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Sexual Development in Female Rats Treated with Cortisone.* (21200)

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An excess of endogenous adrenal oxysteroids as found in Cushing's Disease is associated with sexual dystrophy(1). Hench *et al.*(2) found that adolescents treated with cortisone suffer menstrual disturbances more frequently than adults. While cortisone treatment in women with regular menstrual cycles leads to irregularities, it may lead to establishment of menses in amenorrheic individuals(3). The observation of Sohval and Soffer(4) and of Maddock *et al.*(5) that patients on cortisone excrete excessive amounts of gonadotrophin suggests that these menstrual changes may be related in part to alterations in pituitary function and that cortisone may stimulate the production of gonadotrophin. Antopol(6) found that the ovary and accessory reproductive organs of immature mice treated with 1.25 mg of cortisone weighed less than normal. Byrnes and Shipley(7) found that Lipo-adrenal Cortex and Adrenal Cortex Extract inhibited gonadotrophin secretion in ovariectomized females in parabiosis with an immature female while daily doses of 0.5 or 2.0 mg of cortisone were ineffective. Fawcett and Deane(8) found no difference in water content or stroma development of uteri of ovariectomized rats treated with cortisone and/or estrogen. However, Szego(9) found a decreased hydration of uterus 4 hours after intravenous injection of estrogen to cortisone-treated rats, but no depression of wet weight. Talalay(10) reported that 2.5 mg of cortisone daily for 3 days partially prevented the increase in uterine weight produced by estrogen in ovariectomized rats. With administration of 0.5-5.0 mg of cortisone daily to young rats, Moore(11) found an increase in weight of the ovary accompanied by variable changes in uterine weight. It appears, therefore, that there is no agreement among investigators on the effects of cortisone on the female reproductive system and that only limited information is available on its effects on production and release of pituitary gonadotrophin.

The purpose of this investigation was to study the effects of a small dose of cortisone on the development of the reproductive system and on the gonadotrophic hormone content of the hypophysis of immature female rats.

Materials and methods. Four groups of 23-day-old female rats, 6 pairs to each group, were pair-fed with Rockland Milled Stock Diet. Another 4 groups of 23-day-old female rats, 8 pairs to each group, were fed *ad libitum*. One member of each pair was treated daily with 1 mg cortisone acetate† subcutaneously. At the end of 2 and 4 weeks of treatment as well as 3 weeks after cessation of treatment, 6 or 8 pairs of rats, respectively, were autopsied. Tissues were fixed in Bouin's solution or alcoholic-formalin and stained with hematoxylin and eosin or periodic acid-leucofuchsin, respectively. The pituitary of each animal was frozen individually for subsequent gonadotrophin assay. The uteri of 3 pairs of animals in each group were dried to constant weight at 110°C to determine their water content.

Results. Body weight gain was 22.8 to 48.0% less in the treated animals than in the controls. With cessation of treatment, the

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† Cortisone acetate was generously supplied by Doctor E. Alpert of Merck and Co.

TABLE I. Reproductive System in Pair-Fed Female Rats* Treated with Cortisone.

Treatment,† wk	Ovary mg/100 g body wt	Uterus drained	Days of vaginal opening 36	38	40	42	46
2	47.1‖	110.6	1				
C‡	22.2	71.5	3				
4	32.5‖	195.9‖	0	0		1	6
C	18.9	61.8	0	4		6	6
2 + 3§	38.6‖	209.8‖	0	3	6		
C	30.1	122.6	4	6	6		
4 + 3§	40.5‖	201.3‖	0	1	4		6
C	29.7	112.3	3	4	5		6

* 6 pairs in each experiment.
† 1 mg cortisone acetate daily starting at 23 days of age.
‡ C = Controls.
§ Autopsied 3 wk after cessation of treatment.
‖ P = <.001.

animals resumed an increased rate of growth, so that the final weight of the treated animals sacrificed 3 weeks after cessation of treatment was only 6.8 to 33.5% less than the weight of their controls.

