The Effect of Cortisone on Uterine Growth in Ovariectomized Rats receiving Estradiol

By DON W. FAWCETT AND HELEN WENDLER DEANE

(From the Department of Anatomy, Harvard Medical School, Boston, Massachusetts)

With one plate

SUMMARY

The growth of the uterus in ovariectomized rats treated with estradiol was compared with that of similar animals given both cortisone and estradiol.

Examination of histological sections of the uteri of rats treated in these two ways revealed no significant differences in general histological topography or cytology.

The results of one of the two experiments performed suggested that cortisone may antagonize the increase in interstitial fluid of the uterine wall but does not interfere with the growth of the uterus which results from administration of an estrogen.

These results are compared with those on skin and other organs in which cortisone has been found to inhibit the growth and repair of mesenchymal tissues.

SEVERAL investigators have observed delayed development of granulation tissue in cutaneous wounds of rodents treated with cortisone (Ragan et al., 1949; Spain, Molomut, and Haber, 1950; Layton, 1951). Thus it has been suggested that cortisone inhibits growth of all connective tissue elements of the skin. The present investigation was designed to study its effects upon growth of connective tissue in an organ other than skin.

The rapid growth of the uterus in ovariectomized rats treated with estradiol has been thoroughly studied and well standardized (Dorfman, Gallagher, and Koch, 1935). This form of growth provided an excellent opportunity in the present study to evaluate the effect of cortisone upon connective tissue growth induced by hormonal stimulation rather than trauma. The uterine stroma was therefore studied in adult ovariectomized rats treated with estradiol and with cortisone. The results were assessed by examination of histological sections and by weighing the uteri.

MATERIALS AND METHODS

Thirty female albino rats of the Sprague-Dawley (Hisaw) strain were used. Bilateral ovariectomies were performed when they weighed 100 to 125 grams. Treatment was started 40 days later. Two experiments were performed.

Experiment 1. Sixteen rats were divided into 4 groups. Two controls received no treatment. Four controls received injections of 0.5 ml. physiological salt solution twice daily (9 a.m. and 5 p.m.). Four rats received a daily injection of estradiol (5 p.m.). Six rats received cortisone twice daily (9 a.m. and 5 p.m.).
and 5 p.m.) as well as estradiol once daily (5 p.m.). The saline or cortisone injections were begun 24 hours before the estrogen injections. Alpha-estradiol benzoate (ovocylin benzoate, Ciba) was diluted with peanut oil to a concentration of 3.3 μg. per ml. Each injection of estradiol consisted of 0.1 ml., or 0.3 μg. of the hormone (2 rat units). The 17-hydroxy-11-dehydro-cortico-sterone (cortisone acetate, Merck) was suspended in physiological salt solution at a concentration of 5 mg. per ml. Each injection consisted of 0.5 ml. of this preparation, or 2.5 mg. of the hormone. All injections were made subcutaneously. The dosages of estrogen and of cortisone employed are believed to be within the physiological ranges (Greep and Jones, 1950; Stebbins, 1950).

Half of the animals were killed in the afternoon 4 days after injections were started (when the animals had received 8 injections of saline or cortisone, 3 of estradiol). Half were killed after 7 days (14 injections saline or cortisone, 6 of estradiol).

Experiment 2. Fourteen rats were divided into 3 groups. Group 1 (4 rats) received no treatment; group 2 (5 rats) received estradiol alone; and group 3 (5 animals) received both cortisone and estradiol. The injection schedule was identical with that in the first experiment, and all the animals were killed at noon, 7 days after treatment was begun (14 injections of cortisone, 6 of estradiol).

Methods of Study

In both experiments blood was drawn from the tail immediately before death, and eosinophil counts were made (Recant, Hume, Forsham, and Thorn, 1950). The uterus, two adrenal glands, and thymus were dissected free of fat and weighed.

In the first experiment a piece of the uterus from each animal was fixed in Zenker's fluid, while in the second experiment the uterus of only one rat in each group was fixed. The blocks were embedded in paraffin and sectioned at 5 μ.

