In this paper the effect of streptozotocin-induced diabetes on the activity of hepatic microsomal glucuronyltransferase is reported.

Glucuronidation is, quantitatively, the most important conjugation reaction, glucuronyltransferases (EC 2.4.1.17) converting a wide range of xenobiotics and endogenous compounds to biologically inactive glucuronides which are readily eliminated. The glucuronyltransferases are membrane bound microsomal enzymes, the activity of which can be readily influenced by perturbations of the membrane environment, such as exposure to detergents, storage or sonication.

The glucuronidation of 4-nitrophenol has been shown previously to be deficient in freshly prepared untreated ("native") liver microsomes from male streptozotocin diabetic rats [11, 12]. The defect was abolished by maximal detergent activation of the microsomes, whereas allosteric activation of the transferase enzyme by UDP *N*-acetylglucosamine did not alter the diabetes-induced effect on glucuronidation. It was suggested that increased latency of the transferase enzyme in streptozotocin treated animals in responsible for the defect.

In this study the observations on 4-nitrophenol glucuronidation are extended to investigate the substrate specificity of the streptozotocin-induced defect. The ability of phenobarbitone and 3-methylcholanthrene to induce glucuronyltransferase activity in diabetic rats was also investigated.

The substrate specificity of the glucuronyltransferases has been characterized both in terms of induction [13, 14] and perinatal development [15], nevertheless, the heterogeneity of the enzyme is still the subject of much debate [16, 17]. In the present study 1-naphthol and 4-nitrophenol were chosen as substrates for the 3-methylcholanthrene inducible form (GT 1) of the transferase [13, 14] and phenolphthalein and paracetamol as substrates for the phenobarbitone inducible transferase (GT 2) [13, 14].

MATERIALS AND METHODS

Materials

UDP-glucuronic acid, tetrabutylammonium hydrogen sulphate, 4-nitrophenol glucuronide, phenolphthalein glucuronide and 1-naphthol glucuronide were obtained from Sigma Chemical Company (Poole, U.K.). Saccharo-1,4-lactone was obtained from Calbiochem (C.P. Laboratories, Bishops Stortford, U.K.) and sodium phenobarbitone and Triton X-100 were obtained from BDH Chemicals Limited (Poole, U.K.). 3-Methylcholanthrene was supplied by Fluka Chemical Company, Germany. Protamine-zinc insulin from Wellcome Medical Division was used to treat the animals. The HPLC packings, Spherisorb 5 ODS and Lichroprep RP18 were obtained from HPLC Technology Limited (Macclesfield, U.K.).

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Methods

Abbreviations

When referring to buffer solutions in microsomal experiments (2-amino-2-hydroxymethyl)-propane-1,3-diol is abbreviated to Tris.

Treatment of animals

Male and female Sprague–Dawley rats (180–220 g) were used.

Streptozotocin treatment. Rats were starved for 24 hr prior to receiving 60 mg/kg streptozotocin i.v. in acetate buffer, pH 4.5, on day 1. Controls received an equivalent volume of the vehicle (0.1 ml/100 g). On day 6 the rats were divided into two groups. The first group was killed, liver microsomes prepared and glucuronyltransferase activity measured. The second group received daily treatment with 16 IU insulin, subcutaneously, for 6 days before hepatic glucuronyltransferase activity was measured on day 12.

Phenobarbitone treatment. Sodium phenobarbitone was administered as an 80 mg/kg i.p. dose daily for 3 days before glucuronidation was measured. Rats were killed 24 hr after the last phenobarbitone injection.

3-Methylcholanthrene treatment. 3-Methylcholanthrene was administered once as an 80 mg/kg dose, i.p., in 0.8 ml/100 g corn oil. The corn oil itself had no effect on glucuronyltransferase activity. Three days after treatment the rats were used for experiments.

The dosage-regimens for phenobarbitone and 3-methylcholanthrene are those used previously to induce microsomal enzymes, including glucuronyltransferase [13, 18, 19].

Assessment of the induced diabetes

Blood and urinary glucose concentrations were determined by a specific enzymatic method using *O*-dianisidine as the chromogen according to the Sigma Technical Bulletin No. 310. Clinitest tablets and Dextrostix were also used to obtain estimations of urine and blood glucose concentrations respectively. These diagnostic aids were obtained from Ames Co. Miles Laboratories (Slough, U.K.).

