The Relation of the Antifibromatogenic Activity of Certain Steroids to Their Molecular Structure and to Various Actions of These Hormones*†‡

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Three different synthetic steroids, progesterone, desoxycorticosterone acetate, and testosterone propionate, have been shown to have the capacity of preventing abdominal fibroids elicited by estrogens. The question arises as to which are the special molecular structures responsible for the antifibromatogenic activity of these compounds. Different structures are of interest here (Fig. 1): first, the ketonic oxygen in position 3 and the double bond, △ 4-5, in the ring I which are common to the three steroids; second, the short side chain of carbons 20 and 21 attached in position 17 by which progesterone and desoxycorticosterone differ from testosterone. The importance of these molecular conditions will be discussed by comparing the antifibromatogenic activity of these three steroids and of a fourth, androstanediol (Fig. 1, IV), in which the double bond and the ketonic oxygen in position 3 are lacking.

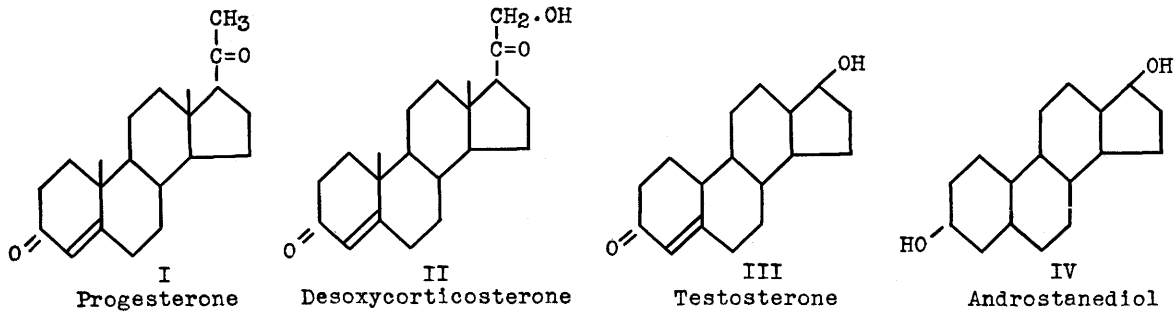

FIG. 1

EXPERIMENTAL

A total of 173 animals was used for comparison (Tables I and II). The control group was of animals treated with subcutaneous tablets of estradiol only; other groups were of animals in which tablets of estradiol and of one of the above-mentioned four steroids were simultaneously present. The 27 animals of the control group (a) of Table II were chosen from different experimental series; only animals in which absorption of estradiol per day was not greater than in the remaining groups were used. On account of the great variation one meets in this kind of experiment, a second series of 20 animals, group (b) in Table II, was included in the control group. This series (b) was chosen because it has given so far a lower *average* tumoral effect with tablets of estradiol than any found in similar control groups; for details of series (b) see Table I of the thesis of Nuñez (17). The desoxycorticosterone group is the same as that of the preceding paper (13). As to the progesterone, testosterone, and androstanediol groups, greater details will be found in the thesis of González and Vera (in preparation). The animals of the testosterone-treated group were also cited partly from previous work by Lipschütz, Vargas, and Nuñez (16). The weight of the tablets, or fragments, of the fibromatogenic estradiol and the antifibromatogenic steroids used in the 173 animals of Tables I and II was between 0.6 and 90 mgm. In this way the necessary variation of quantities absorbed was assured, since absorption depends on the surface.

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Comparison was made on the basis of the following four criteria:

1. The daily threshold quantity of the antifibromatogenic steroid, or the minimum quantity above which individual spherical fibroids of class 1 (about 1 mm. in diameter) were absent. The fibrous reaction consisted in these animals of small nodules only, "tumoral seed," or fibrous strands. This type of extragenital reaction was always present in our work with antifibromatogenic steroids. Indeed we are as yet not sure whether the minimum quantity is likely to give an exact expression of the antifibromatogenic faculty of the steroid. But, as explained in the preceding paper (13), neither can the ratio between the fibromatogenic and antifibromatogenic steroids we have as yet used for this purpose (15-17) be considered as an exact measure in this kind of experiment.

