Technical Papers

Comparative Antifibromatogenic Action of Cortical Steroids[1]

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Antitumorigenic actions of steroids have been studied since 1936, first in laboratory animals and later in patients. It is not intended to summarize this work; a tabulated summary for 1936–1950 has been given elsewhere (*1*). The purpose of this paper is to discuss results obtained in the course of the past year in our work with the prevention of estrogen-induced abdominal fibroids by cortical steroids, or corticoids. Special emphasis will be laid upon those findings which, seemingly, are implicated in any concept of the antitumorigenic action of steroids in general.

Progesterone. We may start from the well-known fact that estrogen-induced abdominal fibroids are prevented when progesterone is administered simultaneously with estradiol (*1*). The antifibromatogenic action is not linked with the primary physiological faculty of progesterone, or its progestational potency. This is shown by the following facts: Compared to progesterone, testosterone has both relatively poor progestational and antifibromatogenic faculties. Yet the progestational potency of testosterone is enhanced by a side chain of two carbons at C_{17}; however, therewith its antifibromatogenic potency is not increased. Thus, ethinyl-testosterone, vinyl-testosterone, ethyl-testosterone are not more—and indeed are even less—antifibromatogenic than is testosterone (*2*). Likewise, androstenediol acquires progestational faculty by substitutions at C_{17}; ethinyl-androstenediol and methyl-androstenediol are progestational, but they have been found inactive against estrogen-induced fibroids, as was androstenediol (*3–5*). These new experimental findings give definite evidence that antifibromatogenic action is not dependent on the progestational action of progesterone. It is an "independent" action of this steroid, to use a term of Selye's.

Does this mean that progesterone, as a steroid of a certain chemical structure, is endowed with antitumorigenic faculties in general? This fundamental question has to be answered in the negative. Indeed, like estrogen-induced abdominal fibroids, estrogen-induced epithelial and conjunctive proliferation of the uterine tract and of the prostatic region in the guinea pig also can be prevented with progesterone (*6*). In this species estrogen-induced proliferation of the mammary gland was not prevented; in the rat, estrogen-induced tumoral growth of the anterior lobe of the hypophysis did not diminish when progesterone was given (*1*). Growth of transplantable lymphosarcoma in mice was not inhibited by progesterone (*7*). Here we find what is certainly a disappointing aspect in antitumorigenic actions of steroids: they act on *specific territories,* and not on others. But this disappointment is by no means prohibitive, as best shown by clinical experiments with progesterone in humans. Indeed, no regression was obtained in cases of uterine fibroids in women, possibly because the quantities administered were too small (*8*). Yet there was some antitumorigenic action in prostatic carcinoma (*9*) and in cancer of the cervix (*10*).

According to the classical work of the Pincus-Hechter group, progesterone appears to be an intermediate in cortical steroidogenesis (*11*). But when reference is made to corticoids, certainly desoxycorticosterone and cortisone are meant in the first place.

Desoxycorticosterone and cortisone. Desoxycorticosterone is second only to progesterone with respect to antifibromatogenic activity (*12*). It also prevents seemingly transplantable leucemia in the rat (*13*). Interest has recently been aroused by antitumorigenic action of cortisone, which counteracts the growth of transplantable lymphatic tumors (*14–18*). Of especial interest in our studies is the fact that cortisone inhibits cicatrization of wounds (*19–21*); it also inhibits hepatic fibrosis induced by CCl_4 (*22–23*) and periarteritis induced by desoxycorticosterone (*24*); cortisone even counteracts spontaneous arteriosclerosis in fowl (*25*). The fibrolytic action of cortisone in many of these cases is possibly a complex one; but this does not alter the fact that in all these cases cortisone has been shown capable of interfering as an inhibitor in proliferative phenomena of the mesenchyma (*5*).

We were rather surprised to find that cortisone was unable to prevent the growth of estrogen-induced abdominal fibroids, even with quantities several times those of progesterone or desoxycorticosterone (*5, 26*). Very large quantities of cortisone, about 25–50 times those of progesterone, must be administered to prevent fibroids, and even then fibroids may appear in some animals. It would be idle to try to explain why estrogen-induced fibroids do not respond to cortisone in the same way as other types of mesenchymatic proliferation do. One may mention in this connection the differential response of specific territories, but it is more likely that the weak response of abdominal

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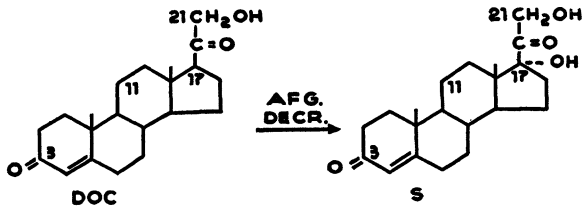

FIG. 1.

fibroids to cortisone is due to the special stimulus that is responsible for this mesenchymatic tumoral proliferation. This explanation introduces a new and most important problem into the discussion of the antitumorigenic action of steroids—the question of the special and complex mechanisms underlying this action. It is certainly a fundamental question to which we shall return; but first we must persevere in the discussion of the comparative antifibromatogenic action of corticoids.

