Changes in Drug-Metabolizing Activities in the Livers of Suckling Rats as a Result of Treatment of the Lactating Mothers with Phenobarbitone and Chlorpromazine

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(Received 26 October 1970)

1. Lactating female rats were treated with phenobarbitone or chlorpromazine. 2. Hepatic microsomal fractions from the suckling offspring were studied to see if changes had occurred in the rates of drug metabolism. 3. Treatment with phenobarbitone significantly increased the cytochrome *P*-450 concentration, the activity of NADPH-cytochrome *c* reductase (EC 1.6.2.3) and the rates of metabolism of aniline, ethylmorphine and [2-¹⁴C]pentobarbitone. 4. Treatment with chlorpromazine produced only small increases in the cytochrome *P*-450 concentration and the rate of hydroxylation of aniline.

The individual administration of a very wide range of drugs is well known to cause increases, often large, in the rates of metabolism by mammalian liver microsomes of many organic compounds, including drugs (see Conney, 1967; Mannering, 1968; Remmer, 1969). It is important to know if such adaptive increases can arise as a result of transfer of drugs to infant animals via maternal milk secretion, in view of the widespread use of antibiotics, hormones, sedatives and other pharmaceutical preparations in veterinary science and in medical practice. That drugs do pass from the systemic circulation into mammalian milk is well established (Sisodia & Stowe, 1964; Rasmussen, 1966).

The present paper describes changes in several parameters of drug metabolism in cell-free supernatant and microsomal preparations from the livers of nursing rats, after treatment of the lactating mothers with phenobarbitone or chlorpromazine. The parameters chosen for measurement were the rate of hydroxylation of aniline, the rate of demethylation of ethylmorphine, the rate of hydroxylation of [2-¹⁴C]pentobarbitone, cytochrome *P*-450 content, and NADPH-cytochrome *c* reductase activity (EC 1.6.2.3). Fouts & Hart (1965) have briefly described increases in the rates of hydroxylation of hexobarbitone, demethylation of ethylmorphine and reduction of *p*-nitrobenzoic acid in homogenates of livers from young rabbits suckled by mothers treated with phenobarbitone.

MATERIALS AND METHODS

Animals. The rats used were of the albino Wistar strain (Scientific Products Farm Ltd., Ash, Kent, U.K.). After mating, the females were placed in separate cages with

sawdust and woodstraw for bedding. At 4 days after parturition, the litters were decreased to four in number, two male and two female, and daily weight gains were recorded until they were killed. Intraperitoneal injections of phenobarbitone sodium (Gardenal; May and Baker Ltd., Dagenham, Essex, U.K.) or chlorpromazine (Largactil; gift of May and Baker Ltd.) into the mothers were made at doses of 50 and 10 mg/kg body weight respectively as solutions in 0.9% NaCl, which was 0.01 M with respect to potassium phosphate, pH 7.4. Phenobarbitone administration was made only at 14 and 16 days after birth; chlorpromazine was injected daily from 4 to 17 days (inclusive) after birth. Two control groups were also set up with the mothers being sham-injected appropriately.

A further control group was set up so that the urine- and faeces-impregnated sawdust and shavings from the cages of phenobarbitone-treated families were transferred daily to one group of non-injected families. This was a precaution in case offspring could be affected by nibbling solids containing drug or metabolites.

The same types of bedding materials were used for all animals throughout the investigation, because of reports (Vesell, 1967; Wade, Holl, Hilliard, Molton & Greene, 1968) that the rates of drug metabolism could be affected merely by changing the kind of bedding material present.

Chemicals. NADP⁺, NADPH, glucose 6-phosphate, cytochrome *c*, albumin and glucose 6-phosphate dehydrogenase were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Ethylmorphine (Evans Medical Ltd., Speke, Liverpool, U.K.), aniline (British Drug Houses Ltd., Poole, Dorset, U.K.), and [2-¹⁴C]pentobarbitone (New England Nuclear Corp., Boston, Mass., U.S.A.) were used without further purification. The [2-¹⁴C]pentobarbitone was converted with NaOH into the sodium salt for solution and was diluted with unlabelled sodium salt (Nembutal; gift from Abbott Laboratories Ltd., Queenborough, Kent, U.K.) before use.

