Placental Transfer of Pentobarbital in the Rat

DAVID P. S. MORK*, WAYNE V. KESSLER, and STANLEY M. SHAW

Abstract □ The placental transfer of pentobarbital and/or metabolites was determined in rats using ^{14}C-labeled pentobarbital. The level of pentobarbital and/or metabolites in fetal blood was greatly influenced by the circulating level of pentobarbital and/or metabolites in the mother. Of the total pentobarbital and/or metabolites transferred, the percentage of unbound pentobarbital in fetal blood plasma was influenced by the pentobarbital dose level administered to the pregnant rat. A greater percentage of total pentobarbital and/or metabolites was present as unbound pentobarbital in fetal blood plasma than in maternal blood plasma. Chronic administration of pentobarbital during gestation decreased sensitivity to the pharmacological effects of further drug administration to the newborn. The changes induced by pretreatment were not permanent.

Keyphrases □ Placental transfer, rat—pentobarbital, metabolites □ Pentobarbital, labeled—fetal blood □ Fetal blood concentration, pentobarbital—maternal blood concentration □ Scintillometry—analysis □ TLC—analysis, radioactivity

Placental transfer of pentobarbital in the human at term was reported by Flowers (1). Transfer was determined by measuring pentobarbital in the arterial and venous blood of the umbilical cord. By observing a decrease in the respiratory rate of the newborn, Dreisbach and Synder (2) determined that pentobarbital crosses the placenta of the rabbit at term. In this investigation, ^{14}C-labeled pentobarbital was employed to determine the placental transfer of pentobarbital and/or metabolites, as well as unbound pentobarbital in the rat. In addition, the influence of chronic pentobarbital administration to the pregnant rat on the sleep time and the blood level of bound and/or unbound pentobarbital required for sleep was studied in the newborn.

EXPERIMENTAL

Animal Care and Mating—Sprague-Dawley[1] albino rats (200–210 g.) were housed individually in screen wire-bottom hanging cages in a controlled light environment. Animals were allowed food[2] and water *ad libitum*. Prior to being used for experimental study, the animals received 5 min. of handling daily for 2 weeks to reduce stress.

For mating, one male was placed with one female at 5:00 p.m. The male was removed at 7:00 a.m. the following morning and a vaginal smear was taken. The slides were fixed in 95% methanol, stained with Giemsa's blood stain,[3] and examined microscopically to determine the estrous cycle of the rat. When sperm were present during estrous, the female was assumed to be mated (3). On Day 18 of gestation, wood shavings were added for bedding to satisfy the nesting instinct.

Labeled Pentobarbital—The labeled compound used was 5-ethyl-5-(1-methylbutyl)barbituric-2-^{14}C acid.[4] It was converted to the sodium salt with 0.01 *N* sodium hydroxide, and the resulting solution was used throughout this study. Chemical purity and radiochemical purity were determined chromatographically using unlabeled authentic pentobarbital and the two solvent systems to be described later. No chemical impurities were found; the radiochemical purity was greater than 99.5%. The specific activity of the labeled pentobarbital injected into rats was 4 μc./mg.

Determination of Radioactivity—Individual tissue samples were placed in tared counting vials containing 1 ml. of a solution of a quaternary ammonium compound,[5] weighed, and dissolved by heating at 60° for 2 hr. with gentle agitation. The vials were cooled; the samples were decolorized with 0.5 ml. of 30% hydrogen peroxide. Glacial acetic acid, 0.5 ml., was added to minimize protein phosphorescence. After 10 min., 15 ml. of XDC scintillator[6] was added to each sample. The samples were counted in a Tri-Carb[7] using the internal standard method (5) to determine the absolute disintegration rates. The maximum counting error was 1%.

Chromatography—TLC plates were prepared with a distilled water slurry of equal parts of two commercial adsorbents.[8] The plates were air-dried, activated in an oven at 100° for 1 hr., and cooled to room temperature prior to spotting. The plates were developed in either a chloroform–acetone (9:1) solvent or a dioxane–benzene–ammonium hydroxide (4:15:1) solvent (6, 7).

Compounds were visually located using UV light. The chromatograms were then sprayed[9] and exposed to Kodak No Screen medical X-ray film[10] for a time period which allowed approximately 10^8 disintegrations/cm.^{2} to occur. The films were developed with Kodak chemicals according to manufacturer's instructions. Labeled compounds on a chromatogram were quantitatively removed for counting by scraping the adsorbent containing the compound from the plate. The adsorbent was placed in a counting vial containing 15 ml. of XDC; colloidal silica[11] was added to form a thixotropic gel. The total radioactivity on the developed plate was summed and considered as 100% of the radioactivity applied. The radioactivity found in a labeled compound was expressed as a percentage of the total applied.