In our work with progesterone and other progestational steroids we recognized that antifibromatogenic action was not dependent on their progestational potency. Antifibromatogenic action was not linked with the androgenic faculty of certain steroids, nor is the antifibromatogenic action of desoxycorticosterone due to its corticoid faculties. This conclusion was reached in work with steroids that can replace desoxycorticosterone, to a certain degree, in regard to its corticoid action. It was found that 21-acetoxypregnenolone, which has some corticoid action, is not antifibromatogenic; on the contrary, pregnenolone-3-acetate with very poor cortical replacement potency, is antifibromatogenic (*2, 27–29*). Thus, the fact that cortisone is not antifibromatogenic corroborates our concept that antifibromatogenic action is not dependent on any classical physiological faculties of corticoids.

From the latter statement it can be inferred that a comparative study of the antifibromatogenic action of corticoids cannot be centered around their hitherto known functional characteristics. In screening cortical steroids for antitumorigenic action other distinctive characteristics of these compounds must be used—namely, their structural particularities. We feel that in this way considerable progress can be achieved.

Dehydrocorticosterone (A) and 17-hydroxydesoxycorticosterone (S). When comparing a corticoid of high antifibromatogenic activity, such as desoxycorticosterone, with a corticoid endowed with but very poor antifibromatogenic potency, like cortisone, we have to envisage two structural particularities: the ketonic group at C_{11} and the hydroxyl group at C_{17}. Which of these two is responsible for the loss of antifibromatogenic activity?

Dehydrocorticosterone, or Kendall's compound A, is antifibromatogenic, but less so than desoxycorticosterone (*30*, and unpublished work). In other words, the ketonic group at C_{11} diminishes the antifibromatogenic potency but not to a very considerable degree. This makes it probable that the great loss of antifibromatogenic potency as evidenced by the comparative behavior of desoxycorticosterone and cortisone is due to the OH at C_{17}. This conclusion has been proved right in recent experiments of our group with 17-hydroxy-desoxycorticosterone, or Reichstein's compound S. With quantities of S about seven times those of desoxycorticosterone no antifibromatogenic action was obtained (Fig. 1).

One may recall here that other steroids with OH at C_{17}, such as testosterone and dihydrotestosterone, as well as methyltestosterone and methyldihydrotestosterone, are antifibromatogenic, although they are certainly much less so than progesterone or desoxycorticosterone. The fact that the androgens mentioned are antifibromatogenic notwithstanding their OH at C_{17} may be due to the β-position of the latter; on the contrary, the OH at C_{17} in cortisone and compound S are in α-position. This explanation is suggested especially by our finding that an isomer of testosterone with OH at C_{17} in α-position, formerly known as cistestosterone, showed no antifibromatogenic action with quantities three times those of testosterone (*5*).

Antifibromatogenic vs. antilymphomatogenic action of corticoids. Comparative work with four corticoids, desoxycorticosterone, compound A, compound S, and cortisone, brings us back to the question of the differential response of specific territories. This becomes fully evident when studying the comparative bearing of structural peculiarities for the antifibromatogenic and antilymphomatogenic actions of corticoids.

The keto group at C_{11}. Whereas cortisone is antilymphomatogenic, S has no comparable action (*16*). From the point of view of the structural peculiarities of antitumorigenic steroids, this means that the ketonic group at C_{11} is fundamental for antilymphomatogenic action. On the other hand, antifibromatogenic action, as we have seen, diminishes from desoxycorticosterone to dehydrocorticosterone (Fig. 2 *A*). We encounter

