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LIPOPHILICITY OF ACCEPTOR SUBSTRATE AS A FACTOR IN "LATE FOETAL" RAT LIVER MICROSOMAL UDP-GLUCURONOSYLTRANSFERASE ACTIVITY

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Abstract—The UDP-glucuronosyltransferase activity towards phenolic compounds, as measured by initial velocity, has been directly related to lipophilicity of acceptor substrates, as obtained by measurement of octanol–buffer or octanol–water partition. The acceptor substrates examined include 17 compounds, all probably conjugated by the "late foetal" enzyme activity. Rat liver microsomal enzyme activity towards five acceptor substrates of the "late foetal" enzyme was examined under different activation conditions. A statistically significant, partition-dependent increase in activity was observed when the effects of ageing or Triton X-100 treatment were studied. With *n*-pentane, phospholipase C or UDP-*N*-acetylglucosamine, although the enzyme activity depended on the partition coefficient of the acceptor substrate, activity towards each substrate was enhanced by a similar amount. Mild trypsin treatment (which did not itself activate the enzyme) or ageing converted the *n*-pentane dependent general activation into a partition related form by reducing the activity of the enzyme towards the less lipophilic substrates. Removal of phospholipid from the membrane by *n*-pentane or hydrolysis by phospholipase C resulted in the partition independent activation. Protein release, which by itself did not activate the enzyme, was also required for a partition-dependent effect. As enzyme activity towards the five substrates was induced by 3-methylcholanthrene, but not by phenobarbital, the "late foetal" enzyme was being studied. The induced enzyme activity appeared similar to the non-induced activity.

An enormous number of drugs and other xenobiotics are conjugated with glucuronic acid [1–3]. UDP-Glucuronosyltransferase(s) (E.C. 2.4.1.17) is the only enzyme so far considered responsible for the conjugations *in vivo* [4]. Recent investigations [5–9] on the development and induction of the enzyme(s) have suggested that at least two UDP-glucuronosyltransferases or two conformations of UDP-glucuronosyltransferase exist in rat liver. One enzyme activity belongs to the "late foetal" group of enzymes developing over births. The second activity is in the "neonatal" group of enzymes [7, 8], which develop immediately after birth.

Studies [7, 8] on the acceptable structures for the "late foetal" enzyme indicate that it conjugates both planar molecules and phenols with alkyl groups posessing one carbon atom adjacent to the hydroxyl group and two carbon atoms in the 3 and 4 positions. Larger molecules in general are conjugated by the "neonatal" enzyme. While these gross differences in the two groups of activities have been distinguished, other structure–activity relationships are much less understood.

Partition coefficients have been considered as an *in vitro* method of assessing the lipid solubility (lipophilicity) of a compound. Octanol–water has been shown to be a good model system for *in vivo* partitioning [10]. Uptake of xenobiotic into liver cells, binding to cytochrome P450 and phase 1 (oxidative) metabolism of drugs are all partition-dependant [11–14]. This paper, therefore, investigates the possible relevance of acceptor substrate lipophilicity as a factor in the activity of UDP-glucuronosyltransferase, a phase II (conjugation) enzyme. As enzyme activities are usually measured at a known pH, a better estimate of lipophilicity might be obtained using buffer rather than water. Some partition coefficients have therefore been determined at pH 7.4 [15]. Since UDP glucuronosyltransferase can be activated using a number of agents [4], the effects of some of these procedures were also examined.

A preliminary communication on part of the findings reported here has appeared [16].

EXPERIMENTAL

Substrates, chemicals and animals. Nitrophenols were purchased from Aldrich Chem. Co. (Gillingham, Dorset, U.K.). Phenol and 1-naphthol were obtained from Fisons (Loughborough, Leics., U.K.). UDP-Glucuronic acid (NH_4^+), UDP-*N*-acetylglucosamine and potassium hydrogen glucarate were from Sigma (Poole, Dorset, U.K.). ^{14}C-Phenol (specific activity 35 mCi mmole^{-1}) and 1-^{14}C-naphthol (specific activity 20 mCi mmole^{-1}) were purchased from The Radiochemical Centre (Amersham, Bucks, U.K.), and diluted with non-labelled material prior to use.

