THE EFFECT OF EARLY POSTNATAL INJECTION OF TESTOSTERONE PROPIONATE ON THE STRUCTURE OF THE ANTERIOR HYPOPHYSIS OF MALE AND FEMALE RATS*

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Previous studies have demonstrated that the administration of testosterone propionate to female rats soon after their birth induces structural and functional defects in the reproductive system which persist after the treatment has been discontinued.[1, 7, 8, 10] Wilson and his associates[8, 10] found that such injections, initiated on the 1st, 5th, or 10th day of life and continued over a 28-day period, resulted in pronounced ovarian abnormalities that were still observable 2 to 4 months later. The ovaries were small and, although they contained both normal and atretic follicles, corpora lutea were absent. In a group of 12 rats in which daily vaginal smears were made, constant vaginal cornification was noted. However, the uteri were small and exhibited a decreased sensitivity to injected estrogen. Mating behavior was obtained only with difficulty.

The findings of Bradbury[1] were essentially similar, in that ovulation failed to occur and vaginal cornification was continuous. In addition, this author presented evidence which indicated that the ovarian abnormalities were secondary to dysfunction of the anterior lobe of the hypophysis. When the dormant ovaries from rats which had received androgenic treatment early in life were transplanted into normal female rats they proved to be capable of ovulation and of forming corpora lutea. In contrast, ovaries from normal female rats which were transplanted into rats which had received androgen early in life showed no further cyclic activity, i.e., ovulation and corpus luteum formation ceased. These findings led Bradbury to postulate that early postnatal injections of testosterone propionate induced a condition similar to that already reported by Pfeiffer,[4] who found that female rats, which at birth had received

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transplants of testes from newborn males, exhibited at maturity constant vaginal estrus associated with a failure of ovulation and luteinization. On the basis of these and other studies in which ovaries transplanted into male rats formed follicles but no corpora lutea, Pfeiffer suggested that the anterior hypophysis of the male rat releases only follicle-stimulating hormone, whereas that of the female releases both follicle-stimulating and luteinizing factors. He concluded that transplantation of testes into newly born females induces a masculinization of the anterior lobes of these females so that they released only the factor stimulating growth of follicles but not that which causes corpus luteum formation. This view is strengthened by his later findings[5] which revealed that in the anterior lobes of the host females carrying the testicular transplants the cellular pattern resembled that of normal male rats rather than the type found in normal female rats. The fact that the cellular patterns in the anterior lobes of male and female rats are different had been previously demonstrated by McQueen-Williams[3] and by Wolfe and Ellison.[11] In the glands of male rats eosinophils and basophils are more numerous, and both types of cells are usually larger and show less evidence of degranulation than those in glands of the female rats. Later studies of Wolfe and Hamilton[12] demonstrated that administration of testosterone propionate to female rats over a period of 90 days resulted in a cellular pattern in the anterior lobe which differed widely from that of normal females, and which in fact closely approached that found in the glands of normal male rats.

In more recent observations, Wilson and Wilson[9] have studied the effects on male rats of early postnatal injections of testosterone propionate. If injections were started on the first day of life, observations made several months later showed that libido was slight, the testes small although normal histologically, and the prostate and seminal vesicles atrophic. No tests for hypophyseal function were undertaken, but these defects were thought to be attributable, in part at least, to deficient hypophyseal gonadotrophic activity.

Since the available evidence seems to indicate that early postnatal injection of testosterone propionate into male and female rats results in permanent anterior lobe dysfunction which in turn results in structural and functional abnormalities in the reproductive system, a histological study of the anterior hypophyses of such male and female rats has been made in an attempt to ascertain if a structural basis for these functional defects exists.

Materials and methods

Two series of rats were used. In series 1, 5 female and 5 male rats were given 36 mg. of testosterone propionate,* distributed in 12 injections between the 1st and 28th days of life. Autopsies were carried out at the age of 110 days. In series 2 a like number of rats received similar injections except that the total dose was 6 mg. and autopsy was done at 131 days. Equal numbers of untreated male and female rats of like ages were used as controls. At autopsy body and certain organ weights were obtained. The means of these weights are recorded and subjected to a statistical analysis in table 1.

The pituitaries were fixed in Regaud's fluid and prepared for study by the method of Cleveland and Wolfe.[2] During the course of histologic study, cell counts were made; these are recorded in table 1 and analyzed statistically. The reproductive organs were preserved, but of these, only the vaginae of the experimental females were studied histologically.

