# THE EFFECTS OF ADRENAL STEROIDS ON CERTAIN VAGINAL RESPONSES TO ESTROGEN STIMULATION IN THE IMMATURE ADRENALECTOMIZED AND OVARIECTOMIZED RAT

by

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A THESIS

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# INTRODUCTION

In 1917, Stockard and Papanicolaou (28) described a periodic cornification of the vagina in the guinea pig and found that this cylic variation could easily be recognized and followed by microscopic examination. This report was followed by a paper by Long and Evans (21) which described a similar vaginal cycle in the rat. These observations provided the foundations for the vaginal smear technique which has become the routine tool for following sexual cycles in most laboratory rodents.

In 1923, Allen and Doisey (1) obtained a hormone from the follicular fluid of sow's ovaries which induced cornification of the vaginal epithelium in the mouse. This discovery, combined with the isolation of pure crystalline estrone in 1929, provided the foundation for much subsequent research into the actions and interactions of various hormones in the female genital tract.

In the earlier days of hormone research, it was considered by some that the cyclic rhythmicity of the vagina was regulated solely by the ovary (4). However, Del Castillo and Calatroni (7) demonstrated that in ovariectomized female rats cycling of the vagina could be re-induced with daily injections of estrogens. The cycling consisted of an estrus period separated by a diestrus period of several days length, and in general, the length of the diestrus period was determined by the dose of estrogen employed. The conclusion drawn from this work was that some extra-ovarian factor was regulating the periodicity of vaginal estrus by decreasing the vaginal sensitivity to estrogen stimulation during the refractory periods. Zuckerman (37), repeated the above work with rats and agreed with their conclusion, as did Emmen (11) with mice.

Observations of changes in water metabolism resulting from estrogenic

stimulation led Zuckerman (38) to suggest that the adrenal gland may be the extra-ovarian factor which cyclically alters the estrogen threshold of the female reproductive organs in the rat. This hypothesis was investigated further by Zuckerman (39), and it was demonstrated that ovariectomized female rats who were cycling normally with the aid of exogenous estrogens exhibited abnormal vaginal cycles when bilaterally adrenalectomized. The conclusion was drawn that if an endocrine mechanism was involved in the cyclical changes of sensitivity of the rat vagina to estrogenic stimulation, that the adrenal cortex would be the gland most likely to be involved. The reports of Del Castillo and Di Paola ( 8) provided further support for Zuckerman's conclusion. These investigators observed that ovariectomized female rats, maintained on a level of exogenous estrogen which was just below that required to produce vaginal estrus (0.5 International Benzoic unit), went into a typical estrus period following bilateral adrenalectomy. After the above experiment, these investigators increased the dose of estrogen to one international benzoic unit. Prior to adrenalectomy, the animals treated in this fashion had two periods of vaginal estrus separated by a single diestrus period, but after adrenalectomy all of the animals went into a prolonged estrus period, demonstrating the absence of a cyclic reduction in vaginal sensitivity to estrogen.

The pituitary was also studied to find what role, if any, it played in the maintainence of the cyclical changes in the vagina epithelium of the rat. Zuckerman (39), investigating this facet of the problem in hypophysectomized, ovariectomized rats, demonstrated that the cyclic response continued despite the absence of the hypophysis. Zuckerman concluded that the pituitary was not necessary for the main-

tainence of the cyclic alterations of the vaginal epithelium. This work was confirmed by Del Castillo and Di Paola ( 8), and Courrier ( 5).

Shortly after the suggestion by Zuckerman that the adrenal gland was involved in maintaining the periodic variations in vaginal sensitivity to estrogens in the rat, investigators began to test the effects of desoxycorticosterone acetate on the stimulatory action of estrogen in the vagina. Robson (24) reported that daily doses of 0.1 or 0.2 mg. of desoxycorticosterone inhibited estrus in the normal mouse. Del Castillo and Di Paolo ( 8) confirmed this work using rats. In addition, these investigators demonstrated that by varying the dosage of this adrenal cortical steroid, they could alter the vaginal cycle at will, from complete blockage of estrogen stimulation to a partial antagonism of the estrogen effect. This work suggested that a relation exists between the dosage of adrenal steroid employed and the degree of estrogen antagonism obtained. However, during this same period Salmon ( 26) reported that 5 to 10 mg. of desoxycorticosterone given three times in one week to ten post-menstrual women whose vaginae exhibited typical estrogen deficiency smears, induced full cornification in all the women at the end of 8 days.

The above seemingly discrepant results were reconciled by Courrier ( 5), who concluded from investigations conducted with rats that cornification of the vagina caused by desoxycorticosterone is an early transitory effect which is followed by mucification. In ovariectomized rats, 3 mg. of desoxycorticosterone acetate caused vaginal cornification on the third day, but with continuation of the treatment leucocytes appeared in the smears and mucification became established. Cornification failed to appear when the animals were given 2.5 gamma estrogen

daily as long as the desoxycorticosterone was continued. With cessation of the administration of the adrenal steriod, cornification immediately appeared in response to the estrogen.

Although many authors are convinced that adrenal gluco-corticoids also antagonize the stimulatory effects of estrogen on the vagina, the results published in recent years have not always supported this concept. Koller (19) using normal female rats demonstrated that 5 mg. of ACTH administered at 28-day intervals would produce suppression of the estrus cycle, indicating a decreased estrogen effect. Alloiteau and Courvoisier (2 ) reported that constant estrus induced by hypothalamic lesions was suppressed by cortisone. Then Courrier ( 6) demonstrated that estrogen-induced vaginal changes in monkeys were antagonized by desoxycorti-costerone acetate but not by cortisone, while on the other hand, Takewaki (32) employing spayed rats, noted that both DCA and cortisone antag-onized estrone-induced vaginal estrus. Edgren ( 9), however, has re-ported that DCA, in doses ranging from 100 gamma to 1000 gamma, and cortisone acetate in similar dose levels had no marked effect as anta-gonists of vaginal changes produced by 5 gamma of estrone. Pliske (23) reported similar results from experiments using cortisone locally applied to the rat vagina. Also Martin and Claringbold ( 22) have observed that cortisol, in doses ranging from 5 gamma X $10^{-4}$ to 500 gamma X $10^{-4}$, failed to antagonize the estrogen induced increase in mitotic activity of the spayed mouse vagina.

As can be seen from the above review, very little conclusive work has been done concerning the interaction of adrenal gluco-corticoids with estrogen in the vagina. The small amount of work which has been done, has exclusively delt with the morphological changes occurring in

the vaginal epithelium. This particular hormonal interaction, however, has been extensively studied in the uterus using other parameters ( 10,29,30,34,35,36 ). It is the purpose of this investigation to examine the effects of hormonal interaction of some adrenal glucocorticoids and estrogens on rat vagina by gravimetric, biochemical, and histological techniques.

## METHODS AND MATERIALS

Described in the following section are two sets of experiments. The first, a pilot study, was performed to grossly explore the effects of the interaction of estrogen and adrenal glucocorticoids on the genital tract of intact adult female rats. Because the results of this experiment demonstrated that adrenal glucocorticoids altered the normal vaginal estrus cycle and since this indicated the possibility of a glucocorticoid-estrogen antagonism, a more definitive second study, as outlined below, was undertaken. In order to facilitate presentation of these experiments, the methods and materials employed in each will be presented separately.

A. Pilot Study.

1. Animals

The animals used in this study were intact adult female rats of the Long-Evans strain weighing between 150 to 220 gms. Vaginal smears were taken each day for 30 days to determine the regularity of the estrus cycle. Thirty animals exhibiting regular vaginal cycles occuring at predictable intervals, were selected to be used in this experiment. All animals were maintained before and during the experimental period on standard lab chow and tap water.

2. Experimental Treatment

The animals were divided into three groups consisting of 10 animals each; a control group and 2 experimental groups. The control group received one subcutaneous injection of 0.2 ml. 0.9% sterile saline solution in the flank region each day for 12 days. One experimental group received 1 mg. and the other 5 mg. of a

micro-crystalline suspension of hydrocortisone acetate in normal saline by subcutaneous injection in flank area. Vaginal smears from these animals were obtained each day following the injection, and the result of the smear recorded. Also, a slide of each day's smear for all animals was prepared and stained for a permanent record.

3. Preparation of Hydrocortisone Suspension

The hydrocortisone acetate[1] employed in this experiment was in a micro-crystalline form. The hormone was weighed on a Christian-Becker beam balance and a suspension prepared using 0.9% sterile saline as the vehicle and Tween 80 as the wetting agent to keep the micro-crystals in suspension. Two concentrations were prepared: one containing 1 mg. of hormone per 0.2 ml. of solution and the other 5 mg. of hormone per 0.2 ml. of solution.

4. Autopsy Procedure

Immediately after taking the final vaginal smear, the animals were sacrificed by an overdose of ether. The uteri were removed through a ventral incision and then weighed in toto on a torsion balance. Both tissues were preserved for future histologic examination in Carnoy's fixative. (See Appendix.)

B. Adrenal glucocorticoid - Estrogen study

1. Animals

Immature female rats of the Sprague-Dawley strain weighing between 40 and 50 Gms. were used in this experiment and were obtained from a commercial supplier.[2] The decision to use immature animals

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1. The Nutritional Biochemical Supply Co.
2. Northwest Rodent Company, Pullman, Washington

in this investigation was based on the observation of Rubin et. al. (25) who reported that the vagina of immature rats are more sensitive to estrogen stimulation. In view of the high dosages of adrenal glucocorticoids, reported by other investigators, necessary to alter the stimulatory effects of estrogens on the rat uterus (33), it seemed essential that in the following investigation the amount of estrogen employed should be kept as low as possible. It was concluded, therefore, that the immature rat with its vaginal hypersensitivity to estrogen stimulation was the obvious choice as an experimental animal.

In order to eliminate the production of natural steroids in the test animal, the adrenals and ovaries were removed prior to treatment with exogenous steroids.

One to two days following the animals arrival at this laboratory a bilateral ovariectomy and adrenalectomy was performed employing intraperitoneal nembutal as the anesthesia (4 mg. per 100 Gms. body weight). The adrenals and ovaries were removed through a stab incision of the flank muscles after these muscles were exposed through a mid-line lumbar skin incision. Following the conclusion of surgery, the skin was closed using metal skin clips. The animals were allowed a recovery period of seven days before the experimental treatment started.

Prior to operation, the animals were maintained on standard lab chow and tap water. Following the bilateral ovariectomy and adrenalectomy, the tap water was replaced with 0.9% saline solution.

2. Experimental treatment

Seven days following their operation, the animals were injected

subcutaneously in opposite flanks with: 1. a placebo, 2. an estrogen, or 3. estradiol and one of the adrenal glucocorticoids. These injections were administered each day for three days at twenty-four hour intervals. Seventy-two hours following the initial injection, the animals were autopsied. The animal group designations, the number of animals in each group, and the treatment they received is compiled in the table below.

| Animal Group | Number of Animals | 3 Subcutaneous Injections in Each Flank at 24 hour Intervals |
|---|---|---|
| A | 19 | 0.2 ml. of physiologic saline in each flank |
| B.05 | 11 | 0.05 gamma estradiol in saline |
| B.1 | 22 | 0.1 gamma estradiol in saline |
| B.2 | 7 | 0.2 gamma estradiol in saline |
| B.5 | 7 | 0.5 gamma estradiol in saline |
| BO.05 | 10 | 0.05 gamma estradiol valerate in oil |
| BO.1 | 19 | 0.1 gamma estradiol valerate in oil |
| BO.2 | 14 | 0.2 gamma estradiol valerate in oil |
| BO.5 | 6 | 0.5 gamma estradiol valerate in oil |
| C | 14 | 10 mgm. hydrocortisone and 0.1 gamma estradiol |
| D | 9 | 10 mgm cortisone and 0.1 gamma estradiol |
| E | 22 | 10 mgm prednisolone and 0.1 gamma estradiol |

3. Preparation of steroid suspension and solutions

A commercial micro-crystalline suspension of 17-beta-estradiol and a commercial sesame oil solution of estradiol valerate were diluted with 0.9% sterile saline solution and peanut oil respectively to obtain suspensions and/or solutions containing 0.05 gamma, 0.1 gamma, 0.2 gamma, and 0.5 gamma estrogen per 0.2 ml. of

volume.[1]

Cortisone, hydrocortisone, and prednisolone were the adrenal corticoids employed in this experiment.[2] Cortisone was administered by subcutaneous injection in a 10 mg. dose from an undiluted commercial suspension, which contained 25 mg. of hormone per ml. The hydrocortisone and prednisolone were obtained in dry microcrystalline form and the injection material was prepared with 0.9% sterile saline solution, so that 0.2 ml. contained 10 mg. of steroid. Subcutaneous 10 mg. dosages of the adrenal glucocorticoids were employed because it has been reported elsewhere (16) that this amount of steroid and site of injection insured maximal effect as measured by body weight loss and provided a residual depot of the steroid at the site of injection. Such a depot was deemed necessary to insure maintenance of a high blood level over the entire experimental period. The importance of this aspect of experimental treatment will be noted in the results of the hydrocortisone treated animals, presented later, this steroid being used in a form that was rapidly absorbed and produced no residual depot.

