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EFFECT OF PHENOBARBITAL ON THE METABOLISM OF PENTOBARBITAL AND MEPERIDINE IN FETAL RABBITS AND RATS*†

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Abstract—Treatment of pregnant rats or rabbits with phenobarbital during the last week of pregnancy stimulated the activity of hepatic enzymes that metabolize pentobarbital and meperidine in the newborn. However, considerable variability was observed for the induction of pentobarbital and meperidine metabolism in the newborn and an occasional litter obtained from mothers treated with phenobarbital did not have enhanced microsomal enzyme activity. The rate of pentobarbital metabolism observed *in vitro* in the newborn rabbit paralleled the rate of pentobarbital metabolism *in vivo*.

NEWBORN animals of a number of species including man are deficient in hepatic microsomal enzymes necessary for the metabolism of a wide variety of drugs and certain naturally occurring substrates such as bilirubin.[1–4] The deficiency of microsomal drug-metabolizing enzymes in the newborn may play a role both in the depression that occurs in infants born to mothers that are heavily medicated with barbiturates or analgesics during childbirth and in the transient elevation in unconjugated bilirubin that occurs in the plasma of the newborn.

The present study was undertaken to determine whether treatment of pregnant rats and rabbits with phenobarbital during the last few days of pregnancy would increase the metabolism of meperidine and pentobarbital *in vitro* and *in vivo* in the newborn. The results show that livers from newborn rabbits and rats have low meperidine *N*-demethylating and pentobarbital hydroxylating enzyme activity and that the activity of these enzymes can be increased by treatment of the mother with phenobarbital during the last few days of pregnancy. These experiments also show that the increase in pentobarbital-metabolizing activity of liver from newborn rabbits born to mothers treated with phenobarbital is reflected in increased ability of these animals to metabolize pentobarbital *in vivo*.

METHODS

Animals. Pregnant New Zealand white rabbits were obtained from Camm Research Institute, (Wayne, N.J.) and were maintained on Rockland rabbit ration (Teklad, Inc.; Monmouth, Ill.) with free access to water. They were treated with sodium

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phenobarbital (40 mg/kg/day) administered subcutaneously from day 26–30 of gestation. When necessary, 20 mg/kg/day was administered for an additional day or two until the litter was born. This was found to be the maximum dose of phenobarbital that could be given for this length of time without unduly sedating the pregnant rabbits. Newborn rabbits 3–18 hr old were either sacrificed for studies *in vitro* or injected with drug for studies *in vivo*. Dams were sacrificed at the same time as their litters.

Pregnant Sprague–Dawley rats were obtained from Blue Spruce Farms, Inc. (Altamont, N.Y.) and were maintained on Rockland rat and mouse diet complete (Teklad, Inc.; Monmouth, Ill.) with free access to water. They were treated with sodium phenobarbital (75 mg/kg/day) administered subcutaneously from day 19–21 of gestation and with 40 mg/kg on day 22 of gestation. Newborn rats were killed 6–18 hr after birth. Dams were killed at the same time as their litters.

Enzyme assays. Adult rabbits were killed by the intravenous injection of air, adult rats were killed by a blow on the head, and all newborn animals were killed by decapitation. The liver was immediately excised, placed in a beaker immersed in ice, and was homogenized with 4 vol. of cold 0·1 m phosphate buffer (pH 7·4) in a teflon-glass homogenizer. The homogenate was centrifuged at 9000 *g* for 30 min, and the supernatant fraction was used as a source of enzyme.

Meperidine *N*-demethylation was determined by measuring the amount of formaldehyde formed. A typical reaction mixture contained enzyme preparation obtained from 200 mg liver, 20 μmole meperidine hydrochloride, 100 μmole semicarbazide (neutralized to pH 7·4), a cofactor mixture consisting of 0·2 ml of 0·3 M glucose 6-phosphate, 0·1 ml NADP solution (4 mg/ml), 0·2 ml of 0·01 M adenosine triphosphate, 0·2 ml of 0·6 M nicotinamide, 0·1 ml of 2 M KCl, 0·1 ml of 0·1 M $MgCl_2$ and 0·1 M phosphate buffer, pH 7·4, in a final volume of 6 ml. The mixture was incubated in air with shaking at 37° for 60 min for experiments with rabbit liver or for 15 min with rat liver. The shorter incubation time for rat liver was necessary because the reaction rate remained linear for only 15 min. Under these conditions, the reaction rate was linear with respect to enzyme concentration and time. Semicarbazide, in the concentration used, completely trapped the formaldehyde formed.