2. The average tumoral effect in the respective group. We have emphasized that the tumoral effect is very variable. The standard deviation of the average and the significant difference also have been calculated according to the method of Burn (1).

3. The number of animals which reached in the respective group the average tumoral effect of the control group.

4. The number of animals which had individual tumors of class 1 (see above).

The results are summarized in Table II. Details referring to experiments with progesterone, testosterone, and androstanediol are to be found also in Fig. 2 of the present paper; for details referring to desoxycorticosterone see Fig. 2 of the previous paper (13).

As seen from Table II, androstanediol behaved differently from the other 3 steroids: abdominal fibroids were produced even when 224 μgm. of androstanediol daily were given against 77 μgm. of estradiol, or 167 μgm. against 40 μgm. The average tumoral effect in the androstanediol group was higher than in the control series (b); the difference between the androstanediol group and the control series (a) was not significant. Neither was there any difference between the androstanediol and control group as to the number of animals which reached the average tumoral effect of the control series especially when compared with the series (b), or as to the number of animals with tumors class 1. It is evident that there was no inhibitory effect with androstanediol under the present experimental conditions. This does not exclude the possibility that greater quantities of this steroid

TABLE I: WEIGHT OF TABLETS AND QUANTITIES ABSORBED IN THE DIFFERENT GROUPS

Group	Tablets of estradiol: Initial weight, mgm.	Tablets of estradiol: Absorbed, mgm.	Tablets of antifibromatogenic steroid: Initial weight (esterified steroid), mgm.	Tablets of antifibromatogenic steroid: Absorbed (free steroid), mgm.
Control	0.6-24.0	0.3- 4.1	.....	.....
Progesterone	8.4-28.1	1.2- 4.5	6.8-35.0	3.4-13.6
Desoxycorticosterone acetate	3.0-38.5	1.3-13.5	1.3-38.5	1.2-14.6 *
Testosterone propionate	3.2-27.0	1.1- 4.2	10.8-32.6	4.5-14.0 *
Androstanediol propionate	9.0-22.0	1.9- 4.3	10.5-89.8	1.5-12.5 *

* Values obtained by dividing the original figures by 1.13, 1.19, and 1.19 respectively.

TABLE II: SUMMARY OF RESULTS

Substances and quantities absorbed per day, μgm.*	Anti-fibromatogenic threshold, μgm.	Average total tumoral effect, (T.T.E.)	Significant difference ‖	Number of animals: Total	Number of animals: With tumors, class 1 †	Number of animals: Reached average T.T.E. of control group	Duration, in days
Estradiol-control 5-63 (a)	.....	4.9 ± 0.56 §	..	27	22 (2)	12	50-62
[35-74] (b)	.....	[3.0 ± 0.54]	..	[20]	[14]	[10]	[54-62]
Estradiol 19-62 and progesterone 54-220	± 65	1.3 ± 0.07	6.3 [3.1]	28	0 (4)	0 [0]	62-64
Estradiol 18-214 and desoxycorticosterone acetate 20-231	< 90	1.6 ± 0.22	5.5 [2.4]	42	10 (3)	2 [7]	57-64 ‡
Estradiol 17-75 and testosterone propionate 70-225	200	1.4 ± 0.35	5.3 [2.5]	25	7	3 [4]	55-65
Estradiol 34-77 and androstanediol propionate 26-224	> 224	3.5 ± 0.59	1.7 [ ] ¶	11	8	3 [8]	55-57

* Quantities of free hormones are given.
† Figures in parentheses: animals intermediate between 0.5 and 1.
‡ Four animals 70 to 73 days.
§ $\epsilon = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$ (standard deviation of the average).
‖ $\frac{m_{ctr} - m_{exp.}}{\sqrt{\epsilon^2_{ctr} + \epsilon^2_{exp.}}}$ (significant difference); m = average.
¶ $m_{exp.}$ greater than $m_{ctr.}$.

