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Nitrofurans and enzyme systems of the neonatal animal

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THE UDP GLUCURONYL transferase enzyme system is poorly developed in the human newborn. This has led to impaired detoxification of drugs which are excreted as glucuronide conjugates[1, 2] and to an increase in neonatal hyperbilirubinemia when drugs which interfere with the enzyme function are used.[3, 4] Fouts and Adamson[5] have shown that a number of drugs that are metabolized in the adult by enzymes found in the hepatic microsomes are not metabolized by the livers of newborn rabbits. The present study is concerned with the effect of certain nitrofurans on glucuronyl transferase of rat liver homogenate and with a comparison of the ability of maternal and neonatal liver and kidney preparations of rats and rabbits to degrade three nitrofurans: nitrofurantoin, 1-[(5-nitrofurfurylidene)amino] hydantoin; furazolidone, 3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone; and nifuradene, 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone.

METHODS

Formation of bilirubin glucuronide was studied in a system similar to that used by Lathe and Walker.[6] Novobiocin and chloramphenicol, known inhibitors of glucuronyl transferase[3, 4] and the sodium salt of nitrofurantoin were added as solutions in alkaline KCl.[7] Because of limiting solubility, furazolidone and nifuradene were added as 1 mg dry powder per flask, and their concentrations were determined spectrophotometrically[8] after incubation in control samples from which the bilirubin had been omitted.

For the nitrofuran degradation studies, the liver and kidney samples were removed rapidly from neonatal and maternal rats after death by decapitation. The maternal rats were stock animals about 11 months of age. The neonatal rats ranged from 2 hr to 3 days in age. Tissues were placed in chilled 0·067 M phosphate buffer, pH 7·4 and processed as rapidly as possible. Livers and kidneys from each litter of rats were pooled. Attempts to slice the neonatal tissue resulted in a mince, and the corresponding maternal tissue was subdidvided to a similar surface area. The incubation medium was 0·067 M phosphate buffer, pH 7·4, containing 100 mg glucose/100 ml (control) or a solution of nitrofuran (22–46 mg/l.) in the control medium. Maternal tissue weights were matched to neonatal tissue weights for each experiment. Liver samples ranged from 320–429 mg/flask and were incubated in 2·5 ml of the appropriate medium. Kidney samples ranged from 143–277 mg and were incubated

in either 1·5 or 2 ml of medium. Samples were removed at zero time and, after 40 min of incubation in air in a Dubnoff shaking apparatus, were inactivated in 95% ethanol, and the nitrofuran concentration was determined spectrophotometrically[8] by using the control medium similarly incubated with tissue for correction.

Liver and kidney preparations from maternal and neonatal rabbits were made by the same technique as for rats except that 500-mg samples of tissue were available and these were incubated in 3 ml of the medium used with the rat preparation. Samples were removed and the nitrofuran concentration was determined[8] as described above. The rabbit litters were either 1 or 2 days of age.

The 9000 *g* supernatant fractions from adult male rats and from male, maternal, and neonatal rabbit liver were prepared as described by Fouts and Brodie[9] with minor modifications. The fractions were held at 0° and used within 3 days. Enzyme preparations were added so that the *p*-nitrobenzoic acid flasks contained the equivalent of 100 mg rat liver or 200 mg rabbit liver and the nitrofuran flasks contained the equivalent of 12·5–100 mg liver in the final 2·5-ml volume. Incubation was carried out in Warburg flasks under nitrogen for 30 min for nitrofurans and for 2 or 3 hrs for *p*-nitrobenzoic acid. Cofactors (NADP, 0·5 μmole; nicotinamide, 50 μmole; glucose 6-phosphate, 25 μmole) were added in 1 ml of 0·2 M phosphate buffer, pH 7·4. The nitrofurans were dissolved in *N-N* dimethylformamide (DMF) (Matheson, Coleman and Bell), 1 mg/ml; this was diluted 1 → 5 with 0·2 M phosphate buffer and added in 0·5 ml quantity to give about 0·45 μmole/flask at zero time. Similarly diluted DMF was added to control correction flasks. The *p*-nitrobenzoic acid (mol. wt. 167) was added in aqueous solution to give 0·6 μmole/flask.

The method of Fouts and Brodie[9] was used for the determination of *p*-aminobenzoic acid (PABA), the end-product of the reduction of *p*-nitrobenzoic acid. Nitrofuran concentrations were determined as described above at zero time and after 30 min of incubation. The absorption maxima of these nitrofurans (nitrofurantoin, furazolidone and nifuradene) are 3675 Å, 3670 Å, and 3865 Å and the corresponding $E^{1\%}_{1\,cm}$ values are 753, 747, and 786. The water solubilities per liter at 25° are: nitrofurantoin 190 mg; furazolidone 40 mg; nifuradene 88 mg. The corresponding molecular weights are 238, 225, and 224.

RESULTS

The data in Table 1 (from 5 experiments) show little or no inhibition of glucuronyl transferase as evidenced by measurement of bilirubin conjugation with the three nitrofurans studied at concentrations which approach saturation and which are several times the blood levels of 1–5 mg/l. experienced

TABLE 1. EFFECT OF NOVOBIOCIN, CHLORAMPHENICOL, AND THREE NITROFURANS ON BILIRUBIN CONJUGATION*

	Conc. (M)	Bilirubin conjugation (μg/g liver tissue/hr)					Average inhibition (%)
		Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	
Control		235	275	458	358	222	
Novobiocin	$3·2 \times 10^{-4}$		7	45	54	0	91
Chloramphenicol	$6·2 \times 10^{-4}$		214	244	247		33
Nitrofurantoin	$8·4 \times 10^{-4}$		318	364	374		0
Furazolidone	$3·1 \times 10^{-4}$	235	261		362	212	3
Nifuradene	$7·6 \times 10^{-4}$	259				187	3

