EFFECT OF UNILATERAL CASTRATION ON THE TESTIS OF SEXUALLY MATURE RATS RECEIVING REPEATED INJECTIONS OF ESTRADIOL DIPROPIONATE

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Injection of 2 mg estradiol dipropionate leads to a decrease in weight of the testis in sexually mature rats, inhibits spermatogenesis at the early spermatid stage, and causes disappearance of the Leydig's cells, atrophy of the accessory sex glands, and an increase in the permeability of the blood-testicular barrier to rivanol. All these changes are reversible in character and cannot be found 2 months after the final injection of estradiol dipropionate. Unilateral castration of previously estrogenized rats, as also of the control animals, was not accompanied by the development of compensatory hypertrophy of the residual testis 10 and 60 days after the operation. The results confirm the hypothesis that the ability of animals to develop compensatory hypertrophy of the testis is interlinked with the level of spermatogenesis attained and the permeability of the blood-testicular barrier.

The starting point for the present investigation was the results of experiments which showed that noninbred rats become incapable of developing compensatory hypertrophy of the testis at the age of 23-24 days, which is also the time of completion of meiosis, of loss of sensitivity of the pituitary to unilateral castration [as shown by the secretion of follicle-stimulating hormone (FSH)], and completion of the formation of the blood-testicular barrier (BTB) [5]. Comparison of these data suggested that compensatory hypertrophy of the testis observed in young rats aged from 1 to 22 days takes place as the result of increased FSH secretion by the pituitary as a sequel to lowering of the blood concentration of a factor produced by the spermatogonia and inhibiting FSH secretion [2, 11], arising after unilateral castration. After the function of the BTB has become stabilized, the factor liberated by the sex cells cannot penetrate into the blood stream, the pituitary does not respond by a change in FSH secretion to unilateral castration, and the latter does not induce compensatory enlargement of the testis. Sexually mature rats and other mammals do not respond by a change in weight of the testis to removal of the paired organ [3, 4, 8]. Injection of estrogens is known to have an inhibitory effect on the secretion of pituitary gonadotropic hormones in sexually mature animals, and it induces reversible inhibition of spermatogenesis [10].

In the investigation described below the effect of unilateral castration was studied on the testis of previously estrogenized sexually mature rats in order to determine whether the degree of completion of spermatogenesis, the permeability of the BTB, and the development of compensatory hypertrophy of the testis in response to unilateral castration are interdependent.

EXPERIMENTAL METHOD

Experiments were carried out on 99 noninbred rats weighing 180-200 g. The animals were divided into two approximately equal groups. Three times a week the rats of the experimental group received a sub-

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TABLE 1. Effect of Preliminary Estrogenization on the Testis of Unilaterally Castrated Rats

Character of treatment	At time of operation		After end of experiment					No. of animals
	body wt. at time of operation	wt. of testis (% of body wt.)	time after operation (days)	body weight (g)	wt. of testis (% of body wt.)	wt. of pituitary (% of body wt.)	wt. of seminal vesicles (% of body wt.)	
Injections of estradiol dipropionate + unilateral castration	200,6±4,7	1,5±0,1	10	202,8±5,1	1,8±0,1	0,106±0,007	0,50±0,02	14
Injections of estradiol dipropionate	186,2±5,8			204,0±4,8	2,0±0,1	0,086±0,05	0,52±0,05	12
P	0,63			0,84	0,13	0,03	0,68	
Injections of olive oil + unilateral castration	267,4±6,3	5,2±0,2	10	299,6±7,7	4,8±0,2	0,034±0,001	3,1±0,2	8
Injections of olive oil	266,7±5,5			311,9±12,0	4,6±0,1	0,034±0,001	3,5±0,2	8
P	0,95			0,43	0,5	1,0	0,21	
Injections of estradiol dipropionate + unilateral castration	206,8±4,3	1,2±0,0	60	335,7±12,5	3,7±0,08	0,047±0,004	4,1±0,2	15
Injections of estradiol dipropionate	205,9±3,7			357,0±10,4	4,0±0,1	0,041±0,003	4,2±0,2	12
P	0,88			0,1	0,13	0,31	0,76	
Injections of olive oil + unilateral castration	310,6±6,6	4,7±0,1	60	392,7±13,8	4,1±0,1	0,026±0,001	3,8±0,2	15
Injections of olive oil	310,0±5,9			389,5±14,5	4,0±0,1	0,026±0,002	4,2±0,2	15
P	1,0			0,76	0,61	1,0	0,16	