Sections were stained by Masson's trichrome stain for connective tissues (Goldner, 1938), by Pap's ammoniacal silver nitrate method for reticulum fibres (Mitchell and Wislocki, 1944), and by a progressive eosin and methylene blue method developed in this laboratory (Singer, personal communication). For the latter, deparaffinized sections were treated with iodine to remove mercury precipitate and then with sodium thiosulphate solution. All slides were then stained together for 24 hours at 25°C in a large staining bath containing M5 x 10^-5 eosin and M5 x 10^-4 methylene blue plus 0.02 M acetate buffer, pH 5.13. The sections were then dehydrated in tertiary butyl alcohol to prevent decoloration, passed through xylene, and mounted in clarite (Singer and Morrison, 1948; Singer and Wislocki, 1948). This use of eosin and methylene blue as a progressive stain permits a more valid comparison of the basiphilia in different sections than does the routine eosin-methylene blue method of Mallory, wherein each slide is overstained with methylene blue and then differentiated.
In the second experiment the uteri, other than those used for histological study, were weighed in tared vessels and then dried to constant weight in an oven at 110° C.

**OBSERVATIONS**

**Experiment 1.** The results of the first experiment are presented in Table 1. Ovariectomized rats which received no treatment had atrophic uteri and their adrenal and thymus weights were typical of the castrate state. The organ weights of those given injections of saline twice daily were not significantly different from untreated controls, indicating that there was little or no effect of endogenous adrenal steroids released as a result of the 'alarm' incident to handling and injection. The groups which had received estrogen showed considerable growth of the uterus. The average uterine weight of those treated with both cortisone and estrogen was somewhat less than for those given estrogen alone. On the seventh day, the estrogen-treated rats showed a fourfold increase in uterine weight compared to saline-injected, ovariectomized controls, while those given estrogen and cortisone had increased approximately threefold. This suggested a partial inhibition of the estrogen effect by cortisone.

**TABLE I. Average data for the Rats in Experiment 1**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. rats</th>
<th>Final body wt., g.</th>
<th>Eosinophils per cu. mm.</th>
<th>Adrenal wt., mg.</th>
<th>Thymus wt., mg.</th>
<th>Uterine wt., mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2</td>
<td>235</td>
<td>220</td>
<td>68.9</td>
<td>890</td>
<td>38.1</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>205</td>
<td>103</td>
<td>55.5</td>
<td>625</td>
<td>45.7</td>
</tr>
<tr>
<td>Estradiol</td>
<td>2</td>
<td>242</td>
<td>221</td>
<td>66.9</td>
<td>1,025</td>
<td>142.7</td>
</tr>
<tr>
<td>Estradiol &amp; cortisone</td>
<td>3</td>
<td>192</td>
<td>7</td>
<td>40.2</td>
<td>267</td>
<td>115.5</td>
</tr>
<tr>
<td>Saline</td>
<td>2</td>
<td>214</td>
<td>256</td>
<td>61.8</td>
<td>683</td>
<td>53.8</td>
</tr>
<tr>
<td>Estradiol</td>
<td>2</td>
<td>194</td>
<td>185</td>
<td>58.3</td>
<td>445</td>
<td>231.0</td>
</tr>
<tr>
<td>Estradiol &amp; cortisone</td>
<td>3</td>
<td>211</td>
<td>75</td>
<td>48.8</td>
<td>113</td>
<td>171.6</td>
</tr>
</tbody>
</table>

The rats receiving cortisone showed partial atrophy of the adrenal glands (Ingle, Higgins, and Kendall, 1938) and marked atrophy of the thymus. They also evinced a sharp decline in eosinophil count.

The increase in uterine weight in response to estrogenic hormone involves hypertrophy, hyperplasia, and increased tissue hydration (Allen, Hisaw, and Gardner, 1939), and it was expected that examination of histological sections would reveal which of these processes was principally affected.