Results

Reference to table 1 will show that the mean weight of the pituitary glands of the injected females was significantly less than was that of the untreated control animals. In contrast, the average weight of the pituitaries from experimental males was not appreciably different from that of their controls. The ovaries of the experimental rats were markedly smaller and lighter in weight than were those of the control females, the difference being highly significant. The weights of the testes and of the prostates and seminal vesicles of the experimental males were less than were those of the control males and the differences were great enough to be statistically significant. The observations on the effects of early postnatal treatment with androgen on the weights of the reproductive organs of male and female rats confirm the earlier findings of Wilson and his associates.

The results of cell counts made on anterior lobes of experimental and control male and female rats are given in table 1. The early postnatal injections of testosterone propionate into female rats resulted in a cellular pattern in the anterior lobe which was similar to that of normal male rats. The percentages of eosinophils and basophils were much greater than were those found in control females, but they were almost identical with those of normal males. Correspondingly, the proportion of chromophobes in the experi-

* The testosterone propionate was furnished under the trade name of Perandren through the courtesy of Ciba Pharmaceutical Products, Inc., Summit, N. J.

mental females was markedly less than in the control females, but again it was practically the same as in the control male rats.

In the anterior lobes of the females which had received the androgen, the eosinophils ranged in size from small to large cells, and these cells were well filled with granules, a condition usually typical of eosinophils in the glands of male rats. When observed, the negative image of the Golgi apparatus was usually applied close to the nucleus, and in most instances this body was not enlarged. In contrast, many eosinophils in the anterior lobes of the female control rats were in various stages of degranulation, and in such cells the negative image of the Golgi body was often enlarged.

The basophils in the anterior lobes of the experimental females were generally large and well granulated, again a condition typical of normal male rats. Lesser numbers of degranulated and partially degranulated cells were observed. In general, the negative image of the Golgi apparatus was larger and more prominent in the granular than in the non-granular basophils. Basophils were less numerous and smaller in the glands of the control females and a much greater proportion of the cells were degranulated (table 1).

TABLE 1

A STATISTICAL ANALYSIS OF THE ORGAN WEIGHTS AND OF

Rat groups	*No. of rats*	*Rat Wt. Gm.*	*Pituitary Wt. Mg.*	*Ovary Wt. Mg.*	*Testes Wt. Gm.*
Injected females	10	M. 242±3.8 SD. 17±2.7	M. 8.9±0.4 SD. 1.8±0.3	M. 22.8±2.4 SD. 9.5±1.7	....
Control females	8	M. 202±6.4 SD. 25±4.5	M. 11.1±0.3 SD. 1.3±0.2	M. 69.6±3.5 SD. 13.8±2.5	....
Injected males	9	M. 286±5.5 SD. 21±3.8	M. 9.0±0.2 SD. 0.6±0.1	....	M. 1.7±0.1 SD. 0.5±0.1
Control males	7	M. 315±5.7 SD. 20±4.1	M. 9.6±0.3 SD. 1.0±0.2	....	M. 2.9±0.1 SD. 0.4±0.1
S. R.* of the difference between the means of:					
injected females and control females		5.4	4.2	11.1	....
injected males and control males		3.6	1.8	....	8.0
injected females and control males		10.7	1.4	....	....

* Significance Ratio $= \frac{\text{Mean 1} - \text{Mean 2}}{\sqrt{(\text{PE Mean 1})^2 + (\text{PE Mean 2})^2}}$

There were also cytologic differences in the chromophobes in the anterior lobes of the experimental and control females. In the latter, these cells were generally small and relatively inconspicuous; often only a scant amount of cytoplasm was found about the nucleus, and frequently the cell membrane was indefinite. When the negative image of the Golgi apparatus was seen, it was generally small and inconspicuous. The chromophobes in the control females usually had more abundant cytoplasm and often they were quite large. In both the smaller and larger chromophobes, enlarged negative images of the Golgi bodies were frequently noted. The general appearance of these cells in the experimental females was quite similar to that found in the control males.

