On the Antifibromatogenic Activity of Synthetic Progesterone in Experiments with the 17-Caprylic and Dipropionic Esters of α-Estradiol*

Alexander Lipschütz, M.D., and Jorge Grismali, M.D.

(From the Department of Experimental Medicine, National Health Service, Santiago, Chile)

(Received for publication October 25, 1943)

INTRODUCTION

Through the work of Biskind (2), Segaloff and Nelson (18), and of Lipschütz and his students (1, 3, 7, 8) considerable evidence has been accumulated in favor of greater resistance of *esterified* estrogens against hepatic inactivation when compared with *free* estrogens. Esterification protects estradiol against inactivation in the liver, though this protection is a limited one.

The question arises whether esterification protects estradiol also against the antagonistic action of progesterone and other steroids in preventing fibroids elicited by estrogens. This question is of considerable interest. Should esterification prove unable to give any protection at all against the antifibromatogenic action of steroids, this would be a new proof in favor of the assumption that antagonizing of one steroid by another is an event *per se* independent of hepatic events. In other words, antagonization of steroids might then be considered as representing a special means of adjustment of the steroid balance supposed to be one of the endocrine aspects of antitumorous autodefense in the body (9, 10).

In former experiments with the simultaneous injection of the benzoic ester of estradiol and of progesterone, abdominal fibroids were prevented (13, 14, 17). Huge quantities of progesterone were used; they were 150 times greater than the quantity of the esterified estradiol administered. In the meantime, however, it has been found in experiments with simultaneous implantation of tablets of estradiol and progesterone that fibroids can be prevented with a quantity of progesterone even smaller than the amount of free estradiol simultaneously absorbed (4). It was thought that the question whether esterification protects the estrogen against antagonization by other steroids might best be studied by establishing the quantity of progesterone that would still prevent fibroids induced by subcutaneously implanted tablets of a fibromatogenic ester of estradiol as potent as the 17-caprylate (11) or the dipropionic ester, which has been shown to be especially resistant against intrahepatic inactivation (18).

* This investigation has been aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research and The Rockefeller Foundation. Thanks are due to Dr. Karl Miescher and to Dr. C. Oppenheimer, Ciba Pharmaceutical Products, for furnishing steroids.

EXPERIMENTS

Results with 54 castrated female guinea pigs are given in Table I. Each of these animals had a subcutaneously implanted tablet, or fragment of tablet, of esterified estradiol on one side of the body, and a tablet of progesterone on the other side. All these experiments lasted from 61 to 65 days, with the exception of three that lasted from 67 to 68 days. The tablets of the dipropionate were 2 to 11 mgm.; those of the caprylate were 20 to 50 mgm. Absorption from subcutaneously implanted tablets of the caprylic ester is about 5 times less than that from tablets of the dipropionic ester (15); for this reason the surface has to be greater with the caprylic ester so as to guarantee absorption of equal quantities of both esters. The tablets, or fragments, of progesterone were 8 to 15 mgm. in the series with the caprylic ester. In most of the experiments with the dipropionic ester, mixed progesterone-cholesterol tablets were used, so as to slow down absorption and thus allow absorption of small quantities of progesterone. The tablets contained 40 per cent of progesterone and 60 per cent of cholesterol. According to unpublished work of Fuenzalida, absorption of progesterone from similar tablets is not selective; in our experiments Fuenzalida found no increase of the cholesterol percentage in the course of 60 days. The mixed tablets or fragments of these weighed 8 mgm. and upwards.

The daily absorption was calculated from the loss of weight of the tablet dried *in vacuo*. The fibrous tumorous effect (F.T.E.) was classified according to rules explained in former papers.

