THE EFFECT OF OESTRIN ON THE TESTIS OF THE ADULT MOUSE

By MARJORIE ALLANSON.

(Harold Row Research Scholar, King's College, London.)

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(With One Plate.)

I. INTRODUCTION.

The effect of the oestrus-producing hormone on the male animal has been the subject of investigation by various workers, and the results are conflicting. It was decided to repeat this work, using the pure solution of crystalline oestrin now available.

Severe "anti-masculine" effects of varying degree were obtained by nearly all of the earlier workers. Herrmann and Stein (7) injected young rats and rabbits with ovarian lipoids and found puberty delayed. Heavier doses led to degenerative changes in the testis similar to those caused by X-rays. Gould and Doisy (see Allen and Doisy (1)) confirmed these results. Fellner (4) found that the injection of ovarian and placental lipoids into adult rabbits caused a decrease in size of the testis, due to complete degeneration of the germinal epithelium, the testis tubules attaining a prepubertal condition. Fels (5), using blood serum of pregnancy, obtained the same results. Laqueur and co-workers (2), using a comparatively pure preparation of oestrin, found in rats, rabbits, and guinea-pigs, that the primary and secondary male characters were retarded in development in the young animal, the testes of injected animals being half the weight of those of control animals. Golding and Ramirez (6) injected young rats with water-soluble preparations of follicular hormone: the growth of the genital organs was retarded and the testes remained in the abdomen. Spermatogenesis was inhibited. Bugbee and Simond (3), using much smaller amounts of the extract than Golding and Ramirez, found that injections of follicular hormone had no effect on the genital organs, although the curve of the body weight showed a decided falling off compared with the controls, the injected animals never reaching full size.

II. TECHNIQUE.

(*a*) Since spermatogenesis is readily affected by changes in diet and other conditions, one testis was removed from each animal a fortnight before the commencement of injections and examined microscopically. Only those animals showing normal spermatogenesis were used for experimental purposes.

(*b*) *Histological Technique.* Half of each testis was fixed in alcoholic Bouin's fluid and half in Flemming's strong solution. The epididymis and vas deferens were fixed in alcoholic Bouin. Sections were cut at 7μ and stained in Ehrlich's haematoxylin and eosin.

(*c*) *Oestrin.* The oestrin used was prepared by Dr G. F. Marrian. An aqueous solution (170 m.u. per c.c.) was used of crystalline oestrin containing 8000 m.u. per mgm. All injections were made subcutaneously, the daily dosage being given in two halves, night and morning.

III. EXPERIMENTS.

Adult albino male mice were injected daily with 2 c.c. of the oestrin solution. The experiments were divided into two series. In the first series three experimental animals and three controls were used. Injections were carried out for fifteen days, and the animals killed at the end of this period. The experimental males were mated with two females each at the end of the first week, and the females examined each day for vaginal plugs. OT 6 and OT 10 copulated with both females during the week and litters were produced in the normal time. OT 12, however, did not copulate with either female. The testis and epididymis in this animal were, however, similar histologically to those of OT 6 and OT 10. In the second series injections were carried out for three weeks, the animals being mated with two females each at the end of a fortnight. In all three cases vaginal plugs were observed for both females. Five of the females produced litters.

The average diameter of the testis tubules was calculated for the experimental animals and the controls, in order to see whether there was a decrease in the activity of the germinal epithelium not discernible from histological examination. While there is a certain amount of variation in size of tubule, this occurs equally in the injected and the normal animals. The average diameter was obtained by drawing with the aid of a camera lucida all the tubules in a section taken approximately through the centre of the testis at right angles to the long axis, and the smallest diameter of each tubule then measured.

Table I.

