Acute Toxicity and Lethal Brain Concentration of Pentobarbital In Young and Adult Albino Rats. (31928)

JOSEPH R. BIANCHINE* AND FRANK C. FERGUSON, JR.
Department of Pharmacology, Albany Medical College of Union University, Albany, N. Y.

It is well documented that young animals are generally more susceptible to the central nervous system depressant effect of barbiturate than adult animals(1,2). Brodie and associates found that one factor contributing to this age difference was the inability of young animals to detoxify administered barbiturates as efficiently as adults, because of specific enzymatic deficiencies(2).

Young rats, unlike adult rats, have an anatomically and functionally incomplete pial-glial (blood-brain) barrier(3,4). This deficit might allow a more rapid and excessive entry of barbiturate into brain tissue of young rats, thus inducing a more profound depression of CNS function in younger animals. Consequently, the increased sensitivity of young rats to barbiturate might also be a reflection of this incomplete pial-glial barrier.

To investigate these hypotheses, the LD_{50} of pentobarbital was determined in groups of rats of varying ages. The concentration of pentobarbital present in the brain tissue of these animals at death was determined and correlated with the LD_{50}.

Method. Albino rats (Wistar strain) were used. Sodium pentobarbital was given by the intraperitoneal route. Adult rats (14-15 weeks old) weighing 180-220 g were deprived of food overnight but allowed free access to water. Both sexes were equally represented in all adult groups. Toxicity studies on young rats were performed on litters of the following ages: newborn (less than 1 day), 5 days, 10 days, 20 days and 30 days old.

Litters containing less than 6 young were not used in this study in order to insure representation of all 6 pentobarbital dosages in each litter. The young rats were removed from the mother just before the start of the toxicity study. No attempt was made to determine the sex of young rats. Collodion was applied to injection sites in young animals to prevent leakage of drug.

Following pentobarbital administration, all animals were observed either until they were dead or until righting reflexes were recovered in adults or spontaneous limb activity was regained in young rats. The LD_{50} and its standard error were determined by the logarithmic probit method of Litchfield and Wilcoxon(5).

The whole brain of each rat which died under the acute influence of pentobarbital was removed from the calvarium promptly, weighed on a Roller-Smith torsion balance, and homogenized in a Stadie Riggs glass homogenizer. Pentobarbital content was determined according to the ultraviolet spectrophotometric method of Goldbaum(6). Brain tissue of rats not receiving pentobarbital was extracted according to the method of Goldbaum and served as controls. A Beckman DU spectrophotometer with ultraviolet attachment was used for all determinations.

Results. The LD_{50} of pentobarbital administered intraperitoneally was determined in 6 age groups of white albino rats ranging from newborn to 15 weeks old. These data are summarized in Table I. The LD_{50} for animals between 20 and 105 days of age is essentially identical, ranging between 80 and 87 mg/kg body weight. However, the LD_{50} for newborn rats was only about 30% that for the "adult" animals. In addition, the newborn rats became anesthetized approximately twice as rapidly as rats 20 days of age or older, as judged by loss of reaction to irritating stimuli.

The LD_{50} for rats 5 and 10 days of age was approximately 55% and 70%, respectively, that for the older rats.

The concentrations of pentobarbital in the brain tissue are summarized in Table I.

Discussion. An early study(7) of the influence of age on the degree of depressant action by barbiturates on rats failed to detect a difference between "young" and "adult" rats.

*Present address: Department of Medicine, Division of Clinical Pharmacology, Johns Hopkins Univ. School of Med., Baltimore, Md.

TABLE I. Acute Toxicity of Sodium Pentobarbital in Young and Adult Rats.

Rat age (days)	No. of rats	Mean body wt (g)	Mean brain wt (g)	Acute toxicity LD_{50} (mg/kg body wt) ± S.E.*	Lethal concentration of pentobarbital in brain tissue† (μg/g wet wt) ± S.D.
<1	56	5.1	.34	23 ± 3.9	27.1 ± 5.0
5	55	11.0	.60	38 ± 4.0	46.7 ± 4.8
10	62	19.7	1.07	55 ± 3.1	71.4 ± 4.4
20	48	35.5	1.39	80 ± 5.0	88.0 ± 9.1
30	55	60.9	1.42	87 ± 2.7	95.4 ± 5.2
Adult	72	204.1	1.78	80 ± 3.4	108.5 ± 7.1

* Estimated according to the method of Litchfield and Wilcoxon(5).
† Determined according to the method of Goldbaum(6).

Since the single "young" groups consisted of rats weighing from 25 to 275 g and were "a few weeks to nine months old," it is not surprising that this initial study failed to detect differences in toxicity to barbiturates later reported by Etsten and associates(1) and confirmed in the present study.

Brodie and associates(2) found that intraperitoneal administration of hexobarbital (10 mg/kg) in newborn mice was followed by sleeping time of about 6 hours, while the same dose in 21-day-old mice resulted in only 27 minutes of sleep. Adult mice slept only about 5 minutes with this dose. Hexobarbital, 50 mg/kg, killed all newborn animals tested, yet failed to kill animals older than 7 days of age, and induced sleep for only a short period of time in adult animals. Brodie concluded that a cause of this prolonged effect in young animals is the inability of newborn mice to detoxify hexobarbital. He demonstrated that no hexobarbital is metabolized by the intact newborn mouse within 3 hours, while 21 to 33% disappears from the intact 21-day-old mouse in the same time.

