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ALTERATION OF REPRODUCTIVE FUNCTION IN THE RAT BY CORTISOL ACETATE

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INTRODUCTION

Adrenal hyperplasia in laboratory animals has been associated with depressed reproductive function by several investigators. Thus increased social pressure and population density in mice induce adrenocortical hyperactivity, which is associated with varying inhibition of reproductive activity. Chronic administration of ACTH to mice abolished ovulation and luteinization, reduced ovarian weight and size of follicles, and increased follicular atresia (Christian, 1964a, b). Jarett (1965) obtained essentially similar results, and furthermore, contrary to the results reported by Christian (1964a), demonstrated that the effects of ACTH were mediated via the adrenal gland.

The mechanisms by which ACTH produced these effects are uncertain, though these workers have suggested direct action of corticosteroids on the ovaries, inhibition of gonadotrophin secretions by adrenal androgens, progesterone, or directly by ACTH. Exogenous gonadotrophin administration also results in diminished ovarian response, but this does not necessarily mean reduced sensitivity of the ovary, since such induced ovulations may involve neuroendocrine mechanisms, as in the rat (Quinn & Zarrow, 1964). Small doses of adrenal androgens do, however, induce most of the effects observed following ACTH (Roy, Mahesh & Greenblatt, 1962; Varon & Christian, 1963), but some of these steroids appear to increase ovarian weight and induce cystic changes. This may well result from modification of the FSH/LH ratio in such a way as to inhibit ovulation and thus expose the ovaries to a continuous preponderance of FSH stimulation.

Glucocorticosteroids have been adequately demonstrated to be necessary for the maintenance of normal reproductive function in male and female laboratory animals. Limited experiments involving the administration of high doses have revealed few specific interactions with the reproductive system. Soon after cortisone became available, some workers reported variable suppression of spermatogenesis and also smaller ovaries in rats, but, though Moore (1953) concluded that cortisone does not have a gonadotrophic effect in rats, he found a consistent increase in ovarian weight which was attributed to increased numbers of follicles. Most impressive is the inhibitory influence of glucocorticosteroids on the uterine response to oestrogen (Velardo & Sturgis, 1956) and the inhibition of progesterone action on experimentally induced decidual tissue formation (Hisaw & Velardo, 1951; Velardo, 1957).

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Several reproductive syndromes in various species specifically involving adrenal function have been discussed by Van Rensburg (1965). These include cystic ovaries, ovarian inactivity, gestational failure, prolonged gestation in females and seminal changes in males. Considerable evidence of unusual gonadotrophic stimulation exists in a number of conditions characterized by hyperadrenocorticism, such as the occurrence of follicular growth and ovulation in anoestrously stressed ewes. This phenomenon is compatible with the observation in man that ACTH and glucocorticosteroids increase urinary gonadotrophin secretion (Sohval & Soffer, 1951; Brown, Thorburn & Crooks, 1963), but does not concur with the suggestion that increased circulating gonadotrophins following ACTH could be a consequence of reduced ovarian sensitivity (Jarett, 1965). No doubt many species differences in the adrenal-gonad axis are due to divergent patterns of adrenocortical steroid biosynthesis, including the sex hormones secreted from the adrenal under stimulation of ACTH. This work is an attempt to clarify more precisely the actions of cortisol on the reproductive system of rats at levels which may be encountered in the upper physiological or pathological range.

Material and Methods

Animals

Wistar rats bred in the laboratory and purchased, immature Long-Evans rats were used in these experiments. The strain is indicated in the tables by the designation W or L respectively. Each animal was identified by ear notches and all groups were randomly caged to prevent possible synchronization of cycles. Rats were randomized into experimental groups with the aid of numbered cards and in no instance did the body weight between groups differ significantly at the commencement of an experiment. Immature rats were weighed daily and mature rats three times weekly; the mean interval using the latter system is represented as 2·33 days. Mature animals were thoroughly accustomed to careful handling and the use of sharp hypodermic needles elicited little or no antagonism.

Cortisol acetate (Steraloids, LTD) was administered subcutaneously as a homogenized saline suspension at a constant volume of 0·1 ml per 100 gm of rat; controls were always treated concurrently with an equal volume of saline. Vaginal smears were made daily with sterilized steel spatulas and examined directly in a drop of saline.

Surgical techniques

Ether anaesthesia was employed and sterile precautions were observed during hysterectomy and with pituitary transplantations. The uterus together with a small portion of the cervix was removed using a midventral approach and the completeness of the operation was confirmed at autopsy. Pituitaries were removed from healthy etherized donors of the same age, sex, and strain as the recipients, placed in sterile saline at 37°C, and then rapidly inserted under the left renal capsule of the oestrous recipient. At autopsy, normal hyperaemic homografts were clearly visible.

Autopsy

Rats were sacrificed with ether and the ovaries, uterus, spleen, thymus, liver, kidneys and testes quickly removed, cleaned of extraneous tissue on moistened filter paper, and weighed to the nearest 0·1 mg. Tissues for steroid analyses were

frozen on solid CO_2 and stored at $-15°C$, those for histological examination were fixed in Bouin's fluid. Tubal ova were counted by the methods mentioned by Quinn & Zarrow (1964). The uteri of pregnant rats were excised on the 17th day of gestation, weighed intact and then incised longitudinally. Foetal membranes were opened and the fluid removed with blotting paper; the difference in weight was regarded as the amniotic fluid weight. Foetuses and placentae were dissected free and the weight for each rat obtained. The uterus with the implantation sites was then weighed and the " uterine weight increase per foetus " calculated by comparison of this value with the mean weight of the uterus in six dioestrous controls.