Preparation of 10 000g supernatant and microsomes. Litters from control and drug-treated mothers were

handled simultaneously as far as possible, offspring from the two groups being killed alternately and the livers placed in ice-cold buffered 1.15% KCl, pH 7.4, which was 0.005 M with respect to potassium phosphate. Livers from the four animals of one litter were pooled and homogenized in the cold with a glass Potter-Elvehjem homogenizer with a motor-driven (up to 3000 rev./min) polytetrafluoroethylene pestle. The homogenates were adjusted with the ice-cold buffered 1.15% KCl so that 1 g of wet-liver equivalent was suspended in 4 vol. of liquid (final volume), then centrifuged at 10000g_{av} for 20 min at 4°C. The supernatants were decanted, leaving behind the 'fluffy layer'; these supernatants were used as such in some experiments ('10000g supernatant'). Otherwise, they were centrifuged at 105000g_{av} for 60 min at 4°C. The resulting reddish gelatinous pellets were resuspended in the buffered 1.15% KCl, pH 7.4, so that the final concentrations were between 100 and 200 mg of wet-liver equivalent/ml. Within these limits, rates of drug metabolism were linear with volume of suspension.

Assay of drug metabolism. Incubation mixtures contained 2.0 ml of 10000g supernatant or microsomal suspension, 2.0 ml of 0.1 M-potassium phosphate buffer, pH 7.4, 0.05 ml of 0.2 M-MgSO₄, 0.5 ml of 0.2 M-nicotinamide, 0.25 ml of NADPH-generation mixture (glucose 6-phosphate, 12 μmol; NADP⁺, 0.5 μmol; glucose 6-phosphate dehydrogenase, 3 units) and the appropriate drug. The final volume was made up to 5.0 ml with water. Ethylmorphine (5 μmol), aniline (6 μmol) and [2-¹⁴C]-pentobarbitone sodium (1.5 μmol, 2.46 μCi) were added as aqueous solutions. Incubations were carried out in duplicate for 15 min at 37°C with shaking. Appropriate control flasks were run simultaneously to determine blank and metabolite recovery values.

p-Aminophenol formed from aniline. This was measured by the method of Wills (1969), modified as follows: 5.0 ml of incubation mixture was poured into 2.5 ml of 15% (w/v) trichloroacetic acid and the precipitated protein filtered off. To 5.0 ml of filtrate, 1.0 ml of 1% phenol in 0.5 M-NaOH was added, followed by 2.0 ml of 2 M-Na₂CO₃. After the mixture had stood for 30 min at 37°C, the blue colour of indophenol was measured spectrophotometrically at 620 nm, and the concentration of p-aminophenol calculated by direct comparison with the values obtained for the recovery flasks, to which 20.0 μg of p-aminophenol in acetone had been added at the end of the incubation.

Formaldehyde formed from the demethylation of ethylmorphine. This was determined by the method of Nash (1953) by using the double-strength acetylacetone-ammonium acetate-acetic acid mixture recommended by Cochin & Axelrod (1959). The incubation mixtures were poured into 2.0 ml of 20% (w/v) ZnSO₄ and protein was precipitated by adding 2.0 ml of saturated Ba(OH)₂ and centrifuging. Supernatant (5.0 ml) was added to 2.0 ml of Nash (1953) reagent and the yellow colour formed after standing for 15 min at 60°C was measured spectrophotometrically at 415 nm. The concentration of formaldehyde was calculated by comparison with the values obtained for the recovery flasks, to which 15.4 μg of formaldehyde in water had been added at the end of the incubation time. The stock standard solution of formaldehyde was itself standardized by reference to the acid hydrolysis of hexamethylenetetramine in a sealed vessel. In this procedure, the NH₃ liberated is assayed by back-titration with NaOH

of the HCl into which the NH₃ has been steam-distilled. The formaldehyde simultaneously liberated is thus of known concentration.

