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.

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

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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

TABLE I. Prevention of Estradiol Benzoate-Induced Sterility in 5-Day-Old Male Rats by Injecting Thymocytes at the Age of 1 Day.

Treatment			Index of spermatogenesis ± S.E. (No. of observations)	Body wt, g ± S.E.	Tissue wt, mg ± S.E.		
Thymocytes	Estradiol benzoate, μg	No. of rats			Testes	Ventral prostate	Seminal vesicles
Autopsy at 50 days of age‡							
—	0	21	87 ± 1.3 (23)	197 ± 5.9	2490 ± 40	141.8 ± 7.2	127.3 ± 4.8
+	0	16	92 ± 1.8 (8)	184 ± 4.2	2130 ±130	116.2 ± 8.2	120.0 ± 7.0
+	120	14	77 ± 3.1 (20)	184 ± 6.8	1540 ± 89	98.2 ± 7.0	46.1 ± 4.4
+	240	15	19 ± 5.3 (25)	184 ± 6.6	1010 ± 88	81.1 ± 7.8	25.9 ± 2.3
Autopsy at 160 days of age§							
+	0	8/8 †	94 ± 2.0 (8)	380 ±14	2890 ±109	*	*
—	120	2/10†	51 ± 7.0 (10)	367 ±13	1920 ± 96	*	*
+	120	9/9 †	99 ± .8 (9)	398 ± 7	3330 ± 97	*	*

* Not determined. † No. of fertile rats/total No. of rats. ‡ Sprague-Dawley strain. § Wistar strain.

centrifuged for 10 minutes at 1500 rpm. The supernatant was discarded, fresh cold solution was added, and the suspension was again centrifuged. This washing procedure was repeated 3 times. The final cell concentration was adjusted so that each 0.05 ml of medium contained approximately 5 million cells. The concentration was checked in a hemacytometer after staining with trypan blue. Each animal was injected intraperitoneally with 0.05 ml within 24 hours after birth.

Testosterone propionate, 100 or 500 μg and estradiol-17β benzoate (120 or 240 μg) dissolved in 0.05 ml of sesame oil were injected at day 5 of life. Control groups were injected with sodium chloride-sodium citrate medium and oil on days 1 and 5 respectively. The day of birth was counted as day one. In the first experiment fertility of treated males was established by breeding when they were 100 days old. The observation period was 30 days. In subsequent experiments fertility was not studied and the treated animals were sacrificed at the age of 50 days. In males the status of spermatogenesis was evaluated microscopically as previously described (3). Several sections were prepared from each testis and 200 tubules were counted in each section. Those showing the absence of free sperm in the lumen or more marked changes were considered as pathological and the percentage found was expressed as the index of spermatogenesis. In females, the presence or absence of corpora lutea was used as the index of ovulation. The weights of gonads, accessory sex tissues, pituitary, adrenals and thymus were also included in the study.

Wistar strain rats were used in our laboratory in Mexico. Other experiments were performed with Sprague-Dawley rats at the Endocrine Laboratories, Madison, Wis., under the direction of Dr. Elva G. Shipley following similar protocol.

Results. Table I records fertility data, organ weights and index of spermatogenesis of male rats autopsied at the age of 50 and 160 days respectively. When thymic cell suspension was injected on day 1 and estradiol benzoate on day 5, the male rats were protected against the deleterious effect of estradiol benzoate. This was apparent from fertility data and also from microscopic examination of the testes. In the 50-day-old group, males which received both thymic cell suspension and 120 μg of estradiol benzoate had a spermatogenetic index of 77 ± 3.1 (S.E.) as compared to 87 ± 1.3 in controls. In the group autopsied at the age of 160 days all males (9 of 9) injected with thymocytes and 120 μg estradiol benzoate were fertile whereas in the group injected only with the estrogen 2 of 10 males sired litters during the 30-day test period. Decreased infertility in the latter group was further reflected in low index of spermatogenesis, which was 51 ± 7.0 as compared to 99 ± 0.8 and 94 ± 2.0 in thymocyte-estradiol benzoate and control groups, respectively.

Testes of the rats injected with 240 μg of estradiol benzoate had an average of only 19% of tubules with free sperm in the lumen, but normally developing spermatocytes were

TABLE II. Cell Count of Various Elements in the Seminiferous Epithelium of 50-Day-Old Male Rats Injected Prepubertally with Estradiol Benzoate and Thymoeytes.

Treatment	No. of observations	Sperm	Spermatids	Spermatocytes	Spermatogonia
	23	87.3 ± 1.3	94.7 ± .9	99.9 ± .1	99.9 ± .1
Thymocytes	8	91.9 ± 1.8	96.3 ± 1.0	100.0 ± .0	100.0 ± .0
Thymocytes + estradiol benzoate					
120 μg	20	76.7 ± 3.1	86.3 ± 2.2	99.2 ± .3	99.2 ± .3
240 "	25	18.6 ± 5.3	22.6 ± 5.7	82.7 ±3.4	96.3 ± .7
Estradiol benzoate*					
120 μg	15	35.1 ±10.6	39.3 ±10.0	76.3 ±4.8	88.3 ±2.9
240 "	18	4.7 ± 1.2	2.6 ± 1.2	35.0 ±5.3	53.4 ±6.1

* Data from another experiment.

present in 83% of the tubules. Rats injected with estrogen only showed marked damage to the seminiferous epithelium (Table II).