After the incubation, the protein was precipitated by addition to the incubation mixture of 2 ml of 20% $ZnSO_4$, which was followed by 2 ml of a saturated solution of barium hydroxide. The sample was thoroughly mixed and centrifuged. A 5-ml aliquot of the protein-free supernatant was treated with 2 ml of double strength Nash reagent and incubated at 60° for 30 min. This solution was then cooled and the colored product was extracted into 5 ml isoamyl alcohol. The color intensity at 515 mμ was determined with a Beckman DU spectrophotometer.[5–7] Known quantities of formaldehyde (analytical reagent grade, Mallinckrodt) were added to the incubation mixture in place of meperidine and served as standards. It was found that formaldehyde was not metabolized by liver under the conditions of our experiments.

For meperidine *N*-demethylation by rabbit liver, an O.D. twice the blank was equivalent to the formation of 0·1 μmole formaldehyde/g liver/hr. For experiments with rat liver, an optical density twice the blank was equivalent to 0·4 μmole formaldehyde formed/g liver/hr.

The metabolism *in vitro* of pentobarbital-2-C^{14} during a 10-min incubation was determined as previously described.[8] In this method, unmetabolized pentobarbital-

2-C^{14} was extracted from the incubation mixture with 1·5% isoamyl alcohol in petroleum ether, and the pentobarbital alcohols were then extracted into ethyl acetate and quantified in a liquid scintillation spectrometer without chromatography. Radioactivity twice the zero time values correspond to the metabolism of 0·1 μmole pentobarbital/g liver/hr.

Measurement of pentobarbital metabolism in vivo. Rabbits, 3–18 hr old, were injected i.p. with a subhypnotic dose (4 mg/kg; 20 μc/kg) of pentobarbital-2-C^{14} (Tracerlab). Environmental temperature was maintained at 27–29°. At the end of 4 hr, each animal was homogenized in a Waring-blendor with 4 vol. of 0·1 M citric acid solution. Two g NaCl was added to a 5-ml aliquot of the homogenate, and the unmetabolized pentobarbital-2-C^{14} was extracted into 30 ml benzene containing 1·5% isoamyl alcohol. The radioactivity of an aliquot of the benzene phase was determined in a scintillation spectrometer.

RESULTS

Newborn rabbits have very low levels of hepatic meperidine *N*-demethylating enzyme activity (Table 1). Newborn rabbits born to mothers treated with pheno-

TABLE 1. EFFECT OF CHRONIC ADMINISTRATION OF PHENOBARBITAL TO PREGNANT RABBITS ON THE HEPATIC METABOLISM OF MEPERIDINE *in vitro* BY THE NEWBORN AND DAM*

Phenobarbital administration to pregnant rabbit	Meperidine *N*-demethylase (μmole CH_2O formed/g liver/hr)	
	Newborn	Dam
No	0·00	3·99
No	0·00	4·31
No	0·09	4·15
No	0·10	2·48
No	0·10	4·03
No	0·18	
No (av.)	0·08	3·79
Yes	0·00	13·95
Yes	0·16	8·42
Yes	0·17	
Yes	0·68	9·55
Yes	1·29	9·86
Yes	2·28	12·15
Yes	3·21	
Yes (av.)	1·11	10·78

* Sodium phenobarbital (40 mg/kg/day, s.c., once daily) was administered to pregnant rabbits from day 26–30 of gestation, and 20 mg/kg/day was administered thereafter until the birth of the litter. Newborn rabbits were sacrificed at 3–18 hr after birth. Dams were sacrificed at the same time as their litters. Each value for meperidine *N*-demethylase activity in the newborn represents the average for the litter determined by using pooled livers from 2–7 animals.

barbital had a mean level of *N*-demethylase considerably higher than rabbits born to untreated mothers. Moreover, they showed an extremely wide variation in enzyme activity. While *N*-demethylase activity for some litters of the newborn rabbits was markedly increased by phenobarbital administration, no increase in enzyme activity

was observed in other litters. Enhanced meperidine *N*-demethylase activity was observed, however, in the livers of all mothers that were treated with phenobarbital.

