

THE ACTION OF VARIOUS STEROID HORMONES ON THE OVARY

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IN COMPARISON WITH the extensive literature concerning the gonadotropic functions of the pituitary, relatively little has been written about the effects of the steroid hormones on the ovary.

Adrenal cortical hormones. Earlier investigators working with a variety of animal species obtained conflicting results concerning the actions of their preparations on the female gonad. Some (1-3) could find no effect on the development of the immature mouse ovary, while others (4-9) obtained positive effects. Similarly in the immature (10-20) and mature rat (18, 19, 21-30) the reported findings were contradictory. At the present time, however, knowing that the adrenal cortex contains a host of active steroids it appears probable that the differences in the results of the earlier investigators were due to differences in their crude extracts, or—if they used transplants—to the fact that these produced several hormones.

As regards pure cortical preparations there are only a few reports on desoxycorticosterone. Hoffmann (31-33) obtained no effect on the ovary with this substance although previously he had noted gonad stimulation following administration of crude extracts. Selye (34) found, on the other hand, that large doses of desoxycorticosterone acetate caused a moderate stunting of somatic growth and marked inhibition of gonadal development in the immature rat. He also noted that this inhibition lasts for a considerable length of time after the hormone injections are discontinued, and that in the adult rat a slight atrophy of the ovary is produced in the case of prolonged treatment with moderate doses.

Corpus luteum hormone. Beard (35) and Prenant (36) were the first to advance the theory that the corpus luteum of pregnancy is in some way responsible for the fact that no follicles mature and no corpora lutea are formed as a rule during the gestation period. However here again the experimental work of subsequent investigators yielded contradictory results (37-44). More recently, Selye et al. (45) administered 4 mg. of synthetic progesterone daily to normally cyclic adult rats and noted immediate cessation of the vaginal cycles, while autopsy on the thirteenth day of treatment revealed atrophic ovaries. On the other hand, McKeown and Zuckerman (46) claimed that daily administration of 1 mg. of progesterone during 9 to 11 days elicits the formation of new corpora lutea; however, as they used post pubertal rats there is no reason to believe that these corpora were formed as a result of their treatment rather than in spite of it. We shall see later that in our experiments, this dose was never sufficient to produce such an effect.

Testosterone. Somewhat more agreement is found in the literature regarding the action of this hormone. In the rat, ovarian atrophy was obtained by some workers (47-48) while others observed stimulation of corpus luteum formation (46, 48-49). Korenchevsky (50) has put forward a compromise view with which the above observations would be in agreement, viz., that short treatment with testosterone increases the size of the ovaries eliciting the formation of numerous corpora lutea, while prolonged treatment results in ovarian atrophy. More recently, Selye (68) produced follicular cysts with testosterone in mice.

Estrin. In the mouse, it has been fairly well established that large doses given over a short period of time lead to corpus luteum formation (51-53), while continuation of the same high dose, or chronic low dose treatment result in follicular atresia and corpus luteum involution (54-55). Similarly in the rat if small doses (2 to 4 u) are given daily, ovarian

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atrophy ensues, although after a time adaptation seems to occur with the restoration of normal appearance and function of the ovary (56-57). On the other hand, the ovaries of mature rats respond to large doses of estrin given for a short time (about 2 weeks) with the formation of large corpora lutea similar in appearance to those seen during gestation (58-62). Continued administration of the large doses cannot maintain these corpora which involute after a few weeks (60).

Combined administration of several steroids. Selye and Stevenson (63) noted in the mouse that the ovary became atrophic when the animal was treated with estradiol alone, while no atrophy was seen if progesterone was simultaneously administered. Selye (64-65) confirmed this for the rat and found also that testosterone propionate could likewise prevent the gonadal atrophy caused by estradiol. On the other hand, he noted that testosterone propionate failed to prevent the ovarian atrophy caused by progesterone.