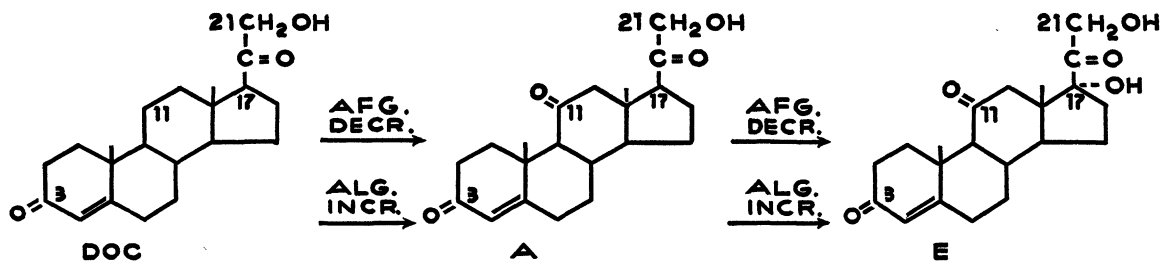

FIG. 2.

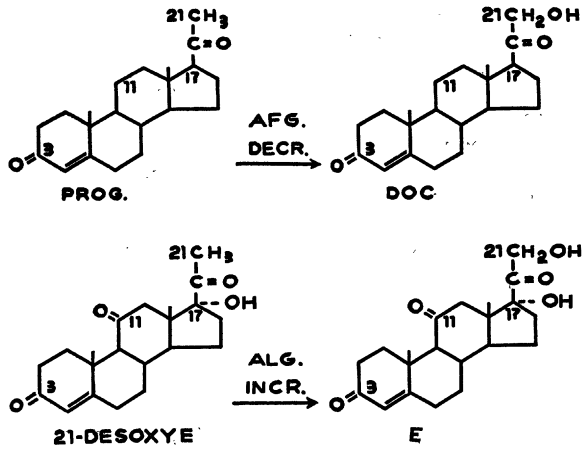

FIG. 3.

here a diametrically opposed structural particularity of antifibromatogenic and antilymphomatogenic actions of steroids. There is actually some knowledge of two other structural characteristics diametrically opposed in these comparative tumorigenic actions.

The hydroxyl group at C_{17}. Although it causes so marked a decrease of antifibromatogenic potency, antilymphomatogenic action is increased: cortisone (Fig. 2 *E*) has greater antilymphomatogenic potency than dehydrocorticosterone (Fig. 2 *A*) (*16*).

The hydroxyl group at C_{21} diminishes antifibromatogenic potency, as shown by the fact that desoxycorticosterone is less active than progesterone. However, OH at C_{21} is fundamental for antilymphomatogenic potency: 21-desoxycortisone is not antilymphomatogenic (*16*) (Fig. 3).

Comparative structural particularities of antifibromatogenic and antilymphomatogenic corticoids. We can characterize antitumorigenic actions of steroids, and especially of corticoids, by various structural particularities which are summarized in Table 1.

It is remarkable that the first three peculiarities are common to both antifibromatogenic and antilymphomatogenic actions, whereas the fourth, fifth, and sixth, as explained above, are diametrically opposed in the two types of antitumorigenic actions.

We must discuss now some conflicting findings which refer especially to corticoids.

After having examined various steroids with an OH at C_3, we were convinced that the ketonic group at C_3 was essential for antifibromatogenic potency. But, contrary to our expectations, pregnenolone, esterified or free, turned out to be antifibromatogenic when large quantities were given (*27*). The emphasis is not on "large quantities" but on "pregnenolone;" this can be inferred from the fact that 21-acetoxypregnenolone is not antifibromatogenic, even when quantities much superior to those of pregnenolone are given. The same is true for large quantities of various 3-keto-steroids, which are not antifibromatogenic (*1, 5*). It is evident that pregnenolone occupies, as to antifibromatogenic action, a unique position among steroids with OH at C_3. One must assume that this is due to the role of pregnenolone as a precursor of progesterone in the adrenal cortex, as evidenced by the brilliant work of Pincus, Hechter, *et al.* (*11*). Along similar lines one may tentatively explain why Δ^{16}-dehydropregnenolone is not antifibromatogenic: if oxidized at C_3 in the adrenal cortex, the result would be Δ^{16}-dehydroprogesterone, a steroid that has no antifibromatogenic faculty (*32*).