4. Autopsy Method

Seventy-two hours following the initial injection the animals were sacrificed by decapitation. The vaginas were then removed through a midline ventral incision, cleaned of extraneous connective tissue and weighed on a torsion balance. The organs

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1. The estradiol valerate was obtained from E. R. Squibb and Sons, and the 17-beta-estradiol was obtained from Schering Corp.

2. The water soluble hydrocortisone was obtained from the Upjohn Co., and the prednisolone and cortisone was obtained from Schering Corp.

were then split longitudinally, the lumens mopped dry by filter paper and weighed again. Finally, a small portion of the vagina was removed for histological study, the remainder of the vagina again weighed, and immediately frozen for later beta-glucoronidase determinations.

5. Beta-Glucoronidase Determination.

Within twenty-four hours of autopsy, the beta-glucuronidase activity levels of the frozen vagina's was determined in the following manner. The vaginal tissue was placed in an ice-chilled glass homogenizer and 5 ml. of cold distilled water was added. The tissue was homogenized for 1-2 minutes at a rapid speed. The homogenate was then centrifuged and the supernatant decanted off. One ml. portions of the supernatant was assayed in duplicate.

Into each of the test tubes 0.5 ml. of 0.1 M acetate buffer (pH 4.5) was pipetted. Following this, 0.5 ml. of 0.0015 M phenolphthalein glucuronidate was added to the two experimental tubes, but not to the control tube. All of the test tubes were then stoppered and placed in a water bath at 38 degrees centigrade till they came to temperature. Then the tubes were removed from the water bath and 1.0 ml. of the vaginal tissue extract was added to each tube at timed intervals. The contents of the tubes were mixed by whirling, stoppered, and allowed to incubate at 38 degrees centigrade for one hour in a water bath. Following incubation the tubes were opened and 5 ml. of 0.4 M glycine buffer (pH 10.45) was added to each tube including the control. This material arrests the reaction and developes the color. Also, at this time 0.5 ml. of 0.0015 M phenolphthalein glucuronidate solution

was added to the control tube. If any precipitation of tissue proteins occurred, the tubes were centrifuged for 15 minutes at a rapid rate. The contents of the test tubes were then decanted into colorimeter tubes and the optical density read in a Bausch and Lomb colorimeter at 540 mu using the control tube as reference. (For composition of solution See Appendix.)

To prepare the phenophthalein calibration curve, 0.5 ml. of phenolphthalein alcohol standard (1mg. phenolphthalein per ml.) of varying dilutions was added to colorimeter tubes already containing 1.5 ml. of 0.1 M acetate buffer (pH 4.5) and 5.0 ml. of 0.4 M glycine buffer. Then the optical density of the solutions were read on a Bausch and Lomb colorimeter at 540 mu using a water blank as the reference. The optical densities were then plotted against the amount of phenolphthalein contained in each tube.

6. Histological Technique

Following the removal of a central portion of the vagina, it was fixed in Carnoy's fixative. After adequate fixation, the tissues were embedded in paraffin and the resulting blocks were cut at 8-10 microns in thickness and sections mounted. The slides were stained with the standard hematoxylin and eosin stain, McManus's alcein-blue periodic-acid-Schiff reaction and the alcein-blue-nuclear fast red stain, and examined for histologic alterations.

## RESULTS

To ascertain the relative significance of the observed organ, body weight changes, and other quantitative measures the mean value differences were tested for significance using the "t" value distribution method. Values of P (0.001 were considered highly significant, P(0.01 significant, P(0.02 probably significant, and P) 0.02 not significant. The values in the following presentation are all significant at levels of P(0.02 unless stated to the contrary.

The experimental animals of the estrogen-adrenal steroid study have been classified into twelve groups, as described in the methods and materials section, to facilitate the description of the observed results. As a brief review, animals injected with estradiol are designated by the capital letter B. The number which follows "B" refers to the type and amount of estrogen received (B0.1=0.1 gamma estradiol valerate in oil, B.1=0.1 gamma 17-beta-estradiol in saline). The letters C, D, and E represent the groups receiving adrenal glucocorticoids in addition to 17-beta-estradiol in saline (see table in methods and materials section for explanation of which letter represents which glucocorticoid). Letter A is used to designate the control group.

### Pilot Study

A) Vaginal Estrus Cycle

In the presentation of the following results the conventional diestrus, proestrus, estrus terminology has been employed to describe the vaginal cycles. However, in the day to day vaginal smear recording, as presented in the raw data, different terminology has been employed.

In this method, the diestrus stage was characterized by leukocytes and epithelial cells (Le Stage), or leukocytes, epithelial, and cornified epithelial cells (Le C Stage). Proestrus was characterized by epithelial and cornified epithelial cells (EC Stage), and estrus is represented by the presence of only cornified epithelial cells in the smear (C Stage).

In the control group only one animal exhibited variation from the predicted vaginal cycle. This variation consisted of a lengthening by one day of one vaginal cycle. However, as can be observed in figure 1, a positive correlation exists between the number of animals exhibiting a variance in their vaginal cycles during the experimental period and the dose of hydrocortisone employed. Also demonstrated by the results plotted in figure 1, is that the change in the vaginal cycles began earlier in the experimental period with the higher dose levels of hydrocortisone.

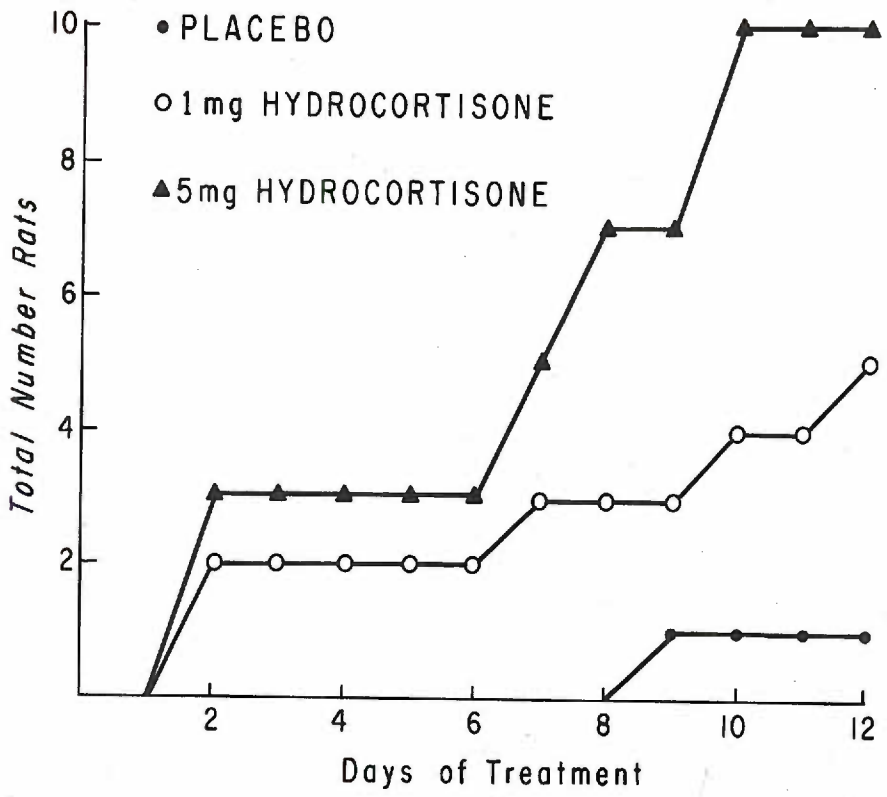
Examination of the raw data in the appendix, reveals that two animals from both the 1 mg. and 5 mg. hydrocortisone groups went into persistant diestrus stages about the eighth day of the experiment. Another abnormality of vaginal cycling exhibited by the experimental groups was the lengthening of their cycles by 1-2 days. Three animals in the 1 mg. group and eight animals in the 5 mg. group, in addition to those animals who went into persistent diestrus, demonstrated this abnormality.

B) Body Weight

The hydrocortisone treated animals exhibited a marked reduction in body weight during the treatment period. The reduction in body weight was progressively greater as the dose of hydrocortisone was

FIGURE I

Total number of animals exhibiting a variation from the expected vaginal cycle during the treatment period.


• PLACEBO
○ 1 mg HYDROCORTISONE
▲ 5 mg HYDROCORTISONE
Total Number Rats
10
8
6
4
2
2
4
6
8
10
12
Days of Treatment

increased, whereas, no change was noted in average net body weights of the controls. This figure however, is deceptive being primarily influenced by one animal which lost 34 Gms. of body weight during the experimental period as the result of a respiratory infection. Without this animal the controls would have a net average weight gain of 4 Gms. The average net body weight change during the 12 day treatment period for the 3 groups were as follows:

| Animal Group | Average Net Body Wt. Change (Gms.) | Range (Gms.) |
|---|---|---|
| Control | 0 | -34 to + 15 |
| 1 mg. Hydrocortisone | -17 | -35 to -10 |
| 5 mg. Hydrocortisone | -30 | -41 to -20 |

C) Uterine Weight

Observation of the results summarized in figure 2 reveals highly significant uterine weight reductions in the 5 mg. hydrocortisone group. Also it is noted that an increase in uterine weight occurred in the 1 mg. hydrocortisone group, however this rise was statistically insignificant when compared to the control group.

It was observed in the preceding section that hydrocortisone produced a marked decrease in gross body weight. Since this drop in gross body weight seemed to be due to a generalized catabolic effect it is possible that the drop in uterine weight in the 5 mg. hydrocortisone group was not a selective effect. In order to offset this general catabolic effect of hydrocortisone a uterine-body weight ratio was employed to study the uterine weight changes. Again, the data presented in figure 2 demonstrates that a significant selective reduction in

FIGURE 2

Summary of the average organ weights and uterine-body weight ratios in intact adult female rats treated with hydrocortisone acetate.

*1 mg. Hydrocortisone $P > 0.90$

*5 mg. Hydrocortisone $P < 0.05$

| Treatment One S.C. Injection at 24 hr. intervals for 12 days | No. Animals | Av. Body Wt. (gm) | Av. Thymic Wt. (mg) | Av. Uterine Wt. (mg) | Uterine Wt. (mg) / Body Wt. (gm) | |
|---|---|---|---|---|---|---|
| | | | | | Av. | S.E. |
| Placebo | 10 | 249 | 165 | 465 | 1.85 | 0.22 |
| 1 mg Hydrocortisone | 10 | 225 | 65 | 483 | 2.10 | 0.19 |
| 5 mg Hydrocortisone | 10 | 219 | 26 | 288 | 1.32 | 0.11 |

uterine weight did occur in the 5 mg. hydrocortisone group and that the rise in uterine weight in the 1 mg. hydrocortisone group was statistically not significant.