On day 6 streptozotocin treated rats had a blood glucose concentration of 380 ± 26 mg/100 ml (N = 8) compared with 99 ± 3 mg/100 ml (N = 8) in control rats. Between 4 and 5 days after streptozotocin treatment rats excreted approximately 16 g glucose/24 hr in the urine. If insulin treatment was not initiated on day 6, the physical condition of the rats deteriorated steadily. The main signs observed were polydipsia, polyuria, glycosuria, elevated blood glucose concentration (>350 mg/100 ml), loss of abdominal fat and considerable body weight loss. All the signs that developed in response to streptozotocin treatment were readily reversed within 48 hr by insulin administration. The insulin dose administered to the diabetic rats was chosen such that urinary glucose concentrations were within the normal range (3–6 mg glucose excreted in 24 hr) on day 12 and blood glucose concentrations were 103 ± 5 mg/100 ml at this time.

Preparation of hepatic microsomal fractions and incubation conditions for measuring glucuronidation

Hepatic microsomes were prepared by conventional ultracentrifugation techniques [20]. In order to stabilize cytochrome P450 15% (v/v) glycerol was added to the buffers in which the microsomes were prepared [21]. Glucuronide formation was measured within 30 min of preparing the microsomal fractions. For activation the microsomes were pre-incubated with 0.25% (v/v) Triton X-100 at room temperature for 10 min. This concentration of detergent was found to cause maximum activation of glucuronide formation with each substrate.

Table 1 shows the incubation conditions used to measure the formation of glucuronide from each of the four substrates. The conditions were selected such that the formation of glucuronide was linear with respect to time and protein concentration. Incubations were carried out in 0.1 M Tris–HCl buffer, pH 7.4. The phenolphthalein incubations contained 0.25 moles/l methanol; this has been shown previously to have no significant effect on glucuronidation [22].

Analytical methods

Glucuronidation of 4-nitrophenol was measured by the disappearance of substrate using the spectrophotometric method [23]. The values for glucuronide formation obtained using this method were not significantly different from those obtained using a direct

Table 1. Incubation conditions

	4-Nitrophenol	1-Naphthol	Phenolphthalein	Paracetamol
Substrate (mM)	0.2	0.1	0.1	1.5
UDP-glucuronic acid (mM)	2	3	5	5
Saccharo-1,4-lactone (mM)	5	5	5	5
$MgCl_2$ (mM)	—	—	—	10
Protein (mg)	3–5	2–3	3–5	3–5
Total volume (ml)	2.5	1.0	1.0	1.0
Incubation time (min)				
"native"	30	5	15	30
activated	15	2	5	15

The final volume was made up using 0.1 M Tris–HCl buffer, pH 7.4.

Table 2. "Native" glucuronyltransferase activity (nmoles glucuronide formed/mg protein/min)

	4-Nitrophenol	1-Naphthol	Phenolphthalein	Paracetamol
Control male rats	1.24 ± 0.13 (7)	5.70 ± 0.44 (5)	0.62 ± 0.04 (6)	0.24 ± 0.01 (6)
Streptozotocin treated male rats	0.61 ± 0.09* (7)	2.56 ± 0.15* (5)	0.53 ± 0.07 (6)	0.25 ± 0.02 (6)
Streptozotocin + insulin treated male rats	1.18 ± 0.07 (6)	5.98 ± 0.30 (4)	—	—
Control female rats	0.84 ± 0.04† (7)	4.00 ± 0.25† (5)	0.59 ± 0.06 (4)	0.28 ± 0.02 (4)

Results are expressed as mean ± S.E. mean, with the number of animals used given in parentheses.
* $P < 0.005$; † $P < 0.05$.
P values refer to differences between control male rats and treated animals for each substrate.

high pressure liquid chromatographic (HPLC) method for quantification of glucuronide formed.

Formation of paracetamol, 1-naphthol and phenolphthalein glucuronides was routinely quantified using reversed phase HPLC. For measurement of 4-nitrophenol, 1-naphthol and phenolphthalein glucuronides, tetrabutylammonium hydrogen sulphate was used as the ion-pairing agent to prolong the retention of the conjugates on the column [24].

