

## Effect of Anabolic Androgens on Granulation Tissue Production in the Rabbit

The experimental studies carried out in order to assess the effect of anabolic androgens on inflammation have given contrasting results<sup>1-4</sup>. Likewise, the interaction of these steroids and corticosteroids on inflammation is still an open question<sup>3-8</sup>. The discrepancies in the results may be attributed to differences in the test procedures employed, as well as to the variable chemical structure of the steroid assayed and the different dosage. Hence, a further investigation has been planned on this subject, using different anabolic androgens at various dose levels, administered alone or together with a very active corticosteroid, such as fluprednisolone. As experimental test, artificially produced wounds were used, since this procedure has been already employed with good results to establish the inhibitory effect of corticosteroids on the normal granulation processes<sup>9,10</sup>.

**Material and Methods.** The present investigation was carried out on rabbits of both sexes, weighing 1800-2000 g and maintained on a well balanced diet. Aseptic skin wounds were made on both ears, using a method similar to the one employed by HOWES<sup>11</sup>.

The following anabolic androgens were tested: 17 $\alpha$ -methyl-17 $\beta$ -hydroxyandrost-1,4-dien-3-one (methandrostenolone, Dianabol Ciba), 17 $\alpha$ -ethyl-4-estrene-17 $\beta$ -ol (oestrenol, Orgabolin, N.V. Organon), 4-hydroxy-17 $\alpha$ -methyl-17 $\beta$ -hydroxyandrost-4-en-3-one (oxymesterone, Oranabol Farmitalia). As corticosteroid, 6 $\alpha$ -fluoroprednisolone (fluprednisolone, Etadrol Farmitalia) was used. The steroids were dissolved in Tween 80 and injected subcutaneously; daily doses varied according to the experimental series. Control animals received Tween 80 alone. Single and combined treatments were carried out, starting three days before the wounds were made and continuing for 8 days thereafter until the time of sacrifice.

At histological examination, the maximal thickness of the granulation tissue formed was calculated and the results evaluated statistically. Infected wounds were not considered. The standard error of the means (' $\epsilon$ ') was calculated according to the formula  $\epsilon = \sqrt{\epsilon^2/n(n-1)}$ , and the statistical significance of the difference (' $P$ ') according to the Student test (' $t$ '); values of ' $P$ ' < 0.001 were considered to be significant. Three experimental series were carried out.

**Results.** In a first experimental series the effect of the three anabolic androgens administered alone, 5 mg daily per animal, on production of granulation tissue in ear wounds was studied. As shown by Table I, such a high dose neither stimulated nor inhibited the amount of granulation tissue. Histologically, no differences were observed between control and steroid-treated animals; actually, in all groups under experiment, collagen and reticulin network, fibroblastic proliferation, new formation of blood vessels, and amount of PAS-positive ground sub-

stance were practically the same. At the end of the experimental period, the epithelization of the wounds was completed both in treated and untreated animals (Figures 1 and 2).

In a second experimental series, the effect of the association of anabolic androgens and fluprednisolone was tested. 5 mg of anabolic androgens were injected daily. The corticosteroid, which when given at 1 mg daily per rabbit resulted in a significant inhibition of the growth of granulation tissue, was injected at a daily dose as low as 0.25 mg daily, i.e. at a dose which did not appreciably affect the height of granulation tissue, or produced only a minimal inhibition of no statistical significance. As shown by Table II, the combined treatment led to a significant reduction of the thickness of granulation tissue when compared to control animals, the inhibitory effect being practically of the same degree for all the anabolic androgens tested.

Table II. Effect of fluprednisolone alone and combined with anabolic androgens (high dose) on production of granulation tissue in ear wounds of rabbits

No. wounds	Group	Dose mg daily	Thickness of the granulation tissue mm $\pm \epsilon$	'P'
14	Controls	—	0.600 $\pm$ 0.044	—
10	Fluprednisolone	1	0.331 $\pm$ 0.049	< 0.001
8	Fluprednisolone	0.25	0.510 $\pm$ 0.058	> 0.05
8	Fluprednisolone Oxymesterone	0.25 5	0.382 $\pm$ 0.057	< 0.001
10	Fluprednisolone Methandrostenolone	0.25 5	0.288 $\pm$ 0.062	< 0.001
8	Fluprednisolone Oestrenol	0.25 5	0.275 $\pm$ 0.038	< 0.001

Histologically, rabbits injected with 1 mg daily of fluprednisolone showed a striking depression of new-growth of all elements of the connective tissue and a lack of epithelization of the wounds, whereas a daily dose of 0.25 mg produced no appreciable effect. The addition to such a low dose of corticosteroid of 5 mg daily of anabolic androgen led to a marked depression of the granulation tissue: few new blood vessels could be seen, fibroblasts in compact arrangement were present around old blood vessels, amorphous ground substance was scarce and elaboration of reticulin and collagen fibrils was consistently reduced. Frequently the wounds were open without epithelization (Figure 3).

