Glucuronyl-transferase activity in uraemic rats

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Whether glucuronidation in uraemia is normal, or otherwise, is important because some uraemic toxins, such as the phenols, are inactivated in this way. Antipyrine clearance studies in man have produced differing findings varying from normal (Lichter et al 1973), to increased values (Maddocks et al 1975) and a reduction when patients have been subjected to long term protein deficiency and have low serum tri-iodothyronine values (Wardle 1978). In fact antipyrine clearances in man are very dependent on genetic and environmental influences. Clearances are increased by smoking and prior exposure to a variety of drugs. We have therefore performed studies in vitro and in the rat liver perfusion circuit of naphthol detoxification by glucuronyl-transferase in the liver of normal and nephrectomized rats. Since glucuronidation can also occur at the gut mucosa, we have also studied the absorption of labelled naphthol by the gut.

Liver perfusion. Rats were made uraemic by bilateral nephrectomy through a mid-line abdominal incision under pentobarbitone anaesthesia and were used for study two to three days afterwards. At the same time control 'normal' rats were subjected to a similar laparotomy and re-suture of the abdominal wall. In order to prevent infection each rat was given gentamycin (1 mg kg^{-1}) subcutaneously. By day 2 blood urea concentrations were at least 30 mM and if left to the third day animals had uraemic twitching. Rat liver perfusion studies were performed by the technique of Hems et al (1966). The volume of perfusate in each circuit was 150 ml. The fluid at pH 7·4 contained 25 mM bicarbonate, bovine serum albumin 2·6 g %, and washed human bank red cells giving a haemoglobin concentration of 2·5 g %.

Thirty minutes after the onset of perfusion 10 ml of a 0·1 mM solution of naphthol (originally made at 5 mM in absolute ethanol and diluted with 0·9% NaCl (saline) containing 1 μCi of [^{14}C]naphthol was added to the reservoir of the circuit. At the same time 2 mg of bromsulphthalein (BSP) was added. Aliquots (3 ml) of perfusate were removed thereafter at 3,5,10,15,20,30 min. BSP clearances were measured by adding 0·5 ml aliquots of serum to 4 ml saline plus 0·2 ml of 2·5 M NaOH and quantitating the absorption at 580 nm. The rate of clearance of free naphthol was measured isotopically by extracting 0·5 ml of each perfusate sample with 2 ml toluene, which was then added to liquid scintillation medium. This consisted of Analar toluene 250 ml, ethanol 750 ml and P.P.O 6 g $litre^{-1}$. The water-soluble conjugates remain unextracted in the serum but the total counts were then measured by dissolving 0·5 ml perfusate in 2 ml 1·0 M hyamine in methanol to which scintillation fluid was added. This is similar to the method of Bock & White (1974). Additionally, the total naphthol disappearance rate could be plotted by diluting 0·5 ml perfusate with 2 ml saline and assaying the concentration in a fluorimeter using extinction at 330 nm and emission at 460 nm (Verity et al 1964).

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Microsomal studies. Rat liver microsomes were prepared from rats that had had uraemia for 3 days and from normal rat livers. The renal vessels were cut in order to bleed each animal and then each liver was removed and cooled, and a homogenate was made in 0·25 M sucrose. The homogenate was spun at 800 *g* for 10 min to remove mitochondria. To the supernatant was added 0·25 M sucrose-24 mM calcium chloride to achieve a final concentration of 8 mM calcium chloride. This caused precipitation of the microsomes and enabled quick separation by centrifugation at 27 000 *g* for 15 min (Cinti et al 1972). The pellet was suspended in 0·15 M potassium chloride and was re-centrifuged at 27 000 *g*. It was then made up in 0·25 M sucrose, 5 mM EDTA, 50 mM Tris-HCl pH 7·4 and was treated with a 0·75% solution of digitonin. After further washing and recentrifugation it was finally dispersed in buffer for the assay.

For the in vitro glucuronyl-transferase assay, the reaction mixture consisted of 2 mg of microsomal protein, 0·5 ml 50 mM Tris-HCl pH 7·4, 0·2 ml of 1·0 mM uridine diphosphoglucose (omitted from the blanks), 0·1 ml of 1 mM glucaro-1,4-lactone and 0·2 ml of 0·25 mM *p*-nitrophenol. Samples were incubated for 10 min at 37°C and thereafter the reaction was terminated by addition of 0·2 ml 5% trichloroacetic acid. After centrifugation, 1 ml of supernatant was mixed with 0·5 ml of 0·1 M NaOH and 1 ml water. The yellow colour of each assay sample was read at 400 nm in comparison with its blank. The protein content of the original microsomal preparation was determined by the method of Lowry et al (1951).