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may be active. But our results show that the antifibromatogenic activity is reinforced by the double bond in ring I or the ketonic oxygen in position 3 (see especially Fig. 1, III and IV). It cannot be said whether reinforcement of antifibromatogenic activity is effected through the simultaneous presence of both these structural conditions or whether only one of both is relevant. This question is under investigation.[1]

Testosterone has considerable antifibromatogenic activity. The difference between the average tumoral effect in the testosterone and the control groups is significant; the number of animals in the testosterone group reaching the average of the control groups is small. Is there also a difference between the testosterone-treated group and those treated with progesterone and desoxycorticosterone? The determination of the antifibromatogenic threshold was certainly not an exact one. But the distance between the threshold values for testosterone on one side, and for progesterone and desoxycorticosterone on the other, was considerable. As to the other criteria, the difference between testosterone and progesterone also was remarkable. All this points to a lesser antifibromatogenic activity of testosterone as compared with that of progesterone. One may suppose that the difference was due to the absence of the short lateral chain of carbons 20 and 21 (Fig. 1, I and III).

According to our results the antifibromatogenic activity of progesterone was apparently also greater than that of desoxycorticosterone acetate. This would raise the question of whether oxidation in position 21 by which desoxycorticosterone differs from progesterone (Fig. 1, I and II) attenuates the antifibromatogenic activity. At present we do not feel warranted in expressing a definite opinion on this subject on account of the difficulties of the exact determination of the threshold quantity (13).

DISCUSSION

Notwithstanding the difficulties which present themselves when one tries to interpret our quantitative results with the antifibromatogenic action of different steroid hormones, three fundamental observations attract attention: first, steroid hormones prevent fibroids elicited by estrogens; second, preventive activity of steroids differs quantitatively and these differences coincide with those of molecular structure; third, a cortical hormone competes quantitatively with progesterone as a powerful antifibromatogenic agent. The descending order of antifibromatogenic activity is most likely the following: progesterone > desoxycorticosterone > testosterone > androstanediol. Since it was in experiments with estrogens that the antifibromatogenic action of certain steroids and their descending order was studied one must raise the question whether this inhibitory action is coincident with an antiestrogenic action in general.

Antifibromatogenic and antiestrogenic activity.— Antifibromatogenic action of steroids in our experiments was certainly concomitant with an inhibitory action against several other effects due to estrogens, such as the increase of the uterine weight, uterine bleeding, glandular hyperplasia, and formation of polyps which also were partly counteracted (unpublished results). Development of the mammary gland was not diminished. But the most essential question is whether antifibromatogenic and antiestrogenic effects follow the same descending order; *i.e.,* whether greater antiestrogenic activity is coincident with greater antifibromatogenic action. So far this can be discussed quantitatively only for the following antiestrogenic actions: 1. inhibition of the cornifying action of the vaginal mucosa of the rat and mouse, and 2. inhibition of the increase of uterine weight and of the opening of the vagina in the guinea pig. According to Courrier and Cohen-Solal (2, 3) and to Robson (18) the quantities of progesterone which were necessary to inhibit the cornifying action of estrogens (estrone, estradiol) on the vaginal mucosa of the rat or mouse were greater than those of testosterone. On the contrary, in our work with guinea pigs, greater antifibromatogenic activity of progesterone and desoxycorticosterone when compared with testosterone was coincident with a more effective inhibition of the increase of uterine weight and of the opening of the vagina as shown by Lipschütz, Vargas, and Nuñez (16), and likewise with a more pronounced inhibiting action against atypical proliferation of the uterine epithelium (unpublished results).

Androstanediol inhibited cornification of the vaginal mucosa in the mouse, according to Robson (18); it was many times less active than testosterone but superior to progesterone. In our experiments with guinea pigs androstanediol, whose antifibromatogenic activity was nil, had neither an inhibiting action against the increase of the uterine weight nor against the opening of the vagina.