Table I. Effect of anabolic androgens on production of granulation tissue in ear wounds of rabbits

No. wounds	Group	Dose mg daily	Thickness of the granulation tissue mm $\pm \epsilon$	'P'
14	Controls	—	0.600 $\pm$ 0.044	—
8	Methandrostenolone	5	0.420 $\pm$ 0.032	> 0.05
9	Oestrenol	5	0.424 $\pm$ 0.045	> 0.05
10	Oxymesterone	5	0.563 $\pm$ 0.076	> 0.05

<sup>1</sup> K. KOWALEWSKI and G. GORT, *Acta Endocrinol.* 30, 273 (1959).

<sup>2</sup> A. RUBENS-DUVAL and J. VILLIAUMEY, *Ann. Endocrinol.* 23, 648 (1962).

<sup>3</sup> G. JASMIN, P. BOIS, and A. MONGEAU, *Exper.* 16, 212 (1960).

<sup>4</sup> V. BARBERA, L. POLLICE, and L. MAZZARELLA, *Exper.* 18, 424 (1962).

<sup>5</sup> W. V. B. ROBERTSON and E. C. SANBORN, *Endocrinol.* 63, 250 (1958).

<sup>6</sup> L. A. BAVETTA, J. BEKHOR, R. SHAH, P. O'DAY, and M. E. MINNI, *Endocrinol.* 71, 221 (1962).

<sup>7</sup> F. TANI, G. BALDRATTI, and G. SALA, *Intern. Congr. Horm. Steroids, Excerpta Med. Found.* 1962, 213.

<sup>8</sup> A. A. FRENZI and J. J. CHART, *Proc. Soc. exp. Biol. Med.* 110, 259 (1962).

<sup>9</sup> C. RAGAN, E. L. HOWES, C. M. PLOTZ, K. MEYER, J. W. BLUNT, and R. LATTES, *Proc. Soc. exp. Biol. Med.* 72, 718 (1949).

<sup>10</sup> C. CAVALLERO, M. BORASI, G. SALA, and A. AMIRA, *Arch. int. Pharmacodyn.* 86, 43 (1951).

<sup>11</sup> E. L. HOWES, *Surg. Gynec. Obst.* 76, 738 (1943).

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

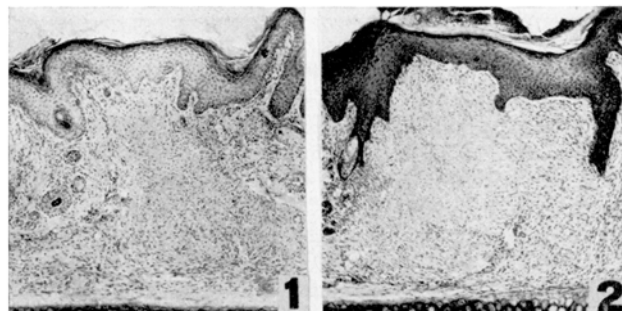


Fig. 1. Untreated control rabbit.

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

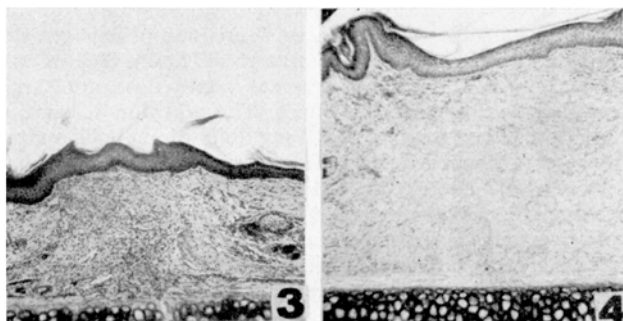


Fig. 3. Rabbit treated with fluprednisolone 0.25 mg/daily, and methandrostenedione 5 mg/daily.

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

### 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

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 $\pm$ $\epsilon$	'P'
14	Controls	–	0.600 $\pm$ 0.044	–
8	Fluprednisolone	0.25	0.510 $\pm$ 0.058	> 0.05
10	Fluprednisolone	0.25		
	Oxymesterone	0.50	0.534 $\pm$ 0.062	> 0.05
10	Fluprednisolone	0.25		
	Methandrostenedione	0.50	0.621 $\pm$ 0.059	> 0.05
10	Fluprednisolone	0.25		
	Oestrenol	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<sup>2,3,7,8</sup>, 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<sup>7,8</sup>, 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 tissu de granulation. L'association avec corticostéroïde, la fluprédnisolone, a donné des résultats différents selon la dose employée: en dose élevée, les anabolisants ont potentialisé l'effet inhibiteur de la fluprédnisolone, en dose faible, par contre, ils n'ont eu aucun effet.

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breeding season (WARWICK<sup>1</sup>; KAMMLADE et al.<sup>2</sup>; and HUTCHINSON and ROBERTSON<sup>3</sup>). SANTOLUCITO et al.<sup>4</sup>,

<sup>1</sup> E. J. WARWICK, Proc. Soc. exp. Biol. Med. 63, 560 (1946).

<sup>2</sup> W. G. KAMMLADE JR., J. A. WELCH, A. V. NALBANDOV, and H. W. NORTON, J. Animal Sci. 11, 646 (1952).

<sup>3</sup> J. S. M. HUTCHINSON and H. A. ROBERTSON, Nature 188, 585 (1960).

<sup>4</sup> J. A. SANTOLUCITO, M. T. C. CLEGG, and H. H. COLE, Endocrinology 66, 273 (1960).