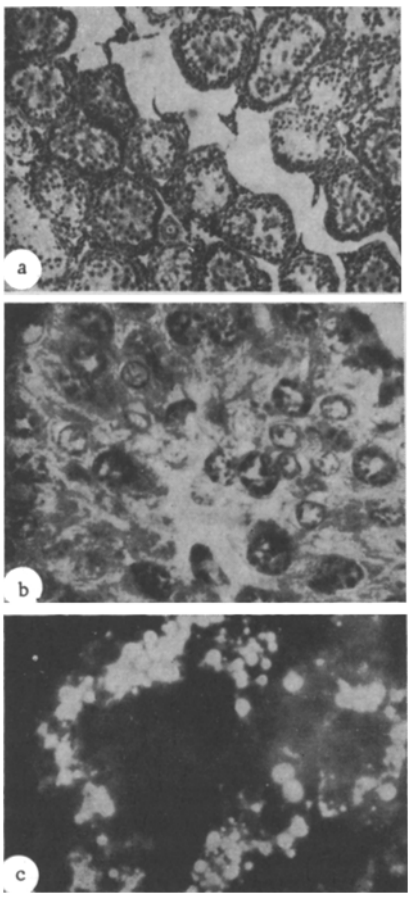

Fig. 1. Section through testis of rat receiving 2 mg estradiol dipropionate immediately after end of injections of the hormone: a and b) marked constriction of seminiferous tubules, inhibition of spermatogenesis at the early spermatid stage, disorganization of spermatogenic epithelium, disappearance of Leydig's cells. Hematoxylin–eosin, a) 100 ×; b) the same, 450 ×; c) luminescence of rivanol inside seminiferous tubules, drops of the dye in cytoplasm of Sertoli cells, 120 ×.

cutaneous injection of 0.2 ml of 0.1% estradiol dipropionate solution, ten injections altogether, with a total dose of 2 mg of the hormone. The rats of the control group received the corresponding dose of olive oil. An operation was performed on all the animals under ether anesthesia, with aseptic precautions, 2 days after the last injection. One testis was removed from half of the rats of the control and experimental groups, while a mock operation was performed on the other animals. The rats were killed 10 (series I) and 60 days (series II) after the operation. Testes removed during the operation and when the animals were sacrificed were weighed and fixed in Carnoy's fluid; paraffin sections 5 μ in thickness were stained with hematoxylin and eosin. The tests of four rats from each group were used to study permeability of the BTB. The state of permeability of the BTB was judged from the penetration of rivanol and endogenous globulins into the seminiferous tubules [1].

EXPERIMENTAL RESULTS

The results given in Table 1 show that injection of 2 mg estradiol dipropionate led to a decrease in the body weight of the animals and in the weight of the testis on the average by 73% of its weight in the control rats. Microscopic examination of the testes removed at operation showed that injection of olive oil into the rats was not followed by any pathological changes in their structure, whereas injection of estradiol dipropionate led to inhibition of spermatogenesis at the early spermatid stage in all the animals (Fig. 1a, b). Marked constriction of the seminiferous tubules (diameter 148.7 ± 4.7 μ in the experimental series, 223.4 ± 1.3 μ in the control) was found in the testes of the estrogenized rats, accompanied by disorganization of the spermatogenic epithelium, desquamation of cells into the lumen of the tubules, vacuolation of the cytoplasm of the Sertoli cells, the appearance of solitary multinuclear spermatocytes, and degeneration or complete disappearance of the Leydig's cells. Swelling and proliferation of the endothelium were detected in the arterioles.

Investigation of the permeability of the BTB showed that rivanol and endogenous globulines do not penetrate into the seminiferous tubules of the control animals. In the estrogenized animals the localization of endogenous globulins in the testis was the same as in the control rats, but the permeability of the BTB to rivanol was modified by injections of estradiol dipropionate, and particles of the dye were found in the cytoplasm of the Sertoli cells between the sex cells in all the experimental rats and formed a yellow rim of luminescence around them (Fig. 1c).

No increase in weight of the residual testis was observed 10 days after unilateral castration in rats receiving repeated injections of estradiol dipropionate or olive oil by comparison with the weight of the testis in rats of the corresponding groups undergoing the mock operation. The body weight and the weights of the seminal vesicles and pituitary in the rats undergoing the mock operation did not differ significantly from these weights of the unilaterally castrated animals whether in the group of previously estrogenized or the group of control animals. Meanwhile the weight of the pituitary in all the estrogenized rats was increased while the weight of the seminal vesicles was sharply reduced compared with that observed in the control animals (Table 1).