The descending order of antiestrogenic activity in the rat and mouse is not identical with that in the guinea pig. This gives evidence that our statements made for one species with reference to the coincidence of antifibromatogenic and antiestrogenic activities are not valid for other species. When making the attempt to bring both types of action into a quantitative agreement one should also not forget that various steroids

[1] In the meantime we have found that the double bond in ring I is not essential for antifibromatogenic activity when the ketonic oxygen in position 3 is present. Dihydrotestosterone (propionate) in which the double bond is lacking was still antifibromatogenic, contrary to androstanediol (propionate) in which both the double bond and the ketonic oxygen are absent.

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such as testosterone and androstanediol have not only an antiestrogenic, but also a "cooperative" action, in the sense that they augment the stimulating action of estrogens on the genital tract (9). An estrogenic action has been demonstrated by Speert (20) even for so as our own work is concerned there is evidence that this is not the case. A transformation of the clitoris into a hypospadic penis-like organ identical with that which has been observed under the influence of a testicular graft (11, 12) was effected with quantities

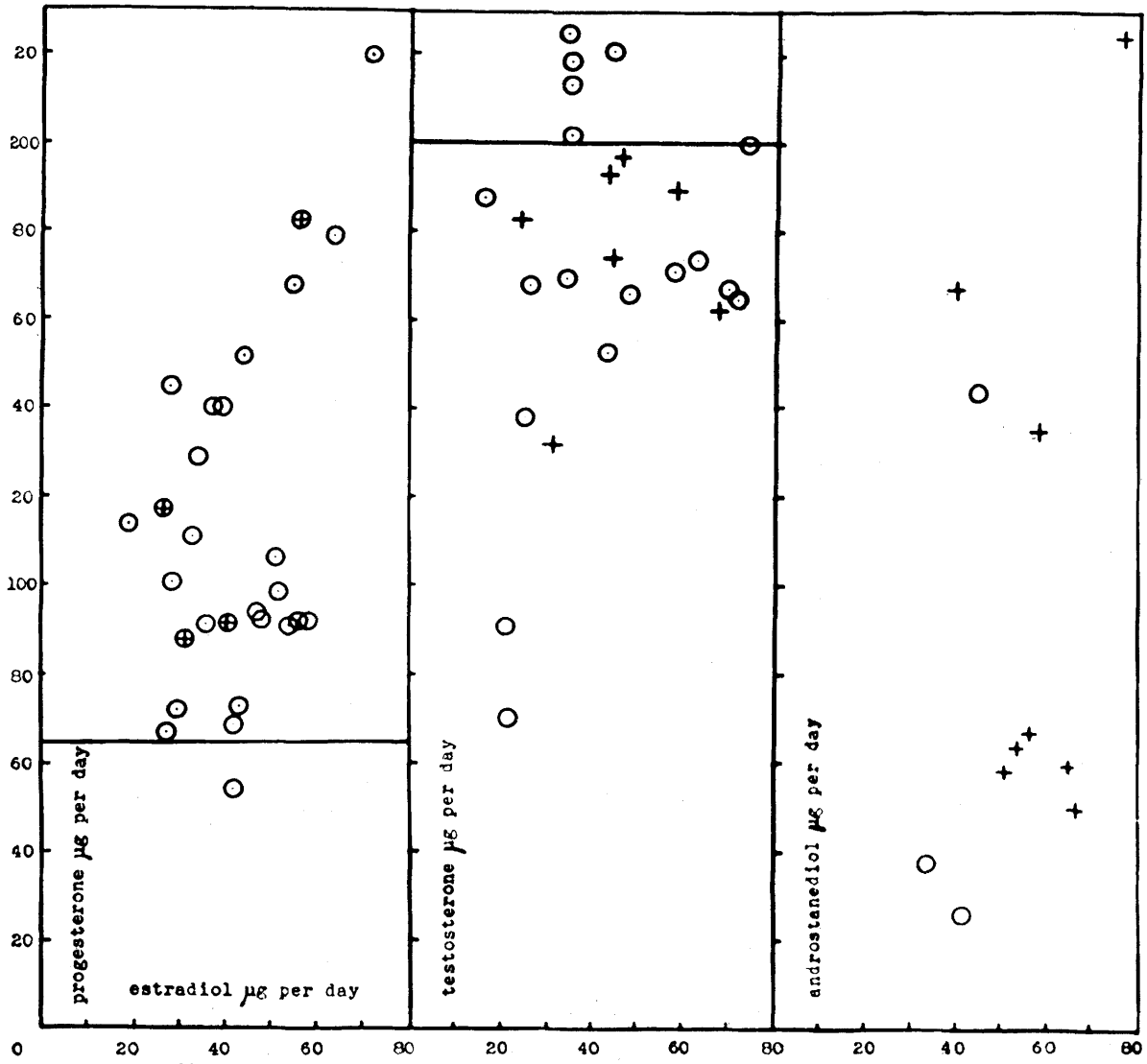

FIG. 2.—Graphical summary of data from Tables I and II showing the tumoral reactions in female guinea pigs with subcutaneously implanted tablets of both estradiol and antifibromatogenic steroids. Varying quantities of the hormones were absorbed.

○ = animals without fibroids.
+ = animals with fibroids.
⊕ = animals with individual fibroids transitional between classes 0.5 and 1.

powerful an antifibromatogenic steroid as desoxycorticosterone.

Antifibromatogenic and masculinizing activity.—One must also question whether the descending order of the antifibromatogenic activity of steroids is coincident with that of their masculinizing activity. As far of testosterone, antifibromatogenic or not (14, 19). This masculinizing effect was never observed with dosages of progesterone or desozycorticosterone much greater than those necessary for antagonizing the fibromatogenic effect of the estrogen (14, 15). It is well known that these steroids can exert a masculinizing

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action; but our results show that the antifibromatogenic threshold is certainly smaller than the masculinizing one with these two steroids.