5-Ethyl-5-(3-hydroxy-1-methylbutyl)-[2-¹⁴C]barbituric acid. This, the probable metabolite of [2-¹⁴C]pentobarbitone, was assayed by a modification of the method of Kuntzman, Ikeda, Jacobson & Conney (1967): 5.0 ml of incubation mixture was poured into a 30 ml stoppered centrifuge tube containing 2.0 ml of 1.0 M-sodium acetate, pH 5, 1.5 g of NaCl, and 10 ml of light petroleum (b.p. 40–60°C) 1.5% (v/v) with respect to 3-methylbutan-1-ol. After vortex mixing for 15 s, and centrifuging, the petroleum layer was discarded. This process was repeated twice to extract the bulk of the unchanged drug. Then two extractions were made with 10 ml of ethyl acetate each time, with vortex mixing and centrifuging as before. The ethyl acetate layers were pooled, evaporated to dryness under nitrogen, and the whole extract was streaked on to an analytical t.l.c. plate (5 cm × 20 cm) (E. Merck A.-G., Darmstadt, Germany), coated with silica gel G254. The plate was developed for 1½–2 h with chloroform-acetone (9:1, v/v). After drying, radioactive areas were detected with a Panax radiochromatogram scanner. Unchanged [2-¹⁴C]pentobarbitone (*R_F* 0.42–0.56) always ran with a good separation from the more polar metabolite (*R_F* 0.04–0.10), which, in control experiments, ran as a single spot with authentic 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid (see below), after recrystallization to constant specific radioactivity. The areas of the plates corresponding to the metabolite were scraped into vials for liquid-scintillation counting with Bray's (1960) dioxan-based solution in a Packard model 3320 liquid-scintillation spectrometer. The machine settings used (gain 9%; window 10–1000 V) gave counting efficiencies of 76–78%, and no quench corrections were found to be necessary. The counting rates obtained with recovery flasks were subtracted from those obtained as a result of 15 min incubation, before calculation of molar yields.

Synthesis of 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid. This compound was necessary as a chromatographic reference, and synthesis was attempted by the method of Dickert, Shea & McCarty (1966). Two modifications were found to be necessary.

1. The reduction and cyclization of diethylethyl(1-methyl-3-oxobutyl)malonate (I) to ethylethyl(1-methyl-3-hydroxybutyl)malonate lactone (II) were accomplished by using NaBH₄ instead of Raney nickel/H₂ (3 atm) as follows: 29.5 g of compound (I) was dissolved in 60 ml of dry ethanol, the solution was cooled to 0°C, and 1.6 g of NaBH₄ was rapidly added. The flask was stoppered and swirled until all the solid had dissolved. After 2½ h at 0°C the mixture was poured into 1½ litres of water, and the whole was extracted with 300 ml of diethyl ether. The ether layer was dried over anhydrous MgSO₄ and the ether was then evaporated off at 40°C until a yellow oil was left. This oil was distilled under vacuum, the fraction corresponding to compound (II) being collected at 130–133°C at 1.5 mm Hg.

2. Condensation of compound (II) with urea to give the required product proceeded smoothly, except that no crystallization occurred after the acidification of the aqueous extract of the reaction. Two extractions with ethyl acetate gave the product in a yellow solution. After drying of the pooled ethyl acetate fractions over an-

hydrous MgSO_4 , the ethyl acetate was driven off slowly by a stream of N_2 , and a light-yellow precipitate formed when the volume was small. This precipitate was filtered off, leaving a viscous yellow oil. The precipitate was several times recrystallized from boiling water as fine off-white plates which, after drying, were found to have all the properties reported for 5-ethyl-5-(3-hydroxy-1-methylbutyl)barbituric acid by Dickert *et al.* (1966).

