connection. Following hypophysectomy the RNA content of the liver decreases, but is restored again by somatotropic pituitary hormone(11). Korner(12) noted an increase in an RNA fraction, possibly messenger RNA, in hypophysectomized animals following the administration of somatotropic hormone. It might be possible that the pituitary promotes the synthesis of specific enzymes involved in DNA synthesis by affecting messenger RNA formation, which would accelerate the beginning of DNA synthesis in partially hepatectomized animals.

Summary. DNA synthesis in regenerating liver following partial hepatectomy of normal and hypophysectomized rats was measured following administration of thymidine-6-T(n). Such DNA synthesis in the hypophysectomized animals exhibits a delayed start and a prolonged period before its peak is reached, as compared with intact animals. However, there is a prolongation of maximal incorporation of DNA precursor, and a slower decline of DNA synthesis in hypophysectomized rats. The promotion of enzyme activity involved in DNA synthesis may be linked to the pituitary effects upon messenger RNA formation.

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Received May 17, 1965. P.S.E.B.M., 1965, v120.

Interactive Effects of X-Irradiation and Barbiturates.* (30500)

V. NAIR, S. FINER AND D. SHAH
Neuropharmacology and Biochemistry Laboratories, Michael Reese Hospital Psychiatric Institute, and Department of Pharmacology, Chicago Medical School, Chicago, Ill.

Previous studies have shown that the anticonvulsant action of acetazolamide was enhanced with respect to the potency, onset and duration of action in head X-irradiated rats(1). It was also found that the enhanced pharmacological effects were temporally related to increased entry of drug into specific brain regions. The present investigation was undertaken to ascertain whether the pharmacological effects of other centrally acting drugs are enhanced or modified in head X-irradiated animals. This report describes our studies with the barbiturates, thiopental, pentobarbital and barbital. These 3 barbiturates differ in their spectrum of action. Thiopental is an ultra short acting drug with a rapid onset of action. Pentobarbital has a short to intermediate duration of action. With barbital, the onset of action is slow and duration of action long. These 3 drugs are well suited to differentiate the radiation effects on the onset and duration of barbiturate hypnosis.

Materials and methods. A total of 140 male Sprague-Dawley rats weighing 200-250 g were employed in these studies. Food and water were given *ad libitum* except when otherwise indicated. Pentobarbital and thiopental were obtained from Abbott Laboratories and sodium barbital from Merck and Co.

* This investigation was supported in part by a grant from the State of Illinois Mental Health Fund 1711.

X-Irradiation. The head alone was exposed to X-irradiation from a 250 kv-30 ma GE Maxitron X-ray machine. The filters employed were 0.5 mm cu. and 1 mm Al. The half value layer of the beam was 1.4 mm cu. While being irradiated, the animals were in plastic holders, with a lead shield (6 mm thick) over the entire body except the region of the head, and they were held on a platform which was rotated continuously (3½ rpm) to ensure equal exposure throughout the head. The experiment was performed in groups of 6 animals and the radiation dose was 500 r (Gr. I), 1,000 r (Gr. II), 5,000 r (Gr. III), or 10,000 r (Gr. IV), given as a single dose at 295 r/min at a focal distance of 57.7 cm. Group V served as sham irradiated control. After irradiation, the animals were transferred to air-conditioned quarters and housed 2 to a cage.

Barbiturate sleeping time. The barbiturate was injected intravenously in a tail vein and either one or both of the following effects were recorded depending on the barbiturate under study.

1) Onset of 'hypnosis':—For the present study, this is taken as the time elapsed after injection of drug to produce loss of static righting reflexes in both fore and hind quarters, *i.e.,* the animal, when placed on its back does not assume its normal posture.

2) Duration of 'hypnosis':—The time elapsing between the onset of hypnosis and the time when the animal righted itself spontaneously.

For thiopental, only the duration of hypnosis was recorded since onset of hypnosis was immediate. For pentobarbital, both 1 and 2 were noted and for barbital only the onset of hypnosis.

Thiopental studies. Fifteen mg/kg of thiopental sodium were administered intravenously by a tail vein to the control group and to each of the irradiated groups (Groups I-IV). The irradiated animals received the drug at 1 hour post-irradiation and at 24-hour intervals thereafter until 144 hours post-irradiation.

Pentobarbital studies. For these experiments, only rats receiving 10,000 r were used. At 72 hours post-irradiation they were injected with pentobarbital sodium at a dose of 30 mg/kg. Both onset and duration of hypnosis were noted. Sham irradiated rats served as controls.