An increase in the period of observation to 60 days did not affect the main result of the experiment: in the control and experimental rats unilateral castration was not accompanied by an increase in weight of

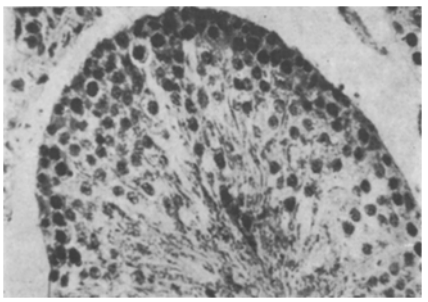

Fig. 2. Testis of rat 62 days after last injection of estradiol dipropionate and 60 days after unilateral castration. Active spermatogenesis, hematoxylin–eosin, 400×.

the residual testis compared with that of the animals undergoing the mock operation. In the time elapsing after the operation the relative weight of the testis and seminal vesicles increased and reached the weight of the corresponding organs in the control group of rats. The weight of the pituitary in all estrogenized rats was still a little higher than the weight of the pituitary in the control (Table 1).

Microscopic examination showed that the testes of rats undergoing unilateral castration or the mock operation after previous estrogenization were identical in structure (Fig. 2) with the testes of the control rats undergoing the corresponding operations. The diameter of the seminiferous tubules in the testes of the estrogenized, unilaterally castrated rats was $248.2 \pm 9.3\ \mu$, and the sex cells in the wall of the tubules were arranged in regular, concentric layers in accordance with the stages of the cycle of the spermatogenic epithelium, with ripe spermatozoa in the center of the tubules. Leydig's cells were arranged in small groups around the vessels in the interstitial tissue.

The permeability of the BTB to rivanol and to endogenous globulins was the same in the rats of the experimental and control groups: specific luminescence inside the tubules was absent 2 months after the last injection of hormone.

Injection of 2 mg estradiol dipropionate into sexually mature rats thus induces a sharp decrease in weight of the testis, inhibition of spermatogenesis in the early spermatid stage, disappearance of the Leydig's cells, atrophy of the accessory sex glands, and hypertrophy of the pituitary gland, and these changes are accompanied by increased permeability of the BTB to rivanol. After injections of the hormone are discontinued, restoration of the structure and function of the testis and of normal permeability of the BTB takes place. However, unilateral castration of previously estrogenized rats, if performed at the beginning of development of regeneration in the testis, does not induce a compensatory enlargement of the residual organ.

Nevertheless, these results do not contradict, but rather confirm the hypothesis of a link between the degree of completion of spermatogenesis, the permeability of the BTB, and the ability of the rat to develop compensatory hypertrophy of the testis. Inhibition of spermatogenesis at the early spermatid stage, induced by injection of 2 mg estradiol dipropionate into the animals, in fact corresponds to the stage of completion of spermatogenesis in noninbred rats at the age of 26–28 days, at which time they are no longer capable of responding by enlargements of the testis to removal of the opposite organ, and the disturbance of permeability of the BTB to rivanol is limited in the estrogenized rats to penetration of the dye into the cytoplasm of the Sertoli cells, whereas in rats under 23 days old staining of the nuclei of the developing sex cells with rivanol is observed [1]. Presumably the more marked inhibition of spermatogenesis and disturbance of permeability of the BTB would lead to restoration of the ability of the sexually mature animals to respond by enlargement of the testis to unilateral castration.

An indirect argument in support of this hypothesis is the recovery of the power of the testis to undergo compensatory hypertrophy observed by Reiter [9] in sexually mature hamsters previously blinded and then subjected to pinealectomy and unilateral castration. In Reiter's experiments spermatogenesis was halted by inhibition of the secretion of pituitary gonadotropic hormones as a result of activation of the pineal gland after blinding.

LITERATURE CITED

1. A. I. Davydova, Byull. Éksperim. Biol. i Med., No. 10, 103 (1972).
2. S. E. Levina, Ontogenez, 1, No. 3, 271 (1970).
3. V. V. Poltorak, State of the Testis after Hemicastration and Some Changes in the Hormonal Balance. Author's Abstract of Candidate's Dissertation, Donetsk (1966).
4. S. S. Raitsina, in: Compensatory Hypertrophy of Mammalian Organs [in Russian], Moscow (1963), p. 180.
5. S. S. Raitsina, S. E. Levina, and V. F. Kudinova, Zh. Obshch. Biol., 33, 230 (1972).

6. A. H. Coons and M. H. Kaplan, J. Exp. Med., 91, 1 (1950).
7. V. Figarova, Folia Morphol. (Prague), 17, 348 (1969).
8. T. Passouant-Fontaine and C. Flandre, C. R. Soc. Biol., 162, 2175 (1968).
9. R. J. Reiter, Experientia, 26, 1153 (1970).
10. E. Steinberger, Physiol. Rev., 51, 1 (1971).
11. R. S. Swerdloff, P. C. Walsh, H. S. Jacobs, et al., Endocrinology, 88, 120 (1971).