No. of animal	Time injected (days)	Total oestrin (m.u.)	No. of females mated	No. of copulations	No. of litters	Total young	Average diameter of testis tubules (μ)
OT 6	15	5100	2	2	2	10	615
OT 10	15	5100	2	2	2	10	754
OT 12	15	5100	2	0	0	0	737
OT 3	Control	None	—	—	—	—	860
OT 11	,,	,,	—	—	—	—	710
OT 13	,,	,,	—	—	—	—	769
OT 16	21	7140	2	2	2	13	721
OT 17	21	7140	2	2	2	4	920
OT 19	21	7140	2	2	1	8	754
OT 14	Control	None	—	—	—	—	771
OT 15	,,	,,	—	—	—	—	714

Histologically there was no difference between the testis of the injected and the control animals. Spermatogenesis was active in the great majority of seminiferous tubules and the epididymis was distended with spermatozoa. Careful comparison was made with sections of testes from mice fed for a short period on a diet of bread and milk only, in which degeneration of the tubules was commencing. While there is always a certain proportion of tubules in the mouse testis in a condition of quiescence and of degeneration the injected animals showed no greater variation than the controls.

It was not thought necessary from the above results to carry out injections on the immature male.

IV. DISCUSSION.

Since injection of a pure preparation of oestrin in the adult male animal is seen to have no adverse effects upon the testis, even using such a heavy dose as 340 m.u. a day, it would seem that the "anti-masculine" action obtained by other workers has been due to either (1) the use of extracts containing large amounts of foreign lipoids—Fellner indeed admitted that he obtained similar degenerative changes by the injection of testis lipoids—or (2) the influence of other factors, *e.g.* diet, not detected before the commencement of the injections. Most previous workers have neglected to demonstrate by the preliminary removal of one testis the normality of the animals used. This, of course, cannot apply to the very marked decrease in weight of the testes of injected animals obtained by Laqueur and other workers, but these results are most striking in the case of the injection of young animals, and the falling off in body weight of these compared with the controls might be an indication of the injurious nature of the extracts used.

The fact that these injections have brought about no degenerative changes in the mouse testis is particularly remarkable, since it has been found very sensitive to changes in conditions: quite severe degeneration of the seminiferous tubules will follow a slight change in diet, and recovery from such a condition may take as long as five or six weeks after a return to normal diet.

The occurrence of oestrin in male urine and in the testis (Fellner(4)) was formerly irreconcilable with the results of oestrin injection in the male: the results obtained above show that oestrin or an oestrin-like substance might occur normally in the male without causing degenerative changes in the testis.

I wish to thank Dr A. S. Parkes, at whose suggestion this research was undertaken, for help and advice while the work was in progress and for reading the paper. I am indebted also to Dr G. F. Marrian for generously supplying the oestrin required.

The histological expenses and the cost of maintaining the mouse colony were defrayed by a grant by King's College for which my thanks are due.

SUMMARY.

1. Two groups of adult male mice were injected daily with 340 m.u. of an aqueous solution of crystalline oestrin for fifteen days and twenty-one days respectively.

2. Matings with these injected mice were fertile.

3. The testis showed no degenerative changes, spermatogenesis being active in the large majority of seminiferous tubules. The diameter of the tubules showed no decrease in size. The epididymis was distended with spermatozoa.

4. The anti-masculine effects obtained by previous workers have not been confirmed.

REFERENCES.

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(3) BUGBEE and SIMOND (1926). *Endocrin.* **10**, 360.
(4) FELLNER (1921). *Pflügers Arch.* **189**, 199.
(5) FELS (1928). *Zeits. f. Geburts. u. Gyn.* **93**.
(6) GOLDING and RAMIREZ (1928). *Endocrin.* **12**, 804.
(7) HERRMANN and STEIN (1916). *Wien. klin. Wochen.* **29**, 50.

EXPLANATION OF PLATE IV.

Fig. 1. Right testis of OT 6 removed before commencement of injections.
Fig. 2. Left testis of OT 6 after injection of oestrin for fifteen days, showing active spermatogenesis.
Fig. 3. Right testis of OT 19 removed before commencement of injections.
Fig. 4. Left testis of OT 19 after injection of oestrin for twenty-one days; tubules show active spermatogenesis.

All figures × 100. Photographs by Mr D. Kempson.