Barbital studies. In these experiments also, only rats receiving 10,000 r were employed and they were examined at 72 hours post-irradiation. Barbital sodium (180 mg/kg) was injected intravenously and the time of onset of hypnosis recorded. Sham irradiated rats were employed as controls.

Results. Thiopental studies. The onset of hypnosis was immediate (within seconds after injection) in both control and irradiated groups. However, there were significant differences in duration of hypnosis between irradiated and control rats, the effect depending on the dose of irradiation and the time post-irradiation. These results are summarized in Fig. 1. A prolongation of hypnosis was noted in rats receiving 10,000 and 5,000 r at 48, 72 and 96 hours post-irradiation intervals, the maximal effect being at the 72-hour interval at which time the 10,000 r irradiated group showed a mean sleeping time of 16 minutes ($p<0.001$) and the 5,000 r irradiation group 9.6 minutes ($p<0.02$) in contrast to 3 minutes in the controls. The very high sleeping time seen at late post-irradiation intervals (144, 168 and 192 hours) may be a nonspecific effect related to the terminal condition(2). This possibility is further supported by the similar effects noticed in starved (food and water deprived) controls at the preterminal and terminal period (Fig. 1). Since the head X-irradiated rats (Groups I and II) lose weight (Fig. 2) due to minimal or no food and water consumption, it is pertinent to assess the role of starvation in the prolongation of hypnotic time noted here. The weight loss profile and the survival time of irradiated and starved animals are essentially similar (Fig. 2). However, no significant prolongation of thiopental hypnosis was seen in the starved animals until about 7-8 days of starvation. The starved animals died in 8-10 days. Thus the radiation effect on the duration of hypnosis at 72 hours post-irradiation is a specific effect and is not

related to starvation. Lower doses of X-irradiation (1,000 and 500 r) and 3-4 days starvation had no significant effect on the mean sleeping time of rats.

Pentobarbital studies. At 72 hours post-irradiation, both the onset and duration of pentobarbital hypnosis were enhanced in the 10,000 r group. While, in the controls, the loss of righting reflex did not occur until 2-3 minutes after the drug administration, in the irradiated group, the loss of righting reflex occurred immediately after injection. The effect on the duration of hypnosis was more impressive (Fig. 3A). The sleeping time of the irradiated animals was 2½-3 times longer than that of the controls ($p<0.005$).

Since the pentobarbital hypnosis was not brief even in the control animals, it was decided to record body temperature periodically to determine whether the drop in body temperature was contributing to the extension of hypnosis time. For this purpose, one group of animals was placed inside an incubator (100-102°F) immediately after injection of drug. Another group of animals remained outside the incubator at room temperature (78-79°F). Both control animals and irradiated ones were examined in this manner. The rectal temperature of each rat was taken with a telethermometer attached to a thermistor sensing unit. The temperature was recorded before, immediately after injection of drug, and at periodical intervals thereafter until the rat righted itself.

The results are shown in Fig. 4B. It can be seen that placing the rats inside the incubator has prevented the drop in body tem-