D) Thymic Weight

In this study a method for ascertaining the systemic activity of the adrenal glucocorticoids was needed to be sure the steroids were active. Observation of the thymic weights was selected as the method of choice, since Stephenson ( 27) has demonstrated that the amount of involution is directly proportional to the dose of adrenal steroid employed.

The average thymic weights for the 1 mg. and 5 mg. hydrocortisone groups were significantly reduced, demonstrating a good level of systemic activity for this steroid (See figure 2 for thymic weights). Involution of the thymus was most pronounced in the animals receiving the largest amounts of hydrocortisone.

Estrogen-Adrenal Steroid Study

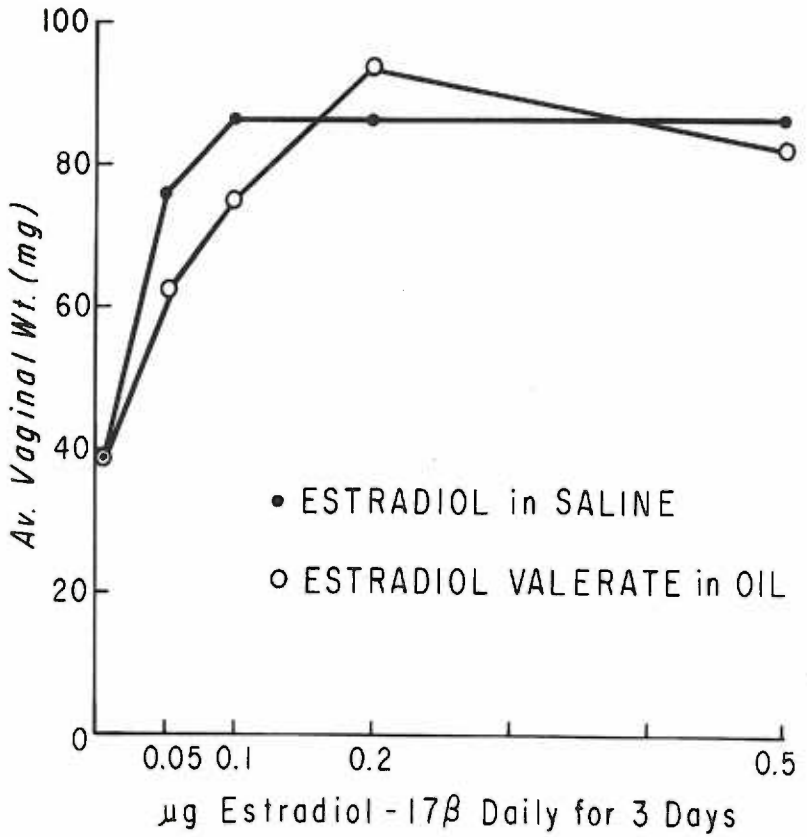
A) Vaginal Weight Response to Estradiol

To initiate this study it was deemed necessary to test two vehicles and two forms of 17-beta-estradiol to ascertain the most effective estrogen treatment. The criteria of this test was the production of a maximal vaginal weight gain within the experimental period at the lowest possible dose.

All of the estradiol treated animals in this study demonstrated statistically significant vaginal weight increases over control values. Examination of figure 3, however, reveals that a maximal vaginal weight gain was obtained with 0.1 gamma estradiol in saline and 0.2 gamma

FIGURE 3

Average vaginal weights produced by various doses of estradiol in the immature, adrenalectomized, and ovariectomized rats.


100
80
60
40
20
0
Av. Vaginal Wt. (mg)
• ESTRADIOL in SALINE
○ ESTRADIOL VALERATE in OIL
0.05
0.1
0.2
0.5
μg Estradiol - 17β Daily for 3 Days

estradiol in oil. The vaginal weight gain obtained with 0.2 gamma estradiol in oil was greater than that obtained by the lower dosage in saline, but this difference statistically is not significant. Since 0.1 gamma estradiol in saline was the lowest dosage of estrogen which produced a maximal vaginal weight gain, this dose level was chosen for the following experiments. The average vaginal weights and standard errors for the various groups are summarized in figure 3.

B) Vaginal weight response to estradiol and adrenal glucocorticoids.

The average vaginal weights for animals receiving the combined treatment of adrenal steroids and estradiol (groups C, D, and E) were below those obtained from the animals treated with estradiol alone (group B). Comparison of the figures compiled in figure 4 indicate that the reductions in vaginal weight in groups C, D, and E were significantly reduced when compared with those obtained from group B. As stated in the pilot study, however, adrenal glucocorticoids in the dosages employed here also produce a reduction in body weight which is believed to be an expression of a general catabolic effect. To adjust for this general effect on the body weight the vaginal weights are compared on the basis of a vaginal-body weight ratio. This analysis demonstrated that the reduction in vaginal weight in group C is not statistically significant. On the other hand the reductions in vaginal weights in groups D and E are significant. It is important to note that the animals in group C were treated with hydrocortisone sodiumsuccinate, a more soluble compound than the prednisolone acetate or cortisone acetate administered to the animals in groups D and E. The greatest reduction in vaginal-body weight ratios occurred in group D.

In addition the data presented in figure 5 demonstrates that each

FIGURE 4

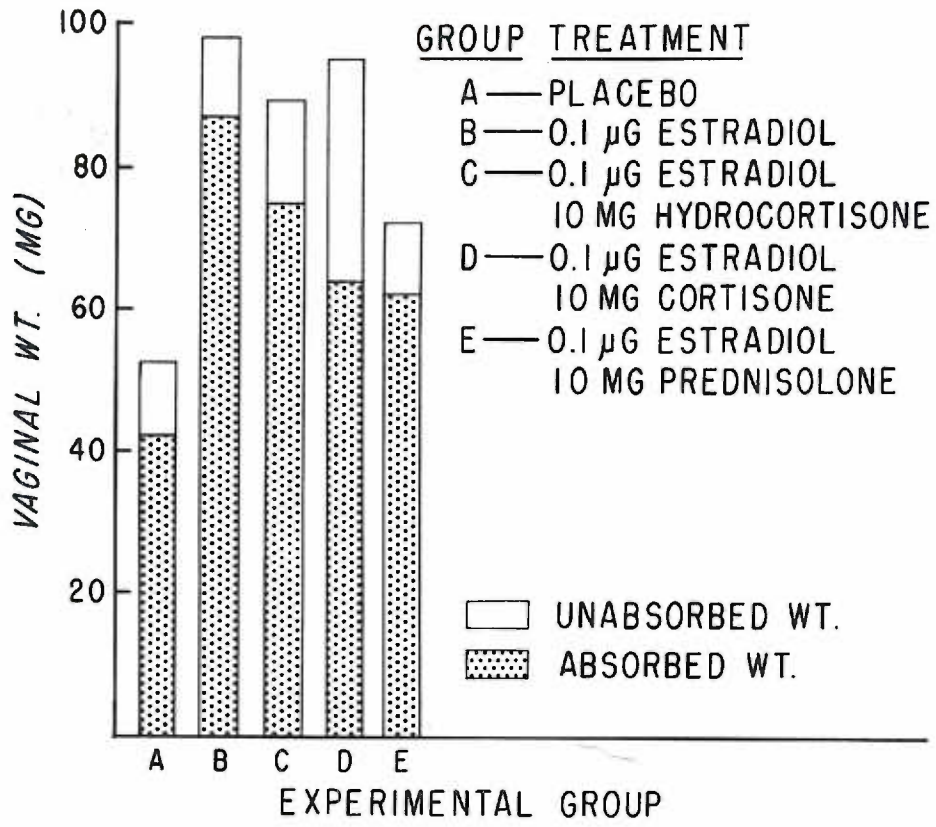
Summary of average body weights, vaginal weights, and vaginal-body weight ratios in immature, adrenalectomized, and ovariectomized rats.

| | |
|---|---|
| *Group C | $P < 0.10$ |
| *Group D | $P < 0.01$ |
| *Group E | $P < 0.01$ |

| GROUP | TREATMENT 3 S.C. INJ. AT 24 HR INTERVALS | NO. ANIMALS | AV. BODY WT. (GM) | AV. VAGINAL WT. (MG) | AV. VAGINAL WT.(MG) / BODY WT. (GM) | S.E. VAGINAL WT. (MG) / BODY WT. (GM) |
|---|---|---|---|---|---|---|
| A | PLACEBO | 19 | 82 | 42 | 0.50 | 0.017 |
| B | 0.1 μG ESTRADIOL | 22 | 77 | 87 | 1.12 | 0.040 |
| C | 10 MG HYDROCORTISONE 0.1 μG ESTRADIOL | 14 | 74 | 75 | 1.01 | 0.070 |
| D | 10 MG CORTISONE 0.1 μG ESTRADIOL | 9 | 73 | 64 | 0.87 | 0.050 |
| E | 10 MG PREDNISOLONE 0.1 μG ESTRADIOL | 22 | 64 | 62 | 0.96 | 0.020 |

FIGURE 5

Average vaginal weight response to estradiol combined with various adrenal cortical steroids in the immature, adrenalectomized, and ovariectomized rats.


100
80
60
40
20
VAGINAL WT. (MG)
GROUP TREATMENT
A — PLACEBO
B — 0.1 µG ESTRADIOL
C — 0.1 µG ESTRADIOL
10 MG HYDROCORTISONE
D — 0.1 µG ESTRADIOL
10 MG CORTISONE
E — 0.1 µG ESTRADIOL
10 MG PREDNISOLONE
UNABSORBED WT.
ABSORBED WT.
A B C D E
EXPERIMENTAL GROUP

group, except group D, had about the same volume of fluid in the vagina at autopsy. The increase in fluid contained within the vagina's of group D is readily explained by the observation that fewer animals in this group had open vaginal plates at the time of autopsy, thus preventing the escape of uterine and vaginal secretions.

C) Response of vaginal tissue beta-glucuronidase to estradiol-adrenal glucocorticoid administration.

Data presented in figure 6 demonstrates that the vaginal beta-glucuronidase activity is significantly reduced below placebo levels with estradiol treatment. Again, as in the vaginal weight study, the maximal response was first obtained with the 0.1 gamma estradiol dosage. It should be noted, however, that the differences in beta-glucuronidase activity in all the estradiol treated groups are not statistically significant.

With the addition of an adrenal glucocorticoid to the treatment schedule the vaginal beta-glucuronidase activity did not deviate significantly from the placebo level. These observations are presented in figure 7 and it will be noted that it occurred with all of the adrenal glucocorticoids employed. The vaginal beta-glucuronidase activity obtained by treatment with the adrenal steroids used in these experiments revealed no significant differences, although variations in the average values are apparent.

By plotting individual vaginal beta-glucuronidase activity levels against vaginal weights regardless of treatment, an inverse relationship is observed (figure 8). Although considerable variation in beta-glucuronidase activity levels at any given vaginal weight is revealed in this scatter graph, the inverse relationship is strikingly apparent.

FIGURE 6

Average vaginal beta-glucuronidase activity level changes with estradiol treatment in the vaginas of immature, adrenalectomized, and ovariectomized rats. (Activity measured by gamma of phenolphthalein liberated from 100 mg. of wet vaginal tissue in one hour at 38 degrees centigrade.)