Newborn rabbits also have low levels of pentobarbital hydroxylating activity in the liver (Table 2). The mean level of this activity in the newborn animal was substantially increased after treatment of the mother with phenobarbital during the last

TABLE 2. EFFECT OF CHRONIC ADMINISTRATION OF PHENOBARBITAL TO PREGNANT RABBITS ON THE HEPATIC METABOLISM OF PENTOBARBITAL *in vitro* BY THE NEWBORN AND DAM*

Phenobarbital administered to pregnant rabbit	Pentobarbital hydroxylated *in vitro* (μmole/g liver/hr)	
	Newborn	Dam
No	0·00	0·80
No	0·01	0·45
No	0·01	0·34
No	0·02	
No	0·02	0·15
No	0·07	0·15
No (av.)	0·02	0·38
Yes	0·08	
Yes	0·15	2·64
Yes	0·22	1·23
Yes	0·32	1·59
Yes	0·65	
Yes (av.)	0·28	1·82

* Sodium phenobarbital (40 mg/kg/day, s.c., once daily) was administered to pregnant rabbits from day 26–30 of gestation and 20 mg/kg/day was administered thereafter until the birth of the litter. Newborn rabbits were sacrificed at 3–18 hr after birth. Dams were sacrificed at the same time as their litters. Each value for pentobarbital metabolism in the newborn represents the average for the litter obtained by using pooled livers from 2–7 animals.

few days of pregnancy. As was the case with meperidine, the newborn animals born to treated mothers showed an extremely wide variation in their response to phenobarbital. Although pentobarbital hydroxylase activity for some of the newborn animals was markedly increased by phenobarbital administration, no increase in enzyme activity was observed in others, despite good induction in their mothers.

Studies in rats revealed that the newborn of this species were deficient in hepatic enzymes that *N*-demethylate meperidine (Table 3) and oxidize pentobarbital (Table 4). All newborn rats born to mothers treated with phenobarbital for 4 days, as described in Table 4, showed an increase in both enzyme activities to levels found in the adult female. In another experiment where phenobarbital was administered to pregnant rats for 8 days, no enzyme induction occurred in the newborn despite good induction in the mothers.

Experiments were done on newborn rabbits to determine whether changes in pentobarbital-metabolizing activity *in vitro* were correlated with changes in ability to metabolize pentobarbital *in vivo* (Table 5). At the same time that litter-mates were sacrificed for determinations of enzyme activity *in vitro*, several animals from the same litter were injected i.p. with pentobarbital for studies *in vivo*.

Litters that metabolized pentobarbital rapidly *in vivo* had high levels of pentobarbital-metabolizing enzymes in the liver. Newborn rabbits born to mothers that had been treated with phenobarbital were able to metabolize pentobarbital at substantially faster rates than animals from control litters.

TABLE 3. EFFECT OF CHRONIC ADMINISTRATION OF PHENOBARBITAL TO PREGNANT RATS ON THE METABOLISM OF MEPERIDINE *in vitro* BY THE NEWBORN AND DAM*

Phenobarbital administered to pregnant rat	Meperidine *N*-demethylase (μmoles CH_2O formed/g liver/hr)	
	Newborn	Dam
No	0·23	1·63
No	0·44	2·34
No	0·47	1·31
No	0·49	1·49
No (av.)	0·41	1·69
Yes	1·98	5·98
Yes	2·24	8·72
Yes	2·28	6·28
Yes	3·06	5·42
Yes (av.)	2·39	6·60

* Sodium phenobarbital (75 mg/kg/day, s.c., once daily) was administered to pregnant rats from day 19–21 of gestation and 40 mg/kg was administered on day 22 of gestation. Newborn rats were sacrificed at 6–18 hr after birth. Dams were sacrificed at the same time as their litters. Each value for meperidine *N*-demethylase activity represents the average for the litter determined by using pooled livers from 9–12 animals.

TABLE 4. EFFECT OF CHRONIC ADMINISTRATION OF PHENOBARBITAL TO PREGNANT RATS ON THE METABOLISM OF PENTOBARBITAL *in vitro* BY THE NEWBORN AND DAM*

Phenobarbital administered to pregnant rat	Pentobarbital hydroxylated *in vitro* (μmole/g liver/hr)	
	Newborn	Dam
No	0·05	0·21
No	0·09	0·35
No	0·10	0·35
No	0·13	0·49
No (av.)	0·09	0·35
Yes	0·32	2·14
Yes	0·39	2·84
Yes	0·45	3·64
Yes	0·46	1·97
Yes (av.)	0·40	2·65

* Sodium phenobarbital (75 mg/kg/day, s.c., once daily) was administered to pregnant rats from day 19–21 of gestation and 40 mg/kg was administered on day 22 of gestation. Newborn rats were sacrificed 6–18 hr after birth. Dams were sacrificed at the same time as their litters. Each value for pentobarbital oxidation represents the average for the litter determined by using pooled livers from 9–12 animals.