Paracetamol and its glucuronide were quantified using the HPLC method described by Adriaenssens and Prescott [25]. Detection of all the substrates and conjugates was by ultraviolet absorbance, at the wavelength of optimum absorbance of the glucuronides. Quantification was by peak height ratios, using appropriate internal standards, and for all eluents measured the standard curves were linear over the range of concentrations used.

Statistical analysis

Means of groups were compared using one way analysis of variance, with Bonferroni probabilities used for the *t*-tests [26].

RESULTS

The glucuronyltransferase activities towards each substrate using untreated "native" microsomes and Triton X-100 activated microsomes are shown in Tables 2 and 3 respectively. With 1-naphthol and 4-nitrophenol as substrates, "native" glucuronyltransferase activity was significantly decreased ($P < 0.005$) in diabetic rats compared with controls. This decrease in glucuronidation in diabetic rats was abolished by treating the animals with insulin. If insulin treatment was not initiated on day 6, then the glucuronidation of both 4-nitrophenol and 1-naphthol in diabetic rats 14 days after streptozotocin treatment was still significantly lower than that in insulin-treated rats (data not shown). No decrease in "native" glucuronyltransferase activity occurred in diabetic rats when paracetamol or phenolphthalein was used as a substrate.

There was a sex difference observed in the formation of 1-naphthol and 4-nitrophenol glucuronides by "native" microsomes. The control male rats produced significantly more glucuronide than did females ($P < 0.05$). The formation of paracetamol and phenolphthalein glucuronides was similar in both sexes. After optimal detergent activation the sex difference in the formation of both 4-nitrophenol and 1-naphthol glucuronides was abolished (Table 3).

There was no significant difference in the glucuronidation of 4-nitrophenol in diabetic rats compared with controls after maximal detergent activation. After activation the glucuronidation of 1-naphthol was still significantly lower in diabetic rats ($P < 0.01$), although the difference in activated enzyme activity between the control and diabetic group of rats was less than in "native" preparations.

It therefore appears that only the 3-methylcholanthrene inducible GT 1 type substrates studied show sex differences and streptozotocin-induced differences in glucuronyltransferase activity.

The ability of phenobarbitone and 3-methyl-

Table 3. Activated glucuronyltransferase activity (nmoles glucuronide formed/mg protein/min)

	4-Nitrophenol	1-Naphthol	Phenolphthalein	Paracetamol
Control male rats	5.78 ± 0.31 (7)	13.54 ± 1.23 (5)	2.14 ± 0.06 (6)	1.02 ± 0.04 (6)
Streptozotocin treated male rats	5.25 ± 0.54 (7)	9.52 ± 0.44* (5)	1.87 ± 0.12 (6)	1.06 ± 0.07 (6)
Streptozotocin + insulin treated male rats	5.87 ± 0.30 (6)	13.24 ± 0.56 (4)	—	—
Control female rats	5.14 ± 0.35 (7)	11.18 ± 0.38 (5)	2.01 ± 0.10 (4)	—

Results are expressed as mean ± S.E. mean with the number of animals used given in parentheses.
* $P < 0.01$.
P value refers to difference between control male rats and streptozotocin-treated male rats.

Table 4. The effect of inducers on "native" 4-nitrophenol glucuronyltransferase activity in male rats (nmoles glucuronide formed/mg protein/min)

	Phenobarbitone		3-Methylcholanthrene	
	Non-induced	Phenobarbitone-induced	Non-induced	3-Methylcholanthrene-induced
Control	1.38 ± 0.08 (4)	1.70 ± 0.11 (8)	1.55 ± 0.09 (4)	4.17 ± 0.24† (6)
Diabetic	0.77 ± 0.04* (4)	1.17 ± 0.03* (4)	0.96 ± 0.07 (4)	4.14 ± 0.18† (6)

Results are expressed as mean ± S.E. mean, with the number of animals used given in parentheses.
* $P < 0.01$; † $P < 0.001$.
P values refer to differences between control rats and streptozotocin treated rats (*) and between control rats and 3-methylcholanthrene induced rats (†).

cholanthrene to induce male diabetic rat glucuronyltransferase was investigated using 4-nitrophenol as substrate (Table 4). 3-Methylcholanthrene induced 4-nitrophenol glucuronidation by the "native" form of the enzyme to a greater extent than phenobarbitone. After phenobarbitone induction there was still a significant difference in the formation of glucuronide between control and diabetic rat microsomes, whereas 3-methylcholanthrene induction abolished the difference in glucuronidation between control and diabetic rat microsomes.