In contrast to the situation noted in the female rats, the early postnatal injection of testosterone propionate in male rats failed to alter materially the structure of the anterior lobe, either quantitatively (table 1) or cytologically, from the condition found in normal males. In both treated and untreated males the eosinophils and basophils were numerous and there was little evidence of degranulation. A few vacuolated basophils were found in the anterior lobes

THE CELL COUNTS IN THE ANTERIOR PITUITARY GLANDS

Prostate & Sem. Ves. Wt. Gm.	*Percentages of cell types*				
	Eosino-phils	*Gran. Baso-phils*	*Non-Gran. Basophils*	*Chromo-phobes*	*Vacuolated basophils*
....	M. 49.1±0.9 SD. 4.3±0.7	M. 4.6±0.4 SD. 1.6±0.3	M. 2.2±0.2 SD. 1.0±0.5	M. 44.1±0.9 SD. 4.3±0.7	...
....	M. 25.6±0.9 SD. 3.7±0.7	M. 1.4±0.1 SD. 0.5±0.1	M. 2.7±0.2 SD. 0.6±0.1	M. 70.3±0.9 SD. 3.8±0.7	...
M. 0.6±0.1 SD. 0.3±0.1	M. 56.2±0.9 SD. 3.9±0.7	M. 4.8±0.3 SD. 1.3±0.2	M. 2.1±0.1 SD. 0.4±0.1	M. 36.8±0.8 SD. 3.3±0.6	M. 0.04 ...
M. 2.8±0.1 SD. 0.3±0.1	M. 47.9±2.8 SD. 10.2±1.9	M. 4.7±0.2 SD. 0.9±0.2	M. 3.1±0.2 SD. 0.8±0.2	M. 43.9±2.6 SD. 9.5±1.9	M 0.32 ...
....	19.5	8.4	1.7	21.8	...
22.0	2.8	0.2	4.1	2.6	...
....	0.2	2.8	2.8	0.8	...

When the ratio equals 3 or over it is significant.

of both groups. In the glands of both the experimental and normal males the chromophobes were small and presented little evidence of cellular activity. There were two slight but noteworthy differences in the structure of the anterior lobes of the two groups. Eosinophils were more numerous, but not significantly so (table 1), in the glands of the injected rats, and although there were only a few vacuolated basophils in the glands of either group, they were more numerous in those of the controls (table 1).

Discussion

These studies seem definitely to establish the fact that early postnatal injection of relatively small amounts of testosterone propionate into female rats results in the production of a cellular pattern in the anterior hypophysis which is quite similar to that found in the male rat, but which differs widely from that found in the anterior lobe of the normal female. Previously, such a condition has been induced by transplanting testicular tissue into newborn female rats[5] or by injecting, for a period of 90 days, testosterone propionate into rats which were approximately 30 days of age at the initiation of treatment.[12] In the studies of Pfeiffer[5] and in those of Wolfe and Hamilton,[12] the source of androgen was maintained in the body until the time of autopsy. However, the present observations seem to indicate that such prolonged androgenic action is not necessary, since administration of the hormone for the relatively short period of 28 days was sufficient to produce a persisting alteration in the cellular ratios of the anterior pituitary gland. There is no evidence to indicate that androgens from an endogenous source might have continued to act after injections were stopped. Therefore, it seems most likely that the male type of cellular pattern in the anterior lobe was developed during the course of the injections and persisted after the stimulating effects of androgen were removed.

Constant vaginal cornification has been observed[4, 5] in rats receiving testicular transplants immediately after birth as well as in rats treated similarly to those used in the present studies.[8] Although vaginal smears were not made, the vaginae of 8 of the 10 injected rats were sectioned and studied. The epithelium was stratified and cornified in 5 of these, and mucified in 3. These findings, together with those quoted above, make it appear likely that at least some of the rats used in these studies experienced constant vaginal

cornification, and that the ovaries of the others were producing at least sub-cornifying amounts of estrogen at the time of autopsy.

It is now well recognized that estrogen administration in rats induces degranulation of eosinophils and basophils and decreases the relative percentage of these cells in the anterior lobe while increasing the proportion of the chromophobes. However, since the cellular pattern typical of males is maintained in the anterior lobes of the female rats receiving androgen as well as in those receiving testicular implants at birth,[5] it seems that levels of estrogen in the animal sufficient to stimulate vaginal cornification do not induce degranulation of the chromophilic cells to a degree necessary to distort the male type of cellular pattern in the anterior lobe. It is interesting to note that Pfeiffer[4] has found that in female rats bearing testicular transplants and exhibiting constant vaginal estrus, the degree of estrogenic stimulation is not sufficient to induce full estrous changes in the uterus.

However, it should be noted here that if sufficient amounts of estrogen (200 r.u. daily for 10 days) are administered to male rats, the percentages of the eosinophils and basophils in the anterior lobe are reduced to the levels of these cells as found in the glands of female rats.[13] Therefore, it seems possible that useful information might be obtained by administration of estrogens to female rats which have an experimentally produced male type of cellular pattern in the anterior lobe. For instance, Wilson and his associates[10] found that the uteri of rats which had received androgen early in life were less sensitive to estrogen than were the uteri of normal rats. It would be interesting to know if the anterior lobes of such rats exhibit the same characteristic of reduced sensitivity to estrogen.