In view of the foregoing experiments, it appeared of interest to investigate the gonadotropic effects of desoxycorticosterone acetate about which little is known, and of progesterone, about which there is controversy. We also wished to extend

TABLE I

Treatment	No. animals	Body wt.	Duration	Ovary wt.	P	Histology
Cholesterol	4	gm. 18 (16-20)	days 10	mg. 3.5 (3.0-4.0)		Corpora lutea present. Normal ovarian structure.
Cholesterol	5	17 (16-18)	20	3.9 (3.7-4.0)		
Desoxycorticosterone acetate	4	19 (18-21)	10	4.0 (3.0-5.0)	0.3	No corpora lutea in any ovary. Very many follicles undergoing cystic atresia. Stroma atrophic.
Desoxycorticosterone acetate	4	18 (16-19)	20	3.1 (3.0-3.2)	<0.01	

the observations of Selye et al. on the effects of combined administration of steroid hormones with the view of finding an explanation for the apparently paradoxical effects which they noted.

Desoxycorticosterone Acetate

Experiment I. Female, non-pregnant, adult mice of the strain designated as DBA (Little's dilute brown strain) were used. Four animals received daily subcutaneous injections of 3 mg. of desoxycorticosterone acetate dissolved in 0.2 cc. of pure peanut oil, while 4 controls were treated with the same amount of cholesterol in oil. A similar second series was used for a more chronic experiment. The significance of the apparent differences between the treated and control groups was evaluated by "Student's" method for small samples and is expressed in the table in terms of probability, estimated by graphic interpolation in Fisher's table of *t*. In accordance with the usual convention, differences between series' cannot be accepted as significant when *P* is greater than 0.05. In calculating the significance of the apparent changes, the 10-day cholesterol treated animals served as controls for the 10-day desoxycorticosterone acetate treated animals, and similarly the 20-day desoxycorticosterone acetate group was compared with the 20-day controls. The results of both experiments are summarized in table I.

From this experiment, we conclude that brief treatment results in complete absence of corpora lutea, increased number of follicles undergoing cystic atresia, and stromal atrophy. It is accompanied by a slight (non-significant) increase in

weight. Prolonged treatment accentuates these changes, and is reflected in a significant loss of gross weight (fig. 1, 2).

Experiment II. Six newborn female hooded litter-mate rats were selected. Of these 5 animals received daily from the day of birth 1 mg. of desoxycorticosterone acetate in 0.2 cc. of peanut oil, subcutaneously for 30 days, followed by 2 mg. as above for a further 10 days. Two of the treated animals were then killed while 3 were maintained for 30 days without treatment and then killed. In all cases the ovaries were taken for weight determination and histological study.