TABLE 1

	Points of interest	Antifibromatogenic potency (afg)*	Antilymphomatogenic potency (alg) (*16–18, 34*)
1	Keto at C_3	All afg steroids are 3-keto (*1*). Exception: pregnenolone (*27*), but it is transformed into progesterone in the adrenals (*11*).	Cortisone and A but also 17-hydroxycorticosterone and corticosterone (?) are alf (*16*).
2	Δ^4	Fundamental with steroids having a side chain of 2 carbons in C_{17}: pregnanedione and allopregnanedione are not afg (*32*). But with no side chain or only 1 carbon in C_{17} Δ^4 is not fundamental: dihydrotestosterone and 17-methyldihydrotestosterone are afg (*1*).	Cortisone and A but also 17-hydroxycorticosterone and corticosterone (?) are alf.
3	Side chain of 2 carbons at C_{17}	Progesterone and desoxycorticosterone are the most potent afg steroids (*1, 12, 33*).	Cortisone and A but also 17-hydroxycorticosterone and corticosterone (?) are alf.
4	Keto at C_{11}	Decrease of activity: dehydrocorticosterone (A) is less afg than desoxycorticosterone (*30*).	Increase of activity: S is not alg; cortisone is alg.
5	OH at C_{17}	Decrease of activity: 17-hydroxydesoxycorticosterone (S) and cortisone are less afg than desoxycorticosterone and dehydrocorticosterone (A).	Increase of activity; cortisone is more alg than A.
6	OH at C_{21}	Decrease of activity: desoxycorticosterone is less afg than progesterone (*1*).	Increase of activity: 21-desoxycortisone is not alg; cortisone is alg (*16*).

* See especially (*1*), where the formulas are not yet written out as they should have been, according to Fieser and Fieser (*31*).

Our scheme is not a "tentative suggestion"—it summarizes exclusively experimental findings, some of which may indeed be subject to corrections with the further development of our work. However, we feel that our scheme may offer a useful lead in screening antitumorigenic steroids.

The mode of action of antitumorigenic steroids. How do antifibromatogenic steroids act? As already stated, this is certainly a fundamental problem. However, its discussion can be at best tentative. Almost 30 years ago we insisted on what may be called the peripheral antagonism of sex hormones; there were then many vigorous arguments against our concept. Today no one doubts this peripheral antagonism (*1, 35–38,* Bruzzone and Lipschutz, unpublished work). Thus, one might venture to say that prevention of estrogen-induced fibroids of the abdominal serosa by progesterone is due to the peripheral antagonistic action of this steroid against estradiol. However, such a statement, even if established by a direct experimental device, would not settle the question. Progesterone does not antagonize estrogen-induced proliferation of the mammary gland in the guinea pig, or estrogen-induced proliferation of the anterior lobe of the hypophysis in the rat. It would thus seem that antitumorigenic action of steroids is not simply a kind of neutralization or inhibition of some tumorigenic substance, but that it is related to events within the cells. Estrogen, testosterone, progesterone, cortisone, and other steroids interfere in intracellular enzymatic processes, and it is very likely that antitumorigenic action takes place on this intracellular level.

There is also the problem of analogs, which Lacassagne and his group (*39*) have so intelligently applied to the anticarcinogenic action of hydrocarbons. The action of a carcinogenic compound of high potency was, in their work, counteracted by a chemical analog of low potency applied simultaneously. But in similar experiments with azo compounds there was no inhibition but addition of potencies of the two compounds (*39*). A tentative effort to apply the concept of analogs was also made with steroids, combining the highly active estradiol-17-β with its isomer estradiol-17-α of low estrogenic activity; no inhibition of the first by the second was obtained (*40*).

Another group of findings of considerable interest will be mentioned here. Abdominal fibroids so easily induced in female guinea pigs failed to appear in animals receiving a diet poor in ascorbic acid (*41*). The transplantable lymphosarcoma regresses when desoxypyridoxine, an analog of pyridoxine, is given (*42*), or when mice are fed with a diet poor in riboflavin (*43*); regression of the tumor under the influence of cortisone is also enhanced by a similar diet (*18*).

The intermediacy of the hypophysis must also be taken into consideration when discussing the mode of action of antitumorigenic steroids. It is certain that experimental ovarian and suprarenal tumors can be prevented by steroids most probably acting on the hypophysis (*44, 45*). The work of Lacassagne, Buu-Hoi, and others in France who have tried to control tumoral growth by diminishing the gonadotrophic and thyreotrophic functions of the hypophysis with *p*-oxypropiophenone is amply known, although it is still under discussion.

All these findings referring to the mode of action of antitumorigenic steroids, although sometimes conflicting, nonetheless deserve full attention when screening antitumorigenic steroids.

To sum up, we may say that at this moment no steroid is known that can control indiscriminately all tumoral growth as such. Action of antitumorigenic steroids is always limited by what may be called the law of specific territories. Unfortunately, control even of these specific territories has been hitherto only a temporary one. But, on the other hand, it has become evident that steroids with the faculty to regulate tumoral growth can to a certain degree be characterized by some of their structural aspects. This applies especially to corticoids.