In histological sections, the atrophic uteri of the untreated ovariecomicized rats and of the rats receiving saline (fig. 1A) displayed cuboidal epithelium and very small endometrial glands. The connective tissue fibres in the stroma were densely packed; the stromal cells and the smooth muscle cells of the muscularis were small and exhibited small, hyperchromatic nuclei.

After 4 days of estrogen treatment (fig. 1B), there was a marked increase in
the height of the uterine epithelium and an increase in the size and number of endometrial glands. In the endometrial stroma there was a conspicuous increase in argyrophilic fibres and in intercellular fluid. The cells themselves were larger and had vesicular nuclei with prominent nucleoli; their cytoplasm was intensely basiphilic. The smooth muscle cells were hypertrophied, particularly in the inner, circular layer. Mitotic figures appeared in the epithelium, smooth muscle cells, vascular endothelium, and the fibroblasts of the endometrial stroma.

After 7 days of treatment (fig. 1D), the effects of estrogen were still more pronounced. The uterine epithelium was columnar, with small papillary projections of tall narrow cells giving the epithelium an undulating surface. The fibrous elements of the connective tissue stroma subjacent to the epithelium were much more dense than at 4 days. In the basal portion of the endometrium the vascular channels were distended and the stroma appeared oedematous. Tissue eosinophils were abundant, particularly in the circular muscle layer and in the endometrial stroma.

The uteri of rats receiving cortisone as well as estrogen for 4 (fig. 1C) and 7 (fig. 1E) days were scarcely distinguishable from those receiving estrogen alone. The stimulation of the epithelium, stroma, and muscularis was identical. However, in the uteri of the rats treated for 7 days, the number of tissue eosinophils was much smaller, and the amount of tissue fluid in the stroma at the base of the endometrium appeared somewhat less.

These histological observations suggested that the hypertrophy and hyperplasia of the mesenchymal tissues of the uterus were unaffected by cortisone but that the increased hydration which normally follows estrogen treatment may have been partially inhibited.

Experiment 2. To verify this interpretation, a second experiment was carried out as before, except that saline-injected controls were omitted and all animals were killed after 7 days (Table 2). As in the first experiment, all animals receiving estrogen had markedly enlarged uteri.

Those rats receiving cortisone lost 20 per cent. of their body weight during the course of treatment, whereas the control animals and those receiving estrogen alone maintained their body weight. The animals receiving cortisone also displayed lower adrenal and thymus weights and reduced eosinophil

---

**Fig. 1**

Transverse sections of uteri fixed in the Zenker acetic acid mixture and stained progressively with eosin and methylene blue at pH 5.13. Photographed with a green filter (Wratten B5, 58). All × 100.

A. Rat receiving saline for 7 days (14 injections).
B. Rat receiving estrogen for 4 days (3 injections).
C. Rat receiving cortisone (8 injections) and estrogen (3 injections) for 4 days.
D. Rat receiving estrogen for 7 days (6 injections).
E. Rat receiving cortisone (14 injections) and estrogen (6 injections) for 7 days. Compare D and E, noting smaller number of eosinophils in the muscularis and stroma of the latter.
FIG. 1
D. W. FAWCETT and H. W. DEANE
counts. All of these criteria gave evidence of an excellent general response to cortisone.

The uterine weights of rats receiving both estrogen and cortisone did not differ significantly from those of animals given estrogen alone. In both groups the uteri were about five times as large as those of the controls. One uterus from each group was studied histologically; the appearance of the sections was entirely similar to those in the first experiment killed after 7 days (fig. 1 D and E). The rest of the uteri were dried to constant weight to determine whether there was any difference in water content of the uteri in the animals receiving cortisone and estrogen, as compared with those receiving estrogen alone. No difference was found (Table 2).