The 2 animals killed on the 40th day of treatment, having received a total of 50

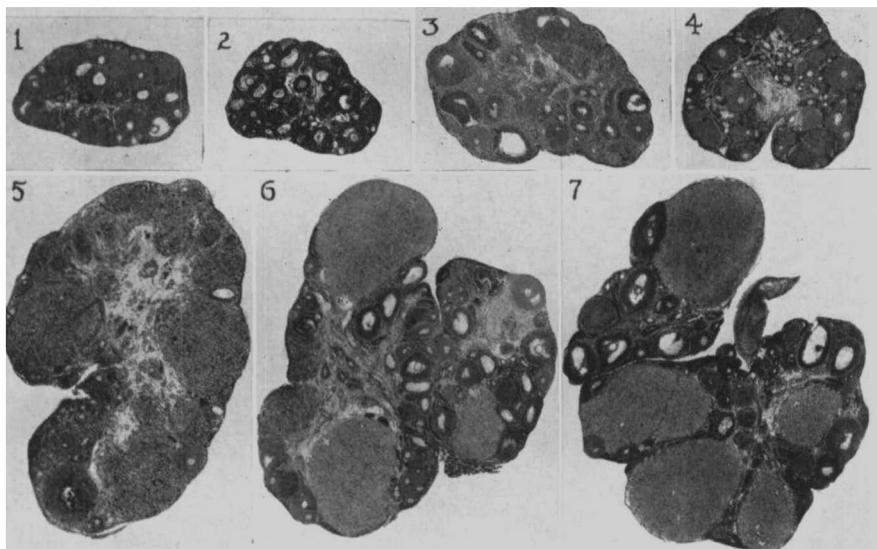


Fig. 1. ADULT MOUSE TREATED WITH 3 MG. OF CHOLESTEROL FOR 10 DAYS. Section shows normal ovarian structure. Fig. 2. ADULT MOUSE TREATED WITH 3 MG. OF DESOXYCORTICOSTERONE ACETATE FOR 10 DAYS. Corpora lutea are absent. There is an increase in the number of follicles showing cystic atresia and the stroma is atrophic. Fig. 3. FORTY-DAY-OLD HOODED RAT NOT TREATED. Section shows normal ovarian structure. Fig. 4. FORTY-DAY-OLD HOODED RAT TREATED WITH A TOTAL OF 50 MG. DESOXYCORTICOSTERONE ACETATE FROM DAY OF BIRTH. Ovary is very small and shows absence of corpora lutea, increase in number of cystic atretic follicles and very atrophic stroma. Fig. 5. ADULT ALBINO RAT, UNTREATED, SHOWING NORMAL OVARIAN STRUCTURE. Fig. 6. ADULT ALBINO RAT, TREATED WITH 10 MG. DESOXYCORTICOSTERONE ACETATE DAILY FOR 20 DAYS. Note the increased number of follicles undergoing cystic atresia, the large corpora lutea and the atrophic stroma. Such large corpora lutea were found only in half of our rats thus treated (compare with fig. 5). Fig. 7. ADULT ALBINO RAT TREATED WITH 10 MG. PROGESTERONE DAILY FOR 20 DAYS. Note the very large corpora lutea occupying most of the ovary, increase in number of follicles undergoing cystic atresia, and atrophic stroma (compare with fig. 5).

mg. of the hormone, showed ovarian weights of 6.3 mg. and 7.2 mg. or an average of 6.7 mg. as against an untreated litter mate killed on the same day which had an ovarian weight of 23 mg. The 3 animals in which treatment was suspended during the next 30 days showed at the end of that time an average ovarian weight of 9.3 mg. (6.0-7.0-15.0 mg.) which is still markedly below the normal ovarian weight of a 70-day-old rat and even markedly below the 23 mg. weight of the 40-day-old litter mate in this series. In all the hormone treated animals, the ovarian stroma was found to be markedly atrophic. No corpora lutea were present in any of the treated animals (fig. 3, 4).

Despite the fact that the data is derived from a small number of observations the weights and histology are striking so that we conclude that desoxycorticosterone