The ovaries of immature rats were serially sectioned at seven microns and every 30th section mounted and stained with haematoxylin-eosin for follicle and corpora lutea counts and measurements. performed with the aid of an ocular micrometer. This procedure resulted in examination of an average of seven sections per ovary (14 per rat) and naturally does not give the true number of follicles and corpora lutea present; nevertheless it should give a fairly accurate reflection for comparative purposes. Pituitary glands were immersed in excess cold acetone and the neurohypophysis removed under a dissecting microscope prior to the Steelman-Pohley assay for FSH as outlined by Parlow (1964). Available material permitted only a three-point assay design.

Progesterone analysis

The ovaries of two to four rats were pooled for each determination using the procedure of Rowlands & Short (1959) with 90 per cent aqueous methanol as the stationary phase and running the chromatograms for three hours. When treated with 20β-hydroxysteroid dehydrogenase as described by Heap (1964) the reaction product exhibited the same R_f value as authentic 20β-hydroxypregn-4-en-3-one. On acetylation, the isolated material and authentic 20β-acetoxypregn-4-en-3-one had the same R_f value on rechromatography and therefore was assumed to be progesterone.

Results

Influence of cortisol acetate on the response of immature rats to gonadotrophin

All rats were treated with 0·6 iu PMS per gram body weight when 24 days old, and therefore according to the consistent results obtained by Quinn & Zarrow (1964) with a similar experimental design, were assumed to ovulate when approximately 26·5 days old. Groups of animals, treated as outlined in Table 1 were examined prior to ovulation, soon after ovulation, and early in the induced pseudopregnancy. As evident from the data in Table 1 the dosages of cortisol acetate used exerted an immediate inhibitory influence on further growth, with concomitant adrenal and thymic atrophy in all treated groups. The dwarfism in treated animals was not due to anorexia, as at the highest dosage used in these experiments food intake was unaffected (Bellamy, 1964). Adrenal steroids, however, induce protein wastage by virtue of a potent effect on gluconeogenesis in rats (Azuma & Eisenstein, 1964); they antagonise the action of growth hormone in rats, and depress serum growth hormone levels in the human (Hartog, Gaafar & Frazer, 1964).

TABLE 1.—*Changes in immature gonadotrophin stimulated rats (means ± SE)*

Group		Treatment	No. Rats	Body weight (gm)	Ovaries (mg)	Uterus (mg)	Adrenals (mg)	Thymus (mg)
Pre-ovulation....	1W	Saline 24–26 days..	6	38·0 ± 2·6	52·0 ± 2·4	69·5 ± 4·6	11·4 ± 0·8	87·1 ± 9·3
	2W	0·5 mg 24–26 days	6	37·0 ± 2·0	64·0 ± 3·5*	74·8 ± 6·1	8·5 ± 0·5*	24·0 ± 2·0†
	3W	1·0 mg 24–26 days	6	34·3 ± 1·3	58·7 ± 2·7	66·6 ± 3·1	8·9 ± 0·4*	19·8 ± 2·8†
Post-ovulation.	4L	Saline 22–26 days..	10	65·4 ± 1·3	79·7 ± 5·1	114 ± 5·3	17·0 ± 0·9	189 ± 17·4
	5L	1·0 mg 26th day...	7	59·0 ± 0·7†	85·2 ± 4·1	84 ± 5·1†	12·9 ± 1·0†	90 ± 13·1†
	6L	1·0 mg 24–26 days	8	54·1 ± 2·0†	88·7 ± 8·4	80 ± 4·3†	11·0 ± 0·6†	44 ± 5·1†
	7L	1·0 mg 22–26 days	8	45·8 ± 1·4†	77·4 ± 5·5	84 ± 4·7†	8·2 ± 0·7†	22·0 ± 3·2†
Pseudopregnant	8L	Saline 25–28 days..	8	77·6 ± 1·8	123·6 ± 8·2	113 ± 6·3	17·1 ± 0·9	206 ± 10·9
	9L	1·0 mg 25–28 days	8	54·4 ± 1·7†	143·5 ± 14·7	113 ± 7·4	9·4 ± 0·5†	23 ± 3·9†

All rats received 0·6 i.u. PMS/gm at 9 a.m. when 24 days old

Groups 1W–3W autopsied 53 hours later

Groups 4L–7L autopsied on morning of 27th day (approx. 12 hr. after ovulation)

Groups 8L–9L autopsied on morning of 29th day (approx. 60 hr. after ovulation)

Treatment—mg cortisol acetate per 10 gm body weight daily

*$P < 0.05$; †$P < 0.01$

TABLE 2.—*Number and diameter of follicles and corpora lutea in immature gonadotrophin stimulated rats (means± SE)*

Groups		No. Rats	Secondary	Tertiary	Class midpoint (mm)				Total	
			<0·15 mm	<0·15 mm	0·225	0·375	0·525	0·675	>0·75 mm	>0·15 mm
Follicles										
Post-ovulation.	4L	8	53·8± 9·1	54·8±13·2	27·9± 6·6	45·6± 6·8	38·9± 5·3	14·0± 2·3	1·6± 0·6	128±14·5
	6L	8	54·4± 5·7	77·9± 7·9	31·0± 4·8	45·8± 6·0	33·1± 4·2	16·1± 3·1	7·9± 2·1‡	139±13·8
Pseudopregnant	8L	6	53·8±10·6	46·8±10·2	59·8± 7·2	29·8± 6·2	17·8± 5·8	5·0± 1·8	1·3± 1·1	114±17·1
	9L	6	48·2± 9·6	44·8±12·8	48·3± 5·1	31·0± 3·3	28·2± 3·8	17·7± 3·5†	18·3± 6·3‡	144± 9·4
Corpora lutea										
Post-ovulation.	4L	8				12·4± 4·2	14·1± 4·3	5·4± 1·8	0·4± 0·2	32± 9·6
	6L	8				12·9± 2·4	19·3± 2·7	13·5± 2·3*	5·6± 0·8†	52± 7·3

