THE EFFECT OF SMALL DOSES
OF PROGESTERONE ON THE UTERUS
OF THE RAT

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It is well known that stimulation, by progesterone, of the formation of a «progestational» endometrium is enhanced by pretreatment with oestrogen. As early as 1928, shortly after the discovery of the corpus luteum hormone, Weichert observed that deciduomata of the traumatized uterus developed only if treatment of the rats with corpus luteum extract had been preceded by the administration of follicular hormone. In 1930, Hisaw & Leonard confirmed these results in rabbits. Shelesnyak (1933) was one of the first to emphasize the importance of an optimal ratio between the doses of both hormones. De Fremery & Geerling (1939) found 0.6 mg. of progesterone daily, preceded by 15 µg. of oestrone or stilboestrol to be the most effective combination. Korenchevsky & Hall (1937) found daily doses of 1 µg. of oestrone followed by 1.5 mg. of progesterone to be even more active than 30 µg. of oestrone followed by 3 mg. of progesterone.

There are other results, however, which show that under certain conditions oestrogen pretreatment is not essential. Moricard & de Senarclens (1949) obtained negative results in spayed, immature rabbits with 2.5 mg. of progesterone over a three days' period; in recently spayed adult animals, however, a typical «dentelle utérine» developed. In this case, oestrogen may have played its part before gonadectomy. Selye (1940) obtained real progestational effects without oestrogen pretreatment by administering very large doses of progesterone (15 mg. daily) to adult spayed rats. Howard & Gengradon (1940) report similar results obtained in mice with 1.5 mg., a high dose for this species. Korenchevsky & Hall (1937) observed in adult spayed rats atypical alterations in the uterine

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epithelium with only 0.5 mg. of progesterone. The cells appeared to be hypertrophic and less stainable.

It appears, therefore, that 0.5 mg. is the lowest dose of progesterone which has some effect by itself. In the course of 1956 we wanted to extend our knowledge about effective combinations of oestrogen and progesterone. We hoped to be able to estimate the amount of oestrogen circulating in the male by means of uterine transplants and progesterone injections. These attempts were not successful, but control experiments in females showed that 1.5 mg. daily stimulated the uterine epithelium. Since one tenth this dose (0.15 mg.) administered to a small number of animals still had a detectable effect, and an action by so small a dose on the uterus had, as far as we are aware, not been described before, we repeated this experiment with a larger series of rats. The results are presented in this paper, together with those obtained with the higher dose (1.5 mg.).

**METHODS**

1. *Experiments with 1.5 mg. progesterone daily:*

   Female rats weighing approximately 200 gm. were spayed and left untreated for two weeks. They were then divided into four groups of five animals each and treated as follows:
   
   A. Arachis oil (solvent), twice daily 0.1 ml. subcutaneously for 4 weeks.
   
   B. Arachis oil for two weeks, followed for the next two weeks by 0.75 mg. progesterone twice daily.
   
   C. Oestradiol benzoate, 0.1 μg. twice daily for 14 days, followed by oil for the next two weeks.
   
   D. Oestradiol benzoate for 14 days, progesterone for the next two weeks.

   At autopsy the uteri were weighed and then fixed in formalin and stained with haematoxyline-eosine.

2. *Experiments with 0.15 mg. progesterone daily:*

   These were carried out on groups of four rats each (A₁ – D₁). The dosage-scheme was similar to that described above. Subsequently, 10 rats which received the same treatment were added to each of the groups A₁ and B₁.

**RESULTS**

1. *1.5 mg. of progesterone daily:*

   Comparison of histological preparations of the uteri from groups C and A revealed that a pronounced oedema of the endometrial stroma still existed 14 days after the last day of the oestrogen treatment. This swelling of the stroma had caused local obliteration of the lumen in some cases, with accompanying pressure atrophy of the epithelium. No signs of stimulation by oestrogen were to be seen in the epithelium apart from a slight increase in the number of poorly stained enlarged cells in those parts of the lumen where the
mucosal membrane is sharply bent. *Burkl et al.* (1954) observed the development of such cells after treatment for a few days with hexoestrol and showed that these cells contained mucin.

The effect of progesterone in oestrogen-pretreated rats can be demonstrated by comparing the groups D and C. Progesterone transformed the epithelium into an uninterrupted series of mucoid cells. The diameter of these cells measured from base to lumen greatly exceeded their width. The stromal oedema caused by oestradiol benzoate disappeared.