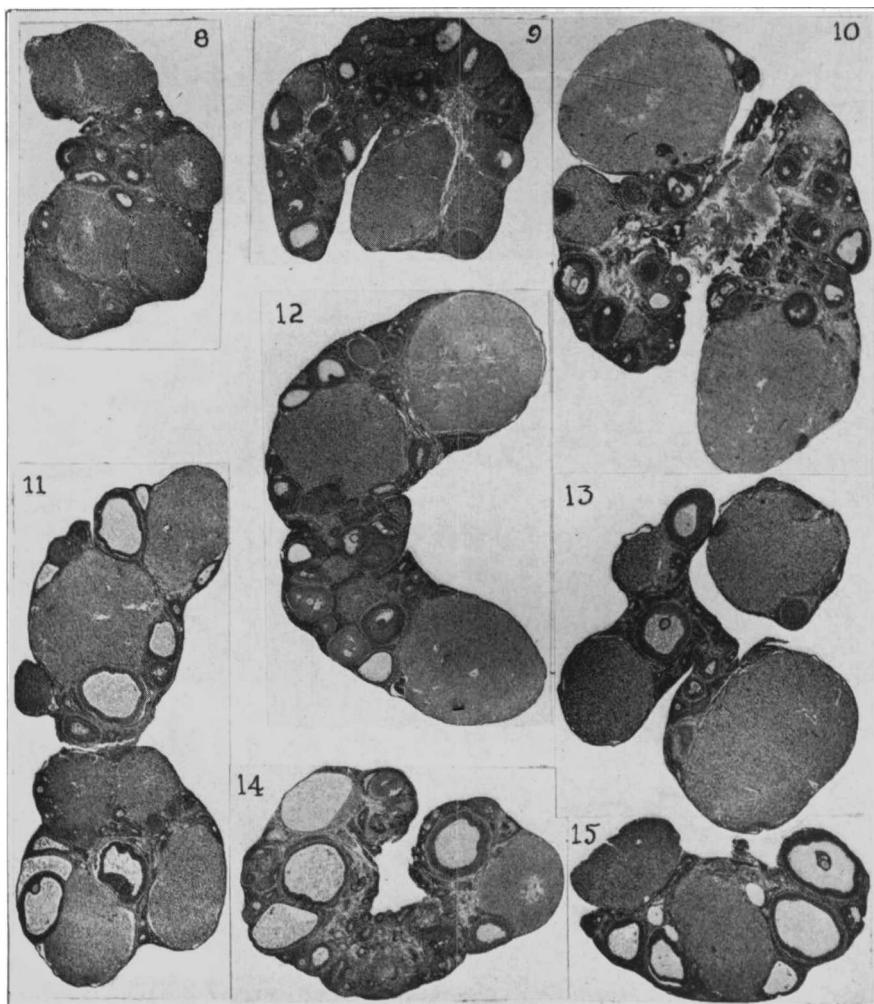


Fig. 8. ADULT ALBINO RAT UNTREATED SHOWING NORMAL OVARIAN STRUCTURE. Fig. 9. ADULT ALBINO RAT TREATED WITH 2 MG. PROGESTERONE DAILY FOR 21 DAYS. The ovary is very small and filled with many follicles showing cystic atresia. The stroma is very atrophic (compare with fig. 8). Fig. 10. ADULT ALBINO RAT TREATED WITH 300 γ OF ESTRIN PLUS 2 MG. OF DESOXYCORTICOSTERONE ACETATE DAILY FOR 21 DAYS. Corpora lutea are very prominent and there is an increase in the number of follicles undergoing cystic atresia. Stroma is very atrophic (compare with fig. 8). Fig. 11. ADULT ALBINO RAT TREATED WITH 300 γ OF ESTRIN PLUS 2 MG. OF TESTOSTERONE DAILY FOR 21 DAYS. The corpora lutea are conspicuous as are the beginning follicular cysts. The stroma is atrophic (compare with fig. 8). Fig. 12. ADULT ALBINO RAT TREATED WITH 300 γ OF ESTRIN PLUS 2 MG. OF PROGESTERONE DAILY FOR 21 DAYS. Note conspicuous corpora lutea, numerous atretic follicles and atrophic stroma (compare with fig. 8). Fig. 13. ADULT ALBINO RAT TREATED WITH 300 γ OF ESTRIN PLUS 2 MG. OF TESTOSTERONE AND 2 MG. OF PROGESTERONE DAILY FOR 21 DAYS. Very large corpora lutea occupy most of the section, 2 beginning follicular cysts are conspicuous. Stroma is very atrophic (compare with fig. 8). Fig. 14. ADULT ALBINO RAT TREATED WITH 2 MG. OF DESOXYCORTICOSTERONE ACETATE PLUS 2 MG. OF TESTOSTERONE DAILY FOR 21 DAYS. Ovary is very small and contains 4 prominent follicular cysts and numerous atretic follicles. The stroma is atrophic (compare with fig. 8). Fig. 15. ADULT ALBINO RAT TREATED WITH 2 MG. OF TESTOSTERONE PLUS 2 MG. OF PROGESTERONE DAILY FOR 21 DAYS. Note the large corpora lutea and prominent beginning follicular cysts. Stroma is atrophic (compare with fig. 8).

acetate inhibits the development of the immature rat ovary producing marked stroma atrophy and preventing the formation of corpora lutea. This effect is still operative for a long time after cessation of the injections.

Experiment III. Six newborn female litter-mate albino rats of an average body weight of 17 gm. at the beginning of the experiment (8th day of life) were selected. Three of the animals received 1 mg. of desoxycorticosterone acetate in 0.2 cc. subcutaneously every 2 days, while the remaining 3 served as untreated controls. One animal in each group was killed on the 30th and the remaining 2 in each group on the 37th day of life. The ovaries in all cases were taken for weight determination and histological study.

On the 30th day of life the ovaries of the treated animal weighed 8.0 mg. (body weight 68 gm.) as against an ovarian weight of 12 mg. (body weight 65 gm.) in the untreated control killed on the same day. On the 37th day of life the treated animals had an average ovarian weight of 9.7 mg. (7.4-12.0 mg.) as against 19 mg. (11.0-27.0 mg.) in the untreated animals killed on that day. All 4 animals had body weights of 80 to 87 gm.

This experiment furnishes further evidence that desoxycorticosterone acetate inhibits the development of the immature rat ovary.