Intestinal absorption. Rats were studied in comparison with normal controls 3 days after bilateral nephrectomy. In each case the abdomen was opened by a mid-line incision and a loop of ileum 10 cm long was mobilized in warm saline swabs. The gut was cannulated and was first washed through with warm isotonic saline and thereafter was filled with 5 ml of fluid made up of 4 ml saline and 1·0 ml of 0·5 mM naphthol containing 5 μCi [^{14}C] naphthol. Exactly 5 min after instillation the main branch of the portal vein was pierced between holding sutures to collect the effluent blood in heparinized glass

tubes. Samples were collected for 30 s periods over another 10 min as in the procedure used by Bock & Winne (1975). During this time the blood volume was maintained by infusion of Haemocel via the femoral vein. The plasma samples were processed so as to be able to count free isotopic naphthol in toluene extract, as already described, and total counts as detailed by Bock & White (1974). It was thus possible to calculate the percentage of free naphthol that was being absorbed from the ileum for each minute over the 5–15 min after instillation of the naphthol.

Results. Successful perfusions were performed on two nephrectomized and two control animals of comparable weight at two and three days respectively after operation. The results in Table 1 show that on each day the BSP clearance did not differ between the groups and yet the half-lives for naphthol clearance were shorter in uraemic animals, and thus the clearance of the naphthol was increased.

The results of the in vitro glucuronidation capacity of liver microsomes were for uraemic animals (n = 16) 15·8 ±4·4 nmol min^{-1} mg^{-1} microsomal protein and for normal animals (n = 16) 6·2 ± 1·9. Thus in vitro glucuronidation was increased in uraemic microsomes. Additionally the total microsomal protein was increased in uraemic animals 260 ± 40 mg/100 g body weight compared with the normals (220 ± 25 mg l 100 g).

Values for the percentage absorption of free unconjugated naphthol are given in Table 2 for six normal animals and for six nephrectomized rats. The figure in parenthesis indicates the duration of time for which the blood samples were actually obtained. In addition it was observed that if gut perfusion deteriorated then free naphthol absorption values rose to 20–30%. Such experiments have been discounted but do demonstrate the importance of viability of the gut mucosa. Two animals that were dying of uraemia also had free naphthol absorptions of 16·7 (± 5·9) and 16·8 (± 3·3)%.

The results show that the capacity of the uraemic rat liver to conjugate phenols (as naphthol and *p*-nitrophenol respectively) is increased both in the perfused liver system and in microsomal preparations. So in acute uraemia in the rat the glucuronyl-transferase system has been induced. It is possible that this effect results from

Table 1. Results of perfusion of rat liver with bromsulphthalein and naphthol.

	$T_{\frac{1}{2}}$ BSP (min)	$T_{\frac{1}{2}}$ Naphthol (mins)	BSP clearance (ml g^{-1} liver)	Naphthol clearance (ml g^{-1} liver)	Liver wt (gs)
2 days					
C1	52·5	6·0	1·98	1·86	9·3
C2	53·0	6·5	1·96	1·98	8·0
U1	52·5	4·5	1·98	3·60	6·4
U2	52·5	5·0	1·98	2·84	7·3
3 days					
C1	55·0	8·5	1·89	1·40	8·6
C2	55·0	8·5	1·89	1·40	8·0
U1	55·0	6·5	1·89	2·27	7·0
U2	55·0	6·5	1·89	2·15	7·4

Table 2. Percentage free naphthol absorption.

	Normal animals	Uraemia
	11·4 ± 3·8 (10)	2·6 ± 0·6 (10)
	9·5 ± 3·2 (10)	4·2 ± 1·1 (17)
	9·5 ± 1·5 (9)	3·6 ± 2·0 (10)
	11·8 ± 1·8 (6)	6·5 ± 3·7 (15)
	8·6 ± 3·0 (13)	5·4 ± 2·4 (11)
	12·6 ± 2·1 (8)	4·6 — 2·1 (18)
mean	10·6 ± 2·6	4·5 ± 2·0

the retention of phenols, for phenol has been shown to be capable of inducing glucuronidation (Takemori & Glowacki 1962). Yet clearly the gut absorption studies indicate that there should be no free phenol in the circulation of uraemic animals and thus some other toxin may account for the induction. Leber et al (1972) did not find any change of the glucuronyl-transferase activity of subtotally nephrectomized animals when using 4-methyl-umbelliferone as substrate. The enzyme does lie behind a lipid barrier for which the different substrates have varying lipid partition coefficients (Illing & Benford 1976).

The concentration of naphthol that was used was high in order to stress the enzyme systems (cf. Wardle 1978). Indeed higher concentrations in the liver perfusion circuit were found to cause red cell haemolysis and impairment of liver perfusion. The operative stress had some slight influence on the findings since both naphthol and BSP clearances were higher at two days compared with three day animals.