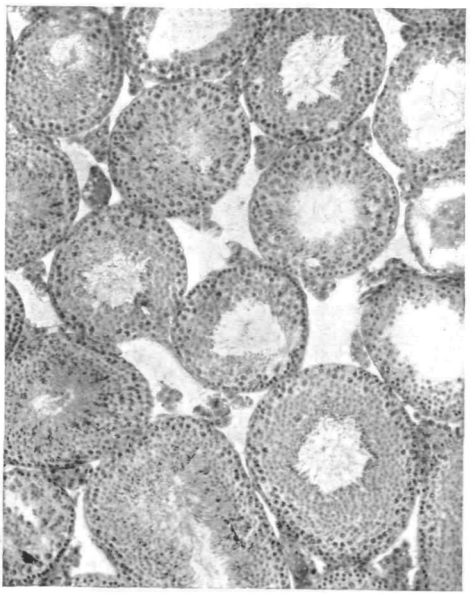

Fig. 1.

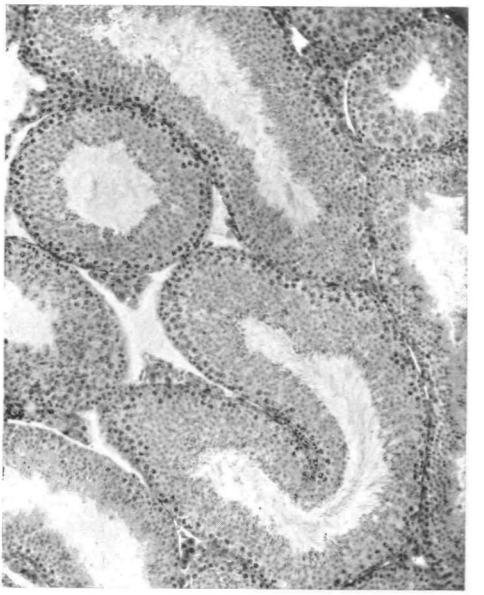

Fig. 2.

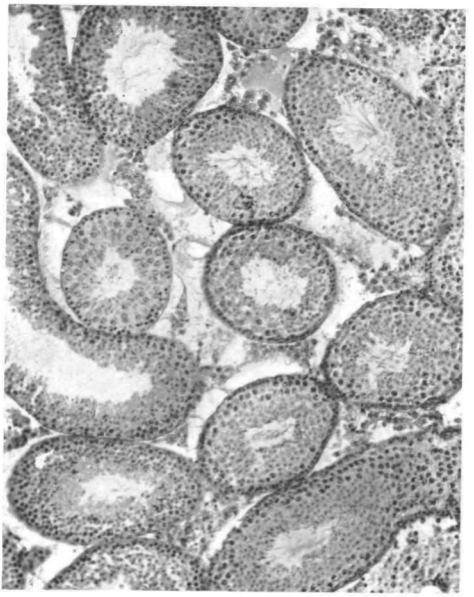

Fig. 3.

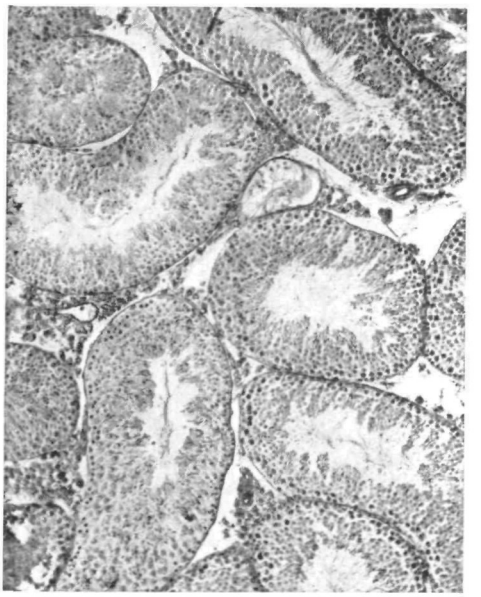

Fig. 4.

ALLANSON—THE EFFECT OF OESTRIN ON THE TESTIS OF THE ADULT MOUSE (pp. 389–392).