Experiment IV. Two groups of 12 adult female albino rats were used. In the first group, 6 animals received daily 2 mg. of desoxycorticosterone acetate subcutaneously and 6 served as untreated controls. In the second group, 6 received 10 mg. of desoxycorticosterone acetate in 0.4 cc. of peanut oil daily subcutaneously while the remaining 6 served as untreated controls. At the end of the period of injection all the animals were killed and the ovaries taken for weight determination and histological study. Table 2 summarizes the data.

TABLE 2

Treatment	No. animals	Body wt.	Ovary wt.	Histology
Desoxycorticosterone acetate 2 mg.—21 days	6	gm. 135	mg. 33.0	Marked increase in number of follicles undergoing cystic atresia. Stroma very atrophic.
Untreated	6	142	36.0	Normal ovarian structure.
Desoxycorticosterone acetate 10 mg.—20 days	5	142	26.0	Same as in above desoxycorticosterone acetate but some enlarged-corpora.
Untreated	6	148	37.0	Normal ovarian structure.

We see that in the adult rat prolonged treatment with relatively small doses of desoxycorticosterone acetate elicits follicle changes with stroma atrophy and is accompanied by an insignificant loss in ovarian weight. In 2 animals of this group the corpora lutea were large so that despite the stroma atrophy, in these the ovarian weights were 52 and 53 mg. respectively. If these were eliminated the loss of weight from the stroma atrophy would be still more apparent.

Prolonged treatment with a high dose of desoxycorticosterone acetate produces the same cystic degeneration of numerous follicles giving the ovary a "Swiss cheese" appearance, and the same stroma atrophy as above. However in this case there is a pronounced loss of ovarian weight. Both in high and low dose treatment cystic atresia of follicles is often seen. In the rat, desoxycorticosterone acetate usually causes no corpus luteum formation in the doses employed here, although definitely large corpora have been noted in some cases (fig. 5, 6).¹

¹ As an incidental observation we noted that in all cases treated with desoxycorticosterone acetate there was an unusually large amount of free peritoneal fluid. An analysis of the mechanism responsible for this phenomenon will be published later.

Corpus Luteum Hormone

Experiment I. Twelve adult female albino rats having an average body weight of 151 gm. were divided into 2 groups. Six received 21 daily subcutaneous injections of 2 mg. of progesterone in 0.2 cc. of peanut oil, while the remaining 6 served as untreated controls. At the end of the injection period, the animals were killed and the ovaries taken for weight and histological study.

In the treated group, the ovaries weighed only 22.9 mg. (11.2-31.6) as compared to 36 mg. (33.5-40.1) in the normal controls. The ovaries of the treated group were noteworthy in that although degenerating corpora lutea were present in about the same proportion and size as in the controls, completion of follicle maturation was markedly depressed because many of them had undergone cystic atresia. The stroma was very atrophic showing beginning 'wheel cell' formation.

We conclude that in the rat prolonged treatment with low doses of progesterone depresses follicle maturation and causes stroma atrophy, but apparently exercises no influence on the corpora lutea. The atrophy is reflected in a significant loss of ovarian weight. ($P = <0.01$).

Experiment II. Five adult female albino rats having an average body weight of 154 gm. received 20 daily subcutaneous injections of 10 mg. of progesterone in 0.4 cc. of peanut oil, while 6 animals of the same weight served as untreated controls. At the conclusion of the period of injection the animals were killed and the ovaries taken for weight determination and histological study. In the treated group the average ovarian weight was 40 mg. (20-50) as compared to 37 mg. (33-43) in the controls. If we omit one animal whose ovaries were abnormally small (20 mg.) the increase in ovarian weight in the treated group is even more significant, as the other 4 had ovaries ranging between 30 and 50 mg. In our rat colony, spontaneous ovarian atrophy is occasionally seen although its cause is not definitely known. This may explain the one instance cited above. In the other ovaries, large corpora lutea accounted for the rise in weight, although these were involuting at the time of autopsy. However, the marked follicular atresia and stroma atrophy, in many cases accompanied by beginning wheel cell formation, were striking (fig. 5, 7).

In the adult rat, a marked depression of follicle maturation and stroma atrophy are seen after treatment with high doses of progesterone. However, since such treatment also causes ovulation and the formation of large corpora lutea, the gross weight of the gonad may be above normal in spite of this.