As to androstanediol, the quantities used which were not antifibromatogenic did not produce the masculinizing effect on the clitoris.

Antifibromatogenic and progestational activity.— The three antifibromatogenic steroid compounds have also a progestational action. Comparative data as to the progestational activity of the three compounds are not very numerous; but it decreases apparently from progesterone to desoxycorticosterone and testosterone according to Klein and Parkes (8) and Leathem and Crafts (10). No data are to be found in the literature on any progestational activity of androstanediol, as indicated by Korenchevsky (9). Though all these quantitative data and likewise those referring to antifibromatogenic activity are by no means exact there is much probability that the descending order of both these activities of progesterone, desoxycorticosterone, and testosterone is the same.

This discussion is summarized in Table III.

It seems contradictory that the descending order of the antifibromatogenic and antiestrogenic activities of certain steroids should not coincide with that of masculinizing activities,[2] whereas the descending order of two activities so contradictory as the progestational and the antifibromatogenic should be the same. Progestational activity is associated with stimulation of proliferation of epithelial and conjunctive cells whereas antifibromatogenic activity means inhibition of proliferation of those cells which give origin to fibroblasts and connective fibers; and it will be shown in subsequent publications that antifibromatogenic action of progesterone and desoxycorticosterone is concomitant also with inhibition of epithelial proliferation. But one must emphasize that both stimulation and inhibition of proliferation may depend upon different quantitative levels of the antagonistic substances in play, as has been recently shown by Crabtree (4) in his work with the administration of different concentrations of chloracetones to the skin of animals painted simultaneously with carcinogenic hydrocarbons.

Our statement that antifibromatogenic activity coincides with the progestational effect is in accord with the findings of Klein and Parkes (8) who established that methylation or ethylation in position 17 reinforces the progesterone-like activity of testosterone and confers progesterone-like activity on androstanediol. Similarly, antifibromatogenic activity, according to our findings, is reinforced by the short side chain of carbons attached in position 17.