Triton X-100 (purified) was obtained from Koch-Light (Colnbrook, Middx., U.K.), *n*-pentane came from Hopkins & Williams (Chadwell Heath, Essex, U.K.). Phospholipase C (type III, from *B.cereus*) trypsin (type 1 from bovine pancreas), trypsin inhibitor (type II-0, from egg white) and 3-methylcholanthrene were purchased from Sigma. Phenobarbital was from Evans Medical (Liverpool, U.K.).

Octanol "specially" pure and all other chemicals were from Fisons.

Male Sprague–Dawley rats (350–450 g), obtained from Charles River (Manston, Kent, U.K.) were used in these studies. These animals were held on the premises for at least a week prior to use. They had access to food (Charles River 4RF diet) and water *ad lib.*

Partition coefficients. Partition of substrates between octanol-saturated 66 mM Tris buffer and buffer saturated octanol at pH 7.4 was performed as previously described [15]. The amount of nitrophenol in the aqueous phase was measured at the appropriate E_{max} (between 395 and 415 nm) after addition of 0.1 ml 5M NaOH to 1 ml sample of nitrophenol solution. Calibration was linear for all the nitrophenols used over the concentration range employed.

Preparation of microsomes. Rats were killed by stunning and cervical dislocation. The livers were rapidly excised and a 33% (w/v) homogenate prepared in 0.25 M sucrose containing 50 mM Tris buffer (pH 7.4) using five stokes of a teflon–glass homogenizer (model S63C, Tri-R Instrument Inc., Rockville, N.Y., U.S.A.; Speed 6). The homogenate was centrifuged in a MSE Superspeed 75 centrifuge for 30 min at 9000 *g* and the pellet discarded. The microsomal fraction was then obtained by centrifugation at 100,000 *g* for 1 hr. This pellet was resuspended in sucrose–Tris (15–25 mg protein ml^{-1}) and used immediately as enzyme source except where stated. All operations were performed at 0–4°.

Measurement of UDP-glucuronosyltransferase activity. Initial velocities of reaction were measured at 37°. Pairs of incubation vessels, each containing 20–50μl of microsomes, 0.2 mM acceptor substrate, 2.7 mM UDP-glucuronic acid and 10 mM Mg^{2+} in 66 mM Tris buffer, pH 7.4 (total volume 0.3 ml), were incubated for 0, 2, 5 or 10 min. The reaction was terminated by addition of 0.5 ml 0.2 M trichloracetic acid and the vessels centrifuged. The colour disappearance of the various nitrophenols was measured as described above, after addition of 1.0 ml 1 M NaOH to 0.4 ml supernatant.

Phenol glucuronidation was measured principally by disappearance of substrate using the procedure previously described [15], except that 1.0 ml 0.1 M NaOH was employed in the second extraction. In later experiments a radiochemical measurement procedure was employed [17]. The ^{14}C-phenol, but no ^{14}C-phenyl glucuronide, was extracted into 10 ml 1% butyl-PBD in toluene. The free phenol radioactivity was then counted in a Packard 2450 scintillation counter. Quench correction was performed using an external standard–channels ratio technique. Direct comparisons of both methods gave essentially the same results (±8%, S.D. for six experiments.) Glucuronidation of 1-^{14}C-naphthol was measured in the same way as phenol conjugation [18].