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Separation and Detection of the Pyrethrin-Type Insecticides and their Derivatives by Reversed Phase Paper Chromatography[1]

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For the purpose of studying the metabolic fate of C^{14}-labeled insecticides of the pyrethrin type a method was required for the separation of these esters and their acid and alcohol products of hydrolysis. It was necessary to separate these materials, under conditions unfavorable to their further decomposition after extraction from insect tissue, etc., on unidimensional paper chromatograms so that they could be assayed radiometrically by the scanning techniques described elsewhere (*1*). The method of reversed phase paper chromatography developed for the separation of the bromine analogs of DDT and its derivatives (*2*) has been found to be applicable, with slight modification as follows:

Whatman No. 1 paper in 1-in. strips is washed by soaking for 30 min in a mixture consisting by volume of 45% ethanol, 50% water, and 5% conc HCl. The strips are then successively soaked in dilute aqueous ammonia and distilled water, and finally drained and dried. For the reversed phase paper chromatography of the esters the washed strips are impregnated with petroleum jelly (USP) by dipping once in a 3% (w/v) solution of petroleum jelly in diethyl ether, draining, and drying. An ethereal solution containing not more than 100 μg of the mixture to be resolved is applied near the bottom of a strip, and the solvent, consisting by volume of 45% ethanol, 50% water, and 5% aqueous ammonia (sp gr, 0.90) allowed to ascend the strip in the usual way in an atmosphere of nitrogen saturated with the solvent vapor. After 24 hr the strips are dried, sprayed with 0.1% neutral aqueous potassium permanganate, immediately rinsed with distilled water until quite free of permanganate, and partially dried. While still damp the strips are sprayed with 0.5% benzidine in dilute aqueous acetic acid (*3*) The MnO_2 formed in the presence of unsaturated compounds of the pyrethrin type appears as intense blue zones, less than 1 μg of the pyrethrins or their hydrolysis products being easily detected.

Allethrin, the allyl homolog of cinerin I, runs with an R_f value of 0.40, whereas the allyl cinerolone and chrysanthemum monocarboxylic acid products of hydrolysis run together with an R_f value of 0.89. When a concentrate of natural pyrethrins, labeled as containing 43% of "pyrethrin I" and 37% of "pyrethrin II" was resolved by this method, two major constituents running with R_f values of 0.23 and 0.72, respectively, and at least three other constituents running with R_f values of 0, 0.12, and 0.90 were detected. The composition of the mixture in terms of the true pyrethrins and cinerins I and II was unknown. The two major zones were believed to be the so-called pyrethrins I and II, respectively, since pyrethrin II is known to have a partition coefficient more favorable to the polar or mobile solvent phase. Evidence in support of this interpretation was obtained in two ways. First, when the zones separated on a second strip were sprayed with 0.05 *N*-ethanolic KOH containing 0.02% thymolphthalein, the faster running major zone required considerably more spraying than the slower running zone before the permanent blue ($pH > 10$) was obtained. This indicated the higher saponification value which would be expected of the dicarboxylic acid esters of "pyrethrin II." Second, a third strip was cut into 1-in. sections, which were rinsed separately in 2 ml petroleum ether containing 2 μl light mineral oil. The rinse of each section was transferred to a 2×4 cm exposure vial and bio-assayed with adult female houseflies by the methods described by Hoskins *et al.* (*4, 5*). Insecticidal activity, as shown by "knockdown" and mortality after 24 hr, was exhibited only by the substances running with R_f values of 0.23 and 0.72, and, qualitatively, the slower running component was the more toxic to houseflies. This is in agreement with Gersdorff (*6*), who found the monocarboxylic acid esters to be the more toxic. Some unsaturated $KMnO_4$-reactive material is present in the neutral ether or acetone extracts of macerated housefly tissue but remains at the point of application in the reversed phase chromatograms and does not, therefore, interfere with the detection of the extracted insecticides.

The alcohol and acid products of allethrin hydrolysis which run together on the reversed phase paper chromatogram may be separated as follows: The mixture is applied near the bottom of a washed strip that has not been impregnated with petroleum jelly. Petroleum ether (boiling range, 35°–60°), saturated with 10% aqueous HCl, is allowed to ascend the strip for about 10 min (atmosphere saturated with the solvent). The strip is dried and then rechromatographed with light

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