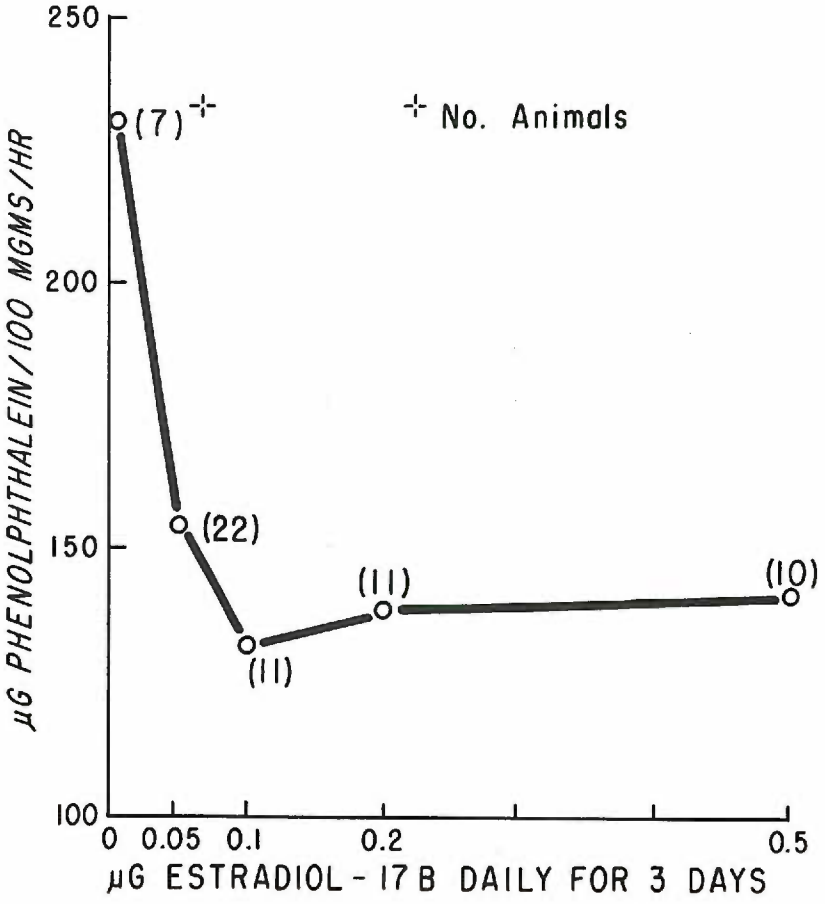

250
200
150
100
µG PHENOLPHTHALEIN/100 MGMS/HR
(7)
(22)
(11)
(11)
(10)
No. Animals
0
0.05
0.1
0.2
0.5
µG ESTRADIOL - 17 B DAILY FOR 3 DAYS

FIGURE 7

Average vaginal beta-glucuronidase activity level responses to combined estradiol, adrenal cortical steroid treatment in immature, adrenalectomized, and ovariectomized rats. (Activity measured by gamma of phenolphthalein liberated from 100 mg. of wet vaginal tissue in one hour at 38 degrees centigrade.)

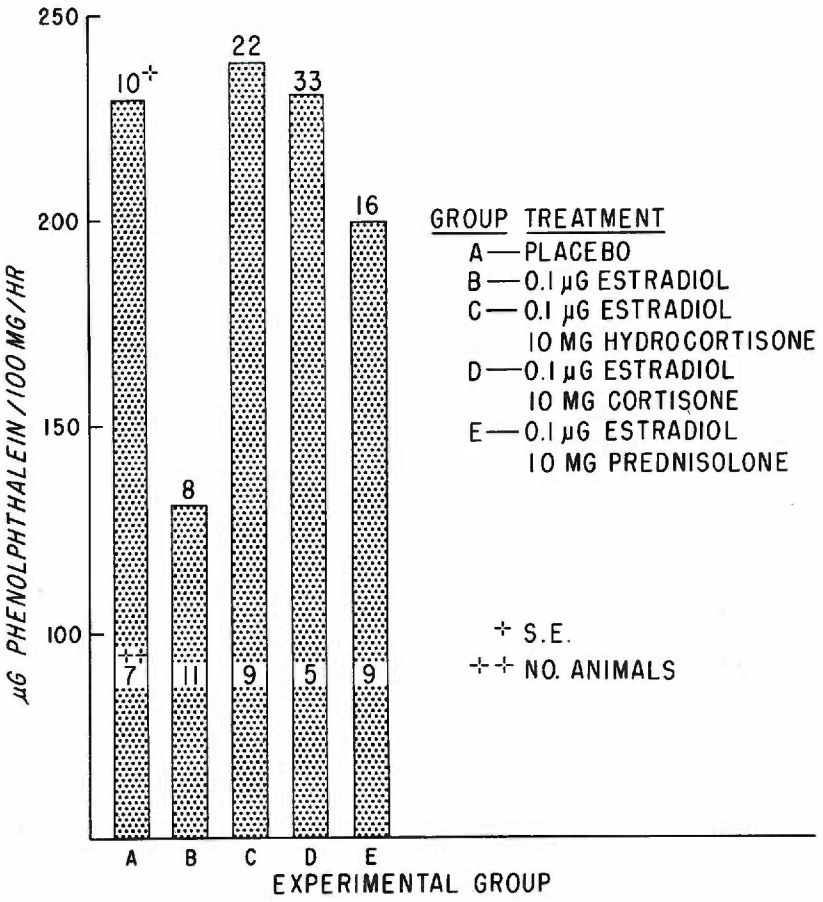
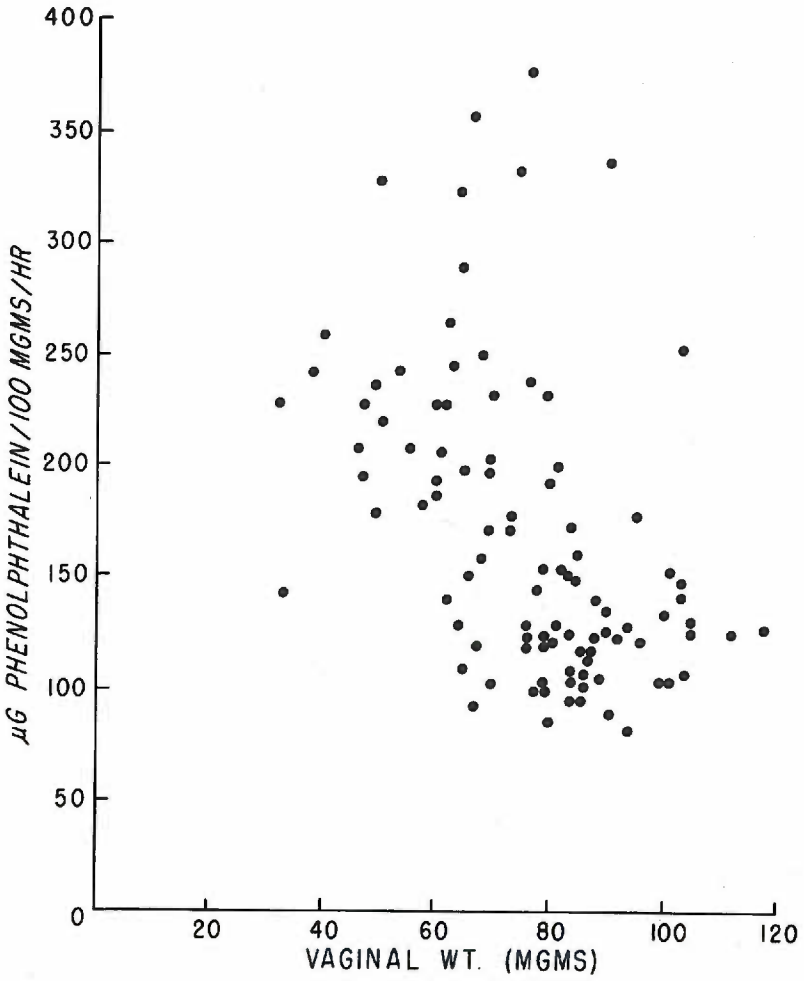

250
200
150
100
µG PHENOLPHTHALEIN/100 MG/HR
10+
22
33
16
8
7
11
9
5
9
A
B
C
D
E
EXPERIMENTAL GROUP
GROUP TREATMENT
A—PLACEBO
B—0.1 µG ESTRADIOL
C—0.1 µG ESTRADIOL
10 MG HYDROCORTISONE
D—0.1 µG ESTRADIOL
10 MG CORTISONE
E—0.1 µG ESTRADIOL
10 MG PREDNISOLONE
+ S.E.
++ NO. ANIMALS

FIGURE 8

Vaginal beta-glucuronidase activity levels and its relationship to vaginal weights in individual animals. (Activity measured by gamma of phenolphthalein liberated from 100 mg. of wet vaginal tissue in one hour at 38 degrees centigrade.)


400
350
300
250
200
150
100
50
0
20
40
60
80
100
120
µG PHENOLPHTHALEIN/100 MGMS/HR
VAGINAL WT. (MGMS)

D) Histological Examination of Vaginal Tissue.

The microscopic preparations of the vagina from the placebo group exhibited a low lying epithelium, which was 2-3 cells thick. The surface cells were macified, and the basal cells of the epithelium were arranged in a relatively even, flat line external to the basement membrane. The submucosa was predominently composed of stellate shaped fibroblasts widely separated by intervening spaces. Capillaries which were observable were few in number (see figure 9).

With estrogen stimulation both the size and number of the vaginal epithelial cells were progressively increased with a positive correlation to the dose employed. Beyond 0.1 gamma estradiol level very little additional change was noted. Keratinization of the epithelium was evident inall the estradiol treated animals with dosages in excess of 0.05 gamma estradiol. The basal cells of the epithelium formed an irregular line when the dose levels of estradiol exceeded 0.05 gamma. In the submucosa the cellularity increased with dosage up to 0.1 gamma estradiol, and the stellate appearance of the cells became more apparent as the result of an increased staining of the cytoplasm. The number of capillaries observed was slightly increased and in general vessels appeared dilated. Also, in the hematoxylin and eosin stained sections there was noticed an increased basophilia in the cytoplasm of the fibroblasts. (See figure 9).

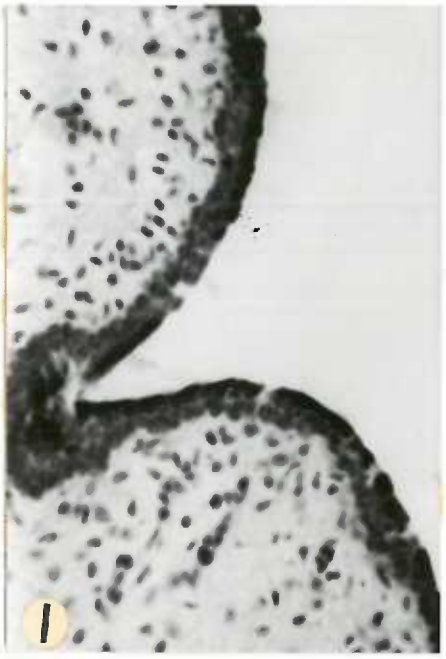
When adrenal glucocorticoids were combined with the 0.1 gamma estradiol treatment the size and number of epithelial cells were reduced. The basal layer of epithelial cells lost some of the irregularity exhibited in the estradiol treated groups. Also, in the groups receiving adrenal glucocorticoids the majority of the vagina's demon-

FIGURE 9

Vaginal sections of estradiol treated immature, adrenalectomized, ovariectomized rats, stained with nuclear fast red-alcian blue (220X).

1. placebo, 2. 0.05 gamma estradiol in saline, 3. 0.1 gamma estradiol in saline, 4. 0.2 gamma estradiol in saline.