### Table 2. Average data for Rats in Experiment 2. All animals were killed 7 days after treatment was begun

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. rats</th>
<th>Initial body wt., g.</th>
<th>Final body wt., g.</th>
<th>Eosinophils per cu. mm.</th>
<th>Adrenal wt., mg.</th>
<th>Thymus wt., mg.</th>
<th>Uterine wt., mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4</td>
<td>217</td>
<td>221</td>
<td>310</td>
<td>45-7</td>
<td>712</td>
<td>40±2</td>
</tr>
<tr>
<td>Estradiol</td>
<td>5</td>
<td>225</td>
<td>223</td>
<td>426</td>
<td>68-9</td>
<td>545</td>
<td>470±2</td>
</tr>
<tr>
<td>Estradiol &amp; cortisone</td>
<td>5</td>
<td>225</td>
<td>180</td>
<td>12</td>
<td>30-8</td>
<td>87</td>
<td>249±0</td>
</tr>
</tbody>
</table>

The explanation for the discrepancy in the results of Experiment 1 and Experiment 2 with regard to uterine wet weight is not entirely clear. In retrospect the only variation in the conduct of the two experiments which might offer a partial explanation was in the time of killing in relation to the final injection of hormones. In Experiment 1 the interval was longer by several hours than in Experiment 2, in which the rats were killed about 18 hours after the last injection of estradiol and 2 to 4 hours after the last of cortisone. It has been shown that the water content of the uterus exhibits a diphasic change following the injection of estradiol (Zuckerman, Palmer, and Hanson, 1950). The water content increases to a maximum in the first 6 hours, falls to a low point at 18 to 20 hours, and then rises to a second peak. Thus, in our second experiment the animals were killed when the water content of the uterus was at a low point and also at a time when the last dose of cortisone had not yet reached its maximal effect. These conditions may have been particularly unfavourable for the demonstration of an effect of cortisone on the water content of the uterus.

**DISCUSSION**

**Connective tissue.** Administration of cortisone has been reported to inhibit the formation of granulation tissue in cutaneous wounds of rabbits, mice, and rats (Ragan et al., 1949; Spain et al., 1950; Layton, 1951). Largely as a result of these observations, it has become the current belief that cortisone specifically inhibits new growth of connective tissue. The results of the experiments reported here are difficult to bring into accord with this generalization. If cortisone exerts a direct inhibitory influence on fibroblasts, then one would
expect it to cause a detectable diminution in the growth of the uterus in ovariectomized rats treated with estrogen. The fact that it fails to do so suggests a fundamental difference between the process of wound healing of the skin and the growth of the connective tissue of the uterus in response to estradiol stimulation.

In interpreting the influence of cortisone upon the complex process of wound healing, one cannot with certainty dissociate its effect upon proliferation of fibroblasts from its effect upon the associated inflammatory reaction. While in such studies physical or chemical injury provides the stimulus which initiates healing, the normal rate of wound repair may depend to some extent upon continued stimulation of fibroblasts by products of the leucocytes normally mobilized at the site of injury. Cortisone is known to reduce markedly the acute inflammation produced by trauma as well as that originating from chemical stimuli (Gross, 1950; Dougherty and Schneebeli, 1950). Hence the delayed granulation of wounds in cortisone-treated animals could be, in part, an indirect result of inhibition of the associated inflammatory reaction. It is pertinent to recall that Carrel in 1922 reported delayed closure of wounds kept completely clean, and he was able to demonstrate the presence of substances in leucocytic exudates which stimulate growth of fibroblasts in vitro and accelerate the healing of wounds.

However, there is evidence of a direct inhibiting action of cortisone upon connective tissues in which these factors of wound healing are not involved. Schneebeli (1950) has described specific morphological changes in fibroblasts 4 hours following cortisone administration, and Castor and Baker (1950) have reported atrophy of collagenous fibres and decreased cellularity of the dermis following prolonged local application of adrenocortical steroids to the skin.

