In a third experimental series it was decided to assay whether also a low dose of anabolic androgen is able to potentiate the inhibitory effect of fluprednisolone. The daily dose of anabolic steroid was reduced to 0.5 mg daily, whereas the one of corticosteroid was maintained at 0.25 mg daily. As shown by Table III, the results have evidently been in contrast to those obtained in the preceding experiment. Actually, the low dose of anabolic androgen, in combination with fluprednisolone given in doses causing a minimal or no response, neither appreciably affected the growth, nor the histology of granulation tissue (Figure 4).

*Discussion.* Analysis of our results reveals that, if anabolic androgens are given alone, they are unable to influence the growth of the granulation tissue in the normal processes of wound healing, independently from their chemical structure. Conversely, fluprednisolone, administered at a sufficiently large dose, is capable, as are other corticosteroids, of inhibiting the growth.

Granulation tissue in ear wounds of rabbits

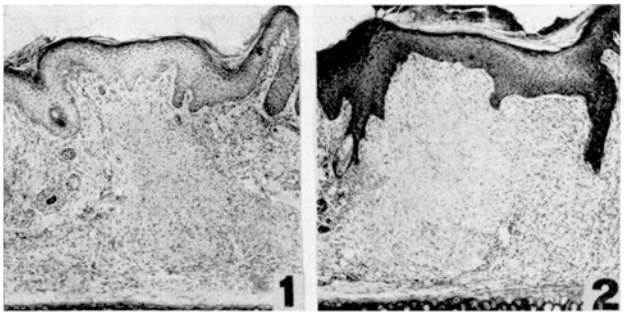


Fig. 1. Untreated control rabbit.

Fig. 2. Rabbit treated with methandrostenolone 5 mg/daily.

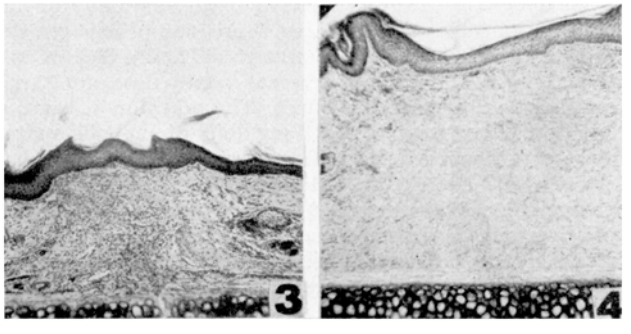


Fig. 3. Rabbit treated with fluprednisolone 0.25 mg/daily, and methandrostenolone 5 mg/daily.

Fig. 4. Rabbit treated with fluprednisolone 0.25 mg/daily, and methandrostenolone 0.5 mg/daily.

Table III. Effect of combined treatment, fluprednisolone and anabolic androgens (low dose), on production of granulation tissue in ear wounds of rabbits

| No. wounds | Group | Dose mg daily | Thickness of the granulation tissue mm $\pm \varepsilon$ | 'P' |
|---|---|---|---|---|
| 14 | Controls | – | $0.600 \pm 0.044$ | – |
| 8 | Fluprednisolone | 0.25 | $0.510 \pm 0.058$ | $> 0.05$ |
| 10 | Fluprednisolone<br>Oxymesterone | 0.25<br>0.50 | $0.534 \pm 0.062$ | $> 0.05$ |
| 10 | Fluprednisolone<br>Methandrostenolone | 0.25<br>0.50 | $0.621 \pm 0.059$ | $> 0.05$ |
| 10 | Fluprednisolone<br>Oestrenol | 0.25<br>0.50 | $0.645 \pm 0.057$ | $> 0.05$ |

In the experimental series where combined treatments were tested, by giving the corticosteroid in doses which caused little or no effect on granulation tissue, it is noteworthy that different doses of anabolic androgens showed different effects without any relationship to their chemical structure. Actually, in the high dose experiments, when the dose ratio between corticosteroid and anabolic androgen was 1:20, a significant additional effect was observed, whereas a dose ratio of 1:2 did not appreciably affect the growth of granulation tissue. For this reason, the inhibitory effect of the anabolic androgens, alone or in combination, observed by other authors using different test procedures[2,3,7,8], appears to be very likely related to the doses employed and not to the chemical structure of the compound. In our experiments, as well as in those of others[7,8], the effective inhibitory dose of anabolic steroid is high in terms of possible human application; however, an interaction between anabolic androgens and corticosteroid in the inflammation and, particularly, in the granulation processes appears to be well established.

*Résumé.* Les auteurs ont contrôlé l'effet de trois stéroïdes anabolisants sur la production du tissu de granulation par la méthode de la plaie cutanée chez le lapin. L'administration isolée de ces stéroïdes n'a modifié ni la quantité, ni la morphologie du tissue de granulation. L'association avec corticostéroïde, la fluprédnisolone, a donné des résultats différents selon la dose employée: en dose élévée, les anabolisants ont potencié l'effet inhibiteur de la fluprédnisolone, en dose faible, par contre, ils n'ont eu aucun effet.

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## Effects of Oestradiol and Progesterone on FSH and LH Contents of the Pituitaries and Blood of Ovariectomized Ewes

Several investigators are attempting to improve breeding efficiency of ewes by bringing anoestrous ewes into oestrus followed by successful fertilization. The results obtained so far showed that total gonadotrophins, FSH and LH contents of pituitaries of ewes during the non-breeding season were not different from those observed during the breeding season (WARWICK[1]; KAMMLADE et al.[2]; and HUTCHINSON and ROBERTSON[3]). SANTOLUCITO et al.[4],

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[3] J. S. M. HUTCHINSON and H. A. ROBERTSON, Nature *188*, 585 (1960).
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however, believe that pituitary FSH plays an active role in the induction of ovulation in ewes. Our findings showed that FSH level in the blood of ewes is greatly increased at prooestrus and LH level is at its maximum during oestrus, suggesting its importance in the ovulation mechanism (NASR et al.[5]).