Optimal Time of Sacrifice—Animals were injected intraperitoneally with a 10-mg./kg. dose of labeled pentobarbital on Day 19 of gestation. Day 19 was selected because most compounds have been shown to cross the placental barrier to the greatest extent near the termination of gestation. Groups of animals were sacrificed with ether 1, 3, 6, 12, or 24 hr. following drug administration. Blood samples were obtained from the heart of the mother and fetus. On the average, the blood sample obtained from each fetus weighed approximately 115 mg. From each mother, certain fetuses were removed, weighed, frozen in liquid nitrogen, placed between sheets of plastic, and pulverized. The powdered fetus was thoroughly mixed and a sample was taken and prepared for counting.

The ^{14}C-radioactivity was determined for blood and tissue samples. In this phase of the investigation, no attempt was made to differentiate between pentobarbital and metabolites. The ^{14}C-radioactivity was expressed as micrograms of pentobarbital per gram of sample.

Effect of Dose Level—Pregnant animals were injected intraperitoneally on Day 19 of gestation with a 5- or 25-mg./kg. dose of labeled pentobarbital. The animals were sacrificed 1 hr. after injection, and samples of various tissues were taken. The ^{14}C-radioactivity was expressed as micrograms of pentobarbital per gram of sample. Maternal and fetal blood were also obtained for determina-

[1] Sprague-Dawley Inc., Madison, Wis.
[2] Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.
[3] Matheson, Coleman & Bell, Norwood, Ohio.
[4] Supplied by New England Nuclear, Boston, Mass.
[5] A methanolic solution of Hyamine hydroxide prepared according to the method of Bruno and Christian (4) from Hyamine 10-X crystals, Rohm & Haas, Philadelphia, Pa.
[6] The XDC scintillator was prepared as follows: *p*-xylene, one part; *p*-dioxane, three parts; 2-ethoxyethanol, three parts; naphthalene, 8%; PPO, 1%; and dimethyl POPOP, 0.5%.
[7] Model 3003, Packard Instrument Co., Inc., Downers Grove, Ill.
[8] Adsorbosil P-1 and Adsorbosil 1, Applied Science Laboratories, Inc., State College, Pa.
[9] With Neatan New, Brinkmann Instruments, Inc., Westbury, N. Y.
[10] Eastman Kodak Co., Rochester, N. Y.
[11] Cab-O-Sil, Godfrey L. Cabot, Inc., Boston, Mass.

Table I—Pentobarbital and/or Metabolites in Tissues of Animals Sacrificed at Various Time Intervals following Administration

Time,[b] hr.	No. of Maternal Animals	Pentobarbital and/or Metabolites, mcg./g.[a]			Fetal Blood[d] / Maternal Blood
		Fetal Blood	Fetal Tissue[c]	Maternal Blood	
1	6	4.67 ± 0.56	6.27 ± 1.15	6.39 ± 0.99	0.74 ± 0.06
3	6	5.07 ± 0.54	6.99 ± 0.98	5.93 ± 1.09	0.93 ± 0.14
6	3	3.71 ± 0.42	4.67 ± 0.33	4.34 ± 0.56	0.86 ± 0.01
12	3	1.93 ± 0.21	2.28 ± 0.18	2.51 ± 0.24	0.77 ± 0.05
24	3	0.67 ± 0.15	0.72 ± 0.32	0.77 ± 0.16	0.88 ± 0.09

[a] Mean ± standard error. [b] Time elapsed between injection and sacrifice. [c] Homogeneous sample of a whole fetus. [d] Ratio of amount of pentobarbital and/or metabolites in the fetal blood to amount in the maternal blood.

Table II—Effect of Dose on Transfer of Pentobarbital and/or Metabolites

Dose Level[b]	No. of Maternal Animals	Pentobarbital and/or Metabolites, mcg./g.[a]					Fetal Blood[d] / Maternal Blood
		Fetal Blood	Fetal Tissue[c]	Fetal Liver	Maternal Blood	Maternal Liver	
5	8	3.91 ± 0.24	4.16 ± 0.16	5.96 ± 0.23	4.43 ± 0.18	12.70 ± 0.44	0.89 ± 0.05
25	6	19.92 ± 0.93	22.13 ± 0.81	29.31 ± 0.73	23.98 ± 1.13	63.14 ± 1.48	0.83 ± 0.03

[a] Mean ± standard error. [b] Milligrams of labeled pentobarbital per kilogram of body weight administered on Day 19 of gestation. [c] Homogeneous sample of the whole fetus. [d] Ratio of amount of pentobarbital and/or metabolites in the fetal blood to amount in the maternal blood.

tion of unbound pentobarbital. Each whole-blood sample was centrifuged for 10 min. in a heparinized capillary centrifuge tube[12] which was sealed with a commercial clay.[13] The capillary tube was broken just above the interface between white blood cells and plasma, and the plasma was spotted on a thin-layer plate. Authentic ^{14}C-labeled pentobarbital was also spotted for reference. The plate was developed; labeled compounds were located and quantified according to procedures previously described. The percentage of the total ^{14}C-radioactivity on the chromatogram that represented unbound pentobarbital was determined.