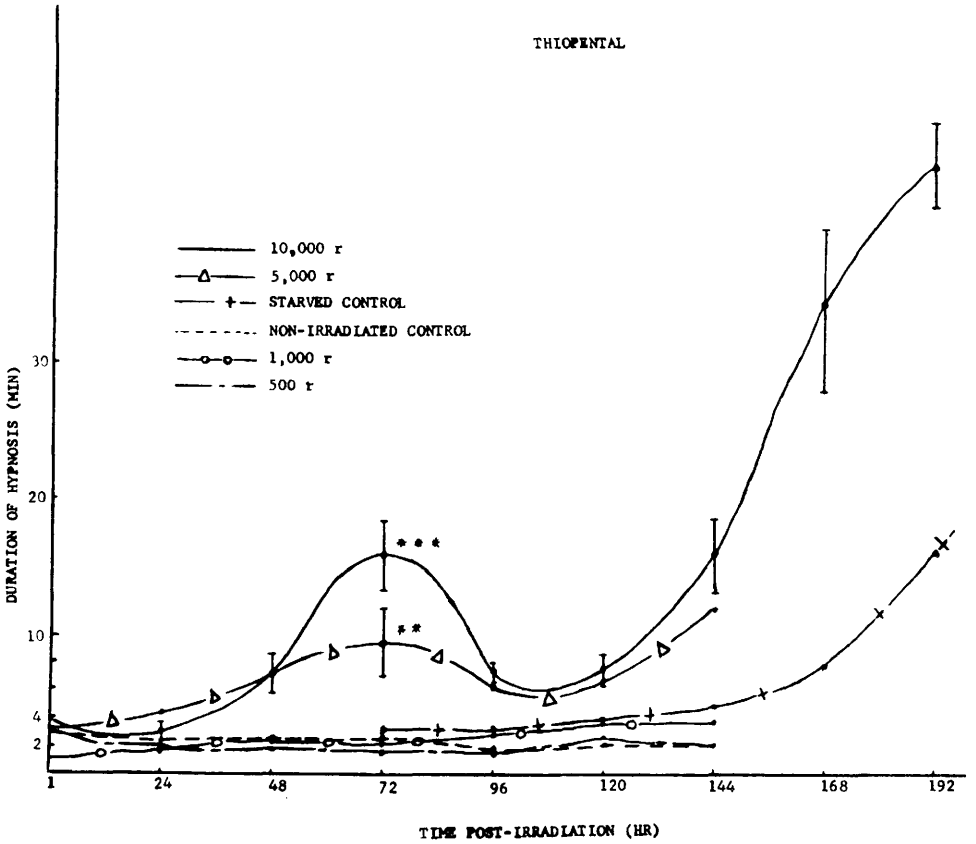

FIG. 1. Duration of thiopental hypnosis in head X-irradiated rats. Each value is the mean time ± S.E. of 6-10 animals except those at 144 hours and beyond for the 10,000 r group. Values for the latter are derived from 3 animals. *** = $p<0.001$; ** = $0.01<p<0.02$. Repeated injections of thiopental (at 24-hr intervals) produced no significant effect on mean sleeping time of control rats.

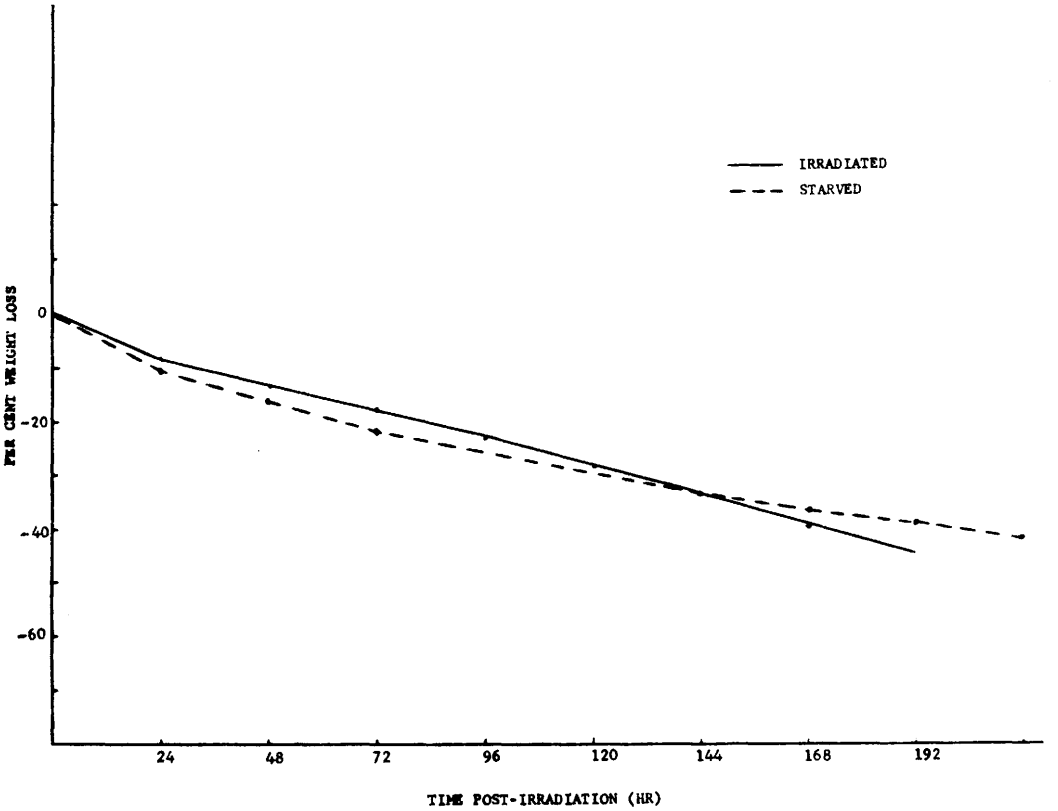

FIG. 2. Comparison of changes in body weights of irradiated and starved rats. Each value is the average of 18 rats in irradiated and of 6 rats in starved group. 'Starvation' refers to complete deprivation of food and water.

perature both in control and irradiated animals. However, this did not affect the duration of hypnosis. No significant differences were noted between the mean sleeping times of animals inside the incubator and those remaining at room temperature. The effect of head X-irradiation alone on body temperature is shown in Fig. 4A. Body temperature was recorded daily until 96 hours after irradiation and there were no significant changes from the controls.