Treatment same as in Table 1

No differences in corpora lutea of groups 8L and 9L

* $P < 0·05$ † $P < 0·01$

Even with these high levels of cortisol acetate, there was no suggestion of inhibition of absolute ovarian weight; in five of the six treated groups mean ovarian weight was appreciably increased but significantly so only in one group examined prior to ovulation. The number of ova found in the fallopian tubes of the treated post-ovulation group did not differ significantly from the controls, indicating that exogenously induced follicular growth and ovulation are unaffected in rats suffering severely from excessive cortisol acetate administration.

Uterus weight was unaffected in the rats examined prior to ovulation; however, soon after ovulation even a single injection of cortisol acetate induced marked inhibition of uterine growth. Treatment did not alter the weight of pseudopregnant rat uteri, suggesting that the uterine weight increase in response to endogenous oestrogen is readily inhibited by cortisol, but that the progestational endometrium is not affected.

The ovaries of treated and control groups sacrificed post ovulation and during pseudopregnancy were serially sectioned for histological examination of the number and size of follicles and corpora lutea (Table 2). No difference in the total number of secondary and small tertiary follicles less than 0·15 mm in diameter was found. However, the ratio of these small follicles exhibiting antral formation was increased by treatment in the post-ovulation group ($P < 0·01$). There were also no significant differences in the total number of follicles larger than 0·15 mm, but examination of the data in Table 2 reveals a divergent pattern of follicle size distribution. Treated groups tended to have fewer intermediate sized follicles and significantly greater numbers of large follicles, some of which may be classed as cystic. Furthermore 26·0 per cent of treated follicles exhibited signs of atresia (changes in thecal layers resembling luteinization with karyorrhexis and disappearance of granulosa layers), whereas atresia was only noted in 4·7 per cent of control follicles ($P < 0·01$). The larger follicles have been subdivided into further classes and the results suggest that the extent of follicle size potentiation is dependent upon the duration of treatment (Fig. 1).

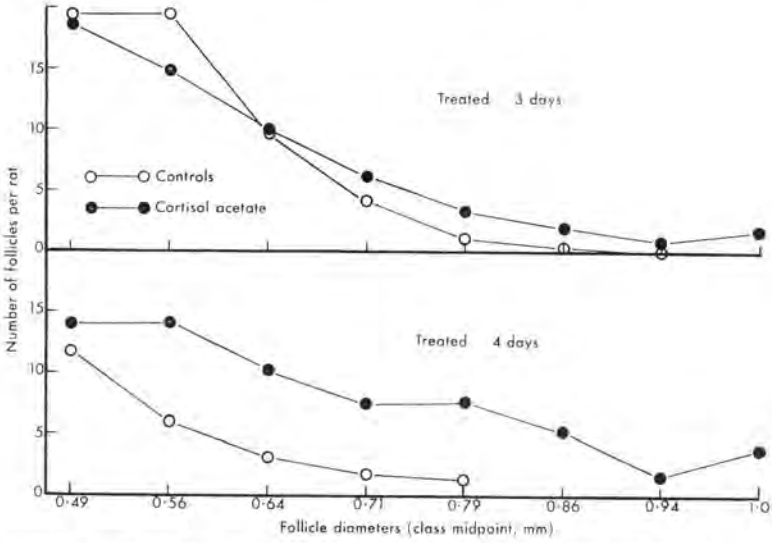

FIG. 1.—Increased numbers of large follicles in immature gonadotrophin treated rats

The total number of corpora lutea seen microscopically tended to be higher in the two treated groups, but not significantly so. Soon after ovulation, however, there were many more corpora lutea larger than 0·6 mm in the treated group ($P < 0·001$, Table 2). Furthermore, within this range there were 2·6 corpora lutea per rat with large central cavities in the controls and 8·9 per rat in the treated group ($P < 0·02$). There were no differences in the total number or size distribution of corpora lutea in Groups 8 and 9 which were examined 48 hours later, but 8·3 corpora lutea per rat were cystic in the controls and 32·1 per rat in the treated group ($P < 0·01$). Thus cortisol acetate appears to induce more rapid luteinization, but many of the corpora lutea exhibit cystic degeneration.