In the present study, growth of the uterus involved proliferation of tissues, including connective tissue and other tissues of mesenchymal origin, without the complicating factors of trauma or inflammation. Under these conditions, administration of cortisone caused no inhibition of growth and no detectable changes in the cells or fibres of the connective tissue. The fibroblasts of the dermis and those of the endometrium have the same appearance, and it is one of the basic assumptions of histology that cells identical in form and in staining properties have the same actual and potential functions. However, if we accept the accumulated evidence for a direct inhibitory effect of cortisone on the connective tissue cells of the skin, then our negative results in the uterus suggest that the fibroblasts in the two sites must differ physiologically—one being sensitive and the other refractory to cortisone.

Physiological differences between morphologically similar connective tissue cells have been demonstrated before. Thus, Parker (1933) isolated fibroblasts from 14 separate tissues and organs of a single chick embryo and found that, when cultivated under identically controlled conditions, these cell-strains differed from one another in rate of multiplication, in rate of change in the pH of the medium, and in fibrinolytic activity. These functional differences were retained throughout months of cultivation. Huggins (1931) observed
that when epithelium from the urinary bladder or gall bladder was transplanted subcutaneously in dogs, the connective tissue cells near the transplant were transformed into osteoblasts which deposited typical bone. Connective tissue from the abdominal wall transplanted to the gall bladder also formed bone. Yet such a transformation was not observed in fibroblasts which occur normally beneath the epithelium of the intact urinary bladder or gall bladder (Huggins and Sammet, 1933). In Huggins's experiments the capacity of fibroblasts in one region to form bone in response to the osteogenic stimulus of certain epithelia, while those in another region are unable to do so, provides another example of physiological differences between fibroblasts of identical appearance.

The proliferation of fibroblasts in the uterus is usually induced by endocrine stimulation, whereas multiplication of subcutaneous fibroblasts commonly follows trauma. Moreover, subcutaneous fibroblasts react to injury with production of a scar, whereas those of the uterus under some circumstances respond to trauma with formation of deciduoma. Hence the connective tissue cells in these two sites are not only normally responsive to different stimuli but may respond differently to the same stimulus. Therefore it is not surprising to find that they differ also in their sensitivity to cortisone.

Eosinophils. It has not been established whether the fall in circulating eosinophils caused by adrenocortical steroids is due to their destruction or their emigration from the blood stream into the tissues. A remarkable increase in number of tissue eosinophils in the uterine wall of animals receiving estrogens has been reported previously (Reynolds, 1949). In the present material, the observation of a decrease in the number of tissue eosinophils in the uteri of cortisone-treated rats concurrent with the fall in circulating eosinophils favours the view that administration of cortisone results in destruction rather than increased emigration of eosinophils.

Body weight. The very considerable loss of body weight in the rats treated with cortisone for 7 days is consistent with previous observations indicating that cortisone in doses of 1 to 5 mg. per day causes a negative nitrogen balance in the rat (Ingle, 1950) and may entirely suppress the growth of young animals (Winter, Silber, and Stoerk, 1950). Indeed, the reported arrest in growth of bone and cartilage (Baker, 1951) and of certain transplantable tumours (Higgins, Woods, and Bennett, 1950; Sugiura et al., 1950) may be a consequence of the increased protein catabolism and general suppression of protein synthesis brought about by cortisone, rather than the expression of a specific inhibiting effect upon tissues of mesenchymal origin.

In view of its effect upon nitrogen balance and upon growth of the animals as a whole, it is particularly interesting that the estrogen-induced increase in uterine dry-weight was entirely unaffected by cortisone.

Acknowledgements

This study was supported in part by an Institutional Grant to Harvard University from the American Cancer Society. The senior author also acknow-
ledges the support of the John and Mary R. Markle Foundation, of which he is a Scholar in Medical Science. The ovocylin benzoate was supplied by Ciba Pharmaceutical Products, Inc., Summit, New Jersey, through the generosity of Dr. Fred E. Houghton. The cortisone acetate was given by Merck and Co., Rahway, New Jersey, through the interest of Dr. Hans Molitor. We wish to thank Dr. Leon P. Weiss for making the eosinophil counts.

REFERENCES