Adrenal and thymus weights were 41.3 to 84.0% less in the treated rats. Three of the 4 groups of animals posted 3 weeks after cessation of treatment showed a 3.2 to 8.4% increase in adrenal weight over the controls instead of a decrease, indicating that during this period of recovery the adrenals had undergone marked growth. This is in agreement with the quick recovery from the inhibitory effect of cortisone on ACTH production and release and consequent adrenal growth noted by others(12). The thymus remained reduced in weight. The thyroid was 11.8 to 16.0% heavier in 3 of 4 groups in both the pair fed and *ad libitum* fed experiments. The effects of cortisone were, in general, more pronounced in the *ad libitum*-fed than in the pair-fed animals.

Reproductive tract. The ovaries of treated pair-fed animals were significantly (P = <0.001) heavier than those of the controls (Table I). The differences are greater in animals posted at the end of the treatment than in those sacrificed 3 weeks after treatment was discontinued. The uteri were heavier in treated than in non-treated animals, but only in the 3 older groups was the difference statistically significant (P = <0.001). In *ad libitum*-fed groups, ovaries were slightly heavier in the treated animals killed at the end of the treatment than in the controls (Table II), but the differences were less marked than in the corresponding pair-fed groups. In animals sacrificed 3 weeks after termination of treatment, ovaries were significantly (P = <0.001) lighter. This is contrary to the results obtained in the pair-fed animals. Compared to controls, uterine weights were slightly less in rats treated for 2 weeks, but heavier in those treated for 4 weeks. This difference in response of the uteri may be due to the longer period of treatment.

Opening of the vagina was delayed in all groups (Tables I and II). The delay was greater in those treated for 4 weeks. When vaginal smears were made of the last group during the last week of the experiment, normal cycles were found.

Histological examination of ovaries revealed a greater degree of follicular development in treated animals than in the controls, due especially to increased size and number of the follicular antra. Three of the pair-fed animals autopsied after 4 weeks of treatment had several corpora lutea, but these were present in only 1 of the pair-fed controls. All rats in the older groups killed at 5 or 7 weeks after start of the experiment, and 3 weeks after cessation of treatment, had corpora lutea indicating that ovulation had occurred. Examina-

TABLE II. Reproductive System in *Ad Libitum*-Fed Female Rats* Treated with Cortisone.

Treatment,† wk	Ovary mg/100 g body wt	Uterus drained	Days of vaginal opening 30	33	36	37	38	40	45-47
2	37.1	172.6	0		0		1		
C‡	33.4	176.3	2		8		8		
4	39.5§	201.4				0		1	8
C	36.4	186.9				2		8	8
2 + 3‖	38.3¶	174.6§		0			1		8
C	40.1	185.0		1			8		8
4 + 3‖	39.9¶	218.1		0			1		8
C	46.0	202.9		1			4		8

* 8 pairs in each experiment.
† 1 mg cortisone acetate daily.
‡ C = Controls.
§ P = <.05.
‖ Autopsied 3 wk after cessation of treatment.
¶ P = <.001.

TABLE III. Pituitary Gonadotrophin Assay of Pair-Fed Rats Treated with Cortisone.

Treatment,‡ wk	Donor* Pituitary	Recipient† Ovary	Recipient† Uterus
	mg/100 g body wt		
2	7.4§	30.4§	138.9
Controls	5.8	28.4	123.7
4	6.1§	28.5§	98.4§
Controls	4.9	26.2	127.5
2 + 3‖	5.3¶	30.5§	134.4
Controls	4.5	28.5	123.5
4 + 3‖	6.5§	27.1	64.3
Controls	4.9	27.0	77.3

* 6 pairs in each experiment.
† 23 days old at start of pituitary inj.
‡ 1 mg cortisone acetate daily starting at 23 days old.
§ P = <.001.
‖ Autopsied 3 wk after cessation of treatment.
¶ P = <.01.

tion of the uteri showed no marked differences in the development of endometrial stroma or glands. Differences in water content of uteri of treated and control animals were less than 4%. This is in agreement with the observation of Fawcett and Deane(8) that in ovariectomized rats, simultaneous treatment with estrogen and cortisone did not significantly modify the effect of estrogen on the water content of the uterus. No significant differences were seen in the distribution and concentration of glycogen in the uterine tissue of treated and control animals.