Table 5 shows the effect of both inducing agents on activated glucuronyltransferase in control and diabetic rats. The inducing effects of both phenobarbitone and 3-methylcholanthrene were less marked in the activated preparations compared with the "native" microsomes. After activation by Triton X-100 there was no significant difference in the formation of 4-nitrophenol glucuronide between control and diabetic rat microsomes from either induced or non-induced livers.

DISCUSSION

In a preliminary study the glucuronidation of 4-nitrophenol was shown to be deficient in "native" microsomes prepared from male streptozotocin-induced diabetic rats [12]. The results presented in this paper have extended these observations and demonstrate that streptozotocin-induced diabetic rats have lower "native" microsomal glucuronyltransferase activity towards GT 1 substrates, but not towards GT 2 substrates.

Triton X-100 minimized the defect in glucuronidation when added to the microsomal incubations to activate the transferase. This detergent is known to interact with, and alter the composition of, the microsomal membrane. It is thought to activate the transferase by decreasing the membrane constraint, thus leading either to a change in enzyme protein conformation or to an increase in the number of active sites on the enzyme that are available to the substrate [27]. Triton X-100 activation was previously shown to abolish the diabetes-induced defect in 4-nitrophenol glucuronyltransferase activity, whereas UDP *N*-acetylglucosamine activation was ineffective in this respect [12]. This latter agent allosterically activates the enzyme in the presence of divalent metal ions [27]. Since activation by membrane perturbation was found to abolish the diabetes-induced defect in 4-nitrophenol glucuronidation, it was suggested that the defect was due to an alteration in the microsomal membrane in diabetic rats.

In the present study the defect in 1-naphthol glucuronidation was not completely abolished by Triton X-100 activation, although it was diminished. The explanation for this residual difference in 1-naphthol glucuronidation is not known. It may be due to a deficiency in an enzyme protein which selectively conjugates 1-naphthol but not 4-nitrophenol. Alternatively, since the rate of 1-naphthol glucuronidation is considerably higher than that of 4-nitrophenol, any slight alteration in the microsomal capacity for glucuronidation will be magnified. Current evidence on enzyme heterogeneity suggests that the latter explanation is more likely. Transferase activity

Table 5. The effect of inducers on activated 4-nitrophenol glucuronyltransferase in male rats (nmoles glucuronide formed/mg protein/min)

	Phenobarbitone		3-Methylcholanthrene	
	Non-induced	Phenobarbitone-induced	Non-induced	3-Methylcholanthrene-induced
Control	5.73 ± 0.18 (4)	6.70 ± 0.46 (8)	7.29 ± 0.67 (4)	12.78 ± 0.60* (6)
Diabetic	5.77 ± 0.15 (4)	6.38 ± 0.19 (4)	6.29 ± 0.30 (4)	13.46 ± 1.14* (6)

Results are expressed as mean ± S.E. mean, with the number of animals used given in parentheses.
* $P < 0.01$.
P values refer to differences between non-induced and induced animals.

towards 1-naphthol and 4-nitrophenol can be co-purified by chromatographic and immunochemical methods [14, 28, 29].

It appears, therefore, that in addition to an alteration in the membrane environment surrounding glucuronyltransferase, another factor also contributes to the defective glucuronidation of GT 1 substrates in streptozotocin-induced diabetes, but only when the system is operating at maximum capacity. There may be an alteration in the protein structure of a transferase enzyme in the streptozotocin diabetic rats similar to that reported by Past and Cook for cytochrome P-450 in alloxan-induced diabetes [30, 31].

The nature of the diabetes-induced alteration in microsomal membrane structure is not known at present. Streptozotocin-induced diabetes causes disturbances in lipid metabolism and cellular lipid composition [32, 33]. Furthermore, Chandramouli and Carter have demonstrated cell membrane changes in chemically-induced diabetic rats [34]. Specific alterations in the lipid composition of liver microsomes from both genetically hyperglycaemic (ob/ob and db/db) mice and streptozotocin-induced diabetic mice have been suggested to be responsible for increased latency of the membrane bound enzyme glutathione-insulin-transhydrogenase and for alterations in monooxygenase activity [35, 36].