Compare height of epithelium, its relative cellularity, cornification of the surface cells and the configuration of the basal surface. The dark staining substance on the surface of the epithelium is alcian-blue positive mucin. Also note increased cellularity of submucosa.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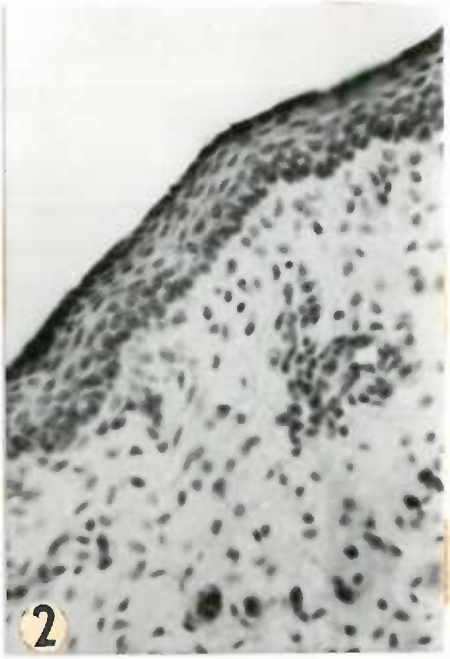
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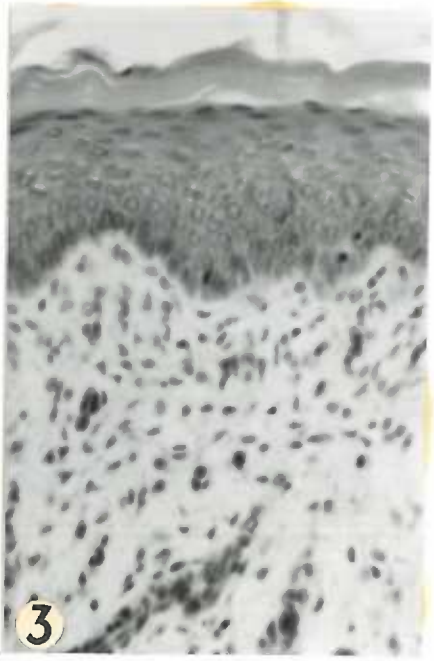
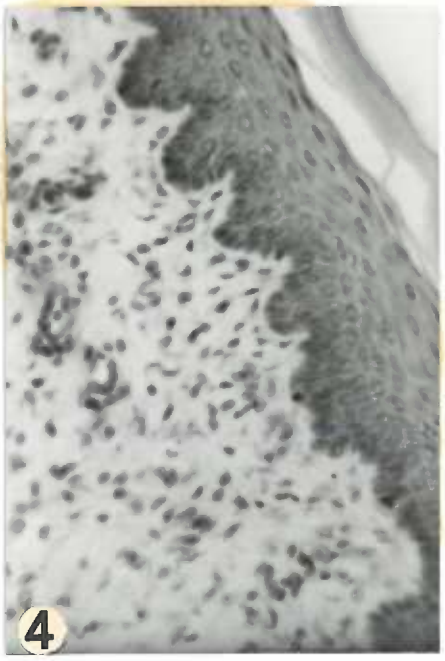

3


4

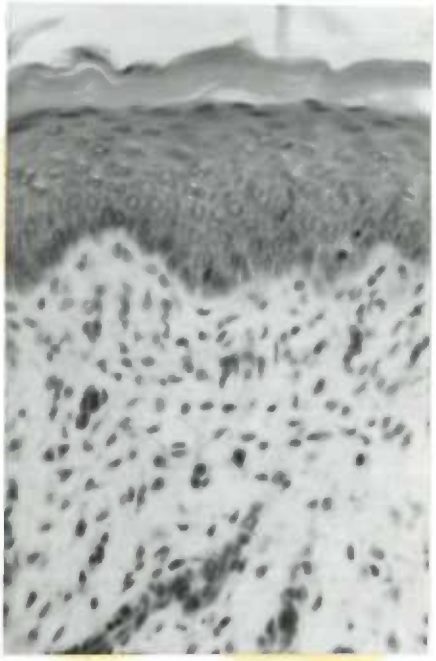
strated mucification of the surface epithelial cells. In the submucosa the number of cells was markedly reduced in all but the hydrocortisone treated group. Prednisolone produced the most marked antiestrogenic effect on the morphologic features of the vaginal tissue, with cortisone producing less effect, and the water soluble hydrocortisone producing the least. The lesser effects of the water soluble hydrocortisone is probably the result of a failure of this steroid to form a residual depot at the site of injection. Also noted in the vaginal sections of the prednisolone group was the presence of clumps of leukocytes in the epithelium and an increase in the number of these cells in the submucosa. (See figure 10.)

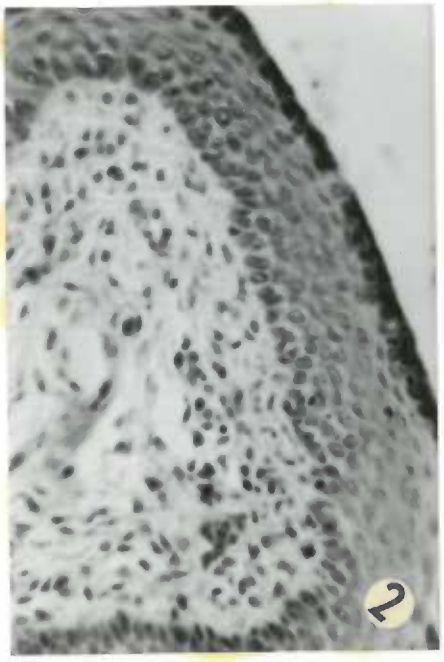
FIGURE 10

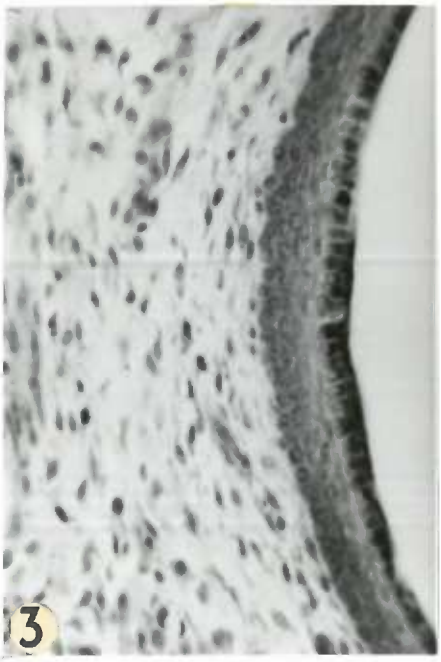
Vaginal sections of estradiol-adrenal glucocorticoid treated immature, adrenalectomized, ovariectomized rats, stained with nuclear fast red-alcian blue (220X).

1. 0.1 gamma estradiol in saline, 2. 10 mgm. hydrocortisone sodium succinate, 3. 10 mgm. cortisone acetate, 4. 10 mgm. prednisolone acetate.

Compare height of epithelium, its relative cellularity, mucinification of the surface cells and the configuration of the basal surface. The dark staining substance on the surface of epithelium is alcian-blue positive mucin. Also note decreased cellularity of submucosa and the clump of leukocytes present in the vaginal epithelium of the prednisolone treated animal.




2


3

## Discussion

The observation that hydrocortisone does produce alterations in the normal vaginal estrus cycles of adult intact rats suggests that adrenal glucocorticoids may interfer in some manner with the estrogen stimulation of the female reproductive tract in the rat. This obeservation is supported by the reports of Koller (19) and Gieger (15). Both of which employed intact female rats. Koller (19) demonstrated that adrenal hyperactivity, induced by daily subcutaneous injections of adrenocorticotrophin, produced suppression of estrus, as evidenced in vaginal smears. Gieger (15), on the other hand, employed hydrocortisone acetate and with this hormone also demonstrated suppression of estrus, which was confirmed by microscopic examination of the stained sections of the vagina. Both of the above authors concluded from their studies that suppression of estrus was an expression of ovarian dysregulation. The failure of Edgren ( 9), Pliske (23), and Martin et. al. (22) to demonstrate inhibition of the estrogen induced effects in the castrate rat and mouse vagina by the administration of adrenal cortical steroids, has been cited as evidence against the conclusion that adrenal cortical steroids inhibit estrogen stimulation of the vagina. It is possible that the failure encountered by these investigators was related to the low doses of steroids employed in their studies. It is of importance to note that Szego (31) has estimated that it takes 3,000 molecules of an adrenal cortical steroid to antagonize 1 molecule of estrogen.

There is an abundance of evidence in the literature, that indicates that adrenal cortical steroids do antagonize estrogen stimulation of the uterus. In 1948, it was demonstrated by Szego and Roberts (30)

that adrenal hyperactivity, induced by adrenocorticotrophin, had a powerful inhibitory influence on the water imbibition response of the castrate rat uterus to estrogen. When hydrocortisone and cortisone became available the above experiments were repeated, employing these adrenal cortical steroids as the test hormones (29). Data from these experiments was presented, which indicated that both hydrocortisone and cortisone posessed to a striking degree the ability to reproduce the inhibition of the uterine water response to estrogen, which had been noted in the earlier cited experiments. This inhibition of estrogenic stimulation was observed in castrate and castrate-hypophysectomized rat uteri. The authors suggested that the observed alterations in the uterus could be secondary to changes in tissue enzyme activity produced by the adrenal cortical steroids employed. It was also mentioned that adrenal cortical steroids may act as competitive inhibitors of estrogens. All of the hormones employed by Szego and associates were administered intravenously with the uterine measurements being made four hours after the injections.

Talalay et. al. (33) examined the uterine weights in castrate rats following three days of treatment with 0.15-0.30 gamma of diethylstilbesterol and/or 7.5 mg. of cortisone acetate. The conclusions of the author were that cortisone acetate in the doses employed suppresses the diethylstilbesterol stimulated uterine weight gain in what appears to be a competitive manner. Contrary to the above work, Fawcett and Deane (12) in 1952 reported that 5.0 mg. of cortisone failed to inhibit the uterine growth response to 0.3 gamma estradiol. Similar observations were also reported by Edgren (10). The discrepancy between the

findings of these authors and those of Talalay may again be the result of dosages of the steroids employed. In recent years, Velardo (34,35,36) has published several reports confirming the adrenal cortical steroid suppression of estradiol stimulated uterine weight gain reported by the earlier investigators. This author employed six different cortisones and hydrocortisones in his experiments. It was also stated by this author that the inhibition of estrogen effects is probably a competitive interaction of the steroids employed.

On the other hand, the suppression of estrogen induced vaginal alterations, morphologically, biochemically, or gravimetrically, by adrenal glucocorticoids has not been reported prior to this paper. A few investigators, however, (as indicated in the introduction) have concerned themselves with this general problem, employing morphologic parameters. It is of interest to compare the results reported here with the previously mentioned investigations and others indirectly related to this problem. To facilitate this part of the discussion, it will be presented in three general sections; gravimetric, morphologic, and biochemical observations.

Vaginal weight has proven to be a sensitive indicator of estrogen effect in this investigation. Adrenalectomized, ovariectomized, immature rats treated with 0.1 gamma estradiol in saline exhibited an increase in vaginal weight which was 108% above placebo levels. Using 0.1 gamma estradiol in oil, the resulting vaginal weight increase was 90% greater than placebo values. Velardo (34), however, employing adult ovariectomized rats treated with 0.1 gamma estradiol in oil observed approximately a 45% increase in wet uterine weight and approximately a 60% increase in dry uterine weight. Comparison of these

figures demonstrate that the vagina is a more sensitive indicator of estrogen stimulation than the uterus at dose levels of estrogen from 0.1 gamma down. Vaginal weights, in addition, reach a maximal response at relatively low dosages of estrogen (0.1 gamma estradiol in saline, 0.2 gamma estradiol in oil), whereas in Velardo's (34) investigation the uterine weight did not reach a maximal level until a dose of 50 gamma estradiol in oil was employed. It appears, therefore, that the vagina of the immature, adrenalectomized, ovariectomized rat would be more sensitive to small changes in estrogen dosage than the uterus of adult ovariectomized rat, as employed by Velardo (34). It must be noted, however, that the vagina exhibits no further significant weight gain when the dosages of estradiol in saline exceed 0.1 gamma.