TABLE 5. EFFECT OF CHRONIC ADMINISTRATION OF PHENOBARBITAL TO PREGNANT RABBITS ON THE METABOLISM OF PENTOBARBITAL *in vitro* AND *in vivo* BY THE NEWBORN*

Phenobarbital administered to pregnant rabbit	Pentobarbital metabolism in the Newborn	
	Pentobarbital hydroxylated *in vitro* (μmole/g liver/hr)	Pentobarbital disappearance *in vivo* in 4 hr (% of dose)
No	0·00	22
No	0·01	25
No	0·01	30
No	0·02	30
No (av.)	0·01	27
Yes	0·08	45
Yes	0·15	65
Yes	0·32	73
Yes	0·65	93
Yes (av.)	0·30	70

* Sodium phenobarbital (40 mg/kg/day, s.c., once daily) was administered to pregnant rabbits from day 26–30 of gestation and 20 mg/kg/day was administered thereafter until the birth of the litter. Newborn rabbits 3–18 hr old were either sacrificed for studies *in vitro* or injected with pentobarbital-2-C^{14} (4 mg/kg, i.p.) for studies *in vivo*. Each value represents the average for the litter and was determined by using pooled livers from 2–7 animals for studies on the metabolism *in vitro* of pentobarbital and 2–5 animals for studies on the metabolism *in vivo* of this barbiturate.

DISCUSSION

The experiments reported here show that treatment of pregnant rabbits and rats with phenobarbital increases the activity of liver microsomal enzymes that *N*-demethylate meperidine and oxidize pentobarbital in the newborn. This treatment enhances the activity of drug-metabolizing enzymes in some, but not all, animals to the normal adult levels. The reasons for the variability in response of the rabbit and rat to phenobarbital are unknown. Pentobarbital metabolism *in vitro* was correlated with the metabolism of this drug *in vivo* and the results indicate that litters of rabbits with elevated levels of pentobarbital-metabolizing enzymes had enhanced rates of pentobarbital metabolism *in vivo*.

A number of investigators have explored the possibility of providing the newborn with an increased complement of hepatic microsomal enzymes by treatment of the mother during the latter part of pregnancy with a drug that can cross the placenta and is capable of inducing the formation of these enzymes in the fetus. This approach was first tried by Inscoe and Axelrod,[9] who treated pregnant rats with 3,4-benzpyrene, but failed to find an increase in glucuronyl transferase activity in liver from the neonate. Hart *et al.*,[10] however, were successful in demonstrating a stimulation in the metabolism of hexobarbital, aminopyrine and *p*-nitrobenzoic acid by liver microsomes from newborn rabbits obtained from mothers that were treated with phenobarbital for several days prior to term. Arias *et al.*,[11] found that the administration of chlorcyclizine or chloroquine to rats during late pregnancy resulted in an

increase in glucuronyl transferase activity in the liver of neonates. Similarly, treatment of pregnant mice with barbital stimulated in the newborn the activity of enzymes that conjugate bilirubin as the glucuronide.[12]

Since newborn humans are deficient in their ability to metabolize a wide variety of drugs and some normal body substrates such as bilirubin, there are certain clinical situations where it may be desirable for the newborn to possess an effective level of these enzymes. Two examples of such clinical situations would be: (1) erythroblastosis fetalis and other diseases marked by elevated unconjugated bilirubin in the plasma, and (2) depression of the newborn by sedative and analgesic drugs administered to the mother during labor and delivery. One approach to providing the newborn with an increased complement of hepatic microsomal enzymes is by treatment of the expectant mother during the latter part of pregnancy with an agent that can cross the placenta and is capable of inducing the formation of these enzymes in the fetus. However, such studies should be done with caution, for inducers of liver microsomal enzymes may produce undesirable effects by altering normal body steroid metabolism, by displacing bilirubin from binding sites on plasma protein, or by increasing in the fetus the formation of polar metabolites which cannot be eliminated from the body until birth.

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