Ovarian weight response appeared to be potentiated in most groups treated with cortisol, though the difference was significant in only one group which received 0·5 mg cortisol and was autopsied 53 hours after the PMS injection, prior to ovulation. PMS has a predominantly FSH-like activity, and it seemed of interest to examine the influence of the same dosage of cortisol on immature rats treated with other gonadotrophins. HCG and NIH-FSH-S2 were used as outlined in Table 3; the results demonstrate a complete lack of any inhibitory or stimulatory action of cortisol under circumstances thought to constitute relatively low FSH stimulation alone, or in combination with low or maximal LH-like stimulation

TABLE 3.—*Influence of cortisol on the ovarian and uterine response to various gonadotrophins in immature rats*

Treatment		Ovarian weight (mg)	Uterine weight (mg)
100 μg NIH-FSH-S2	Saline	23·4 ± 1·0	30·0 ± 1·7
	Cortisol	23·1 ± 0·3	33·7 ± 2·2
25 i.u. HCG	Saline	38·3 ± 1·9	85·6 ± 1·2
	Cortisol	40·9 ± 1·5	85·2 ± 2·4
90 μg NIH-FSH-S2 + 50 i.u. HCG	Saline	57·1 ± 3·8	—
	Cortisol	53·5 ± 3·2	—
100 μg NIH-FSH-S2 + 5 i.u. HCG	Saline	45·0 ± 1·9	90·7 ± 3·5
	Cortisol	39·4 ± 2·1	97·7 ± 4·5

Gonadotrophin treatment is total dose administered in 6 injections over 3 days, animals autopsied on morning of 4th day

Cortisol acetate (0·5 mg/10 gm rat) injected daily during gonadotrophin treatment
Six rats per group

In most of these groups in this experiment the ovarian weight increase did not approach that following the PMS treatment, and in PMS-treated rats it was demonstrated that the total number of follicles was unchanged by cortisol acetate treatment but that the size of the larger follicles was increased. It is possible that the gonadotrophins used here did not induce the growth of sufficiently large follicles to be responsive to cortisol; in any event the results militate against the possibility that cortisol may selectively potentiate or inhibit the action of either gonadotrophin, or cause significant alterations in endogenous gonadotrophin secretion.

Influence of cortisol acetate on the reproductive cycle of mature rats

In the first experiment the pattern of cycles was established in a group of eight Long-Evans female rats. Commencing on the day of the fourth observed oestrus and continuing until a further six cycles had been completed, four rats were injected daily with cortisol acetate at the rate of 1 mg per 100 gm rat daily. The random controls were treated similarly with saline, and both groups included four and five-day cycling rats. Mean duration of the first two treated cycles remained unchanged; however, the following four cycles were prolonged by one to two days in every cortisol treated rat and the mean cycle durations were all significantly different from the controls.

This phenomenon was further investigated in Wistar rats, using four groups of six each and only four-day cycling rats. Prolongation of the interoestrous interval was confirmed; furthermore, a dose-response relationship, both in the duration of the cycle and the time elapsing before an effect is observed, is clear from the data presented in Fig. 2. An additional group not represented in Fig. 2 was observed for three cycles, and injected daily with saline for an additional six cycles; in not a single instance did the duration of the cycle deviate from four days. The levels of cortisol acetate used readily prolonged the cycle to five days, whereas six days was not uncommon but seven days only encountered in animals suffering from grossly excessive dosage. The number of days per cycle that typical proestrus, oestrus, and metoestrus vaginal smears were encountered did not differ from the controls, hence the prolonged cycles were exclusively due to an increased dioestrus or luteal phase period.

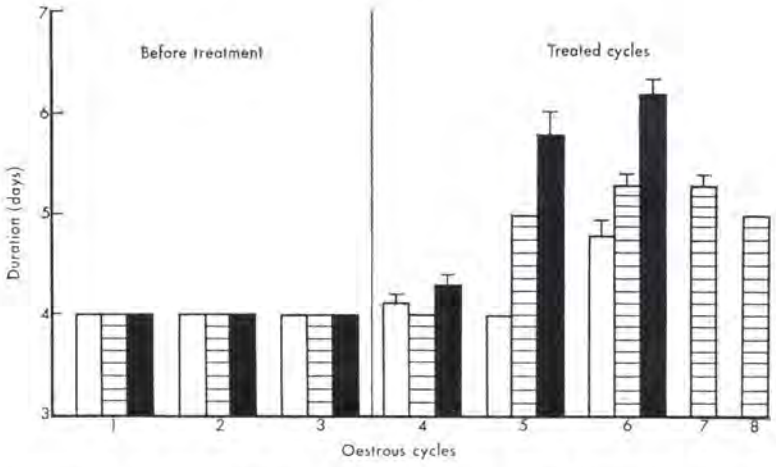

FIG. 2.—Prolongation of sexual cycles by cortisol acetate. Note dose-response relationship in cycles 5 and 6. Clear bars, 0·5 mg; horizontally scored bars, 1·0 mg; black bars 2·0 mg. An additional group not represented exhibited no prolongation during six cycles of saline vehicle treatment

Though hysterectomized rats generally cycle normally, this operation does prolong the duration of pseudopregnancy (Waynforth, 1965). Since cortisol acetate induced the disappearance of the usual heavy endometrial infiltration of eosinophiles,

and modified the weight of the uterus in late dioestrus animals, it was thought of interest to examine the response of hysterectomized rats to cortisol acetate. A group of four controls continued to exhibit four-day cycles when treated for 20 days with saline, but all hormone treated rats (1 mg per 100 gm) exhibited prolonged cycles similar to the same dose-level group of intact rats in Fig. 2.

The question arises as to whether the prolonged cycles are due to some luteotrophic influence of cortisol or retarded follicular growth and ovarian oestrogen synthesis in some way. Even though the vagina clearly remains under cyclical oestrogenic and progestin influence as evident from the smears, the possibility of suppressed follicular growth and perhaps interference with ovulation remains. One group treated for six successive cycles with 1 mg per 100 gm body weight (Fig. 2) was autopsied 75 hours after the seventh cycle had commenced, along with a saline control group. The ovarian weight of these treated cycling animals did not differ from normal (Table 4) but there was marked inhibition of uterine weight in conjunction with expected adrenal and splenic atrophy. Assay of the ovarian progesterone content (Table 6) revealed a slightly higher content in the treated group, which at this stage could be due to incipient regression of corpora lutea in the controls, which were exhibiting shorter cycles. The very high uterine weight of this control group would suggest some active proestrual oestrogenic influence.