Progestins play an important part in regulating the oestorus cycle of ewes as it is believed that they favour the release of gonadotrophic hormone (ALLEN and LAMMING[6]) and in small doses it potentiates oestrogen in order to induce oestrus in ovariectomized ewes (ROBINSON[7] and MELAMPY et al.[8]). The importance of progestins also appears from the observations of SANTOLUCITO et al.[4], who found that in ewes the corpus luteum of one cycle does not regress until after ovulation of the succeeding cycle and the formation of a new corpus luteum. The findings of HUTCHINSON and ROBERTSON[3] showed that the ovaries of anoestrus ewes contain small and large follicles but no corpora lutea.

In the present investigation we planned to study the effects of short term administration of a small dose of oestrogen alone and in combination with progesterone upon the FSH and LH contents of the pituitaries and blood of ovariectomized ewes.

Eight ovariectomized ewes of the Osemi breed, 3–4 years old were used. They were divided into 4 groups of two animals each. Group I was injected intraperitoneally with 1 ml cotton seed oil on two successive days. Group II was injected with 20 μg oestradiol benzoate on two successive days. Group III received 20 μg oestradiol benzoate on the first day followed by another dose of 20 μg oestradiol together with 10 mg progesterone on the second day. Group IV was treated as group III but received 20 mg progesterone instead of 10. The ewes were sacrificed and blood samples and pituitary glands were obtained from them. The proteins of the sera of treated ewes (containing gonadotrophins) were separated by acetone and washed with acetone–ether mixture to get rid of the steroid hormones (SZEGO and ROBERTS[9]). The serum proteins were redissolved in saline to bring them back to the original volume of serum. The pituitaries were dried with acetone and a saline suspension was prepared.

The method of assay of FSH used was that adopted by BROWN[10], which depends upon the HCG augmentation effect on FSH. For this purpose, groups of 20 immature female mice were used to determine the FSH contents of 0.5 ml serum and 1.0 mg fresh pituitary. The LH content of 0.5 ml serum and 2 mg pituitary was determined by using the method adopted by SOLIMAN[11]. For this purpose groups of 20 immature female mice were injected with 20 I.U. of PMS and after 4 days the test material was injected. After 48 h the mice were killed with ether and the average number of corpora hemorrhagica per mouse was used as a criterion of the LH activity.

It appears from the accompanying Table that the administration of 20 μg of oestradiol daily for two days to ovariectomized ewes did not cause any variation in FSH and LH contents of the pituitaries and serum as compared with their levels in control ovariectomized ewes.

The administration of a single dose of 10 mg progesterone into the oestrogen primed ovariectomized ewes resulted in a significant increase in the level of FSH in the blood, suggesting the stimulation of release of this hormone into the blood stream. The administration of 20 mg progesterone to the oestrogen primed ovariectomized ewes caused a significant decrease in the FSH content of the pituitary gland without any apparent change in the level of this hormone in the blood. It is suggested that this high dose of progesterone used stopped the synthesis and release of FSH. It also caused the outpouring of LH from the pituitary gland into the blood stream.

It is concluded then that in ewes both oestrogen and progesterone are necessary for regulating the synthesis and release of FSH and LH during the oestrous cycle. Progesterone seems also to have a double threshold effect when given conjointly with oestrogen; the low levels favour the release of FSH and the higher ones inhibit the synthesis and release of FSH but stimulates the release of LH into circulation[12].

Effects of oestrogen and progesterone on pituitary and serum gonadotrophins of ovariectomized ewes

| | FSH | | LH | |
|---|---|---|---|---|
| | Mouse ovary weights mg/100 g body weight | | Corpora hemorrhagica per mouse | |
| Treatment | Pituitary | Serum | Pituitary | Serum |
| Ovariectomized | 44.06 | 40.61 | 0.72 | 0.33 |
| | 2.09[a] | 3.59[a] | 0.12[a] | 0.10[a] |
| Oestradiol | 39.81 | 42.35 | 0.68 | 0.21 |
| | 2.18[a] | 4.17[a] | 0.15[a] | 0.12[a] |
| Oestradiol | 40.84 | 53.23[b] | 0.76 | 0.83 |
| 10 mg progesterone | 2.56[a] | 3.67[a] | 0.19[a] | 0.32[a] |
| Oestradiol | 35.73[b] | 41.83 | 0.35[b] | 1.19[b] |
| 20 mg progesterone | 1.43[a] | 3.92[a] | 0.02[a] | 0.35[a] |

[a] Standard error.
[b] Significantly different from ovariectomized ewes at 5% level of probability.

*Zusammenfassung.* Zufuhr von 20 μg Östradiolbenzoat an zwei sich folgenden Tagen verursachte bei ovariektomierten Mutterschafen keine Veränderung im FSH- und LH-Gehalt von Hypophyse und Serum. Nach einmaliger Verabreichung von 10 mg Progesteron (nach Östradiolbehandlung) stieg das FSH im Blut an. Ebenso ergab Zufuhr von 20 mg Progesteron bei mit Östrogen behandelten Mutterschafen Anstieg des LH-Gehaltes im Blut.

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[11] F. A. SOLIMAN, Nature *183*, 321 (1960).
[12] Thanks are due to Prof. Dr. N. HELMI, head of the Surgery Department for performing ovariectomy to the ewes.