Barbital studies. As indicated above, in the control animals barbital has a slow onset of action but a long duration. In the irradiated animals (10,000 r), hypnosis was produced in less than half the time that was required for the controls (Fig. 3B; $p<0.01$). Duration of hypnosis was not measured.

Effect of irradiation on enzyme induction. It is known that many drugs (ex: phenobarbital), when given to control rats, en-

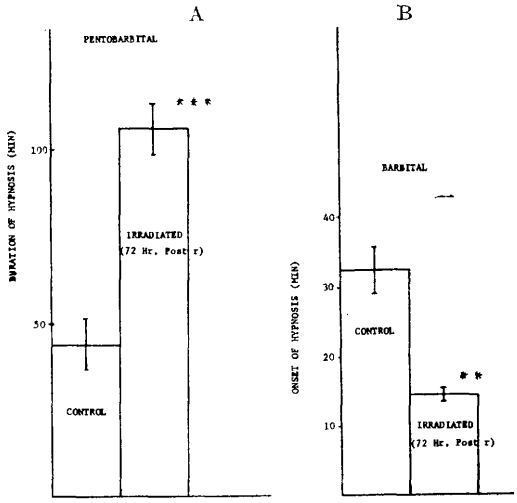

FIG. 3A. Duration of pentobarbital hypnosis in head X-irradiated rats at 72 hours post-irradiation. Each value is the mean ± S.E. of 6 animals. *** = $0.001<p<0.005$. B. Time of onset of barbital hypnosis in head X-irradiated rats at 72 hours post-irradiation. Each value is mean ± S.E. of 6 animals. ** = $p<0.01$.