Combination treatments. In the series of experiments tabulated below, all animals used were adult female albino rats having an average body weight of 134 gm. They were injected subcutaneously with various steroids, the daily dose of which was dissolved in 0.2 cc. of peanut oil in each case. After 21 injections all animals were killed and the ovaries were weighed and taken for histological study. Table 3 summarizes our results. Each ovarian weight and histological description represents the average of a group of 6 identically treated rats. From these data it appears that in 2 mg. doses given 21 days, desoxycorticosterone acetate, testosterone or progesterone do not cause the formation of large, 'pregnancy-type' corpora lutea, but lead to marked involution of the stroma and consequently to a decrease in ovarian weight. Daily administration of 300 μ of estradiol for the same period likewise results in atrophy of the stroma and a decrease in the gross weight of the ovary. However, histological examination of the latter series still shows remnants of fairly large corpora lutea. Direct inspection after 14 days of treatment in this series showed no large corpora lutea in the groups treated with desoxycorticosterone acetate, testosterone or progesterone but revealed the presence of hyperemic, large 'pregnancy-type'

corpora in all groups in which estradiol was given alone or in combination with other hormones. The fact that at autopsy on the 21st day of treatment the ovarian weights were below normal in the groups receiving estrin alone or in combination with desoxycorticosterone acetate is attributed to the circumstance that this dose of estradiol either by itself or even in combination with desoxycorticosterone acetate was unable to maintain the large corpora lutea which it originally produced. Similarly in the groups receiving desoxycorticosterone acetate in combination with testosterone or progesterone, the ovaries were small because the stroma underwent

TABLE 3

Treatment	Ovary weights mg. 36.0	Histology
Untreated	36.0	Normal ovary
Estradiol 300 γ	30.0	Atrophic stroma—corpora lutea of moderate size; involuting.
Desoxycorticosterone acetate 2 mg.	33.0	Atrophic stroma—increase in number of follicles undergoing cystic atresia.
Testosterone 2 mg.	25.0	Atrophic stroma—follicles in all stages with beginning cyst formation.
Progesterone 2 mg.	22.9	Atrophic stroma—scarcity of maturing follicles.
Estradiol 300 γ Desoxycorticosterone acetate 2 mg.	32.0	Atrophic stroma—corpora lutea of moderate size; involuting.
Estradiol 300 γ Testosterone 2 mg.	40.0	Atrophic stroma—large corpora lutea—small follicular cysts.
Estradiol 300 γ Progesterone 2 mg. Testosterone 2 mg.	46.0	Atrophic stroma—large corpora lutea—relative scarcity of follicles.
Estradiol 300 γ Progesterone 2 mg. Testosterone 2 mg.	41.0	Atrophic stroma—large corpora lutea—relative scarcity of maturing follicles—beginning follicular cysts.
Desoxycorticosterone acetate 2 mg. γ Testosterone 2 mg.	22.0	Atrophic stroma—small follicular cysts.
Progesterone 2 mg. Testosterone 2 mg.	26.1	Atrophic stroma—scarcity of maturing follicles—some follicle cysts.

atrophy and no 'pregnancy-type' corpora were formed. On the other hand, in the groups treated with estradiol simultaneously with progesterone, testosterone or both of these latter hormones, the ovarian weight was above normal because in these combinations, this dose of estradiol maintained the large corpora lutea for 21 days. The stroma atrophy produced by all the hormones used in this series was in no way inhibited in any of the groups given combinations of sterols (fig. 8-15). These observations clarify the apparently paradoxical finding that the ovaries may actually become larger than normal in case of combined treatment with two hormones each of which decreases ovarian weight. The fact that progesterone and testosterone given simultaneously each in 2 mg. doses cause no large corpora or increase in ovarian weight is probably due to the circumstance that even 4 mg. of either of these hormones is not sufficient to produce 'pregnancy corpora.' From other experiments, we know however, that in 8 to 10 mg. daily doses these steroids cause the formation of such corpora, so that this is probably merely a matter of dosage.

The question whether the gonadotropic effects of the above steroids are due to their direct action on the ovaries or are mediated through the pituitary is also of importance. That steroids can exert a direct gonadotropic effect was first shown by Selye and Collip (66). They found that in hypophysectomized rats, the ovaries become atrophic and cannot be stimulated by estrone, but if this atrophy is prevented by maintenance doses of pituitary gonadotropic substances, estrone has a direct effect on the ovaries inasmuch as it produces 'pregnancy-type' corpora. Recently Pencharz (67) and others have come to similar conclusions.