Activation procedures. Ageing was achieved by storage of the resuspended microsomes in sealed vessels in a refrigerator for the times stated. For Triton X-100 activation microsomes, Triton (0.3% v/v) was present in the final resuspension medium. UDP-*N*-Acetylglucosamine (final concentration 2.7 mM) was added directly to the incubation medium. *n*-Pentane (3.5% v/v) was mixed with the microsomes by gentle inversion. Phospholipase C (2.5 units ml^{-1} microsomal suspension) was incubated with the microsomes in the presence of 5 mM Ca^{2+} for 30 min at 20° and the reaction inhibited by addition of EDTA (final concentration 5 mM). Preliminary experiments suggested this was the optimal activating condition for our preparation of phospholipase C and microsomes. Sub-optimal levels of trypsin (50 mg g^{-1} microsomal protein) were incubated for 30 min at 37° and the reaction terminated by addition of an excess (1.2 fold) of trypsin inhibitor. After phospholipase treatment the microsomal fraction was reprecipated to prevent interference from any products released.

Protein determinations. Protein concentrations were determined using the Lowry procedure with bovine serum albumin standard [19].

Phospholipid determination. Microsomes and supernatant (1 ml aliquots) were dried in a vacuum oven at 37° and the phospholipids extracted three times with chloroform–methanol (2:1 v/v; 23). The extracts were evaporated to dryness and the phospholipids determined as described by Graham *et al.* [20] or using a standard test system (Boehringer phospholipid kit).

Statistical procedures. Correlation coefficient, and linear regression were determined using a least squares method. The probability for fitting data to the line was determined by *F*-test. Intercepts and slopes were analysed using a procedure for comparison of two regression lines described by Dixon and Massy [21].

The significance of the data in Tables 1, 3 and 4, which is given as mean ± S.D., was assessed using Students *t*-test.

RESULTS

Partition coefficient and enzyme activity. Figure 1 contains results from a comparison of enzyme activity

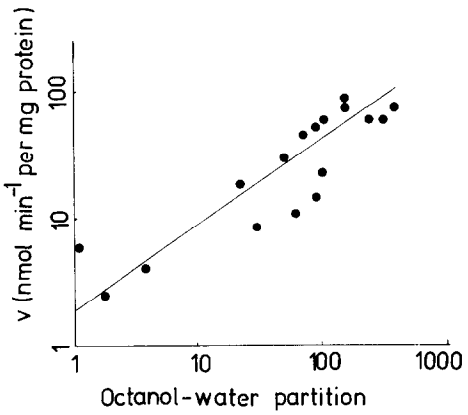

Fig. 1. Relationship between partition coefficient of acceptor substrate and enzyme activity measured by initial volocity. Data for this figure were obtained from refs. 22 (enzyme activity) and 23 (octanol–water partition coefficient). All those compounds for which both values were obtainable were plotted. The equation of the line is $y = 0.4954x + 0.5602$, N = 17, root mean square S.D. = 0.384, correlation coefficient = 0.863, Probability (*F*-test) <0.001 (equation 1).

Table 1. Comparisons of UDP-glucuronoslytransferase activity under different activation conditions

Treatment	Activity (nmoles/min/mg protein) with different substrates				
	Phenol	2-Chloro-4-nitrophenol	4-Nitrophenol	3-Methyl-4-nitrophenol	4-Methyl-2-nitrophenol
Non-activated	1.49 ± 0.52	2.01 ± 1.06	2.08 ± 0.87	3.63 ± 1.44	2.98 ± 0.74
n-Pentane	6.58 ± 1.21†	9.07 ± 2.72†	13.20 ± 3.03†	15.25 ± 1.06†	16.27 ± 6.03†
UDP-*N*-acetylglucosamine	3.73 ± 1.43*	3.16 ± 1.20	4.93 ± 1.79*	8.39 ± 1.80*	7.83 ± 1.53†
Phospholipase C	4.02 ± 3.55	3.06 ± 1.47(6)	9.36 ± 4.71(6)*	17.92 ± 7.01(6)†	9.47 ± 2.53†
Phospholipase C control	1.30 ± 0.67	0.94 ± 0.20	1.98 ± 0.43	2.29 ± 0.79	3.20 ± 0.41
Ageing (9 days, 0°)	2.51 ± 1.31	4.12 ± 1.41	8.44 ± 4.29*	18.38 ± 7.92†	18.05 ± 8.96†
Triton X-100	2.23 ± 1.56	3.61 ± 0.82	6.04 ± 0.60†	10.51 ± 0.96†	18.49 ± 4.33†
Trypsin	1.47 ± 0.62	1.94 ± 0.52	2.21 ± 0.62	3.39 ± 0.74	5.67 ± 3.60
n-Pentane + ageing (9 days, 0°)	4.19 ± 0.85	6.66 ± 1.13*	20.17 ± 3.87	34.00 ± 7.73†	25.82 ± 6.53†
n-Pentane + trypsin	3.05 ± 0.84	5.17 ± 0.99*	13.44 ± 2.67†	15.60 ± 3.34†	24.92 ± 6.90†
Log P (Partition coefficient‡)	0.62	0.73	1.38	1.84	2.15