It was noted in the results that the adrenal glucocorticoids employed in this study produced a decrease in total body weight, which was interpreted as representing a general catabolic effect of these steroids. Therefore, in order to offset this generalized loss in body weight, it was necessary to employ a vaginal-body weight ratio in evaluating the vaginal weight changes. When this was accomplished, it was noted that only prednisolone acetate and cortisone acetate produced significant selective reductions in vaginal weight, thereby indicating estrogen inhibition. The inability of hydrocortisone sodium succinate to inhibit the estrogen induced vaginal weight gain is probably related to the inability of this steroid to form a residual depot in the tissue.

Martin et. al. (22) employed morphological changes produced in the ovariectomized mouse vagina by estrogen treatment as an assay method

for this steroid. The morphologic alterations which these authors examined were the number of mitoses present in the vaginal epithelium and the thickness of this tissue. Estrogen produces an increase in both of these epithelial responses. Employing the above measurements, these investigators reported that hydrocortisone acetate given subcutaneously in doses ranging from 5-500 microgram X 10-4 failed to antagonize the stimulatory effect of estrone which was also given subcutaneously. The dosage of estrone employed varied from 0.04--1.0 microgram X 10-4. It is probable that the doses of hydrocortisone acetate employed in Martin and co-workers ( 22) investigations, according to Szego (31) as previously cited, were much too low to antagonize the estrone stimulation of the ovariectomized, mouse vagina.

On the other hand, in this study, it will be recalled that vaginas of animals receiving adrenal glucocorticoids in addition to estradiol exhibited a reduction in epithelial height, mucification of the surface cells, a reduction in the irregularity of the basement membrane, and similarity in the appearance of the submucosa to that observed in the placebo treated animals. All these changes are indications that the estrogen effect in the vagina was being inhibited.

Examination of the stained slides of vaginal tissue from animals in this study revealed the above described alterations. These morphological changes were present in varying degrees depending on the ability of the adrenal glucocorticoid employed to antagonize the estrogen stimulation of the immature adrenalectomized, ovariectomized rat. Prednisolone acetate exhibited the greatest ability to antagonize the estrogen stimulation, cortisone acetate had less effect, and hydrocortisone sodium succinate had the least effect.

In 1944 Fishman and Fishman (13) published the first report which demonstrated that a relationship existed between estrogen and beta-glucuronidase. These investigators observed that uterine beta-glucuronidase could be reduced in mice by ovariectomy. It was also observed that exogenous estrogen administered to these castrate animals would re-elevate the uterine beta-glucuronidase back to normal levels. This work has been confirmed and extended by other investigators (18,20. Szego and Beylor (3 ) observed that hydrocortisone acetate would antagonize the capacity of estrogen to augment the beta-glucuronidase acitivity.in the uterus of a castrate adult rat.

The observations in this paper indicate that the vaginal tissue beta-glucuronidase reacts in a manner opposite to that of the uterus and other tissues. That is, with estrogen treatment the vaginal beta-glucuronidase activity levels were markedly reduced below placebo levels. Also, all of the adrenal glucocorticoids employed in this study completely inhibited the estradiol suppression of vaginal beta-glucuronidase in the adrenalectomized, ovariectomized, immature rat. Support of the above observations is presented by Fishman (14), who observed in histochemically stained sections of vaginal biopsies obtained from pre- and post-menopausal females that beta-glucuronidase activity is greater in the vaginal epithelium of the post-menopausal women. Also, Kasdon (17) reports that beta-glucuronidase activity in the vaginal fluid, taken from 20 post-menopausal women, is markedly reduced by a single 2 mg. dose of estradiol. Despite the numerous investigations concerned with beta-glucuronidase,its exact relationship to estrogen and the effect of estrogen on the female reproductive tract is unknown.

## Summary

I. Data was presented that indicated that hydrocortisone acetate will produce, in the adult intact female rat, alterations in the normal vaginal estrus cycles and a reduction in uterine weight.

II. Estrogen and/or prednisolone acetate, cortisone acetate, hydrocortisone sodium succinate were administered to adrenalectomized, ovariectomized immature rats. Results are summarized below.

A) Gravimetric Studies

(1) The stimulatory effects of 17-beta-estradiol in oil and in saline were compared. It was found that 0.1 gamma estradiol in saline was the minimal dose of estrogen capable of inducing a maximal weight gain in the vagina.

(2) Exogenous prednisolone acetate and cortisone acetate, administered in 10 mg. doses, antagonized the stimulating effects of 0.1 gamma estradiol in saline.

B) Morphologic studies

(1) Estradiol in 0.1 gamma dose produced vaginal epithelial keratinization, epithelial hyperplasia, and an increased irregularity of the basement membrane. Also the cellularity of the submucosa was markedly increased and the capillaries present appeared dilated.

(2) When 0.1 gamma estradiol treatment was combined with hydrocortisone socium succinate, prednisolone acetate, or cortisone acetate in 10 mg. doses there was produced surface mucification and decrease in the thickness in the vaginal epithelium. The cellularity of the submucosa

approached that of the placebo treated animal and the irregularity of the basement membrane of the vaginal epithelium was reduced.

C) Biochemical studies

(1) Estradiol in 0.1 gamma dose produced a decrease in the vaginal tissue beta-glucuronidase activity.

(2) When 10 mg. of hydrocortisone sodium succinate, prednisolone acetate, or cortisone acetate were added to the 0.1 gamma estradiol treatment the vaginal tissue beta-glucuronidase activity was maintained at placebo levels.

III. Results of this investigation were compared and correlated with the current reports in the literature pertaining to the study of the estrogen-adrenal cortical steroid antagonism.

## Conclusion

It may be concluded that in the immature, adrenalectomized, ovariectomized rat that the adrenal glucocorticoids employed in this investigation in the doses stated do inhibit partially or completely the vaginal response to estrogen stimulation.

# APPENDIX

Section A



Section B



Section C



Section D

# SECTION A

## Composition of Stains

## and Solutions

## Table I

### Alcian Blue-Periodic Acid Schiff Reaction

1. Bring paraffin sections to water.
2. Stain in Alcian blue stain for 30 minutes.
3. Wash in running tap water 2 minutes and rinse in distilled water.
4. Place in 0.5% periodic acid ($HIO_4$) 10 minutes.
5. Wash in running tap water 5 minutes and rinse in distilled water.
6. Place in Schiff's reagent 10 minutes.
7. Wash in running tap water 5 minutes.
8. Dehydrate in graded alcohols, clear, and mount.

Schiff's Solution:

Dissolve 1 gm. basic fuchsin in 200 ml. boiling water; filter, cool and add 2 gm. potassium metabisulfite ($K_2S_2O_5$) and 10 ml. of 1 N HCl. Let bleach for 24 hours and then add 0.5 gms. activated carbon. Shake for about 1 minute and filter through coarse filter paper. If filtrate is not colorless, add HCl a drop at a time, agitating the solution with each drop of acid added, until the solution is water clear.

Alcian blue stain:

Dissolve 1.0 Gms. of alcian blue in 100 ml. of 3% solution of glacial acetic acid.

0.5% periodic acid:

Dissolve 0.5 Gm. periodic acid ($HIO_4$) in 100 ml. of distilled water.

Table 2

Alcian Blue-Nuclear Fast Red Stain

1) Bring paraffin sections to water.

2) Stain in alcian blue stain for 30 minutes.

3) Wash in running tap water for 2 minutes.

4) Counter-stain in Nuclear fast red solution for 5 minutes.

5) Wash briefly in running tap water.

6) Dehydrate in graded alcohols, clear, and mount.

Alcian Blue:

See table 1 for preparation.

Nuclear Fast Red:

Dissolve 0.1 Gm. of nuclear fast red in 100 ml. 5% solution of aluminum sulfate with the aid of heat. Cool, filter, and add crystal of thymol.

TABLE 3

Carnoy's Fluid

| | |
|---|---|
| 1) Absolute alcohol | 60 ml. |
| 2) Chloroform | 30 ml. |
| 3) Glacial acetic acid | 10 ml. |

0.1 M acetate Buffer (pH 4.5)

1) 5.8 gm. of c.p. sodium acetate
2) 3.3. ml. of glacial acetic acid
3) 700 ml. of distilled water
4) adjust to pH4.5 with 10% NaOH or acetic acid.
5) Dilute to 1 liter with distilled water.

0.2 M Glycine Buffer (pH 10.4)

1) 15.0 gm. Aminoacetic Acid (Glycine)
2) 11.7 gm. NaCl
3) 700 ml. distilled water
4) Adjust to pH 10.4 with 10% NaOH
5) Dilute to 1 liter with distilled water.

0.0015 M Phenolphthalein Glucuronidate (pH 7.0)

1) 5.0 ml Phenolphthalein mono-beta-glucuronidate solution (Sigma Chemical Co.), 0.01M, pH. 7.0.
2) 28.3 ml. distilled water

(sufficient for approximately 65 determinations)

## Section B

Autopsy weights, body weights, and estrus cycle records for the Pilot Study.

Table 4

Control

| Animal No. | Body wt. (Gms.) 1 | 2 | Uterine wt. unabsorbed (mgm.) | Thymic wt. (mg.) |
|---|---|---|---|---|
| B.H. 11 | 245 | 240 | 282 | 106 |
| B.H. 14 | 265 | 265 | 550 | 218 |
| B. 18 | 263 | 274 | 490 | 174 |
| G. 22 | 245 | 249 | 340 | 247 |
| B.H. 26 | 235 | 245 | 356 | 140 |
| B. 29 | 255 | 265 | 614 | 172 |
| B. 31 | 250 | 250 | 850 | 164 |
| B. H.34 | 235 | 247 | 441 | 187 |
| B.H. 37 | 275 | 241 | 251 | 63 |
| B.H. 46 | 230 | 220 | 473 | 181 |

Table 4

1 mg. Hydrocortisone

| | | | | |
|---|---|---|---|---|
| B.H. 2 | 230 | 210 | 304 | 56 |
| G. 3 | 260 | 240 | 586 | 71 |
| B.H. 9 | 293 | 260 | 856 | 51 |
| B.H. 19 | 236 | 225 | 427 | 72 |
| B.H. 23 | 200 | 180 | 366 | 47 |
| B.H. 24 | 254 | 240 | 540 | 139 |
| B.H. 25 | 254 | 240 | 507 | 73 |
| B.H. 28 | 240 | 220 | 589 | 35 |
| B. 35 | 250 | 235 | 299 | 54 |
| B.H. 38 | 212 | 205 | 362 | 51 |

Table 4

5 mg. Hydrocortisone

| | | | | |
|---|---|---|---|---|
| B.H. 4 | 270 | 250 | 283 | 33 |
| B.H. 7 | 225 | 192 | 253 | 28 |
| B.H. 10 | 245 | 210 | 429 | 26 |
| B.H. 13 | 230 | 206 | 298 | 25 |
| B.H. 21 | 275 | 235 | 247 | 24 |
| B.H. 27 | 270 | 250 | 248 | 35 |
| B. 30 | 245 | 204 | 303 | 23 |
| G.H. 41 | 235 | 211 | 357 | 21 |
| G. 44 | 270 | 235 | 268 | 30 |
| B.H. 45 | 228 | 200 | 196 | 19 |

Table 5

Control

| Animal No. | Treatment Days 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BH. 11 | EC | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le |
| B.H. 14 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | EC |
| B. 18 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | EC |
| G. 22 | EC | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le |
| B.H. 26 | EC | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le |
| B. 29 | EC | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le |
| B. 31 | EC | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le |
| B.H. 34 | EC | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le |
| B.H. 37 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | EC |
| B.H. 46 | C | LeC | Le | EC | C | LeC | Le | EC | EC | LeC | Le | Le |