Cytochrome P-450. This was assayed as described by Omura & Sato (1967), their value of $91 \text{ cm}^{-1} \cdot \text{mm}^{-1}$ for ϵ being used.

NADPH-cytochrome c reductase. This was measured by the method of Williams & Kamin (1962), the value of $18.5 \text{ cm}^{-1} \cdot \text{mm}^{-1}$ for ϵ being used (Margoliash, 1954; see Gigon, Gram & Gillette, 1969).

Protein. This was determined by the procedure of Lowry, Rosebrough, Farr & Randall (1951) with bovine plasma albumin as standard.

RESULTS

The nutritional state of an animal can markedly affect the rate of hepatic drug metabolism (Kato & Gillette, 1965; Kato, 1967; see Parke, 1968). As a general rule, pair-feeding or force-feeding should therefore be adopted for control and drug-treated animals in case drug administration causes inadequate feeding. This cannot be done in lactational experiments, and daily weighing of the young is absolutely necessary. No significant changes in litter growth rates in fact resulted from administration of either chlorpromazine or phenobarbitone to the mothers.

Phenobarbitone administration to lactating rats causes an increase in the rate of drug metabolism in the livers of the pups (see Table 1). The metabolism of $[2\text{-}^{14}\text{C}]\text{pentobarbitone}$ by 10000g supernatant is increased fivefold relative to the control animals on the basis of molar quantities hydroxyl-

ated per unit of body weight or per unit of supernatant protein. The hydroxylation of aniline and the demethylation of ethylmorphine are not significantly affected, a somewhat surprising result in view of the results in Table 2 on microsomal activities. In addition to results on actual rates of drug metabolism, Table 2 includes results on NADPH-cytochrome c reductase activities and cytochrome P-450 concentrations. Irrespective of the basis on which the parameters are expressed, phenobarbitone administration causes increases in all five of them. However, only when calculated as $\mu\text{mol/kg}$ body wt. is the difference in cytochrome P-450 content highly significant. Expressed as nmol/100mg of protein, or as nmol/total liver equivalent, there is only a trend to statistical significance in the difference in hepatic microsomal cytochrome P-450 content between the litters from control mothers and those of phenobarbitone-treated mothers. There is a twofold increase in NADPH-cytochrome c reductase activity, a five- to six-fold increase in the rate of hydroxylation of $[2\text{-}^{14}\text{C}]\text{pentobarbitone}$, a $2\frac{1}{2}$ –3 $\frac{1}{2}$ -times increase in the rate of demethylation of ethylmorphine, but only a 30–50% increase in the rate of hydroxylation of aniline. Cytochrome P-450 content is increased by 30–70%.

The treatment of lactating mothers with chlorpromazine causes little change in any of the parameters measured. There is a statistically significant elevation of cytochrome P-450 concentration of about 20%, but only in nmol/100mg of protein. The rates of demethylation of ethylmorphine and hydroxylation of $[2\text{-}^{14}\text{C}]\text{pentobarbitone}$ are unaffected by the treatment with chlorpromazine. There is a trend to a 20% increase in the rate of hydroxylation of aniline, when expressed as nmol

Table 1. *Hydroxylation of aniline, demethylation of ethylmorphine and hydroxylation of $[2\text{-}^{14}\text{C}]\text{pentobarbitone}$ by 10000g supernatant preparations from the livers of 18-day-old rats suckled by phenobarbitone-treated mothers*

Numbers of separate families used are given in parentheses. Results are expressed as means $\pm$ S.E.M. Values of P were calculated by using Student's t test. N.S., not significant.