* Each flask contained 0·4 ml of 0·5 M potassium phosphate buffer, pH 7·4; 1·2 ml rat serum containing 1·2 mg bilirubin; 0·1 ml 0·5 M $MgCl_2$; 1·3 ml boiled liver extract; 1 ml rat liver homogenate (20% in alkaline KCl), and alkaline KCl to a total of 5 ml. Incubation, 30 min at 37° in air.

clinically with nitrofurans.[10] The data on novobiocin and chloramphenicol are in agreement with those of other workers.[4, 7, 11]

The spectrophotometric method of measuring the degradation rate of the nitrofurans provides an index of the persistence of the nitrofuran portion of the molecule. The data in Table 2 demonstrate that there is no major difference in rate of degradation between the neonatal and the maternal liver tissue for the three nitrofurans. The rate for the neonatal rat kidney is definitely slower than that for the maternal tissue but is still appreciable. The reduced rates are not surprising since histological

TABLE 2. DEGRADATION OF THREE NITROFURANS BY NEONATAL AND MATERNAL LIVER AND KIDNEY SLICES OF RABBITS AND RATS*

Tissue	Degradation (μg/g fresh tissue in 40 min)					
	Nitrofurantoin		Furazolidone		Nifuradene	
	Neonatal	Maternal	Neonatal	Maternal	Neonatal	Maternal
Rabbit liver (5)†	55 ± 4·9‡	64 ± 12·6	95 ± 11·4	117 ± 8·8	68 ± 14·2	78 ± 9·4
Rabbit kidney (5)	75 ± 7·0	100 ± 13·2	101 ± 6·2	132 ± 13·9	89 ± 10·7	122 ± 9·2
Rat liver (5)	94 ± 13·5	106 ± 2·8	120 ± 10·7	108 ± 12·7	105 ± 3·8	104 ± 11·3
Rat kidney (3)	35 ± 10·7	118 ± 17·7	72 ± 1·5	156 ± 15·7	28 ± 8·7	129 ± 29·8

* Conditions: see Methods.

† Figure in parentheses indicates the number of experiments on different animals, except for rat liver with nifuradene where only 3 animals were used.

‡ Mean ± S.E.

TABLE 3. DEGRADATION OF NITRO COMPOUNDS BY 9000 *g* FRACTION OF MATERNAL, NEONATAL, AND MALE RABBIT LIVER AND OF MALE RAT LIVER

Substrate	Parameter measured	Enzyme activity* in hepatic 9000 *g* fractions from:			
		Neonatal rabbit	Maternal rabbit	Male rabbit	Male rat
Nitrofurantoin	Substrate disappearance	503 ± 98 (5)†	2727 ± 191 (5)	2018 ± 315 (5)	2571 ± 171 (5)
Furazolidone	Substrate disappearance	853 ± 136 (5)	3512 ± 267 (5)	2332 ± 432 (4)	2664 ± 495 (5)
Nifuradene	Substrate disappearance	544 ± 148 (5)	3225 ± 250 (5)	not done	3602 ± 147 (5)
p-Nitrobenzoic acid	PABA formed	0·64 ± 0·47 (3)	9·8 ± 1·7 (3)	27 ± 5·1 (3)	28 ± 6·4 (5)

* The values are μg of nitrofuran (±S.E.) disappearing in 30 min/g wet wt. tissue represented; for *p*-nitrobenzoic acid they represent the PABA formed calculated to the same basis.

† The figures in parentheses are the number of experiments carried out.

studies[12] have shown that the newborn rat kidney and the 5-day rat kidney are not fully formed morphologically. They resemble the embryonic kidneys of certain animals and man in which the process of nephron formation is more nearly complete at birth. Buzard and Conklin[13,14] have found that nitrofurans are more slowly degraded by fetal liver and kidney slices from dogs or guinea pigs than by maternal tissue.

Studies were also made with the 9000 *g* fraction described as an active mammalian nitro reductase by Fouts and Brodie.[9] It is apparent from Table 3 that the adult liver 9000 *g* preparations degrade the nitrofurans much more rapidly than the slice preparations (Table 2). This is presumed to be due to the fact that the 9000 *g* preparations are used under strict anaerobic conditions (Warburg) and the slice preparations under aerobic (air) conditions. When the 9000 *g* preparations were used under aerobic conditions, little or no reduction of the nitrofurans was obtained. This is in agreement with the work of Fouts and Brodie[9] on *p*-nitrobenzoic acid. Since animal tissues do not carry out metabolic processes under anaerobic conditions *in vivo*, it was felt that the slice preparations used aerobically might be more representative of processes in the intact animal for work comparing adult and neonatal animals.

In a long-term study in progress in these laboratories on the effect of age on degradation of nitrofurans, we have found 118 μg nitrofurantoin destroyed/g liver/40 min with stock, virgin, female rats that are 1 yr-old, and 105 μg with stock male rats of the same age. In the present study a comparable value of 106 μg was found for maternal liver from rats about 11 months old.

The 9000 *g* preparations of the neonatal rabbit liver did not reduce *p*-nitrobenzoic acid appreciably (in agreement with the results of Fouts and Adamson[5]), but they did reduce the nitrofurans at about 1/5 to 1/6 the rate of the maternal preparations. The difference between the rate of degradation of nitrofurans and of *p*-nitrobenzoic acid by adult preparations is pronounced. On a molar basis there is at least a 60-fold difference for the male rat or rabbit liver preparations and a 190-fold difference for maternal rabbit liver preparations.

Eaton Research Laboratories,
The Norwich Pharmacal Company,
Norwich, New York, U.S.A.

MARY F. PAUL
CATHERINE HARRINGTON

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