* $P < 0.01$.

† $P < 0.001$ vs appropriate control (unactivated) incubations. The data is obtained from incubations performed as described under Experimental. Mean ±S.D. are shown for 4 experiments unless more are indicated.

‡ Partition coefficient for partition between octanol and pH 7.4 buffer.

Table 2. Effects of activators on the conjugation of a series of phenolic acceptor substrate for UDP-glucuronosyltransferase

Equation No.	Treatment	Equation*	Fit of data to line: Correlation coefficient	Fit of data to line: P (*F*-test)	Comparison with control line (P): Intercept	Comparison with control line (P): Slope	Comment
3	Control	$y = 0.200x + 0.097$	0.881	0.048			
4	*n*-pentane	$y = 0.235x + 0.742$	0.949	0.014	0.001	0.66	Changes largely in intercept, activator increased activity uniformly
5	UDP-*N*-acetyl glucusamine	$y = 0.268x + 0.356$	0.953	0.013	0.07	0.43	
6	Phospholipase C	$y = 0.394x + 0.329$	0.894	0.068	0.08	0.65	
7	Control for phospholipase C	$y = 0.295x - 0.147$	0.947	0.015			
8	Ageing (9 days, 0°)	$y = 0.550x + 0.400$	0.934	0.020	0.18	0.04	Changes largely in slope, activation also partition related
9	Triton X-100	$y = 0.535x + 0.076$	0.949	0.002	0.87	0.007	
10	Trypsin	$y = 0.325x - 0.21$	0.951	0.013	0.48	0.29	No significant change
11	*n*-Pentane + ageing (9 days, 0°)	$y = 0.559x + 0.141$	0.975	0.005	0.77	0.01	Changes largely in slope, activation also partition related
12	*n*-Pentane + trypsin	$y = 0.582x + 0.265$	0.962	0.009	0.31	0.02	

* In equation $y = mx + c$, where y = log "enzyme activity" and x = log partition coefficient.
Data is presented in Table 1. N = 5 for all experiments. A P value <0.1 is taken to indicate a trend.

Table 3. Release of protein and phospholipid from microsomes after treatment with activators*

Treatment	Per cent released from microsomes after treatment and resedimentation	
	Protein	Phospholipid
Control (no treatment)	21.5 ± 2.5(4)	<3
n-Pentane	25.3 ± 9.7(4)	27.8 ± 17.2‡
Phospholipase C	23.9 ± 4.9(4)	82.7 ± 2.8(5)‡
Ageing (9 days, 0°)	60.5 ± 15†	nd
Trypsin	57.2 ± 4.2‡	nd
n-Pentane + trypsin	56.3 ± 11.4(4)‡	nd

* Microsomes were prepared as described in Experimental, and after perturbation, resedimented and resuspended in the same volume of sucrose–Tris. Phospholipid and protein content were measured in both the decanted supernatant and the resuspended microsomes. There was approximately 0.76 mg phospholipid in microsomes corresponding to 1 mg protein. nd—not determined.
† $P < 0.05$.
‡ $P < 0.01$; $N = 3$.