Treatment of lactating mothers	Aniline hydroxylation (<i>p</i> -aminophenol formed/ 15 min)		Ethylmorphine demethyl- ation (formaldehyde formed/15 min)		$[2\text{-}^{14}\text{C}]\text{Pentobarbitone}$ hydroxylation (5-ethyl-5-(3-hydroxy-1-methylbutyl)- $[2\text{-}^{14}\text{C}]\text{barbituric acid}$ formed/15 min)	
	(nmol/100 mg of supernatant total protein)	($\mu\text{mol/kg}$ offspring body wt.)	(nmol/100 mg of supernatant total protein)	($\mu\text{mol/kg}$ offspring body wt.)	(nmol/100 mg of supernatant total protein)	($\mu\text{mol/kg}$ offspring body wt.)
Control (sham-injected twice during 4 days)	377 ± 35 (6)	8.6 ± 0.8 (6)	183 ± 41 (6)	4.20 ± 0.97 (6)	41.6 ± 1.9 (6)	0.94 ± 0.04 (6)
Phenobarbitone (injected twice during 4 days)	471 ± 71 (6)	11.2 ± 1.8 (6)	130 ± 19 (6)	3.10 ± 0.63 (6)	222 ± 16 (5)	5.09 ± 0.39 (5)
P	N.S.		N.S.		<0.001	

Table 2. *Hydroxylation of aniline, N-demethylation of ethylmorphine, hydroxylation of pentobarbitone, cytochrome P-450 content and NADPH-cytochrome c reductase activities in microsomal preparations from the livers of 18-day-old rats suckled by phenobarbitone- and chlorpromazine-treated mothers*

Numbers of separate families used are given in parentheses. Results are expressed as means $\pm$ S.E.M., except for the one row of control values where results from two separate experiments are presented. Values of *P* were calculated by using Student's *t* test. N.S., not significant.

Treatment of lactating mothers	Aniline hydroxylation (<i>p</i> -aminophenol formed/15 min)			Ethylmorphine demethylation (formaldehyde formed/15 min)		
	(nmol/100 mg of microsomal protein)	(nmol/total liver equivalent)	(μ mol/kg of offspring)	(nmol/100 mg of microsomal protein)	(nmol/total liver equivalent)	(μ mol/kg of offspring)
Control (sham-injected twice during 4 days)	914 $\pm$ 54 (5)	747 $\pm$ 77 (5)	5.13 $\pm$ 0.25 (5)	2310 $\pm$ 260 (5)	1860 $\pm$ 210 (5)	12.7 $\pm$ 0.9 (5)
Phenobarbitone (injected twice during 4 days)	1200 $\pm$ 90 (5)	991 $\pm$ 81 (6)	7.75 $\pm$ 0.48 (6)	5990 $\pm$ 510 (5)	5250 $\pm$ 730 (6)	40.1 $\pm$ 4.6 (6)
<i>P</i>	0.05 > <i>P</i> > 0.02	0.10 > <i>P</i> > 0.05	< 0.01	< 0.001	< 0.01	< 0.001
Control (contact with nesting material of phenobarbitone-treated animals) (two litters)	789; 668	659; 684	6.25; 3.86	2404; 1530	1700; 1540	16.2; 8.8
Chlorpromazine (injected for 14 days)	991 $\pm$ 62 (6)	953 $\pm$ 133 (6)	6.04 $\pm$ 0.51 (6)	1860 $\pm$ 130 (6)	1780 $\pm$ 220 (6)	11.3 $\pm$ 0.8 (6)
Control (sham-injected for 14 days)	805 $\pm$ 51 (4)	910 $\pm$ 109 (4)	6.01 $\pm$ 0.63 (4)	1720 $\pm$ 50 (4)	1970 $\pm$ 220 (4)	12.8 $\pm$ 1.0 (4)
<i>P</i>	0.10 > <i>P</i> > 0.05	N.S.	N.S.	N.S.	N.S.	N.S.
[2- ¹⁴ C]Pentobarbitone hydroxylation (5-hydroxy-5-(3-hydroxy-1-methylbutyl)[2- ¹⁴ C]- barbituric acid formed/15 min)						
	Cytochrome P-450 content			NADPH-cytochrome c reductase		
	(nmol/100 mg of microsomal protein)	(nmol/total liver equivalent)	(μ mol/kg of offspring)	(μ mol/min per 100 mg of micro- somal protein)	(μ mol/min per total liver equivalent)	(mmol/min per kg of offspring)
121 $\pm$ 15 (3)	87.2 $\pm$ 4.7 (3)	0.76 $\pm$ 0.09 (3)	105 $\pm$ 9 (5)	84.6 $\pm$ 7.6 (5)	584 $\pm$ 36 (5)	12.9 $\pm$ 1.7 (5)
654 $\pm$ 89 (4)	554 $\pm$ 92 (5)	4.33 $\pm$ 0.58 (5)	133 $\pm$ 9 (5)	129 $\pm$ 17 (6)	987 $\pm$ 108 (6)	22.0 $\pm$ 1.2 (5)
< 0.01	< 0.01	< 0.01	0.10 > <i>P</i> > 0.05	0.10 > <i>P</i> > 0.05	< 0.01	< 0.01
92.5; 172	77.3; 145	0.73; 1.00	71.3; 72.2	59.6; 63.1	566; 417	15.3; 12.9
						12.8; 11.2
						122; 74.3
122 $\pm$ 17 (6)	116 $\pm$ 21 (6)	0.73 $\pm$ 0.09 (6)	81.9 $\pm$ 4.4 (6)	77.8 $\pm$ 8.9 (6)	493 $\pm$ 26 (6)	14.0 $\pm$ 0.9 (6)
102 $\pm$ 4 (4)	116 $\pm$ 14 (4)	0.77 $\pm$ 0.08 (4)	69.8 $\pm$ 2.4 (5)	76.7 $\pm$ 6.0 (5)	504 $\pm$ 32 (5)	14.0 $\pm$ 0.9 (5)
N.S.	N.S.	N.S.	0.05 > <i>P</i> > 0.02	N.S.	N.S.	N.S.