Antifibromatogenic Action of Progesterone Against the 17-Caprylic Ester of Estradiol

As seen in Table I, the antagonistic action of progesterone was evident when the 17-caprylic ester

of α-estradiol was used as a fibromatogenic agent. There was not a single animal with tumors of 1.5 mm. or more in diameter when progesterone was acting simultaneously with this ester. The average recorded fibrous or tumorous effect of 1.5 in the caprylate-progesterone group was due exclusively to fibrous peritoneal strands or to small nodules just visible to the naked eye. Increase of uterine weight also was greatly lowered and there was no genital bleeding.

The antagonizing action of progesterone was still obvious when no more than 58 μgm. were absorbed daily. A graphic representation of the results with 58 μgm. of progesterone is given in Figs. 1 and 2. The ratio between estradiol and progesterone absorbed daily was 1:1.5 in this latter case and 1:1.4 in another case. On the other hand, the same quantity of estradiol when present alone in the body produced tumors in 9 of 10 animals (Table I). Individual results with 10 animals receiving the caprylate of estradiol alone and 10 animals simultaneously receiving progesterone are given in Fig. 3. Not a single animal of the caprylate-progesterone group (B) reached the average F.T.E. of the caprylate group (A).

From what we know from our extensive work with the antifibromatogenic threshold of progesterone acting against free estradiol, we must infer that the antifibromatogenic threshold of progesterone with the 17-caprylic ester is smaller than 58 μgm. With free estradiol and with smaller quantities of progesterone the antifibromatogenic diagram showed a *transitional zone* in which the fibrous tumoral reaction, though present, was seemingly less pronounced than in the total absence of progesterone, as in Fig. 1 of the preceding paper, beneath the division line at 13 μgm. But this transitional zone was not yet reached in our present work with the caprylic ester of estradiol and this suggests that with quantities smaller than 58 μgm. of progesterone there will be prevention of fibroids elicited by the caprylic ester.

TABLE I:* FIFTY-FOUR CASTRATED FEMALE GUINEA PIGS WITH SUBCUTANEOUSLY IMPLANTED TABLETS OF ESTERIFIED ESTRADIOL AND PROGESTERONE. DURATION OF THE EXPERIMENTS 61 TO 68 DAYS

Group		Absorbed per day: Estradiol †, μgm.	Absorbed per day: Progesterone, μgm.	Number of animals: Total	Number of animals: With tumors	Average F.T.E.	Animals with genital bleeding	Uterine weight, gm.
Caprylate	A	32 (13-59) ‡	0	**10**	**9**	3.3	6	4.3 (2.5-10.5)
Caprylate and progesterone	B	32 (17-60)	**58**-120	**10**	**0**	1.5	0	2.1 (1.5-3.2)
Dipropionate	A	23 (11-31)	0	10	9	4.5	2	4.7 (3.1-10.2)
Dipropionate and progesterone	B	27 (12-45)	55-140 §	15	1 ¶	1.0	0	2.0 (1.2-3.7)
"	C	21 (11-31)	**28**-49 ‖	**5**	**0**	1.0	0	1.5 (0.9-2.2)
"	D	29 (23-36)	6-11 ‖	4	3	2.8	0	2.5 (1.8-3.7)

* Selected from the thesis presented for the degree of M.D. by J. Grismali (5) in the Universidad de Chile. Minor changes have been made in the evaluation of F.T.E. (fibrous tumorous effect) in various animals.

† Always calculated as free estradiol.

‡ Figures in brackets indicate range.

§ Five experiments with pure progesterone, 102 to 140 μgm. daily. The remaining experiments with tablets containing 40 per cent of progesterone and 60 per cent of cholesterol.

‖ All experiments with tablets mixed with cholesterol.

¶ With 116 μgm. of progesterone and 42 μgm. of estradiol daily.

ANTIFIBROMATOGENIC ACTION OF PROGESTERONE AGAINST THE DIPROPIONIC ESTER OF ESTRADIOL

In experiments in which the dipropionic ester of α-estradiol (Table I) was used as a fibromatogenic agent, there were tumors in only 1 of 20 animals receiving 28 to 140 μgm. of progesterone, whereas with the dipropionic ester present alone in the body 9 of 10 animals had tumors (Table I). The smallest ratio between estradiol and progesterone, in cases ranging between 28 and 49 micrograms of the latter, was 1:1.4 (Figs. 4 and 5).