in rat liver microsomes under Triton X-100 activated conditions and acceptor substrate partition coefficient. The compounds examined, in terms of structure, fall within the parameters defined for the "late foetal" UDP-glucuronosyltransferase (see introduction). The data shows that the enzyme activity depended on acceptor substrate partition coefficient and this relationship could be described using the equation

$$\log \text{Activity} = \log \text{Partition coefficient} + \text{Constant}. \quad (2)$$

Statistical analysis of the data for Fig. 1 shows that an acceptable correlation was obtained. Thus lipid solubility is a major determinant in the suitability of an acceptor substrate for conjugation by the "late foetal" UDP-glucuronosyltransferase.

Effects of activation procedures on enzyme activity. As enzyme activities from many studies are obtained in the presence of activators of the enzyme, an examination was undertaken on the effects of such activators using five acceptor substrates. These substrates were chosen to give a wide range of octanol–buffer partition coefficients. The study examined the effects of activators on the activity and activation of rat liver microsomal UDP-glucuronosyltransferase towards these substrates, all of which on enzyme induction criteria (using phenobarbital and 3-methylcholanthrene treatments) fall within the "late foetal" group of enzyme activities.

The data obtained is shown in Table 1 and statistical evaluation is given in Table 2. With the marginal exception of phospholipase C, a satisfactory ($P < 0.05$) statistical correlation of activity and partition coefficient was obtained.

The activators studies appeared to fall largely into two groups (Table 2). In one group, activation elevated the activity towards acceptor substrates by a similar amount (equations 4–6). While no differences could be detected in the slopes of the equations in this group, there was a clear trend for the intercepts to increase. In the other group (equations 8, 9, 11 and 12) a statistically satisfactory ($P < 0.05$) correlation for partition dependent activation was

Table 4. Comparisons of UDP-glucuronosyltransferase activity under different induction procedures*

Substrate	Activity (nmoles/min/p mg protein) under different treatments			
	Saline	Phenobarbital	Corn oil	3-Methylcholanthrene
Phenol	5.0 ± 0.3	6.1 ± 0.4†	4.2 ± 0.6	11.2 ± 3.3†
2-Chloro-4-nitrophenol	6.1 ± 2.3	5.0 ± 2.1	8.1 ± 2.0	12.9 ± 1.3†
4-Nitrophenol	10.9 ± 1.9	12.9 ± 1.6	13.8 ± 2.0	24.3 ± 5.0†
3-Methyl-4-nitrophenol	18.3 ± 3.4	19.5 ± 3.3	15.2 ± 0.6(3)	28.3 ± 4.5(3)†
4-Methyl-2-nitrophenol	23.4 ± 3.8	21.9 ± 2.4	16.3 ± 6.0	35.2 ± 5.3†

* All activities were measured in *n*-pentane activated microsomes using procedures described in the Experimental section. Phenobarbital (40 mg kg^{-1} day^{-1} in 0.5 ml saline p.o. for 7 days) and 3-methylcholantharene (20 mg kg^{-1} day^{-1} in 0.3 ml corn oil i.p. for 3 days) pretreatments were used as induction procedures.
† $P < 0.01$ vs appropriate control. $N = 4$ except where stated.

Table 5. Effects of various pretreatments on UDP-glucuronosyltransferase activity towards acceptor substrates of different partition coefficient in *n*-pentane activated microsomes

Equation No.	Pretreatment	Equation*	Fit of data to line: Correlation coefficient	Fit of data to line: P (*F*-test)	Comparison with control line (P): Constant	Comparison with control line (P): Slope	
13	Saline	$y = 0.442x + 0.433$	0.997	<0.001			Change in constant is 0.042, equivalent to an induction of 1.1
14	Phenobarbital	$y = 0.434x + 0.475$	0.977	0.004	0.63	0.77	
15	Corn oil	$y = 0.331x + 0.568$	0.893	0.042			Change in constant to 0.311, equivalent to an induction of 2.1
16	3-Methyl-cholanthrene	$y = 0.320x + 0.879$	0.949	0.002	0.08	0.92	

* In the equation $y = mx + c$, where y = log "enzyme activity" and x = log partition coefficient.