Gonadotrophin content of pituitary. In an attempt to obtain information on the gonadotrophic activity of the pituitary, each frozen gland was homogenized in 3 cc of saline solution (pH = 8). One-half cc portions of the suspension of each gland were injected 2 times per day for 3 days into a 23-day-old female rat which was autopsied on the fourth day. Pituitaries of treated animals were heavier than those from corresponding controls (Tables III and IV). In Table III it is seen that pituitaries from treated pair-fed animals of all except the oldest group caused a significantly (P = <0.001) greater development of the ovaries of the test rats than did the pituitaries of the controls. Ovaries of rats receiving pituitaries from treated animals showed more advanced follicular development as indicated by number and size of antra. Uterine weights were increased in animals receiving pituitaries from pair-fed animals treated for 2 weeks, but decreased when donors had been treated for 4 weeks. In recipients of pituitaries from treated animals fed *ad libitum,* ovaries were heavier in 3 of the 4 groups (Table IV). Uterine weights were increased in animals receiving pituitaries from *ad libitum*-fed donors autopsied at termination of treatment, but were decreased in those autopsied 3 weeks later. The gonadotrophic activity of the pituitary of treated rats was greater than that of the pituitary of pair-fed controls. This is indicated by 1) a generally heavier weight of the ovaries in the former, and 2) a greater development of the ovaries as indicated by the increased number and size of antra in animals receiving pituitaries from treated animals.

Discussion. A reduction in body, adrenal and thymus weights during treatment with cortisone has been previously reported(12,13). The marked growth of the adrenals after cessation of treatment confirms the findings of Patterson *et al.*(12) who found that the adrenals reattained normal histological appearance in 7-10 days after end of treatment.

With administration of 2-5 mg of cortisone daily to young rats, Moore(11) found an increase in the weight of the ovary and greater development of ovarian follicles similar to that observed in our experiments. This could be due to either an increased sensitivity of the

TABLE IV. Pituitary Gonadotrophin Assay of *Ad Libitum*-Fed Rats Treated with Cortisone.

Treatment,‡ wk	Donor* Pituitary	Recipient† Ovary	Recipient† Uterus
	mg/100 g body wt		
2	5.6§	31.9‖	181.2§
Controls	4.9	28.4	100.0
4	5.9§	31.3	81.7¶
Controls	5.2	30.8	70.7
2 + 3**	5.1	26.9¶	73.6§
Controls	5.1	30.8	80.5
4 + 3**	5.5§	28.3¶	103.7§
Controls	5.2	26.2	130.8

* 8 pairs of female rats in each category.
† 23 days old at start of pituitary inj.
‡ 1 mg cortisone acetate daily starting at 23 days of age.
§ P = <.001.
‖ P = <.05.
¶ P = <.01.
** Autopsied 3 wk after cessation of treatment.

ovary to stimulation by gonadotrophic hormones or greater production of gonadotrophins under the influence of cortisone acetate. The increased incidence of corpora lutea in animals autopsied after 4 weeks treatment compared to controls suggests an increased production and/or release of both follicle-stimulating and luteinizing hormones of the hypophysis.

While in all of our pair-fed and in some of our *ad libitum*-fed groups, there was a marked increase in weight of the uteri of treated animals compared to controls on a mg per 100 g body weight basis, no such differences were reported by Moore(11). This may be due either to the differences in dosage and/or periods of treatment. Similarly, the report by Talalay(10) of a depression by cortisone of estrogen stimulation of uterine growth probably represents an effect of differences in treatment regime or experimental design.

Data obtained in pair-feeding experiments probably are more accurate than those obtained in rats fed *ad libitum,* since we found that animals treated with 1.0 mg cortisone daily consumed 30-50% less food than controls. The better uterine growth in *ad libitum*-fed controls is therefore due, in part, to their greater food consumption.

The greater weight of the uteri of pair-fed treated animals probably represents a quantitative increase in growth of the uterus in response to increased production of ovarian hormones by the hypertrophied gland. This hypothesis is supported by the lack of qualitative histological differences in development of endometrial stroma or glands, in water content or glycogen distribution between treated and control animals. Similarly, Fawcett and Deane(8) reported no difference in development of stroma or water content in ovariectomized rats treated simultaneously with cortisone and estrogen as compared to estrogen alone.