The effect of 1.5 mg. of progesterone alone may be determined by comparing groups B and A. Again, progesterone appeared to have increased the height of the epithelial cells, this time without the aid of oestrogen. Poorly stained, enlarged (mucoid?) cells were only present in the bends as described above for the rats treated with oestrogen alone. Again, they appeared to be slightly more numerous than in the untreated control animals. The thickness of the muscular layers had increased, thus corresponding to the larger average uterine weight found in group B: 177 against 92 mg. This may be considered to be a real difference, since the weight of each uterus from B surpassed that of the highest weight from group A. On the other hand, the difference between the uterine weights in C (282 mg.) and D (190 mg.) does not allow of any conclusion because of the wide spread of the individual values.

It follows from the evidence presented above that 1.5 mg. of progesterone daily certainly has some activity of its own. Formation of a fully developed mucous-producing epithelium, however, requires pretreatment with oestrogen. The development of stromal oedema by oestrogen was depressed by progesterone and in this respect the two substances acted as antagonists.

2. **0.15 mg. of progesterone daily:**

Comparison of group C and A1 leads to conclusions similar to those arrived at previously (C and A1: effect of oestrogen alone). By comparing groups D1 and B1 (progesterone with and without oestrogen-pretreatment) it appears that oestrogen did not influence in any way the effect of this low dose of progesterone. The stimulation of the epithelium (see below) was not enhanced. Moreover, the stromal oedema by oestrogen was not counteracted by 0.15 mg. of progesterone.

The effect of 150 μg. of progesterone alone (B1 and A1) was small but clear and was confined to the epithelium. The development of the muscular layers was not influenced by this dose of progesterone and this is in agreement with the absence of a difference between the uterine weights in groups A1 (112 mg.) and B1 (115 mg.) as well as between those of groups C1 (149 mg.) and D1 (152 mg.). The effect of 150 μg. of progesterone alone can best be shown from the results of the more accurate histological examination of the uteri of ten rats which were added to each of the groups A1 and B1:
A member of the laboratory staff unacquainted with the differences to be expected was asked to separate the slides into two groups by microscopic examination. He succeeded in classifying all but one of the preparations rightly into the groups A₁ and B₁, which in itself is an indication that some effect had been produced. Next, we attempted to describe the differences and to summarize them, as follows:

1. In 9 of 10 control uteri the epithelial nuclei as viewed through the microscope overlap each other. This was not seen in the progesterone group.
2. The nuclei were spherical (or nearly so) in 9 out of 10 control rats, whereas in 6 out of 10 progesterone uteri they were oval.
3. In 7 out of 10 progesterone treated uteri a broad zone of cytoplasm was present between the nucleus and the lumen. In the control uteri little cytoplasm was to be seen in this part of the cells.
4. In places where the epithelial membrane was sharply curved the before-mentioned pale and relatively large cells were observed in five control uteri and in nine progesterone treated uteri.

Some of these features (1 to 4) can be seen in Figs. 1 and 2.

Figs. 1 and 2.
2. Uterus of rat (C 2767) treated with 150 µg. progesterone daily from the 15th to the 28th day following ovariectomy. Body weight on the 29th day: 221 gm.; uterine weight: 139 mg. Haematoxyline-eosine. Broad zone of cytoplasm on the lumen side of the epithelial nuclei. Arrow: Large pale cell extruding its content.
Since all the observations so far had the disadvantage of being subjective we measured the height of the epithelium with a micrometer eyepiece. Fifteen measurements were made in each of the 20 (twice 10) uteri (oil-immersion, magnification approx. 1000 times). The mean height expressed in micrometer-units (with standard errors) amounted to 13.3 ± 0.7 in the progesterone group as against 11.0 ± 0.4 in the control group. The difference is significant, Student's t being 2.8.

All this makes it clear that a dose of only 0.15 mg. of progesterone, which is very small indeed, is able to produce certain effects in the uterine mucosa of the rat without any aid from oestrogen pretreatment.

It should be mentioned that we did not observe any enlargement of the stromal nuclei, such as described by Hooker & Forbes (1947, 1949) in the mouse. These authors used still smaller doses which were, however, injected directly into the uterus.

SUMMARY

The uterine epithelium of adult rats spayed 2 weeks previously reacted to treatment for 14 days with only 150 μg. of progesterone by a measurable increase in the cell height. The nuclei became oval. Somewhat more pale enlarged (mucin-containing?) cells were present near the corners of the lumen, an effect which has also previously been observed with oestrogen only.

REFERENCES

Howard, E. & Gengradon, S.: Endocrinology 26, 1048, 1940.