Effect of Pretreatment during Pregnancy on the Sleep Time and Dose Required for Sleeping in the Newborn—Pregnant rats were injected intraperitoneally on Days 11–20 of gestation with 10 mg./kg. of unlabeled pentobarbital. Pregnant control rats received saline solution. The animals were allowed to give birth. Each litter was reduced to six animals to keep maternal care a constant. From each litter, three of the young rats were studied 5 days following birth and the other three were studied 30 days after birth. Rats were weaned 25 days after birth. During the time interval previous to the determination of sleep time, no attempt was made to reduce stress in the 5-day-old animals by daily handling. The 30-day-old rats were handled daily for 10 min. for a period of 6 days prior to sleep studies.

The 5-day-old rats were injected intraperitoneally with 20 mg./kg. of labeled pentobarbital, while the 30-day-old rats received 30 mg./kg. The sleep time was determined by measuring the loss of the righting reflex. Upon return of the righting reflex, blood was removed from the heart to determine the level of bound and/or unbound pentobarbital in the blood which was just below the level required for sleep. Bound and/or unbound pentobarbital was extracted from blood according to the method of Brodie *et al.* (8). This extraction method removes bound and unbound pentobarbital while excluding metabolites of pentobarbital. The petroleum ether layer was added to the XDC scintillator, and ^{14}C-radioactivity was determined. Counting data were expressed as pentobarbital.

Analysis of Results—The Student *t* test (9) was used to test the results of the treatments for significant differences. All results were tested at the 95% level of significance.

RESULTS AND DISCUSSION

Optimal Time of Sacrifice—The results are presented in Table I. Fetal and maternal blood levels of pentobarbital and/or metabolites at 12 and 24 hr. postinjection were significantly different from each other and from the values at 1, 3, and 6 hr. Values at 1, 3, and 6 hr. were not significantly different from each other. In general the measurable level of pentobarbital and/or metabolites in all tissues declined rapidly after 3 hr. One hour after injection was the time selected for sacrifice for the remainder of the study.

For each time interval, the ratio of the fetal blood level of pentobarbital and/or metabolites to the maternal blood level was calculated. No significant difference was found between any of the ratios. The results indicate that by 1 hr. after injection an equilibrium had been established between the pentobarbital and/or metabolite level in the maternal and fetal blood.

Effect of Dose—As may be seen in Table II, an increased dose of pentobarbital administered to the pregnant rat resulted in an increased level of pentobarbital and/or metabolites in all of the tissues studied. The ratios of the amount of pentobarbital and/or metabolites in the fetal blood to the amount in the maternal blood were not significantly different for the two dose levels. Thus, the level of pentobarbital and/or metabolites in the fetal blood was directly proportional to the pentobarbital and/or metabolite level in the maternal blood, regardless of the dose administered to the pregnant rat.

Results of the determination of the unbound pentobarbital level in fetal and maternal blood plasma are shown in Table III. The results are expressed as the percentage of the total ^{14}C-radioactivity on a chromatogram which represented unbound pentobarbital. The data show that a higher percentage of total pentobarbital and/or metabolites was present as unbound pentobarbital in fetal blood plasma than in maternal blood plasma. The relationship was true for both dose levels. The data indicate that unbound pentobarbital transfers through placental membranes more readily than bound pentobarbital and/or metabolites.

There was a significant increase in the percentage of unbound pentobarbital in the maternal and fetal blood when the dose was increased from 5 to 25 mg./kg. As was already shown, an increase in the pentobarbital dose administered to the pregnant rat increased the total pentobarbital and/or metabolites that transferred across the placenta. Of the total compound that transferred, a higher percentage of unbound pentobarbital was present in the fetal blood plasma at the higher dose level than was present at the lower dose level.

Effect of Pretreatment during Pregnancy on the Sleep Time and Pentobarbital Blood Level Required for Sleeping in the Newborn—As may be observed from the results presented in Table IV, there was a significant decrease in sleep time of 5-day-old newborn rats from pretreated mothers compared to 5-day-old newborn rats from control mothers. These results agree with the findings of Hart *et al.* (10) in a study of the stimulation of hepatic microsomal drug metabolism in the newborn and fetal rabbit. The data in Table IV show that a higher blood level of bound and/or unbound pentobarbital was required to induce sleep in the 5-day-old newborn rats from pretreated mothers, which indicated a decreased sensi-

[12] Red Tip Heparinized Capillary Tubes, Division of American Hospital Supply Corp., Evanston, Ill.
[13] Seal-Ease, Clay Adams, Inc., New York, N. Y.