The effect of 3 days, 0.3 ml day^{-1} corn oil was not significant, but the 2 fold induction by 3-methylcholanthrene was. Both the control (0.9% NaCl) and phenobarbital-induced microsomes gave significantly (P = 0.016) different lines to those of *n*-pentane activated microsomes. This was probably because some 3–4 hr storage (in petri dishes on ice) took place prior to preparation of the microsomes and resulted in some ageing [37]. Activity data is given in Table 4, partition in Table 1. A separate P value of <0.1 is taken to indicate a trend.

obtained. Triton X-100 belonged to the second group, hence the results obtained by Illing and Benford [15].

If *n*-pentane treatment of microsomes (first type of activation) is followed by ageing or trypsin treatment, then the combined activation is partition related (see equations 11 and 12). Thus partition-independent activation can be converted to a partition dependent form.

Effects of activation procedures on microsomal structure. From the results in the previous section, two forms of activation of UDP-glucuronosyltransferase could be defined. The effects of some activators on membrane proteins and phospholipids were therefore examined to see if some of these differences could be ascribed to changes in membrane structure.

Treatment with *n*-pentane or phospholipase C did not result in additional protein loss on resedimentation and resuspension of the rat liver microsomes when compared to non-activated microsomes. Ageing microsomes or treatment with trypsin resulted in loss of protein on resedimentation and resuspension of microsomes (Table 3). As Triton X-100 releases enzyme from the microsomes [24, 25], it was thought that these procedures also affected the microsomal proteins.

Little microsomal phospholipid was released in untreated microsomes, while significant quantities were released by *n*-pentane treatment and hydrolysed by phospholipase C treatment (Table 3). Thus *n*-pentane and phospholipase C affected the microsomal phospholipids without releasing proteins.

Effects of induction procedures on enzyme activity. The effects of two inducers of UDP-glucuronosyltransferase, phenobarbital and 3-methylcholanthrene, were investigated using five acceptor substrates of different partition coefficient (Tables 4 and 5). Phenobarbital induction had little effect on conjugation of these substrates in *n*-pentane activated rat liver microsomes. 3-Methylcholanthrene induced the activity towards the substrates in *n*-pentane activated microsomes approximately 2-fold. These results are similar to those obtained generally for the "late foetal' enzyme [6–9]; they confirm that the acceptor substrates belong to this group and that the new enzyme obtained on induction is similar in this respect to the enzyme present in non-induced rats.

DISCUSSION

The data presented in the first sections of this report demonstrates that lipid solubility, as assessed by octanol–buffer or octanol–water partition coefficient, is a factor in the ability of a phenolic acceptor substrate to be conjugated by the "late foetal" rat liver microsomal UDP-glucuronosyltransferase, as defined in the introduction. The data also suggest that use of equation 2 (log Activity = log Partition coefficient + Constant) is a suitable method for relating microsomal enzyme activity towards an acceptor substrate and the lipophilicity of that substrate. Similar comparisons can be carried out using K_m data [26] and, where they exist, octanol-water partition values [23] from the literature.

This type of relationship has been shown to have wide applicability [27], and also applies to the uptake, binding to cytochrome P450 and oxidation of xenobiotics over the range of lipophilicity examined [11–14].

Preliminary, limited data with only four acceptor substrates [8] may indicate that conjugation by the "neonatal" enzyme is not partition related. Some substrates for this group are endogenous compounds (see refs. 5–9); thus possible transport mechanisms to the active centre could be present and obscure physico-chemical parameters.