of *p*-aminophenol formed/100mg of protein. All the results are based on comparisons with the appropriate sham-injected control groups.

The transfer of nesting materials from the cages of phenobarbitone-treated families to non-injected families causes no change in any of the parameters estimated. Table 2 shows that the two families tested gave results very similar to those associated with sham-injection, with respect to all five parameters.

DISCUSSION

In newborn and very young animals, the activity of the hepatic enzyme systems involved in the metabolism of drugs is low, but the degree of increase of this basal activity after the administration of inducing agents such as phenobarbitone is in general much more marked than in adults (Mannering, 1968). The effect on the suckling young of treating the lactating mothers with drugs should then be more pronounced than in control animals.

However, other factors have to be taken into account, such as the strain of animal used, the sex and the nutritional state (see Parke, 1968; Remmer, 1969). The use of different strains of rat may result in altered responses (Cram, Juchau & Fouts, 1965), but the results of Furner, Gram & Stitzel (1969) and Page & Vesell (1969) show that these are of degree and not of kind. Furner *et al.* (1969) have also shown that sex differences in the Wistar strain of rat (used here) are less pronounced for 30-day-old animals than for 100-day-old animals, both intrinsically and in respect of the inductive effect of phenobarbitone on the hepatic microsomal metabolism of aniline, ethylmorphine and hexobarbitone. The same authors have further shown that older male Wistar rats may not be inducible at all, although the kinetic results of Page & Vesell (1969) do not support this. Klinger, Kusch, Neugebauer, Splinter & Ankermann (1968) report that with increasing age, male Wistar rats over 18 days old gradually lose their responsiveness to barbitone with respect to the hepatic microsomal hydroxylation of

phenazone and the demethylations of codeine and aminophenazone.

The effects of sex- and age-differences were minimized in the experiments described here by using litters composed of equal numbers of male and female pups, and by killing all animals at 18 days of age.

As mentioned in the Results section, variations in lactational performances of the mothers were checked by measuring litter weight-gains, but these were found to be unaffected by drug treatments of the mothers.