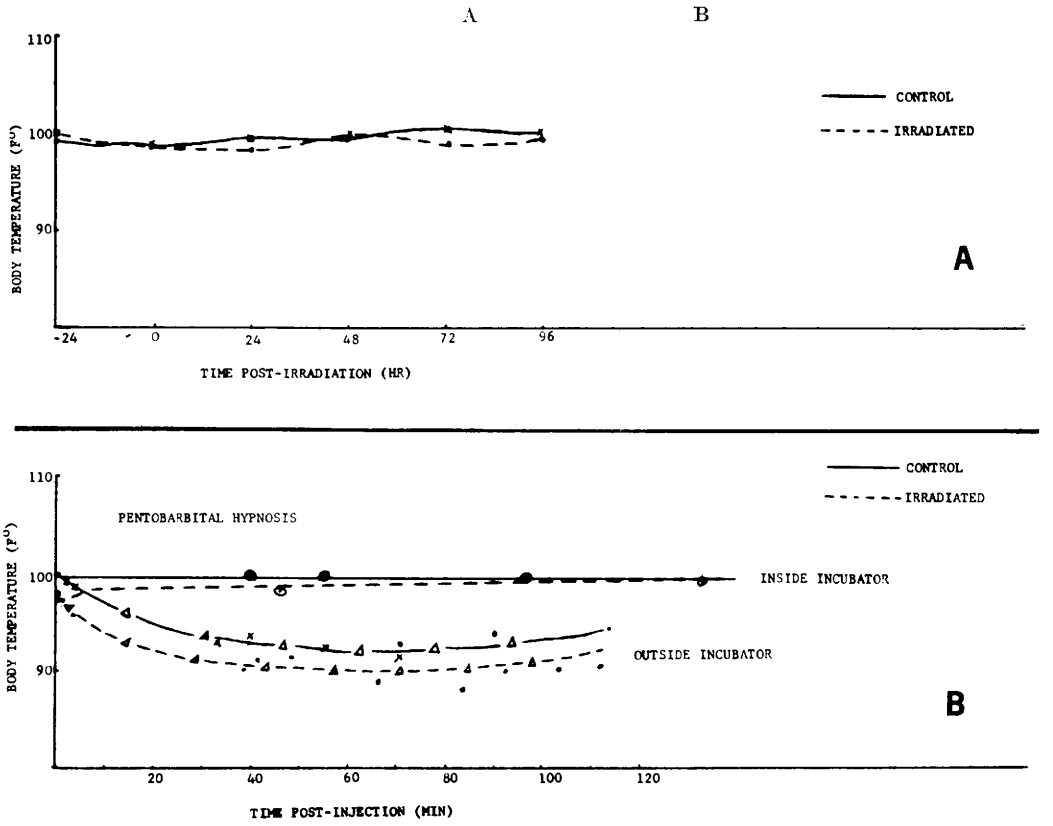

FIG. 4A. Effect of head X-irradiation on body temperature. Control group = 8 animals, irradiated group = 14-18 animals. B. Body temperature during pentobarbital hypnosis. Control group = 9 animals, irradiated group = 24 animals.

hance not only their own metabolism but that of several other drugs also(3,4). Pharmacologically, this is reflected in a decreased duration of action. Several lines of evidence indicate that these stimulators of enzyme activity actually induce the synthesis of drug metabolizing enzymes(3,5). There is also some indication that the central nervous system possibly through the hypophysis, plays a regulatory role in the biosynthesis of microsomal enzymes, at least in the developing rat when the enzyme activity is progressively increasing to attain the adult level. Much of the evidence for this comes from the studies of DuBois and associates(6,7) using irradiated weanling rats. These authors noted no inhibitory effect of irradiation on the enzyme induction process, at doses up to 400 r.

However, since in the present studies higher radiation doses have been employed and especially since radiation was restricted to the head alone, it was decided to examine whether or not the enzyme induction process was affected abscopally. Three doses of radiation were used: 1,000, 5,000 and 10,000 r. Phenobarbital was employed to stimulate enzyme activity and the duration of pentobarbital hypnosis was taken as the pharmacological index of the activity of metabolizing enzymes. Both control and the irradiated group of rats received phenobarbital intraperitoneally (75 mg/kg) for 2 days and on the third day, the duration of pentobarbital hypnosis was determined for each rat as described earlier. It can be seen that (Fig. 5) phenobarbital treatment has decreased the duration of hypnosis to less than half in the controls, and X-irradiation of the head up to 10,000 r has not inhibited this effect of phenobarbital. It is also of interest that the results were the same in all of the following combinations.

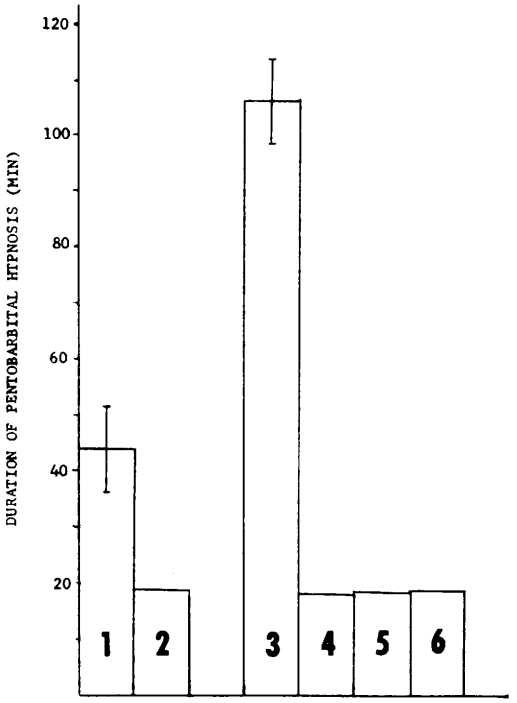

FIG. 5. Effect of varying doses of head X-irradiation on phenobarbital induction of accelerated drug metabolism. Duration of pentobarbital hypnosis was employed as the pharmacological index of the activity of metabolizing enzymes. Each value is mean of 6 animals. 1, non-irradiated control; 2, non-irradiated control (phenobarb. treated); 3, irradiated (10,000 r); 4, 1,000 r (phenobarb. treated); 5, 5,000 r (phenobarb. treated); 6, 10,000 r (phenobarb. treated).

1. 1st phenobarb. injection on day prior to irradiation.
2. 1st phenobarb. injection on day of irradiation.
3. 1st phenobarb. injection on day after irradiation.