1 mg. Hydrocortisone

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B.H. 2 | C | C | LeC | Le | Le | EC | C | LeC | Le | LeC | LeC | Le |
| G. 3 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | EC |
| B.H. 9 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | Le |
| B.H. 19 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | EC |
| B.H. 23 | C | LeC | Le | EC | C | LeC | Le | EC | C | C | LeC | LeC |
| B.H. 24 | C | LeC | Le | EC | C | LeC | EC | EC | C | LeC | Le | EC |
| B.H. 25 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | EC |
| B.H. 28 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | Le | EC |
| B. 35 | C | C | LeC | Le | EC | C | LeC | Le | LeC | LeC | C | LeC |
| B.H. 38 | C | LeC | Le | EC | C | LeC | Le | EC | C | LeC | LeC | EC |

5 mg. Hydrocortisone

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B.H. 4 | C | LeC | Le | EC | C | LeC | EC | Le | EC | C | LeC | LeC |
| B.H. 7 | C | C | LeC | Le | Le | EC | EC | C | EC | EC | LeC | Le |
| B.H. 10 | C | C | Le | EC | EC | EC | C | LeC | Le | LeC | LeC | LeC |
| B.H. 13 | C | C | LeC | Le | Le | EC | EC | C | EC | LeC | Le | Le |
| B.H. 21 | C | LeC | Le | EC | C | LeC | Le | EC | C | C | LeC | LeC |
| B.H. 27 | C | LeC | Le | EC | C | LeC | Le | Le | Le | EC | C | LeC |
| B. 30 | C | LeC | Le | EC | C | LeC | Le | EC | C | C | LeC | LeC |
| G.H. 41 | C | LeC | Le | EC | C | LeC | Le | EC | C | C | LeC | LeC |
| G. 44 | C | LeC | Le | EC | C | LeC | Le | Le | Le | EC | C | LeC |
| B.H. 45 | C | LeC | Le | EC | C | LeC | EC | EC | EC | LeC | LeC | LeC |

## Section C

## Autopsy weights for Estrogen-Adrenal Steroid Study.

Table 6

Group A

| Animal No. | Body Wt. (Gms.) | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic wt. (mg.) | glucuronidase activity (gamma/100mg.) |
|---|---|---|---|---|---|
| 43 | 107 | 34 | 34 | 349 | |
| 44 | 90 | 45 | 37 | 412 | |
| 45 | 80 | 37 | 33 | 356 | |
| 46 | 86 | 45 | 33 | 313 | |
| 47 | 80 | 39 | 36 | 424 | |
| 80 | 86 | 45 | 38 | 325 | |
| 81 | 86 | 36 | 42 | 279 | |
| 82 | 80 | 47 | 54 | 255 | |
| 83 | 85 | 50 | 43 | 338 | |
| 84 | 76 | 47 | 54 | 255 | |
| 85 | 88 | 44 | 45 | 291 | |
| 157 | 80 | 40 | 22 | 385 | 258 |
| 158 | 80 | 38 | 30 | 388 | 241 |
| 159 | 70 | 32 | 23 | 301 | 228 |
| 160 | 80 | 46 | 46 | 252 | 209 |
| 223 | 98 | 49 | 35 | 458 | 178 |
| 228 | 93 | 68 | 80 | 342 | 250 |
| 229 | 85 | 49 | 50 | 329 | 236 |
| 237 | 86 | 33 | 46 | 358 | |
| 239 | 89 | 39 | 31 | 443 | |

Table 7

Group B.05

| Animal No. | Body wt. (Gms.) | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic Wt. (mg.) | glucuronidase activity (gamma/100mg.) |
|---|---|---|---|---|---|
| 138 | 98 | 60 | 86 | 488 | 186 |
| 139 | 87 | 69 | 110 | 372 | 198 |
| 140 | 85 | 80 | 124 | 484 | 192 |
| 141 | 60 | 61 | 104 | 268 | 150 |
| 142 | 68 | 76 | 151 | 350 | 121 |
| 143 | 68 | 73 | 137 | 317 | 178 |
| 144 | 79 | 69 | 91 | 478 | 203 |
| 145 | 91 | 88 | 144 | 338 | 123 |
| 146 | 83 | 76 | 99 | 418 | --- |
| 147 | 76 | 84 | 129 | 323 | 173 |
| 148 | 80 | 65 | 85 | 301 | 199 |
| 150 | 68 | 79 | 142 | 267 | 120 |
| 151 | 68 | 76 | 152 | 330 | 119 |
| 152 | 66 | 79 | 147 | 317 | 121 |
| 153 | 79 | 84 | 174 | 329 | 108 |
| 154 | 83 | 85 | 128 | 237 | 160 |
| 156 | 81 | 83 | 150 | 313 | 151 |
| 162 | 93 | 104 | 172 | 370 | 106 |
| 163 | 97 | 68 | 73 | 401 | 157 |
| 164 | 88 | 84 | 123 | 349 | 125 |
| 165 | 100 | 94 | 140 | 452 | 128 |
| 166 | 78 | 81 | 114 | 357 | 129 |
| 167 | 95 | 62 | 72 | 363 | 256 |

Table 8

Group B.1

| Animal No. | Body Wt. (Gms.) | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic wt. (mg.) | glucuronidase activity (gamma/100 mg.) |
|---|---|---|---|---|---|
| 24 | 88 | 88 | 144 | 315 | ---- |
| 27 | 65 | 77 | 125 | 177 | ---- |
| 28 | 66 | 80 | 143 | 166 | ---- |
| 29 | 72 | 77 | 132 | 266 | ---- |
| 30 | 64 | 102 | 156 | 226 | ---- |
| 31 | 88 | 95 | 198 | 407 | ---- |
| 32 | 64 | 87 | 139 | 266 | ---- |
| 90 | 85 | 86 | 181 | 265 | ---- |
| 91 | 90 | 98 | ---- | 313 | ---- |
| 198 | 105 | 100 | 150 | 441 | 104 |
| 200 | 99 | 103 | 148 | 412 | 253 |
| 201 | 71 | 84 | 154 | 284 | 94 |
| 202 | 77 | 87 | 144 | 306 | 114 |
| 203 | 71 | 79 | 144 | 290 | 154 |
| 204 | 85 | 89 | 141 | 346 | 105 |
| 205 | 75 | 79 | 152 | 364 | 100 |
| 206 | 75 | 94 | 143 | 341 | 81 |
| 208 | 80 | 91 | 153 | 380 | 89 |
| 209 | 90 | 84 | 121 | 365 | 99 |
| 234 | 99 | 90 | 141 | 449 | 125 |

Table 9

Group B.2

| Animal No. | Body wt. (Gms.) | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic wt. (mg.) | glucuronidase activity (gamma/100mg.) |
|---|---|---|---|---|---|
| 178 | 77 | 67 | 146 | 343 | 119 |
| 181 | 68 | 74 | 140 | 311 | ----- |
| 179 | 88 | 100 | 198 | 295 | 134 |
| 182 | 98 | 88 | 172 | 448 | 140 |
| 183 | 108 | 118 | 205 | 410 | 129 |
| 185 | 91 | 81 | 156 | 405 | 200 |
| 186 | 66 | 84 | 179 | 341 | 150 |
| 187 | 106 | 103 | 159 | 383 | 141 |
| 199 | 105 | 112 | 153 | 446 | 125 |
| 207 | 69 | 78 | 150 | 318 | 145 |
| 211 | 73 | 80 | 155 | 357 | --- |
| 214 | 99 | 105 | 162 | 409 | 128 |
| 215 | 87 | 86 | 169 | 370 | 117 |

Group B.5

| | | | | | |
|---|---|---|---|---|---|
| 180 | 76 | 76 | 141 | 256 | 238 |
| 189 | 75 | 73 | 177 | 375 | 173 |
| 194 | 90 | 101 | 241 | 336 | 152 |
| 195 | 107 | 103 | 231 | 417 | 148 |
| 210 | 68 | 62 | 102 | 233 | 140 |
| 212 | 54 | 65 | 116 | 269 | 109 |
| 216 | 80 | 87 | 157 | 329 | 116 |
| 217 | 74 | 70 | 130 | 350 | 103 |
| 219 | 91 | 76 | 147 | 368 | 128 |
| 220 | 98 | 96 | 170 | 410 | 121 |

Group B0.05

| | | | | |
|---|---|---|---|---|
| 124 | 122 | 64 | 58 | 625 |
| 125 | 115 | 67 | 77 | 515 |
| 126 | 130 | 66 | 49 | 588 |
| 128 | 102 | 55 | 50 | 477 |
| 130 | 100 | 54 | 63 | 432 |
| 131 | 120 | 65 | 65 | 610 |
| 132 | 125 | 65 | 57 | 513 |
| 134 | 108 | 62 | 58 | 431 |
| 135 | 102 | 67 | 72 | 430 |
| 136 | 119 | 68 | 68 | 577 |

Table 10

Group BO.1

| Animal No. | Body wt. (Gms.) | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic wt. (mg.) |
|---|---|---|---|---|
| 108 | 103 | 63 | 105 | 473 |
| 109 | 117 | 117 | 191 | 497 |
| 110 | 80 | 69 | 110 | 300 |
| 111 | 101 | 85 | 119 | 482 |
| 112 | 92 | 68 | 93 | 410 |
| 113 | 92 | 64 | 97 | 333 |
| 114 | 84 | 63 | 72 | 451 |
| 115 | 74 | 68 | 103 | 291 |
| 116 | 79 | 86 | 120 | 468 |
| 117 | 88 | 86 | 150 | 369 |
| 118 | 92 | 65 | 85 | 401 |
| 119 | ---- | 76 | 130 | 444 |
| 120 | 103 | 80 | 112 | 528 |
| 121 | 85 | 88 | 101 | 339 |
| 122 | 83 | 87 | 131 | 412 |
| 123 | 93 | 61 | 92 | 442 |
| 127 | 107 | 79 | 107 | 555 |
| 129 | 120 | 73 | 110 | 628 |
| 137 | 116 | 74 | 102 | 515 |

Table 11

Group BO.2

| Animal No. | (Gms.) Body wt. | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic wt. (mg.) |
|---|---|---|---|---|
| 92 | 72 | 82 | 188 | 343 |
| 93 | 70 | 74 | 168 | 299 |
| 94 | 100 | 91 | 180 | 405 |
| 95 | 79 | 94 | 216 | 351 |
| 96 | 98 | 93 | 174 | 406 |
| 97 | 80 | 101 | 183 | 341 |
| 98 | 76 | 106 | 217 | 362 |
| 99 | 80 | 96 | 174 | 416 |
| 100 | 95 | 105 | 182 | 374 |
| 101 | 85 | 83 | 174 | 319 |
| 102 | 93 | 96 | 190 | 342 |
| 103 | 86 | 89 | 191 | 298 |
| 104 | 108 | 94 | 166 | 364 |
| 133 | 91 | 105 | 190 | 402 |

Group BO.5

| | | | | |
|---|---|---|---|---|
| 190 | 76 | 67 | 155 | 297 |
| 191 | 79 | 86 | 163 | 317 |
| 192 | 111 | 105 | 223 | 335 |
| 193 | 74 | 69 | 176 | 290 |
| 196 | 94 | 86 | 168 | 364 |
| 197 | 76 | 84 | 182 | 322 |

Table 12

Group C

| Animal No. | Body wt. (Gms.) | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic wt. (mg.) | glucuronidase activity (gamma/100mg.) |
|---|---|---|---|---|---|
| 4 | 63 | -- | 126 | 58 | |
| 5 | 75 | -- | 98 | 43 | |
| 33 | 73 | 82 | 122 | 68 | |
| 34 | 65 | 64 | 83 | 46 | |
| 35 | 68 | 85 | 119 | 66 | |
| 36 | 52 | 71 | 105 | 46 | |
| 37 | 66 | 74 | 130 | 60 | |
| 39 | 81 | 74 | 100 | 87 | |
| 41 | 85 | 85 | 125 | 93 | |
| 42 | 77 | 83 | 93 | 90 | |
| 168 | -- | 90 | 152 | 149 | 135 |
| 170 | 86 | 90 | 148 | 138 | 179 |
| 172 | 78 | 95 | 75 | 96 | 227 |
| 173 | 84 | 60 | 93 | 126 | 324 |
| 177 | 86 | 40 | 78 | 190 | 327 |