A previous injection of thymic cell suspension prevented, likewise, the sterilizing effect produced by testosterone propionate in female rats (Table III). Injection of thymocytes alone had no inhibitory effect on ovulation. At the age of 50 days 15 of 16 females had luteinized ovaries and the average number of corpora lutea was 13 ± 1.4. In contrast, only 1 of 8 animals ovulated in the group injected with testosterone propionate and the average number of corpora lutea for this group was 2.0. When thymocytes were injected on day 1 and testosterone propionate on day 5, 100% (8 of 8) ovulated in the group injected with 100 μg of the androgen and about 91% in the rats injected with 500 μg. The average number of corpora lutea in both groups was, however, less than seen in controls. When both the thymocytes and the steroid were injected on day 5 of life, the ovaries contained only follicles indicating that under these conditions thymocytes produced no effect.

The "protective" role of thymocytes appeared to be fairly specific. Cell suspensions prepared in a similar manner from the liver, spleen or salivary glands had no influence against testosterone propionate as demonstrated by the absence of corpora lutea in the ovaries of the treated females (Table IV).

In the above described experiments, we had seen no differences in the weights of pituitary, adrenal, or thymus glands between the various treatment groups.

Discussion. We have shown that a dose of 120 μg and especially of 240 μg of estradiol benzoate, injected into male rats within the first few days of life, will produce long-lasting alteration in sexual development manifested by infertility, azoospermia and decreased androgen production(1,3). In females both es-

TABLE III. Prevention of Testosterone Propionate-Induced Sterility in 5-Day-Old Female Rats by Injecting Thymocytes at the Age of 1 Day (Autopsy at the Age of 50 Days).*

Treatment		Body wt, g ± S.E.	Ovarian response†	Avg No. C.L. ± S.E.	Vaginal introitus, days ± S.E.	Tissue wt, mg ± S.E.	
Thymocytes	Testosterone propionate, μg					Ovaries	Uterus
—	0	138 ± 3.8	13/17	9.0 ± 1.3	41 ± 1.3	43.9 ± 4.5	161 ± 8.8
+	0	147 ± 5.4	15/16	13.0 ± 1.4	39 ± .8	51.9 ± 3.7	203 ± 15.2
—	500	158 ± 4.4	1/8	2.0 ± .8	15 ± .2	17.1 ± 1.8	125 ± 44.2
+	100	157 ± 4.0	8/8	4.2 ± 1.2	17 ± 2.6	29.2 ± 2.4	218 ± 19.5
+	500	167 ± 3.9	13/16	6.0 ± 1.0	13 ± 1.3	32.5 ± 4.0	233 ± 19.3
+‡	500‡	139 ±13.6	1/8	1.0 ± .9	12 ± .3	23.3 ± 3.2	129 ± 31.9

* Sprague-Dawley strain.
† Ovarian response = No. of rats with corpora lutea/total No. of rats.
‡ Injected on day 5 of life.

TABLE IV. Lack of Protective Action of Splenic Cells, Liver Cells and Salivary Gland Cells Injected on Day 1 of Life and Testosterone Propionate on Day 5 of Life (Autopsy at 50 Days of Age).*

Treatment	Body wt, g ± S.E.	Ovarian response	Avg No. C.L. ± S.E.	Vaginal introitus	Tissue wt, mg ± S.E. Ovaries	Uterus
0	136 ± 5.8	13/15	8.0 ± 1.1	43 ± .1	37.9 ± 3.1	
Splenic cells (SC)	179 ± 10.0	8/10	20.3 ± 3.4	31 ± 3.4	58.2 ± 6.4	242 ± 2.6
Liver " (LC)	165 ± 3.2	8/8	>20	23 ± .0	82.7 ± 5.2	290 ± 14.7
Salivary gland (SG)	155 ± 11.6	4/4	10.3 ± 4.5	35 ± 1.5	48.8 ± 6.6	183 ± 13.1
Testosterone propionate (TP, 500 μg)	166 ± 2.6	15/38	5.2 ± 1.3	25 ± .7	39.5 ± 2.8	207 ± 9.9
SC + TP	169 ± 5.1	4/13	3.0 ± 1.4	24 ± 2.4	33.6 ± 4.1	208 ± 21.2
LC + TP	167 ± 2.3	0/6	.0 ± .0	23 ± .0	31.5 ± 4.6	266 ± 7.8
SG + TP	155 ± 10.8	0/11	.0 ± .0	25 ± .9	22.8 ± 2.6	224 ± 23.7

* Sprague-Dawley strain.

trogens and androgens inhibit fertility. Testosterone propionate, 100 μg, injected on day 5 of life inhibited luteinization in about 70% of females at the age of 45 days. A dose of 500 μg was effective in almost 100%(4). It has been postulated(5) that androgen treatment in the neonatal female rat affects deleteriously those centers in the suprachiasmatic-preoptic area which are destined to control ovulation in later life.

We were also able to prevent steroid-induced infertility in both sexes by injecting a suspension of thymocytes prior to steroid treatment. From the available literature, there is no evidence which would indicate a dependence between thymus and the hypothalamus, and the mechanism of action by which thymocytes prevent the deleterious effect of gonadal hormones in the neonatal animal remains unknown. Other experiments are now in progress to study this effect.

Summary. A subcutaneous injection of androgenic or estrogenic steroid hormones into 5-day-old rats results in abnormal gonadal development and sterility in the adult animal. Males injected with a suspension of thymic cells and then with estradiol benzoate develop normally and are fertile. Similar protection was obtained in prepubertal female rats injected with testosterone propionate as indicated by the presence of luteinized ovaries. This effect of thymocytes was fairly specific. Cell suspension prepared from spleen, liver or salivary gland did not protect female neonatal rats against the deleterious effect of testosterone propionate.

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