Discussion. The enhancement of barbiturate hypnosis in head X-irradiated animals noted here is compatible with our earlier findings with the anticonvulsant drug, acetazolamide(1). It is of interest that the enhancement of hypnosis is seen only at the relatively higher radiation doses (5,000 and 10,000 r). That this is a specific effect of radiation is established by comparison with starved controls. In rats receiving 500 r to the head, duration of thiopental hypnosis was less than in that of the controls, but the statistical significance of this difference can only be established with more animals. This reduced effect with barbiturates will be expected on the basis of the findings of Rosenthal and Timiras(8), who observed that whole body X-irradiation of rats at 250 and 500 r produced a reduction in the threshold for electroshock seizures and in general increased brain excitability. It is of interest that previously our findings of the irradiation induced anticonvulsant effect and the above findings of Rosenthal and Timiras seemed to be divergent. However, Sherwood and Timiras have now demonstrated that the seemingly divergent findings could be explained by the radiation dose differences. The anticonvulsant effect at high radiation doses has been confirmed by Sherwood(9).

It is significant that the time of maximal anticonvulsant activity as well as that of the pharmacological effects of all the drugs tried coincide, *i.e.*, they all appear about the 3rd day post-irradiation. Blood brain barrier (BBB) permeability changes have also been shown to occur maximally at this time(10). In the case of acetazolamide, the enhancement of its anticonvulsant action in irradiated animals was found to be temporally related to the increased drug entry into brain, which in turn may be a result of the increased BBB permeability. The early onset of hypnosis noted here with the slow penetrating barbital may also be explained by the BBB alterations. The mechanism of the enhanced duration of effect is, however, not clearly understood. The possibility of a longer persistence of the drug in the irradiated brain in comparison to the control brain is considered and the brain levels of the barbiturates are being determined to verify this. For thiopental, preliminary results indicate an increased amount of drug in irradiated brain at 1 and 7 minutes after injection in comparison to the controls at the respective times. Other possibilities include 1) 'sensitization' of the brain by radiation and a lowering of the threshold for drug effect, 2) alteration of the balance of neural transmitters (ex: serotonin, GABA) in brain thus producing interactive or potentiating effects with drugs. Serotonin, for example, has been shown to potentiate barbiturate hypnosis(11, 12). Some serotonin releasing agents have

also been reported to share this property(13).

Our studies on the brain serotonin changes following head irradiation in fact do show a significant rise in the brain amine content at 72 hours post-irradiation(14). However, its exact role in the enhancement of the barbiturate hypnosis in irradiated animals requires further study.

Summary. The effects of varying doses of cephalic X-irradiation on barbiturate hypnosis have been examined in rats. An enhancement of hypnosis was found in the irradiated animals, the effect depending on the dose of radiation and the time post-irradiation. At relatively higher doses (5,000 and 10,000 r), the duration of thiopental hypnosis was significantly prolonged. Similarly, in the 10,000 r irradiated rats, the time of onset of hypnosis for the slow acting barbital was considerably shortened. Using pentobarbital, it was possible to demonstrate the enhancement effect, both with respect to onset and duration of hypnosis. It was also shown that even at the high doses of radiation employed, head X-irradiation produced no inhibition of the phenobarbital induction of microsomal enzyme activity as evidenced by the pharmacological effects.

Grateful acknowledgement is made to the University of Chicago Argonne Cancer Hospital and the Department of Radiology for use of radiation facilities and Mr. James Bland for skillful assistance with the radiation procedure.

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Received May 24, 1965. P.S.E.B.M., 1965, v120.

Prevention of Steroid-Induced Sterility in Neonatal Rats with Thymic Cell Suspension. (30501)

FRED A. KINCL, A. ORIOL, A. FOLCH PI AND M. MAQUEO
(Introduced by R. K. Meyer)
Institute of Clinical Medicine, Syntex Corporation, Palo Alto, Calif., Escuela de Medicina Rural, I.P.N., Mexico, D.F., and Hospital Gineco-Obstetrico No. 1, I.M.S.S., Mexico, D.F.

We have reported previously that estradiol benzoate injected into 5-day-old male rats produced infertility in later life(1). We have also reported that animals injected with a suspension of thymic cells within the first 24 hours of life and then with estradiol benzoate at 5 days of age were fertile(2). This observation suggested strongly that in the neonatal male rat thymocytes interfered in some way with the sterilizing effect produced by estradiol benzoate. Preliminary results of similar experiments in both male and female rats are the subject of this report.

Materials and methods. Thymic cell suspension was prepared from 22- to 30-day-old female rats killed by decapitation. The gland was rapidly removed and placed in cooled (0°C) aqueous solution of sodium chloride, 0.14 molar, and sodium citrate, 0.01 molar. The tissue was cut into small pieces, crushed through a stainless steel 325 mesh sieve with glass pestle into the same cooled solution and