The effects of different activators on the "late foetal" enzyme activity were also examined using rat liver microsomes. Initial rates of conjugation were the measure of enzyme activity. Two types of activation emerged. For one type, activation resulted in a change in constant, such that, while the activity was still partition-dependent, the amount of activation seen was largely independent of the partition coefficient. In the second type the slope changed on activation. In an earlier study [15], the amount of activation by one of the second group of activators, Triton X-100, was shown to be dependent on the partition coefficient for a wider range of substrates.

With the two non-physiological activators, *n*-pentane and phospholipase C, principally the first type of activation (partition independent) resulted and phospholipid release from the microsomes took place without release of protein. The second type (partition related), the type found after ageing microsomes or on treatment of microsomes with Triton X-100, required alterations in the protein structure, possibly changing the nature of the enzyme.

Approximately half the enzyme protein in the microsomes could be removed by very mild trypsin treatment. This amount of trypsinization did not activate UDP-glucuronosyltransferase [28, 29, this work]. However, if prior phospholipid removal had taken place (by *n*-pentane), the same amount of trypsin-induced proteolysis altered the catalytic nature of the enzyme, deactivating it towards the more water-soluble substrates. Ageing *n*-pentane activated microsomes also resulted in a conversion from the first to the second form of activation. Thus the two types of activation are not exclusive, and individual activators may cause a combination of them.

Access to the enzyme *in vitro* may be restricted by the membrane structure of the microsomal vesicles [30–33]. Some change in the phospholipids of the membrane appears to be the minimum required for better access of all acceptor substrates of the "late foetal" enzyme and of trypsin. Thus, in non-disrupted microsomes there is some barrier to the maximal activity towards acceptor substrates. This barrier, which may be regulated by UDP-*N*-acetylglucosamine *in vivo* in rat, does not distinguish acceptor substrates on the basis of lipophilicity, and protects the enzyme active centre from the effects of mild trypsin treatment.

Phospholipids are required for maximal enzyme activity towards acceptor substrates of the "late foetal" enzyme [34–36]. As the enzyme renders acceptor substrates more water soluble by conjugating them, it is reasonable that the lipid solubility of the acceptor substrate is a factor in the requirement for conju-

gation. This effect is more marked in tissue damaged by autolysis (ageing), Triton X-100 treatment, and *n*-pentane and trypsin treatment combined.

The fact that induction by 3-methylcholanthrene, but not by phenobarbital, occurred indicated that the activation studies were on substrates which belonged to the "late foetal" enzyme [6-9]. Comparisons of the induced and non-induced *n*-pentane activated enzyme activity towards the substrates indicated that a similar relationship of lipid solubility for acceptor substrate and enzyme activity existed. Induction implies synthesis of new enzyme proteins; thus in this respect the induced enzyme resembled that originally present in the membrane.

Octanol–water or octanol–buffer partitions are measures of lipid solubility, and when used with equation 1, offer a way of predicting the approximate enzyme activity towards new acceptor substrates of the "late-foetal" UDP-glucuronosyltransferase.