Effects of phenobarbitone treatment on rates of drug metabolism. The fivefold increase in the rate of hydroxylation of [2-¹⁴C]pentobarbitone by hepatic 10000g supernatant of young rats, which occurs (Table 1) as a result of administering 50mg of phenobarbitone/kg body wt. twice to the nursing mothers, is very significant, in view of the report that as little as 0.5mg of phenobarbitone/kg administered intraperitoneally twice daily is sufficient to stimulate the metabolism of hexobarbitone in young rats (see Conney, 1967). More than this dose must then pass into the pups via the milk. The apparent lack of change in the rates of hydroxylation of aniline and demethylation of ethylmorphine could well be a result, first, of high and variable blank values being obtained from the assays, and second, of an unknown inhibitor being present in the 10000g supernatant preparation, which retarded the rates of formation of the coloured compounds finally measured spectrophotometrically (see the Materials and Methods section).

No such problems were encountered with the microsomal preparations by using identical assay methods. There seem to be no other reports in the literature of the same problems with 10000g supernatants. With suckling rats it is plain that drug-metabolism studies *in vitro* should be carried out with microsomal preparations rather than with whole homogenates or supernatants.

Table 2 demonstrates conclusively that the rates of hydroxylation of aniline and demethylation of ethylmorphine are, in fact, increased in the livers of the pups after treatment of their lactating mothers with phenobarbitone. As expected, the increase relative to control litters in the rate of hydroxylation of [2-¹⁴C]pentobarbitone is the same (five- to six-fold) whether microsomes or 10000g supernatant are considered.

In view of the large adaptive increases in the rates of metabolism of ethylmorphine and [2-¹⁴C]pentobarbitone, it seems unlikely that any of the factors described by Fouts (1970) and Sasame & Gillette (1970) as inhibitors of drug metabolism *in vitro* are operating to any but a small degree. These factors include the nicotinamide concentration in the incubation mixtures, the protein concentration, the

rate of shaking and the use of air instead of oxygen as the gas phase.

There are many reports in the literature that hepatic microsomal cytochrome *P*-450 content is markedly elevated (by at least twofold) when male or female rats are treated with phenobarbitone (e.g. Orrenius & Ernster, 1964; Kato, 1966; Guarino, Gram, Gigon, Greene & Gillette, 1969; Remmer, 1969; Sladek & Mannering, 1969). Such increases result from quite large dosages and are associated with simultaneous rises of similar size in drug-metabolizing activities such as the demethylations of aminopyrine and ethylmorphine, the hydroxylation of pentobarbitone and the activity of NADPH-cytochrome *c* reductase. Cytochrome *P*-450 content should then be a guide to inductions of drug-metabolizing activities in general.

It appears from Table 2 that, for suckling rats, measurements of cytochrome *P*-450 concentrations are not nearly such sensitive guides to adaptive changes in the capacity of hepatic microsomes to metabolize drugs as the measurements of actual metabolite formation from drugs themselves. The increases in the rates of demethylation of ethylmorphine and the hydroxylation of [2-¹⁴C]pentobarbitone are relatively large, and it is suggested that either of these drugs can be used successfully on its own as a routine assay for the capacity of tissues to respond to foreign compounds. The rate of hydroxylation of aniline is only slightly increased in the livers of the offspring after treatment of the mothers with phenobarbitone, and comparison of the ratios of the individual values showed that this parameter and cytochrome *P*-450 concentrations and the activities of NADPH-cytochrome *c* reductase were all increased by the same small relative amounts. This is evidence that only minimal quantities of drug pass into the offspring via the milk. Page & Vesell (1969) found no correlation between cytochrome *P*-450 content of liver microsomes and the rate of hydroxylation of aniline and the demethylation of ethylmorphine in studies of the effects of phenobarbitone treatment on hepatic drug metabolism in various strains of rats.