Table III—Unbound Pentobarbital in Fetal and Maternal Blood Plasma

Dose Level[a]	No. of Maternal Animals[b]	Fetal Blood[c]	No. of Maternal Animals[b]	Maternal Blood[c]
5	7	43.21 ± 5.95	6	12.20 ± 3.46
25	6	72.73 ± 3.74	5	28.94 ± 2.58

[a] Milligrams of labeled pentobarbital per kilogram of body weight administered on Day 19 of gestation. [b] Variation in animal number due to difficulty involved in chromatographic techniques. [c] Percent of total ^{14}C-radioactivity which represented unbound pentobarbital. Mean ● standard error.

Table IV—Sleep Time and Pentobarbital Blood Level Required for Sleep[a]

	No. of Maternal Animals	Sleep Time, min.	Pentobarbital Blood Level Required for Sleep, mcg./g. Blood
		Five Day[b]	
Control[c]	6	409.5 ± 4.50[d]	4.05 ± 0.28
Pretreated[e]	6	298.2 ± 5.90	5.23 ± 0.21
		Thirty Day	
Control	5	91.80 ± 10.89	11.93 ± 0.58
Pretreated	6	94.50 ± 2.37	12.19 ± 0.23

[a] Sleep time is expressed as the duration of the loss of the righting reflex. Pentobarbital blood level required for sleep is expressed as the level of bound and/or unbound pentobarbital in the blood, which was necessary for the loss of the righting reflex. [b] Day after birth at which sleep study was conducted. [c] Newborn from mothers treated with saline solution during gestation. [d] Mean ± standard error. [e] Newborn from mothers receiving pentobarbital during gestation.

tivity to the pharmacologic effect of the drug. The 30-day-old pretreated rats exhibited neither a lower sleeping time nor a higher dose required for sleep when compared to 30-day-old rats from control mothers, indicating that in the intervening time interval the effects of pretreatment were not permanent.

SUMMARY

Pentobarbital and/or metabolites reached an equilibrium between maternal and fetal circulation within 1 hr.

An increased dose of pentobarbital administered to the pregnant rat resulted in an increased level of pentobarbital and/or metabolites in all tissues studied. The level of pentobarbital and/or metabolites in fetal blood was greatly influenced by the circulating level of pentobarbital and/or metabolites in the mother. Of the total compound transferred, a higher percentage of unbound pentobarbital was present in the fetal blood plasma at the higher dose level than was observed in animals receiving a lower dose of pentobarbital. A higher percentage of total pentobarbital and/or metabolites was present as unbound pentobarbital in fetal blood plasma than in maternal blood plasma.

The placental transfer of pentobarbital during chronic administration to pregnant rats caused a decreased sensitivity to the pharmacological effects of further drug administration to the newborn. Five-day-old newborn rats from pentobarbital pretreated mothers exhibited a decrease in sleep time, with a higher bound and/or unbound pentobarbital blood level being required for sleep. The changes induced by pretreatment were not permanent and did not exist by the time the rat was 30 days old.

REFERENCES

(1) C. E. Flowers, Jr., *Amer. J. Obstet. Gynecol.*, **78,** 730(1959).

(2) R. Dreisbach and F. F. Synder, *J. Pharmacol. Exp. Ther.*, **79,** 250(1943).

(3) M. X. Zarrow, J. M. Yochim, and J. L. McCarthy, "Experimental Endocrinology—A Sourcebook of Basic Techniques," Academic, New York, N. Y., 1964, p. 37.

(4) G. A. Bruno and J. E. Christian, *Anal. Chem.*, **33,** 1216 (1961).

(5) C. H. Wang and D. L. Willis, "Radiotracer Methodology in Biological Science," Prentice-Hall, Englewood Cliffs, N. J., 1965, p. 294.

(6) A. Weissberger, "Technique of Organic Chemistry," vol. 12, Interscience, New York, N. Y., 1967, p. 504.

(7) P. J. Schechter and L. J. Roth, *J. Pharmacol. Exp. Ther.*, **158,** 164(1967).

(8) B. B. Brodie, J. J. Burns, L. C. Mark, P. A. Lief, E. Bernstein, and E. M. Papper, *ibid.*, **109,** 26(1953).

(9) N. M. Downie and R. W. Heath, "Basic Statistical Methods," 2nd ed., Harper and Row, New York, N. Y., 1965, pp. 138–143.

(10) L. G. Hart, R. H. Adamson, R. L. Dixon, and J. R. Fouts, *J. Pharmacol. Exp. Ther.*, **137,** 103(1962).

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* Department of Biology, Saint Cloud State College, Saint Cloud, MN 56301