Although ovaries of treated donors show a greater degree of development, the opening of the vagina, a secondary sexual character, is delayed. This may indicate a decrease in sensitivity of the vaginal plate tissue to estrogen or a decreased production of estrogen by the ovary under the influence of cortisone. Since both the ovary and the uterus, which is dependent on estrogen for growth, are heavier in the treated animals than in the controls, a decreased sensitivity of the vaginal plate to growth stimuli appears to be the more probable cause for the delay in its opening.

Gonadotrophin content of pituitaries, as determined by ovarian response, appears greater in rats treated for 2 or 4 weeks than in pair-fed controls. This difference declines as the animals grow older and is smaller in those autopsied 3 weeks after cessation of treatment. This decrease in the difference between controls and treated animals in the older groups may be due to a normal process of maturation of the animals resulting in an increased production of pituitary gonadotrophin accompanied by an increased ovarian hormone secretion. The data indicate that there is an increased production of gonadotrophin and not only an increased storage in cortisone-treated animals since such donor animals also show greater development of ovaries. The possibility that the altered development of the reproductive system after cortisone treatment may be the result of a modified metabolism does not appear likely in view of the fact that the glucose and non-protein nitrogen content in blood and urine samples taken at autopsy showed no significant differences from normal.

Our observations suggest that the increased weight and greater follicular development of ovaries in treated animals are due to the action of cortisone on the pituitary rather than on the gonads or reproductive tract. In nearly all cases, the pituitaries taken from animals with the heavier ovaries produced the greatest stimulation of ovaries in test animals. The lack of effect of cortisone on gonadotrophin secretion in parabionts reported by Byrnes and Shipley(7) may be due to the range of dosage employed.

Alterations in pituitary gonadotrophin may be due to the fact that cortisone, through its inhibition of ACTH, could reduce sex steroid production by the adrenal. The ability of exogenous cortisone to inhibit secretion of adrenal sex hormones has been shown by Wilkins *et al.*(14) and others. Decrease of adrenal sex steroid secretion may allow an increased production of pituitary gonadotrophin so as to stimulate gonadal growth and hormone secretion.

Summary and conclusions. 1. Immature female rats were treated with 1.0 mg of cortisone acetate daily for 2 or 4 weeks starting at 23 days of age and were maintained on pair or *ad libitum* feeding regimes. 2. Body weight, adrenal and thymus weights were reduced compared to control animals. These differences were less marked when animals were autopsied 3 weeks after cessation of treatment. 3. The weights of ovary and uterus were generally greater in the treated animals. The increased weight of the ovaries was due to a greater number of follicles and to an increased size of the antra. 4. Pituitary glands removed from animals treated with cortisone appeared to have a greater gonadotrophic potency than normal as indicated by the growth of the ovaries in immature rats injected with these glands. 5. Possible explanations for these changes are discussed.

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A Means of Increasing the Tolerated Dose of Isoniazid in Mice. (21201)

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(Introduced by B. J. Olson.)

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Isoniazid (INH) as a therapeutic agent in human tuberculosis is in widespread use; one limiting factor is its toxicity(1-5). Two well-known detoxifying agents, glycine and sodium glucuronate, administered simultaneously with the INH in mice, have shown a marked effect in increasing toleration to this therapeutic drug. Three types of experiments are reported here: 1) Survival of DBA mice (20-g weight) after single and repeated oral administrations of toxic amounts of INH with different quantities of the 2 chemicals; 2) blood plasma levels of INH after a single oral administration of the 3 chemicals; and 3) an *in vitro* test of the effect of the detoxifying chemicals on the bacteriostatic action of INH on tubercle bacilli.

Inasmuch as the toxicity data were available for white mice only, a preliminary determination of the toxic dose of INH in DBA mice was necessary. According to Grunberg and Schnitzer(6) the LD_{50} by gavage in white mice was found to be 203 mg/kg. In our laboratory this amount in white mice resulted in 57% survival, but in the DBA strain all the mice survived. However, an oral dose of 250 mg/kg (5 mg/20-g mouse) resulted in only 45% survival in a group of 49 DBA mice. In contrast, when 50 mg of sodium glucuron-

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