These results using the neutral phenol, naphthol, differ from those that have been reported for the phenolic acid, *p*-aminobenzoic acid, the conjugation of which is reduced in uraemia (Howie & Bourke 1979). Apart from the terminally uraemic animals, conjugation at the gut mucosa was increased and we have similar findings in animals with obstructive jaundice. Thus neutral phenols that are the product of gut bacteria, or that are ingested in food, can be expected to be detoxified before they actually gain entry to the body. Our figure of 10% free naphthol absorption in normal animals is higher than might be expected but does accord with the results of Bock & Winne (1975) for this particular experimental situation. As in the liver perfusion studies a high concentration of naphthol was presented to the gut mucosa so as to test the maximum capacity of the mucosal glucuronidation system. In this way it has been found that the enzyme system has actually undergone adaptive induction in acute uraemia. Such data have been lacking hitherto (Dutton & Burchell 1977).

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Inhibition of peristalsis in rat jejunum by non-ionic surfactants

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The non-ionic polyoxyethylene alkylether surfactants have been shown to exert both systemic and local anaesthetic effects (Zipf et al 1957, 1964; Benke et al 1977). They may also have an analgesic action (Schulz et al 1953). Recently this class of surfactants was shown to be toxic to goldfish, the compounds based on stearyl, oleyl and cetyl alcohol being much less potent than those derived from lauryl alcohol (Florence et al 1978). In this communication we report the inhibitory effects of some of the 'Brij' non-ionic surfactants on the peristalsis of sacs of rat jejunum in vitro.

Sacs, 4 cm long, were prepared from the lower jejunum of male Sprague-Dawley rats weighing about 350 g as described by Whitmore et al (1979) except that the sacs were not everted. Each sac was filled with 0·2 ml of Krebs' original Ringer phosphate buffer (Dawson et al 1969) at pH 6 and suspended in 5 ml of the same solution maintained at 37°C in a water bath and 'bubbled' with a mixture of O_2/CO_2 (95/5%). The top ligature on the sac was attached to an isometric transducer (Devices Instruments Ltd, Welwyn Garden City, Herts) and the output from the latter was fed through an amplifier to a X-T recorder. Peristalsis was initiated by application of a 4 g tension to the sac and monitored by the recorder. Brij 30 (polyoxyethylene$_4$ laurylether), Brij 35 (POE_{23} laurylether), Brij 58 (POE_{20} cetylether) and Brij 98 (POE_{20} oleylether) were used as received from Honeywill Atlas Ltd, Carshalton.

Fig. 1 shows a typical recording of the peristalsis. The first 20 min were used as a control and then samples (10 μl or less) of the text surfactant were added to the solution bathing the serosal surface at intervals of about 10 min. The surfactant was dissolved in the same buffer solution used for the bathing medium. In the example shown only one addition of Brij was made because the high concentration used was sufficient to cause complete inhibition of the peristalsis. The average amplitude of the pendular movement clearly decreased within 3 min of addition of the surfactant, although the frequency of the contractions did not change significantly. Thereafter the amplitude continued to decrease, with no change in frequency, until no movement was detectable (after about 15 min). Similar responses were recorded when lower concentrations of surfactant were added to other sacs, except that only partial inhibition of movement was produced—that is, the average amplitude decreased but remained measurable. The decrease in the inherent tone of the sac that is apparent in Fig. 1 was not consistently observed.

To quantify the results the average amplitude of the pendular movement during the appropriate periods of time was taken as an arbitrary measurement of peristalsis. The values thus obtained for different sacs were then normalized by conversion to relative peristalsis values:

$$\text{Relative peristalsis} = \frac{\text{Peristalsis in the presence of Brij}}{\text{Peristalsis during the control period}}$$

These relative values are plotted in Fig. 2 as a function of surfactant concentration. A value of 1 shows that peristalsis was unaffected and a value of zero indicates complete inhibition of movement. All four of the Brij compounds tested inhibited peristalsis but they were not

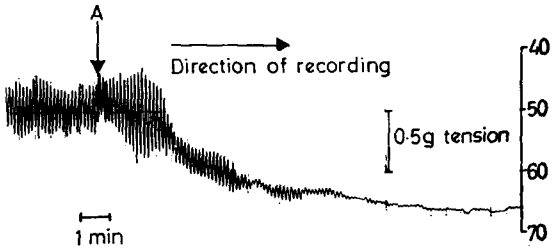

FIG. 1. Recording of isometrically transduced peristaltic movement. The experimental conditions are given in the text. Only the last 3 min of the control incubation period are shown and at the time marked A the surfactant (Brij 30) was added to the solution bathing the serosal surface, giving a final concentration above 20 μM.

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