Hysterectomized rats appear to have a very high ovarian progesterone content. Again there was no significant difference between treated and control rats. These limited progesterone values nevertheless do indicate active luteal function during the dioestrous period in rats with prolonged cycles.

Gestation in rats chronically treated with cortisol acetate

The groups of six rats treated with three levels of cortisol acetate as in Fig. 2 were placed individually with recently proved fertile males on the third day of the third treated cycle. Spermatozoa were found in the vaginal smears in every instance at the following oestrus and conception followed in all females.

Chronic administration of cortisol appeared to have little effect on the activity and disposition of treated individuals, though a staring coat and considerable weight loss was evident. Unbred females continued to exhibit a near-linear weight loss for a period in excess of 30 days. The weight increase due to gestation was not appreciably reduced (Fig. 3). In the group treated with 2 mg per 100 gm daily, two of the six animals developed severe haemorrhagic vaginal discharges accompanied by a sudden loss of weight on the 13th day of gestation. One died the following day and the other was autopsied *in extremis* on the 17th day. The presence of large implantation sites without any signs of the foetus confirmed the supposition that these animals had aborted.

TABLE 4.—*Autopsy results of mature female rats (means ± SE)*

Group	Treatment	No. Rats	Body weight (gm)	Ovaries (mg)	Uterus (mg)	Adrenals (mg)	Spleen (mg)
Cycling	Saline	6	200 ± 3·7	54 ± 3·6	443 ± 18	45 ± 2·2	481 ± 34
	1·0 mg/100 gm	6	149 ± 5·8†	55 ± 4·4	268 ± 12†	15 ± 0·8†	308 ± 18†
Hysterectomized	Saline	4	201 ± 3·8	60 ± 4·5	—	47 ± 3·0	493 ± 36
	1·0 mg/100 gm	4	158 ± 4·4†	49 ± 5·1	—	17 ± 0·4†	323 ± 8†
17th day of Gestation	Saline	6	256 ± 3·0	77 ± 3·0	2500 ± 66	47 ± 1·9	600 ± 27
	0·5 mg/100 gm	6	199 ± 5·4†	77 ± 4·9	1940 ± 251	19 ± 1·7†	432 ± 8†
	1·0 mg/100 gm	6	192 ± 4·2†	77 ± 3·6	1590 ± 828	16 ± 0·7†	425 ± 17†
	2·0 mg/100 gm	5	168 ± 8·2†	77 ± 4·9	1210 ± 720†	16 ± 1·7†	327 ± 42†
Pituitary transplant-pseudo-pregnant	Saline	5	200 ± 8·2	55 ± 5·2	325 ± 13	44 ± 1·5	451 ± 25
	1·0 mg/100 gm	5	173 ± 2·8†	52 ± 3·3	326 ± 12	27 ± 1·2†	327 ± 19†

† $P < 0{\cdot}01$

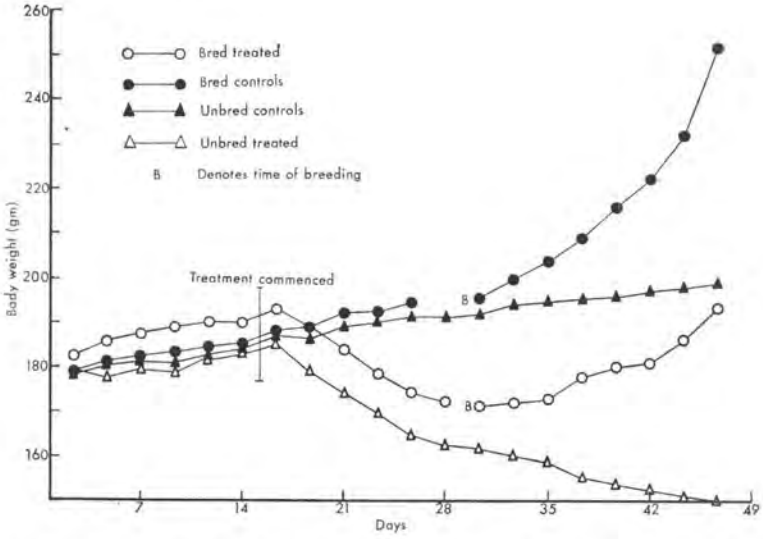

FIG. 3.—Body weight alterations during chronic treatment with cortisol acetate (1·0 mg/100 gm daily)

Autopsy of all remaining animals on the 17th day of gestation revealed a surprisingly consistent mean ovarian weight in all groups (Table 4). Though the ovarian weights of treated groups did not differ from the controls, the limited progesterone analyses possible with these ovaries clearly suggest a reduction of progesterone concentration as a consequence of cortisol acetate treatment in pregnant animals (Table 6). Pituitary FSH potency of the pregnant groups was too low to provide data of satisfactory precision.

In addition to atrophy of the adrenals and spleen, the weight of the liver was significantly reduced in all groups while the total renal weight remained unchanged. The percentage of body weight which the adrenals and spleen constitute was well below the controls but increased in the case of the liver and kidneys.