The failure of early postnatal injection of testosterone propionate to alter materially the structure of the anterior hypophyses of male rats was perhaps to be expected, since these observations indicate that in female rats, the effect of such injections is to produce in the anterior lobe a type of cellular pattern which normally occurs in males. Yet despite the fact that the injections do not appreciably alter the cellular pattern in the anterior lobes of male rats, it is known from the studies of Wilson and Wilson[9] that such injections induce pronounced functional and structural disturbances in male rats. Their findings are confirmed by the decrease in the size of the testes and a marked atrophy of the accessory reproductive organs in the experimental rats of the present series. Since the cellular

pattern in the anterior lobes of the experimental and control male rats was similar, it might be concluded that the actual percentages of the cells in the anterior hypophysis do not play a dominant rôle in the induction of the reproductive defects found in these male rats.

Although these observations, as well as the previous studies of Pfeiffer, associate the experimentally produced male type of cellular pattern in the anterior lobes of female rats with structural and functional abnormalities in the ovaries, the findings made on the anterior lobes of androgen-injected male rats make it appear doubtful that the male type of cellular pattern in the anterior lobes of the experimental females is itself responsible for the ovarian abnormalities. It seems more likely that such a cellular pattern in the anterior lobes of female rats is another manifestation of the endocrine dysfunction induced by the early injection of androgen. The studies of Pfeiffer[4, 6] and of Bradbury[1] indicate that the ovarian defects are due to the failure of the anterior lobe either to produce or to release the factors stimulating ovulation and luteinization. Just why the anterior lobes of female rats subjected to androgen treatment at an early age should fail to release the luteinizing factor is at present obscure.

Summary

During the first 28 days of life, 10 male and 10 female rats received 12 subcutaneous injections, totalling either 6 or 36 mg., of testosterone propionate. An equal number of untreated male and female rats of the same ages were used as controls and the animals were autopsied at either 110 days or 131 days of life.

Identical and permanent results were obtained by temporary treatment with these two dosages of androgen. The mean weight of the pituitary glands of the injected female rats was significantly less than was that of the untreated females. The pituitary weights of the injected males and their controls did not differ materially. The ovaries of the experimental rats were markedly decreased in weight as compared with those of the controls. In the male rats the mean weights of the testes and of the prostates and seminal vesicles were significantly less in the experimental than in the control males.

The percentages of eosinophils and basophils in the anterior hypophyses were significantly higher in the injected female rats than in untreated control females. Correspondingly, the percent-

ages of the chromophobes were significantly lower in the injected females. The eosinophils and basophils of the anterior lobe of the injected female rats showed much less evidence of degranulation than was found in the same cell types in the female control rats. The cellular pattern in the injected female rats resembled closely that of the glands of normal males, but differed widely from that of the control females.

In contrast to the findings in the female rats, early postnatal injection of testosterone propionate failed to induce any appreciable change in the cellular pattern in the anterior lobes of the pituitary glands of male rats.

References

1 Bradbury, J. T.: Endocrinology, 1941, *28*, 101.
2 Cleveland, R., and J. M. Wolfe: Anat. Rec., 1932, *51*, 409.
3 McQueen-Williams, M.: Anat. Rec., Suppl., 1934, *58*, 78.
4 Pfeiffer, C. A.: Am. J. Anat., 1936, *58*, 195.
5 Pfeiffer, C. A.: Endocrinology, 1937, *21*, 812.
6 Pfeiffer, C. A.: Yale J. Biol. & Med., 1942, *14*, 619.
7 Selye, H.: Endocrinology, 1940, *27*, 657.
8 Wilson, J. G., J. B. Hamilton, and W. C. Young: Endocrinology, 1941, *29*, 784.
9 Wilson, J. G., and H. C. Wilson: Endocrinology, 1943, *33*, 353.
10 Wilson, J. G., W. C. Young, and J. B. Hamilton: Yale J. Biol. & Med., 1940, *13*, 189.
11 Wolfe, J. M., and E. T. Ellison: Anat. Rec., Suppl., 1934, *58*, 93.
12 Wolfe, J. M., and J. B. Hamilton: Endocrinology, 1939, *25*, 572.
13 Wolfe, J. M., and D. Phelps: Proc. Soc. Exper. Biol. & Med., 1935, *32*, 1305.