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REFERENCES

1. C. A. Marsh, in *Glucuronic Acid—Free and Combined* (Ed. G. J. Dutton), pp. 4–119. Academic Press, New York (1966).
2. D. E. Hathway, *Foreign Compound Metabolism in Mammals* (Ed. D. E. Hathway), Vol. 4, pp. 77–192. Chemical Society, London (1977).
3. C. T. Bedford, in *Foreign Compounds Metabolism in Mammals* (Ed. D. E. Hathway), Vol. 4, pp. 193–258. Chemical Society, London (1977).
4. G. J. Dutton and B. Burchell, in *Progress in Drug Metabolism* (Eds. J. W. Bridges and L. F. Chasseaud), Vol. 2, pp. 1–70. Wiley, London (1977).
5. G. J. Wishart, *Biochem. J.* **174**, 485 (1978).
6. G. J. Wishart, *Biochem. J.* **174**, 671 (1978).
7. G. J. Wishart, M. T. Campbell and G. J. Dutton, in *Conjugation Reactions In Drug Biotransformation* (Ed. A. Aitio), pp. 179–187. Elsevier/North Holland, Amsterdam (1978).
8. G. J. Wishart and M. T. Campbell, *Biochem. J.* **178**, 443 (1979).
9. G. W. Lucier and O. S. McDaniel, *J. Steroid Biochem.* **8**, 867 (1977).
10. A. Nelson-Smith, C. Hansch and M. M. Ames, *J. Pharm. Sci.* **64**, 599 (1975).
11. Y. C. Martin and C. Hansch, *J. med. Chem.* **14**, 777 (1971).
12. K. A. S. Al-Gailany, J. W. Bridges and K. J. Netter, *Biochem. Pharmac.* **24**, 867 (1975).
13. T. D. Yih and J. M. Van Rossum, *Biochem. Pharmac.* **26**, 2117 (1977).
14. K. A. S. Al-Gailany, J. B. Houston and J. W. Bridges, *Biochem. Pharmac.* **27**, 783 (1978).
15. H. P. A. Illing and D. Benford, *Biochim. biophys. Acta* **429**, 768 (1976).
16. H. P.A. Illing, *Biochem. Soc. Trans.* **6**, 1211 (1978).
17. H. P. A. Illing and E. S. A. House, *Biochem. Soc. Trans.* **7**, 652 (1979).
18. G. W. Lucier, O. S. McDaniel and H. B. Matthews, *Archs Biochem. Biophys.* **145**, 520 (1971).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. A. B. Graham, B. G. Woodcock and G. C. Wood, *Biochem. J.* **137**, 567 (1974).
21. W. J. Dixon and F. J. Massy, *Introduction to Statistical Analysis*, 3rd Ed, pp. 206–211. McGraw Hill, New York (1969).
22. G. J. Mulder and A. B. D. Van Doorn, *Biochem. J.* **151**, 131 (1975).
23. A. Leo, C. Hansch and D. Elkins, *Chem. Rev.* **71**, 525 (1971).
24. O. Hanninen and R. Puukka, *Suomen Kemistilehti* (Finnish Chem. J.) **43**, 451 (1970).
25. A. Winsnes, *Biochim. biophys. Acta* **284**, 394 (1972).
26. G. J. Mulder and J. H. N. Meerman, in *Conjugation Reactions in Drug Biotransformations*, (Ed. A. Aitio), pp. 389–397. Elsevier/North Holland, Amsterdam (1978).
27. W. J. Dunn and C. Hansch, *Chem. biol. Interact* **9**, 75 (1974).
28. J. Wilkinson and T. Hallinan, *Fedn Eur. biochem. Soc. Lett.* **75**, 138 (1977).
29. A. B. Graham, D. T. Pechey and G. C. Wood, *Molec. Pharmac.* **15**, 375 (1979).
30. D. T. Pechey, A. B. Graham and G. C. Wood, *Biochem. J.* **175**, 115 (1978).
31. T. Hallinan, in *Conjugation Reactions in Drug Biotransformations* (Ed. A. Aitio), pp. 257–267. Elsevier/North Holland, Amsterdam (1978).
32. D. Zakim and D. A. Vessey, in *The Enzymes of Biological Membranes* (Ed. A. Martonosi), Vol 2, pp. 443–461. Plenum Press, New York (1976).
33. G. C. Wood, A. B. Graham and K. C. Toogood, in *Conjugation Reactions in Drug Biotransformation* (Ed. A. Aitio), pp. 203–212. Elsevier/North Holland, Amsterdam (1978).
34. B. Burchell and T. Hallinan, *Biochem. J.* **171**, 821 (1978).
35. R. H. Erikson, D. Zakim and D. A. Vessey, *Biochemistry* **17**, 3706 (1978).
36. J. P. Gorski and C. B. Kaspar, *Biochemistry* **17**, 4600 (1978).
37. B. Burchell, J. Leakey and G. J. Dutton, *Enzyme* **20**, 156 (1975).