TABLE 5.—*Uterus and contents of bred rats on 17th day of gestation (means ± SE)*

Item	Saline	0·5 mg	1·0 mg	2·0 mg
No. rats	6	6	6	5
Body weight (gm)	256 ± 3·0	199 ± 5·4†	192 ± 4·2†	168 ± 8·2†
Live foetuses	10·7 ± 0·3	10·3 ± 1·0	7·0 ± 1·8	4·8 ± 1·2†
Resorbed foetuses	0·3 ± 0·3	0·8 ± 0·5	3·0 ± 1·0*	6·8 ± 1·4
Total implants	11 ± 0	11·2 ± 0·7	10·0 ± 1·9	11·6 ± 0·8
Foetal weight (mg)	702 ± 21	648 ± 24	687 ± 21	615 ± 19†
Uterus growth/foetus (mg)	194 ± 4·8	146 ± 5·2†	183 ± 22·4	184 ± 25·6
Mean placenta weight (mg)	290 ± 8·2	246 ± 15·6*	255 ± 19·0	222 ± 19·1†
Amniotic fluid/foetus (mg)	617 ± 15	554 ± 11·8*	576 ± 19	535 ± 19†

*P <0·05 †P <0·01

Data obtained from examination of the uteri and their contents are summarized in Table 5. The number of endometrial deciduoma representing implantation sites was consistent in all groups, demonstrating that follicular growth, ovulation and conception is unimpaired in rats chronically treated with excess cortisol acetate. However, the number of live foetuses decreased with increased dosage; necrotic debris representing " resorbing " foetuses which had died between an estimated gestational age of 5 to 15 days were found to account for nearly all implantation sites, except in the two animals which had aborted. The reduced number of viable foetuses in treated groups caused considerable variation in the " uterine weight increase per foetus ", placental, foetal and amniotic fluid weights, though all these parameters were significantly reduced in some groups. These reductions in weight were approximately in proportion to the loss of body weight exhibited by the treated rats, with exception of the foetus weight which appeared to have some preferential resistance to the depressant effects of cortisol acetate. No doubt this phenomenon was partly due to fewer foetuses in the treated groups, yet the fact remains that such foetuses are sustained by relatively smaller placentae which may contribute to the observed loss of embryos and foetuses.

TABLE 6.—*Mature rat ovarian progesterone assays*

Treatment	Reproductive status	No. of rats per assay	Progesterone μg/gm	Means progesterone
Saline	Cycling	3	5·5	5·2
		3	4·8	
1 mg	Cycling	3	5·7	6·3
		3	6·8	
Saline	Pseudopregnant	2	6·6	7·1
		3	7·5	
1 mg	Pseudopregnant	2	5·9	6·7
		3	7·4	
Saline	Hysterectomized	4	9·1	9·1
1 mg	Hysterectomized	4	8·1	8·1
Saline	Pregnant	3	8·7	9·1
		3	9·4	
0·5 mg	Pregnant	3	5·8	5·5
		3	5·1	
1·0 mg	Pregnant	3	6·0	5·5
		3	4·9	
2·0 mg	Pregnant	4	6·0	6·0

For details see text

Influence of cortisol acetate on pseudopregnant mature rats with pituitary implants

When pituitaries are grafted into intact cycling rats, the sequence of cycles is changed into a series of successive pseudopregnancies (Rothchild, 1960). Such subjects seem suitable to study the possible effects of cortisol on pituitary luteotrophic function and the response of the uterus under prolonged progesterone influence uncomplicated by gestation. In this experiment a pituitary was implanted under the renal capsule in 10 rats on the day when vaginal oestrus was observed and half were treated with 0·5 mg per 100 gm daily for 14 days, commencing on the day following surgery.

Both control and treated groups lapsed into pseudopregnancy and no oestrous smears were found up to the time of autopsy on the 14th day. Ovarian and uterine weights were quite unchanged by treatment (Table 4). These results concur with the findings in immature rats pseudopregnant following gonadotrophin treatment. Unlike pregnant rats treated similarly, there was no suggestion of reduced ovarian progesterone in these pseudopregnant animals (Table 6). These results support the evidence obtained in the earlier experiments that cortisol does not inhibit the endometrial gravimetric response to progestin influence or ovarian progesterone content in non-pregnant rats.

Administration of cortisol acetate to mature male rats

Twelve four-month-old Wistar males were randomized into two groups, one of which was treated with 1 mg per 100 gm for 16 days and the other with saline. Female rats would at this time be commencing their third prolonged cycle. In the males, this treatment induced an insignificant reduction in body weight from a mean of 303 gm to 260 gm. Ventral prostate weights were not reduced significantly, but testes, adrenal, and spleen weights were ($P < 0{\cdot}01$). Adrenal and spleen weights were again reduced proportionally more than body weight, but in the control group testes weight constituted 0·98 per cent of the body weight and in the treated animals 1·00 per cent.

FSH assay of the pooled anterior pituitary glands revealed a relative NIH-FSH-S1 potency of 163 μg per mg anterior pituitary powder for the controls and 159 μg for the treated group; both these values are essentially similar to those found by Parlow (1964) for normal male adult pituitaries. Histologically there did not appear to be any difference in qualitative or quantitative aspects of the testicular interstitial or seminiferous cellular elements; however, they appeared more dense in treated animals and the general impression was one of loss of intercellular fluid from the testes as a consequence of treatment.

It therefore appears that, while administration of cortisol acetate does not alter ovarian weight, testes weight is reduced in proportion to body weight without any obvious impairment of function, according to ventral prostate weight, FSH potency of the adenohypophysis, and histological appearance of the testes.