Group D

| | | | | | |
|---|---|---|---|---|---|
| 169 | 66 | 66 | 111 | 25 | 357 |
| 171 | 69 | 69 | 118 | 32 | 171 |
| 174 | 80 | 69 | 97 | 35 | 231 |
| 175 | 67 | 61 | 88 | 24 | 227 |
| 176 | 75 | 64 | 98 | 41 | 290 |
| 213 | 79 | 47 | 60 | 36 | 228 |
| 218 | 79 | 61 | 81 | 46 | 206 |
| 221 | 70 | 55 | 90 | 32 | 208 |
| 227 | 76 | 82 | 131 | 39 | 152 |

Table 13

Group E

| Animal No. | Body wt. (Gms.) | Vaginal wt. absorbed (mg.) | Uterine wt. absorbed (mg.) | Thymic wt. (mg.) | glucuronidase activity (gamma/100mg.) |
|---|---|---|---|---|---|
| 63 | 69 | 70 | 131 | 36 | |
| 64 | 78 | 73 | 122 | 39 | |
| 65 | 71 | 57 | 88 | 22 | |
| 66 | -- | 70 | 113 | 31 | |
| 68 | 71 | 66 | 104 | 41 | |
| 69 | 76 | 64 | 128 | 35 | |
| 70 | 54 | 70 | 110 | 34 | |
| 71 | 58 | 59 | 121 | 28 | |
| 72 | 54 | 67 | 126 | 20 | |
| 73 | 55 | 49 | 110 | 30 | |
| 74 | 58 | 67 | 132 | 21 | |
| 75 | 57 | 60 | 121 | 25 | |
| 76 | 57 | 54 | 115 | 25 | |
| 77 | 62 | 57 | 101 | 28 | |
| 78 | 57 | 57 | 104 | 21 | |
| 79 | 52 | 52 | 91 | 19 | |
| 86 | 71 | 77 | 138 | 28 | |
| 87 | 65 | 65 | 120 | 30 | |
| 88 | 64 | 59 | 120 | 30 | |
| 89 | 70 | 61 | 155 | 22 | |
| 222 | 74 | 64 | 152 | 29 | 128 |
| 225 | 88 | 80 | 150 | 28 | 121 |
| 226 | 70 | 63 | 109 | 26 | 245 |
| 230 | 71 | 50 | 68 | 30 | 230 |
| 232 | 73 | 53 | 78 | 31 | 244 |
| 233 | 80 | 60 | 115 | 28 | 194 |
| 235 | 63 | 47 | 85 | 23 | 194 |
| 236 | 69 | 58 | 117 | 35 | 183 |
| 238 | 83 | 79 | 182 | 43 | 233 |

# SECTION D

# STATISTICAL DATA

TABLE 14

PILOT STUDY

Uterine - Body Weight Ratio Study

| Group | Mean | S.D. | S.E. | P Values |
|---|---|---|---|---|
| Control | 1.85 | 0.65 | 0.22 | |
| 1 mg. | 2.10 | 0.56 | 0.19 | >0.90 |
| 5 mg. | 1.32 | 0.32 | 0.11 | <0.05 |

Adrenal Glucocorticoid - Estrogen Study

Vaginal Weight Study

| Group | Mean | S.D. | S.E. | P Values (A vs. other groups) |
|---|---|---|---|---|
| A | 42 | 5.6 | 1.3 | |
| B.05 | 76 | 12.1 | 3.3 | < 0.001 |
| B.1 | 87 | 8.2 | 2.3 | < 0.001 |
| B.2 | 87 | 10.7 | 4.5 | < 0.001 |
| B.5 | 87 | 12.8 | 5.3 | < 0.001 |
| BO.05 | 63 | 4.7 | 1.5 | < 0.001 |
| BO.1 | 76 | 13.1 | 3.1 | < 0.001 |
| BO.2 | 94 | 9.0 | 2.5 | < 0.001 |
| BO.5 | 83 | 12.6 | 5.7 | < 0.001 |

Vaginal - Body Weight Ratio Study

| | | | | (B.1 vs. other groups) |
|---|---|---|---|---|
| B.1 | 1.12 | 0.15 | 0.04 | |
| C | 1.01 | 0.23 | 0.07 | < 0.10 |
| D | 0.87 | 0.14 | 0.05 | < 0.01 |
| E | 0.96 | 0.14 | 0.02 | < 0.01 |

Beta - Glucuronidase Study

| | | | | (A vs. other groups) |
|---|---|---|---|---|
| A | 229 | 25.3 | 10.3 | |
| B.05 | 155 | 38.5 | 8.4 | < 0.001 |
| B.1 | 131 | 60 | 19 | < 0.01 |
| B.2 | 139 | 21.8 | 6.9 | < 0.001 |
| B.5 | 143 | 37.7 | 12.6 | < 0.001 |

| | | | | (B.1 vs. other groups) |
|---|---|---|---|---|
| B.1 | 131 | 60 | 19 | |
| C. | 238 | 66.5 | 33.3 | < 0.02 |
| D | 198 | 44.3 | 15.7 | < 0.01 |
| E | 230 | 63.6 | 22.5 | < 0.02 |

REFERENCES

1. Allen, E., & Doisy, E. A. An ovarian hormone. Preliminary report on its localization, extraction and partial purification, and action in test animals. J.A.M.A., 1923. 81, 819.

2. Alloiteau, J. J., & Courvoisier, B. Action anti-oestrale de la cortisone chez la rate. Compt. rend. soc. biol., 1953. 147, 553. (Cited by Edgren, R.A., 1959).

3. Beyler, A. L., & Szego, C. M. Endocrine regulation of beta-glucuronidase activity. Am. J. Physiol., 1951. 167, 768.

4. Burrows, H. Biological actions of sex hormones. Cambridge Eng.: University press, 1949.

5. Courrier, R. L'action keratinisante de las desoxy-corticosterone fur le vagin, et les differentes proprietes des trois substances hormonales progestatives. Comp. rend. Soc. Biol., 1942. 136, 196. (Cited by Burrows, H., 1949).

6. Courrier, R. Cortisone et sexualite femelle. Ann. Endocrinol., 1959. 15, 1. (Cited by Edgren, R. A., 1959).

7. Del Castillo, E. B. & Calatroni, C. Rev. Soc. Argent. Biol., 1930. 2, 108. (Cited by Del Castillo, E. B., 1942).

8. Del Castillo, E. B., & DiPaola, G. Cyclical vaginal response to the daily administration of estradiol in castrated rats. Endocrinology, 1942. 30, 48.

9. Edgren, R. A. The modification of estrogen-induced changes in

rat vaginas with steroids and related agents. Ann. N. Y. Acad. Sci., 1959. 83 (2), 160.

10. Edgren, R. A., & Calhoun, D. W. Estrogen antagonisms: effects of gluco-and mineral-corticoids on estrone-induced uterine growth. Proc. Soc. Exper. Biol. Med., 1960. 103, 294.

11. Emmens, C. W. The duration of action of certain natural and synthetic oestrogens when administered orally or by injections. J. Endo., 1939. 1, 142.

12. Fawcett, D. W., & Deane, H. W. The effect of cortisone on uterine growth in ovariectomized rats receiving estradiol. Anat. Rec., 1952. 112, 110.

13. Fishman, W. H., & Fishman, L. W. The elevation of uterine beta-glucuronidase activity by estrogenic hormone. J. Biol. Chem., 1944. 152, 487.

14. Fishman, W. H., & Mitchell, G. W. Studies on vaginal enzymology. Ann. N. Y. Acad. Sci., 1959. 83 (2), 105.

15. Gieger, J. M. Personal communication.

16. Gunberg, D. L. Personal communication.

17. Kasdon, S. C., Romano, P., & Katzimichael, A. Influence of hormones on the blood-serum enzyme beta-glucuronidase. Obst. Gynec., 1960. 15, 367.

18. Knobil, E. The relation of some steroid hormones to beta-glucuronidase activity. Endocrinology, 1952. 50, 16.

19. Koller, A. Der einflusz des adren corticotropen hormone (ACTH)

auf den schleiden-cyclus der ratte. Archiv. Fur Gynakologie, 1952. 182, 277.

20. Leatham, J. H. Some biochemical aspects of the uterus. Ann. N. Y. Acad. Sci., 1959. 75 (2), 463.

21. Long, J. A. & Evans, H. N. The estrous cycle in the rat and its associated phenomena. Memoirs Univ. of California, 1922. 6.

22. Martin, L. & Claringbold, M. The mitogenic action of estrogen in the vaginal epithelium of the ovariectomized mouse. J. Endo., 1960. 20, 173.

23. Pliske, E. C., Baker, B. L., & Johnson, W. Local action of adrenocortical preparations on secondary sexual organs. Proc. Soc. Exp. Biol. Med., 1953. 84, 510.

24. Robson, J. M. Comparison of the amounts of progesterone and of desoxycorticosterone acetate needed to produce certain progesterone-like actions. J. Physiol., 1939. 96, 21p.

25. Rubin, B. L., Dorfman, A. S., Black, L., & Dorfman, R. I. Bioassay of estrogens using the mouse uterine response. Endocrinology, 1951. 49, 429.

26. Salmon, U. J. Desoxycorticosterone acetate is estrogenic in the human female. Proc. Soc. Exper. Biol. Med., 1939. 41, 515.

27. Stephenson, N. R. The effect of corticosteroid analogues on the thymus gland of the immature rat. J. Pharm. & Pharm., 1960. 12, 411.

28. Stockard, C. R., & Papanicolaou, G. N. The existence of a typical

oestrous cycle in the guinea pig with a study of its histological and physiological changes. Am. J. Anat., 1917. 22, 225.

29. Szego, C. M. Pituitary-adrenal cortical antagonism to estrogenic stimulation of the uterus of the ovariectomized rat. Endocrinology, 1952. 50, 429.

30. Szego, C. M., & Roberts, S. Pituitary-adrenal cortical antagonism to estrogenic stimulation of the uterus of the ovariectomized rat. Am. J. Physiol., 1948. 152, 131.

31. Szego, C. M., & Roberts, S. Uterine metabolism. Recent Progress in Hormone Research, 1953. 8, 419.

32. Takewaki, K., & Hidaka, T. Effect of adrenal corticoids on vaginal response to estrogen in spayed rats. J. Faculty Sci. Univ. Tokyo, Sect. IV. 1958. 8, 347. (Cited by Edgren, R. A., 1959).

33. Talalay, P., Dobson, M. M., Ebersol, C. M., & Huggins, C. Physiological interaction of cortisone acetate and diethylstilbesterol. Lack of androgenic or estrogenic effects of cortisone acetate. Endocrinology, 1952. 50, 574.

34. Velardo, J. T. Steroid hormones and uterine growth. Ann. N. Y. Acad. Sci., 1959. 75 (2), 441.

35. Velardo, J. T., Hisaw, F. L., & Bever, A. T. Inhibitory action of desoxycorticosterone acetate, cortisone acetate, and testerone on uterine growth induced by estradiol-17-beta. Endocrinology, 1956, 59, 165.

36. Velardo, J. T., & Sturgis, S. H. Suppression of uterine growth by purified hydrocortisone acetate, 9 alpha-fluorohydrocortisone acetate and corticotropin. J. Clin. Endo., 1957. 16, 496.

37. Zuckerman, S. Cyclical fluctuations in oestrin threshold. Nature, 1937. 139, 628.

38. Zuckerman, S. Cyclical fluctuations in the sensitivity of the rat to oestrogenic stimulation. J. Physiol., 1938. 92, 12P.

39. Zuckerman, S. Adrenals and pituitary in relation to cyclical changes in the oestrin-sensitivity of the rat. J. Physiol., 1938. 92, 13P.