The present study demonstrated that there was a sex difference in the glucuronidation of GT 1 substrates, the male rats forming more glucuronide than the females. Although sex differences in monooxygenase activity are well documented in the rat, rather less is known about the sex-dependent characteristics of the enzymes of conjugation. Chhabra and Fouts investigated the sex dependence of 4-nitrophenol glucuronidation and found that male rat liver was more active than that of female [23]. These sex dependent characteristics of metabolism are under the control of the sex hormones. Kato and Gillette have observed that alloxan diabetes in castrated male rats did not cause any reduction in the metabolism of sex-dependent substrates [2]. This suggests that experimental diabetes interferes with the stimulatory effect of androgenic hormones on metabolism in male rats. With respect to glucuronyltransferase this interference appears to take place in the liver and not at the level of the pituitary gland since the *in vitro* activation of the transferase by Triton X-100 abolishes the sex difference.

The most important result from the induction experiments was that 3-methylcholanthrene abolished the effect of diabetes on "native" glucuronyltransferase activity whereas phenobarbitone did not. Both phenobarbitone and 3-methylcholanthrene have been shown to cause alterations in the protein–lipid interactions of the microsomal membrane which result in changes in membrane viscosity [37–39]. 3-Methylcholanthrene modifies mainly the lipid areas of the membrane, while phenobarbitone increases the amount of protein in the outer membrane layer [40, 41]. It is thought that 3-methylcholanthrene does not primarily induce the synthesis of new membrane, but changes the environmental conditions surrounding the enzymes [40, 41]. It is probable that 3-methylcholanthrene induction abolishes the effect of streptozotocin-induced diabetes on "native" glucuronyltransferase activity, at least in part, by altering the membrane composition and not by *de novo* synthesis.

In a recent study Warren *et al.* showed that deficient monooxygenase activity occurs in spontaneously diabetic Wistar rats analogous to that observed in streptozotocin-induced diabetic rats [42]. This is an important finding and the implications of the results obtained in diabetic animals must be assessed for their relevance to diabetic patients. Diabetic patients often receive a plethora of different drugs concomitantly with either oral hypoglycaemic or insulin therapy [43]. Any deficiency in the drug detoxifying mechanisms could therefore lead to a greater incidence of adverse side effects of drugs in diabetic patients. Very little is known about the heterogeneity of human glucuronyltransferase, but recently Leakey *et al.* have shown similar substrate specificity for the enzyme in Rhesus monkey liver as for that in rat liver [44]. There is considerable overlap in the substrate specificity of different transferases in rat liver, and drugs, for example morphine, with both planar and non-planar parts in their structure can be conjugated by more than one form [17]. It is important, therefore, to gain further information on the heterogeneity of human glucuronyltransferase before the risk to diabetic patients can be assessed.

REFERENCES

1. R. L. Dixon, L. G. Hart and J. R. Fouts, *J. Pharmac. exp. Ther.* **133**, 7 (1961).
2. R. Kato and J. R. Gillette, *J. Pharmac. exp. Ther.* **150**, 285 (1965).
3. L. A. Reinke, S. J. Stohs and H. Rosenberg, *Xenobiotica* **8**, 611 (1978).
4. L. A. Reinke, S. J. Stohs and H. Rosenberg, *Xenobiotica* **8**, 769 (1979).
5. F. H. Faas and W. J. Carter, *Horm. Metab. Res.* **12**, 706 (1980).
6. K. Chawalit, S. Sretarugsa and A. Thithapandha, *Drug Metab. Disp.* **10**, 81 (1982).
7. B. Müller-Oerlinghausen, A. Hasselblatt and R. Jahns, *Life Sci.* **6**, 1529 (1967).
8. H. Schriefers, R. Ghraf and F. Pohl, *Hoppe-Seyler's Z. Physiol. Chem.* **344**, 25 (1966).
9. Y. Hinohara, S. Takanashi, R. Nagashima and A. Shioya, *Jap. J. Pharmac.* **24**, 869 (1974).
10. E. Rouer, J. L. Mahu, P. Dansette and J. P. Leroux, *Biochim. biophys. Acta* **676**, 274 (1981).
11. G. M. Hawksworth and M. H. Morrison, *Br. J. Pharmac.* **72**, 539P (1981).
12. M. H. Morrison and G. M. Hawksworth, *Biochem. Pharmac.* **31**, 1944 (1982).
13. K. W. Bock, W. Fröhling, H. Remmer and B. Rexer, *Biochim. biophys. Acta* **327**, 46 (1973).
14. K. W. Bock, D. Josting, W. Lilienblum and H. Pfeil, *Eur. J. Biochem.* **98**, 19 (1979).
15. G. J. Wishart, *Biochem. J.* **174**, 485 (1978).
16. G. J. Mulder, *Biochem. Soc. Trans.* **2**, 1172 (1974).
17. B. Burchell, in *Reviews in Biochemical Toxicology*, Vol. 3 (Eds. E. Hodgson, J. R. Bend and R. M. Philpot), p. 1. Elsevier/North Holland, Amsterdam (1981).
18. P. Moldéus, H. Vadi and M. Berggren, *Acta pharmac. Tox.* **39**, 17 (1976).