Discussion

In these experiments the " anti-anabolic " effects of cortisol acetate caused a profound inhibition of further growth in immature rats and loss of weight in mature rats. The usual cushingoid muscular atrophy in conjunction with abdominal fat deposition was obvious at autopsy, and spleen and thymic gland weights were reduced proportionally more than body weight, in accordance with the known selective inhibitory effects of corticosteroids on lymphoid tissue. Kidney weight was not affected and though liver weights were significantly reduced, the reduction was less in proportion to body weight.

Such animals, some of which were chronically treated for periods in excess of one month, consistently failed to show any signs of true inhibition of ovarian function, though certain alterations of function were apparent. The total number of follicles present was unchanged, but the size of the larger follicles was potentiated and many exhibited signs of atresia. A large proportion of the corpora lutea were " cystic ", and the serial histological studies strongly suggest that cystic corpora lutea develop primarily as a consequence of degenerative changes in the granulosa cells.

In man and some animals, much evidence exists that ACTH or corticosteroids increase pituitary gonadotrophic secretion and increase urinary oestrogen excretion (Sohval & Soffer, 1951; Brown *et al.*, 1963; Barlow, 1964; Van Rensburg, 1965), yet in these experiments on the rat the alterations found are not consistent with usual increased gonadotrophic influence. Neither the total number of growing follicles nor the number ovulating was changed in any experiment. Increased ovarian weight was not observed in immature rats treated with low levels of gonadotrophins of varying FSH: LH activities; cortisol appears to act primarily on medium to large follicles, which after treatment are inclined to exhibit cystic degeneration. Similar changes are observed following the administration of adrenal androgens (Roy, *et al.*, 1962). It is unlikely that androgenic metabolites of the administered steroid in these experiments were of much significance as these changes were observed particularly in immature gonadotrophin treated rats, and the ovaries of chronically treated mature rats did not exhibit any decline of weight or obvious macroscopic cystic changes. Furthermore the ventral prostate weight of mature rats was unaffected.

Very rapid luteinization and dose-related prolongation of the luteal phase in cycling rats suggest some luteotrophic action of cortisol. This activity is probably mediated via pituitary prolactin, since similar doses of cortisol increase the pituitary prolactin concentration in rats (Johnson & Meites, 1955) and *in vitro* directly stimulates the secretion of prolactin from pituitary explants (Ben-David, Dikstein & Sulman, 1964). These workers suggested that high doses of cortisol would, like oestradiol, inhibit pituitary prolactin secretion; no such evidence was obtained with these *in vivo* observations. An alternative cause of elevated prolactin secretion in treated females could be increased oestrogen secretion from enlarged ovarian follicles; experiments using the castrate or male would be of interest to clarify this point. Though a variety of stressors, including ACTH, causes acute discharge of pituitary prolactin (Grovenor, McCann & Nallar, 1965), it is unlikely that glucocorticosteroids are solely responsible, due to the exceptional rapidity of pituitary prolactin depletion following some forms of traumatic stress.

On the basis of the few progesterone assays possible, it appears that there is a factor in the non-gravid uterus which limits the ovarian progesterone concentration, since hysterectomy resulted in an increase of cyclical ovarian progesterone to values comparable to those in normal gestation. This inhibitory influence is removed by gestation, but certainly not fully by pseudopregnancy. Cortisol acetate treatment did not influence ovarian progesterone content in intact cycling or pseudopregnant mature rats; however, the elevated values found in normal gestation were consistently reduced but unaffected in hysterectomized individuals. These data suggest that the administered hormone or its metabolites do not act on the uterus directly, but can eliminate the inhibition on this postulated uterine factor usually exerted by the placenta and/or endometrial deciduoma. If such an inhibitory influence of the uterus on ovarian progesterone exists and it could be removed by the presence of decidual tissue, it could explain the mammotrophic influence of artificial deciduoma and the failure of various placental suspensions to alter the decline seen in mammary development of pseudopregnant rats (Wrenn, Bitman, DeLauder & Manch, 1966).

The potent inhibitory influence of glucocorticosteroids on experimentally induced decidual tissue formation is well known (Velardo, 1957; and others). There is, however, no information on the condition of this tissue following natural implantation. In this work we have demonstrated a selective inhibition of the usual gestational increase of combined uterine and decidual tissue weight per foetus, and though placental weight was also significantly reduced, this reduction was less in

proportion to body weight. Remarkably the conception and implantation rate is unaffected by high levels of treatment. The post-implantation embryonic losses appear from the above evidence to be largely due to defective maternal uterine/decidual tissue function, rather than inhibited placental function only. The uterus weight of chronically treated pseudopregnant mature and immature rats was unaffected, suggesting little interference with the progestational endometrium as such. However, when the uterus was under endogenous oestrogenic influence inhibition was found, in conformity with the results of Velardo & Sturgis (1956) who found inhibition of the uterine growth response to administered oestradiol.

Summary

The effects of cortisol acetate were studied on immature gonadotrophin treated rats, mature intact and hysterectomized cycling females, gestating and pseudopregnant subjects with pituitary implants, and mature males. Observations included growth, organ morphology, histological, follicular and corpora lutea counts and measurements, vaginal smears and ovarian progesterone assays.

The diameters of larger follicles were potentiated and the incidence of follicles exhibiting histological atresia increased. Luteinization occurred more rapidly but many corpora lutea were cystic in treated immature rats. The cycles of mature rats exhibited dose-related prolongation of the dioestrous period which was unaffected by hysterectomy. Ovulation, conception, and nidation were unchanged by chronic treatment, but embryonic mortality was increased. The combined uterine-decidual tissue weight was inhibited to a greater extent than the various conceptus elements. Uterine growth was unaffected in pseudopregnant immature and mature rats, but under conditions of oestrogen predomination inhibition was marked. Ovarian progesterone concentration was reduced by treatment only in pregnant rats, and the significance of this finding is discussed.