Our data provide further evidence for the sensitivity of newborn rats to the depressant effects of pentobarbital. Five- and 10-day-old rats exhibited a progressively decreasing sensitivity to the toxic effects of pentobarbital. Rats 20 days old and older had essentially equal sensitivities to pentobarbital. Approximately one-third the adult dose, on a weight basis, was required to produce death in newborn rats.

If the incomplete pial-glial barrier of the newborn rat played a significant role in this obvious sensitivity to pentobarbital, relatively high concentrations of pentobarbital should have been found within the brain tissue of newborn rats dying from pentobarbital. However, the data summarized in Table I indicate a high correlation between the LD_{50} and the mean concentration of drug in the brain tissue of animals dying from pentobarbital in all age groups. There is thus no increased sequestration of pentobarbital in the brain tissue to account for the sensitivity of the young rats to pentobarbital. Rather, it would appear that the change in sensitivity with age is explainable on the basis of sensitivity of the central nervous system to the drug *per se*. These data thus do not support either of the hypotheses previously mentioned.

Conclusions. The acute toxicity and lethal brain concentration of sodium pentobarbital were determined in newborn rats, young rats of several age groups and adult albino rats. The LD_{50} of pentobarbital in newborn rats was approximately one-third that of rats 20 days of age and older. Similarly, the lethal concentration of pentobarbital found in the brain tissue of newborn rats was approximately one-third that found in brain tissue of rats 20 days old. Therefore it is concluded that brain tissue of newborn rats *per se* is more sensitive to depressant effect of pentobarbital than that of adult rats. The incomplete pial-glial barrier of newborn rats does not allow an inordinate sequestration of pentobarbital into brain tissue.

1. Etsten, B., Alexander, E. A. D., Himwich, H. E., J. Lab. Clin. Med., 1943, v28, 706.
2. Jondrof, W. R., Maickel, R. P., Brodie, B. B., Biochem. Pharm., 1959, v1, 352.
3. Tschirgi, R. D., in Biology of Mental Health and Disease, Hoeber, New York., 1952, p. 34.
4. Lajtham, A., J. Neurochem., 1957, v1, 216.

5. Litchfield, J. T., Jr., Wilcoxon, F. B., J. Pharmacol. Exp. Therap., 1949, v96, 99.

6. Goldbaum, L. R., Anal. Chem., 1952, v24, 1604.

7. Carmichael, E. B., J. Pharmacol. Exp. Therap., 1937, v60, 101.

Received December 8, 1966. P.S.E.B.M., 1967, v124.

Liver Regeneration in Rats Exposed to Simulated Changes in Gravity. (31929)

D. D. FELLER, K. S. TALARICO, AND E. D. NEVILLE

Environmental Biology Division, Ames Research Center, NASA, Moffett Field, Calif.

The biological role of gravity is being investigated in many laboratories as a result of the advent of space exploration. Many studies are aimed at predicting the effects of weightlessness on biological processes, but one must not disregard effects of other changed gravitational conditions such as decreased and increased gravity.

Increased gravity can be simulated in the laboratory by changing the accelerative force with a centrifuge. This technique has been used predominantly for following the responses of animals, including man, to increased gravity. Information on how gravity affects cellular development and growth is sparse. Pflüger(1) and Schultze(2) showed that normal gravity influences the direction of cleavage and development in frog's egg.

The liver is an excellent choice as a mammalian tissue for study of the regenerative process. When two-thirds of the rat liver is removed by surgical ablation, the remaining liver grows until it attains its original mass. The completion of this regenerative process takes 10-15 days(3); however, most of the original mass has been regenerated within 7 days. Information from liver regeneration studies may help in the understanding of general processes of regeneration and growth of other tissues, such as muscle or skin, in the process of wound healing. The present study describes effects of simulated changes in gravity on liver regeneration as measured by mitotic count and on the gross appearance of the regenerating tissue.

Methods. A 10-radial-armed centrifuge having an effective operating radius of 4.5 feet was employed in this study. The cages ($20 \times 10 \times 10$ inches) were mounted in the swing-bucket fashion, allowing for one degree of freedom. The cages swung outward upon centrifugation so that the resultant G-vector was always perpendicular to the cage floor. The centrifuge was illuminated with fluorescent light automatically set for on-off cycling at 6:00 A.M. and 6:00 P.M., respectively. Centrifuged as well as noncentrifuged animals were exposed to these light conditions. The centrifuge was run continuously except for stoppages of no longer than 30 minutes each, 3 times a week, to feed the rats and clean the cages. All animals were kept in air-conditioned rooms.

Three-week- and 7-week-old male Sprague-Dawley rats obtained from Simonsen Laboratory, Gilroy, California, were used. The animals were divided into 3 different groups, 2 of which were noncentrifuged and served as controls to the third group, which was centrifuged. One control group was given food and water *ad libitum* and the other control group was pair-fed with the centrifuged group.

Partial hepatectomies were performed according to the procedure of Ralli and Dumm (4) on both control and experimental animals. The operations were carried out between the hours of 8:30 A.M. and 10:30 A.M. in order to control the effects of diurnal variation in mitosis(5). Surgery was performed under ether anesthesia. Normally, an abdominal incision is made to expose the liver(6); however, since the additional stress of increased gravity on the abdominal wall may interfere with the normal healing of such a wound, we chose to make a dorsal incision(4). On the basis of measurements made after autopsy on a separate group of