Expression of all five parameters in Table 2 on the basis of liver microsomal activity or content per unit of litter body weight gives the most statistically significant indications of increases arising from the treatment with phenobarbitone. The reasons for this are obscure, as the microsomal protein content per g of liver is not significantly changed (control value 15.6 ± 1.2 mg/g of liver), and neither are the weight of liver per kg offspring body wt., nor the total liver weight per litter (control values 4.1 ± 0.4 g and 36.2 ± 1.5 g/kg offspring body weight respectively).

Effects of treatment with chlorpromazine on rates of drug metabolism. Treatment of lactating mothers

with chlorpromazine for 14 days produces an increase in nmol of cytochrome *P*-450/100mg of hepatic microsomal protein, and a trend to an increase in the rate of metabolism of aniline per 100mg of protein. No other changes are detectable. Increases of 100–200% were found by Platt & Cockrill (1969) in the rate of demethylation of aminopyrine and in the activity of NADPH-cytochrome *c* reductase in liver microsomes of rats fed on high doses of chlorpromazine for 14 days. Kato, Chiesara & Vassanelli (1964) and Kato (1966) described 40–60% increases in the rates of metabolism of pentobarbitone, aminopyrine, hexobarbitone and meprobamate by an 8500 g supernatant of rat liver as a result of administering chlorpromazine at a dosage of 12–15mg/kg body weight 48–60h previously. It thus seems likely in the experiments reported here that very little chlorpromazine enters the offspring via the milk, and further, that the response to chlorpromazine is completely different from that to phenobarbitone. This is unexpected in the light of the reports mentioned above that chlorpromazine does cause adaptive increases in the rates of metabolism of barbiturates; the assays described here for the rate of hydroxylation of [2-¹⁴C]pentobarbitone are very sensitive.

Some additional experiments were carried out whereby chlorpromazine was administered for 4 days to six lactating mothers to see if the results of treatment for 14 days did in fact represent a small adaptive increase in the rate of hydroxylation of aniline and of cytochrome *P*-450 content. This could not positively be ascertained; there were no significant deviations in the rates of metabolism of aniline, ethylmorphine and [2-¹⁴C]pentobarbitone from the rates shown in Table 2 for control animals sham-injected for 4 days. There was, however, a significant decrease in cytochrome *P*-450 concentration (to 65.2 ± 8.9 nmol/100mg of microsomal protein), and a highly significant increase in the activity of NADPH-cytochrome *c* reductase (to 15.2 ± 1.2 μ mol/min per total equivalent liver) associated with a significant increase in the same enzyme activity expressed in mmol/min per kg equivalent body wt. of offspring (to 94.3 ± 2.2), but no change in the activity expressed as μ mol/min per 100mg of microsomal protein (14.1 ± 1.1). A possible explanation of these findings lies in the fact that treatment with chlorpromazine for 4 or 14 days caused no changes in the microsomal protein content/g of liver, but treatment for 4 days significantly elevated the total liver weight per litter (to 6.0 ± 0.4 g), compared with the appropriate control value (4.1 ± 0.4 g), whereas treatment for 14 days caused no change.

Conclusion. The administration of phenobarbitone during 4 days to the lactating mothers of suckling rats causes adaptive increases in the

capacity of the hepatic microsomes of the young to metabolize aniline, ethylmorphine and [2-¹⁴C]-pentobarbitone. The concentration of cytochrome *P*-450 and the activity of NADPH-cytochrome *c* reductase are also increased. In contrast, the administration of chlorpromazine for 14 days to the lactating mothers causes no significant increase in the rates of metabolism of aniline, ethylmorphine and [2-¹⁴C]pentobarbitone, and no change in the activity of NADPH-cytochrome *c* reductase. The concentration of cytochrome *P*-450 is, however, significantly increased.

I thank the Wellcome Trust for a grant under the tenure of which this work was performed. Professor D. A. Price Evans gave most helpful advice and encouragement. Skilled technical assistance was provided by Mr R. K. Grundy. The maintenance of the animal colonies was carried out with the help of Mr G. Norton and Mr P. Hynes.

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