It is difficult to explain the fact that the various steroid hormones may either stimulate or inhibit the ovary, yet this dual action has also been noted with respect to their effect on the testis (64, 65). In the latter case, the situation is somewhat less complicated because the confusing factor of qualitatively different actions (stroma atrophy and corpus luteum formation) is absent. All our observations may perhaps best be explained by assuming that the gonad inhibitory action of the steroids is indirect and due to the depressing effect which they exert on the gonadotropic hormone production of the hypophysis, while their gonad stimulating action is direct. In good accord with this interpretation, we found that none of the steroid hormones cause atrophy of the gonads in hypophysectomized animals receiving maintenance doses of hypophyseal gonadotropic preparations. It seems probable that estrin for instance, which stimulates the corpora lutea in hypophysectomized rabbits even without gonadotropic hormone treatment, while in the rat simultaneous administration of the latter is essential to secure this effect, acts in this manner because the involution of the ovary is more rapid in the rat and consequently the gonad loses its responsiveness unless it is maintained by a pituitary preparation. The male gonads on the other hand, which involute less rapidly in the hypophysectomized rat, are readily stimulated by such steroids as the various androgens and progesterone. This will receive more detailed consideration in a future publication dealing with the effect of steroid hormones in the male. It also appears that, at least in the female, smaller doses of the various steroids suffice to exert the indirect gonad inhibitory action than are required to demonstrate the direct gonad-stimulating effect. This was true in the case of estrogens, progesterone and androgens. Desoxycorticosterone acetate proved inhibitory in most cases except in some of the rats receiving 10 mg. daily so that essentially its action is the same as that of the other steroids. This interpretation might perhaps help to understand why, despite the great differences in their specific effects, the actions of the various steroid hormones on the gonad are so surprisingly similar.

SUMMARY

Desoxycorticosterone acetate inhibits the development of the immature rat ovary, while in the adult rat and mouse it produces atrophy of the stroma and cystic atresia of follicles. Only in some animals receiving 10 mg. daily did it elicit the formation of enlarged corpora.

Progesterone given in daily doses of 2 mg. causes only atrophy of the stroma and inhibition of follicle maturation in the rat. In doses of 10 mg. per day it leads to the formation of a single set of 'pregnancy-type' corpora lutea, still inhibiting follicle maturation and causing stroma atrophy.

Testosterone elicits only stroma atrophy and the formation of follicle cysts when given in doses of 2 mg. per day. However other experiments performed in this laboratory but not reported here in detail indicate that if 8 daily doses of 6 mg. are administered to the rat, testosterone also leads to the formation of a single set of large corpora lutea. More prolonged treatment even with such large doses results in the gradual involution of these corpora and the formation of follicle cysts.

Estradiol in daily doses of 300 μ g produces only very short-lived 'pregnancy-type' corpora. However, if this amount of estradiol is given in combination with testosterone or progesterone, the life span of the newly formed corpora lutea is greatly prolonged although the dose in which these latter hormones are administered is too small to cause corpus luteum formation by itself. This explains the surprising fact that the ovarian weight increases if the dose of estrin which decreases ovarian weight by itself is administered simultaneously with a dose of testosterone or progesterone which given alone would also cause only ovarian atrophy. The maintenance of the large corpora lutea in such cases overcompensates for the relatively small loss in ovarian weight which results from the stroma atrophy.

It is noteworthy that testosterone retains its follicle cyst forming effect when given in combination with estradiol, progesterone or both these hormones. The ability of estradiol, progesterone and testosterone to form and maintain large 'pregnancy-type' corpora lutea as well as their power to cause atrophy of the ovarian stroma, are summated in case of combined treatment with such hormones, while the follicle cyst-forming effect of testosterone is not significantly influenced by other steroids.

The view is expressed that the various steroid hormones exert a gonad-inhibitory action which is indirect and due to the depression of the hypophyseal gonadotropic hormone production. This common pathway in the mechanism of their action may explain why diverse steroids produce the same type of atrophy. The gonad stimulating effect characteristic of most, if not all, the above mentioned steroids on the other hand, appears to be direct as it is not prevented by hypophysectomy.

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