TABLE III: COMPARATIVE ACTIVITIES OF STEROIDS

Action	Descending order of activity	Authorities
Antifibromatogenic	Progesterone > desoxycorticosterone > testosterone > androstanediol	The present paper
Antiestrogenic (inhibition of increase of uterine weight and of opening of vagina, in guinea pig)	Progesterone and desoxycorticosterone > testosterone > androstanediol	Lipschütz, Vargas and Nuñez (16) Vera (unpublished)
Antiestrogenic (vaginal mucosa of the castrated rat)	Testosterone > androstanediol > progesterone	Courrier and Cohen-Solal (2, 3) Robson (18)
Masculinizing (hypertrophy of the clitoris of the guinea pig)	Testosterone > desoxycorticosterone and progesterone	Lipschütz and Vargas (13, 14) Ruz (16) Vera (unpublished)
Progestational (in the sensitized rabbit)	Progesterone > desoxycorticosterone > testosterone	Klein and Parkes (8) Leathem and Crafts (10)

Our results are in accord also with clinical observation. It has been claimed for a long time that uterine fibroids are more frequent in virgin women than in nonvirgins; and that fibroids are more frequent in childless women as compared with women who have given birth to children (5). The assertion has been made also by Pinaud, quoted from Forgue and Massabuau (6), that their frequency was greater in women who became pregnant only at a more advanced age and had few pregnancies. One may tentatively assume that the longer action of progesterone exerted a preventive influence in women with pregnancies or with a greater number of pregnancies. Intervention of cortical steroids also has to be given attention in consideration of the causes of fibroids in women since it has been shown that desoxycorticosterone is a powerful antifibromatogenic factor.

Two parallel statements have been discussed above: first, that progesterone is seemingly the ideal physiological antifibromatogenic steroid, and second, that the descending order of the antifibromatogenic activity of a group of steroids was coincident with that of their

[2] But one should not forget that the short side chain attached in position 17 which reinforces progestational and antifibromatogenic activity diminishes masculinizing activity. This becomes especally clear when one compares the potency of testosterone and ethinyl-testosterone: addition of an ethinyl group to testosterone reduces its masculinizing activity about 600 times, as shown by Emmens and Parkes (6).

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progestational activity. But it must be kept in mind that the second statement cannot be generalized. The possibility should not be excluded that when a greater number of steroids are examined, antifibromatogenic and progestational activities may be found to diverge and that a steroid may be eventually encountered which could be antifibromatogenic without having progestational activity.

SUMMARY

The faculty of preventing fibroids elicited by estrogens has been studied comparatively for different synthetic steroids with the purpose of relating antifibromatogenic activity to molecular structure.

The antifibromatogenic threshold, or the minimum quantity which must be released per day by a subcutaneous tablet of the antifibromatogenic steroid so as to inhibit fibroids, was lowest with progesterone; desoxycorticosterone (acetate) was next in order; testosterone (propionate) was highly active but less than progesterone and desoxycorticosterone; androstanediol (propionate) had no antifibromatogenic action.

The diminution of the average tumorigenic effect and other quantitative criteria of antifibromatogenic action were in accord with the statements referring to the antifibromatogenic threshold.

The question is discussed whether the descending order of the antifibromatogenic faculty of different steroids is coincident with that of their antiestrogenic and masculinizing activities. This was the case for the antiestrogenic activity in the guinea pig but not in the rat, and not for the masculinizing activity. There was a coincidence between the descending order of the antifibromatogenic activity as studied in the guinea pig and the progestational activity as studied in the rabbit. It would be unwise to generalize these statements on account of differences in the species.

Antifibromatogenic activity of steroids is apparently reinforced by the ketonic oxygen in position 3 or by the double bond, △ 4-5, ring I, or by both of these, and by carbons attached in position 17 (the short side chain of carbons 20 and 21). The latter is in accord with former statements of Klein and Parkes referring to the molecular conditions of progestational activity of steroids.

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