19. H. Vainio, *Xenobiotica* **3**, 715 (1973).
20. L. C. Ericksson, J. W. DePierre and G. Dallner, *Pharmac. Ther.* **2**, 281 (1978).
21. Y. Ichikawa and T. Yamano, *Biochim. biophys. Acta* **131**, 490 (1967).
22. H. Vainio, *Acta pharmac. Tox.* **34**, 152 (1974).
23. R. S. Chhabra and J. R. Fouts, *Drug Metab. Disp.* **2**, 375 (1974).
24. A. Karakaya and D. E. Carter, *J. Chromatogr.* **195**, 431 (1980).
25. P. I. Adriaenssens and L. F. Prescott, *Br. J. clin. Pharmac.* **6**, 87 (1978).
26. BMDP-81, Biomedical Computer Programs, University of California, Berkley, U.S.A. (1981).
27. D. Zakim and D. A. Vessey, in *Enzymes of Biological Membranes*, Vol. 2 (Ed. A. Martonosi), p. 443. Plenum Press, New York (1976).
28. B. Burchell and P. J. Weatherill, in *Conjugation Reactions in Drug Biotransformation* (Ed. A. Aitio), p. 347. Elsevier/North Holland, Amsterdam (1978).
29. P. J. Weatherill, S. M. E. Kennedy and B. Burchell, *Biochem J.* **191**, 155 (1980).
30. M. R. Past and D. E. Cook, *Biochem. Pharmac.* **31**, 3329 (1982).
31. M. R. Past and D. E. Cook, *Res. Comm. chem. path. Pharmac.* **37**, 81 (1982).
32. S. L. Smith, M. Novotny, S. A. Moole and D. L. Felten, *J. Chromatogr.* **221**, 19 (1980).
33. V. K. Murthy and J. C. Shipp, *J. clin. Invest.* **69**, 923 (1981).
34. V. Chandramouli and J. R. Carter, *Diabetes* **24**, 257 (1975).
35. P. I. Varandani, R. M. Darrow and M. A. Nafz, *Proc. Soc. exp. Biol. Med.* **15**, 156 (1977).
36. E. Rouer, P. Dansette, P. Beaune and J. P. Leroux, *Biochem. biophys. Res. Comm.* **95**, 41 (1980).
37. S. C. Davison and E. D. Willis, *Biochem. J.* **140**, 461 (1974).
38. M. Laitinen, M. Lang and O. Hänninen, *Int. J. Biochem.* **5**, 747 (1974).
39. B. V. R. Sastry, C. N. Statham, R. G. Meeks and J. Axelrod, *Pharmacol* **23**, 211 (1981).
40. H. Vainio, *Xenobiotica* **3**, 715 (1973).
41. H. Vainio and O. Hänninen, *Acta pharmac. Tox.* **35**, 65 (1974).
42. B. L. Warren, R. Pak, M. Finlayson, L. Gontovnick, G. Sunahara and G. D. Bellward, *Biochem. Pharmac.* **32**, 327 (1983).
43. The University Group Diabetes Programme, *Diabetes* **24**, 65 (1975).
44. J. E. A. Leakey, Z. R. Althaus, J. R. Bailey and W. Slikker, *Biochem. J.* **214**, 1007 (1983).