No evidence of alterations in the endogenous gonadotrophic secretions was found in any of these experiments. It is concluded that the administration of excess cortisol acetate exerted no true inhibitory influences on gonadal activity, unlike the potent inhibitory effects of adrenocorticotrophin.

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References

AZUMA, T. & EISENSTEIN, A. B., 1964. Effect of adrenal steroids on carbohydrate metabolism in various animal species. *Endocrinology*, 75, 521–526.

BARLOW, J. J., 1964. Adrenocortical influences on estrogen metabolism in normal females. *J. clin. Endocr. Metab.*, 24, 586–596.

BELLAMY, D., 1964. Effect of cortisol on growth and food intake in rats. *J. Endocr.*, 31, 83–84.

BEN-DAVID, M., DIKSTEIN, S. & SULMAN, F. G., 1964. Effect of different steroids on prolactin secretion in pituitary-hypothalamus organ co-culture. *Proc. Soc. exp. Biol. Med.*, 117, 511–513.

BROWN, P. S., THORBURN, A. R. & CROOKS, J., 1963. Urinary gonadotrophin excretion during treatment with corticotrophin. *J. Endocr.*, 28, 125–126.

CHRISTIAN, J. J., 1964a. Actions of ACTH in intact and corticoid-maintained adrenalectomized female mice with emphasis on the reproductive tract. *Endocrinology*, 75, 653–669.

CHRISTIAN, J. J., 1964b. Effect of chronic ACTH treatment on maturation of intact female mice. *Endocrinology*, 74, 669–679.

GROVENOR, C. E., McCANN, S. M. & NALLAR, R., 1965. Inhibition of nursing-induced and stress-induced fall in pituitary prolactin concentration in lactating rats by injection of acid extracts of bovine hypothalamus. *Endocrinology*, 76, 883–889.

HARTOG, M., GAAFAR, M. A. & FRAZER, R., 1964. Effect of corticosteroids on serum growth hormone. *Lancet*, 2, 376–378.

HEAP, R. B., 1964. A fluorescence assay of progesterone. *J. Endocr.*, 30, 293–305.

HISAW, F. L. & VELARDO, J. T., 1951. Inhibition of progesterone in decidual development by steroid compounds. *Endocrinology*, 49, 732–741.

JARETT, R. J., 1965. Effects and mode of action of adrenocorticotrophin on the reproductive tract of the female mouse. *Endocrinology*, 76, 434–440.

JOHNSON, R. M. & MEITES, J., 1955. Effects of cortisone, hydrocortisone and adrenocorticotrophin on mammary growth and pituitary prolactin content of rats. *Proc. Soc. exp. Biol. Med.*, 89, 455–458.

MOORE, C. R., 1953. Adrenal cortical secretion in relation to the reproductive system of rats. *J. clin. Endocr. Metab.*, 13, 330–368.

PARLOW, A. F., 1964. Importance of differential, quantitative bio-assays for pituitary gonadotrophins in the rat. *Endocrinology*, 74, 138–141.

QUINN, D. L. & ZARROW, M. X., 1964. Inhibition of pregnant mare's serum-induced ovulation in the immature rat. *Endocrinology*, 74, 309–313.

ROTHCHILD, I., 1960. The corpus luteum-pituitary relationship: the association between the cause of luteotrophic secretion and the cause of follicular quiescence during lactation; the basis for a tentative theory of the corpus luteum-pituitary relationship in the rat. *Endocrinology*, 67, 9–41.

ROWLANDS, I. W. & SHORT, R. V., 1959. Progesterone content of the guinea-pig corpus luteum during the reproductive cycle and after hysterectomy. *J. Endocr.*, 19, 81–86.

ROY, S., MAHESH, V. B. & GREENBLATT, R. B., 1962. Effect of dehydroepiandrosterone and $\triangle^4$-androstenedione on the reproductive organs of female rats: production of cystic changes in ovaries. *Nature (Lond.)*, 196, 42–43.

SOHVAL, A. R. & SOFFER, L., 1951. The influence of cortisone and adrenocorticotrophin on urinary gonadotrophin excretion. *J. clin. Endocr. Metab.*, 11, 677–687.

VAN RENSBURG, S. J., 1965. Adrenal function and fertility. *Jl S. Afr. vet. med. Ass.*, 36, 491–500.

VARON, H. H. & CHRISTIAN, J. J., 1963. Effects of adrenal androgens on immature female mice. *Endocrinology*, 72, 210–211.

VELARDO, J. T., 1957. Influence of purified adrenal hormones and adrenocorticotrophin on progesterone in decidual tissue formation. *Am. J. Physiol.*, 190, 408–412.

VELARDO, J. T. & STURGIS, S. H., 1956. Suppression of uterine growth by purified hydrocortisone acetate, 9-αflurohydrocortisone acetate and corticotrophin. *J. clin. Endocr. Metab.*, 16, 496.

WAYNFORTH, H. B., 1965. Uterine tissues in the regulation of corpus luteum life span in the rat. *J. Endocr.*, 33, xi–xii.

WRENN, T. R., BITMAN, J., DELAUDER, W. R. & MANCH, M. L., 1966. Influence of the placenta in mammary gland growth. *J. Dairy Sci.*, 49, 183–187.