In the small group with minute quantities of progesterone, 6 to 11 μgm. daily, uterine and extragenital fibroids were not prevented (Table I and Fig. 6), though the fibrous reaction was not very pronounced. With these small quantities of progesterone acting against the dipropionic ester of estradiol, the

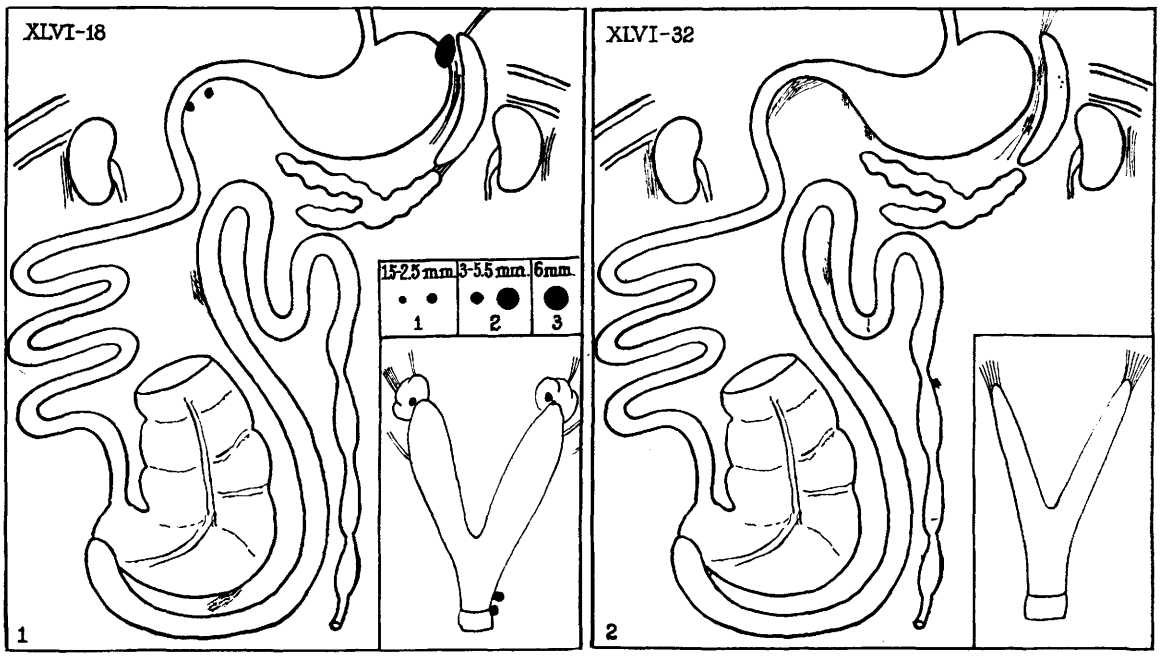

FIG. 1.—Caprylic ester of estradiol, 61 days (XLVI-18). Estradiol daily, 13 μgm. Subserous uterine tumors near the vagina = 1; apical tum. = 1; mesent. tum. of the duodenum = 1; splenic tum. = 2. F.T.E. = 5. Weight of uterus, 3.6 gm.

FIG. 2.—Caprylate of estradiol and progesterone, 64 days (XLVI-32). Estradiol daily, 38 μgm.; progesterone daily, 58 μgm. No tumors: only fibrous strands in the abdominal cavity and small nodules on the surface of the spleen. F.T.E. = 2. *Maximal reaction of this series.* Weight of uterus, 2.8 gm. Diagrams of Figs. 1 and 2, including the scale in Fig. 1, have been reduced to half the natural size.

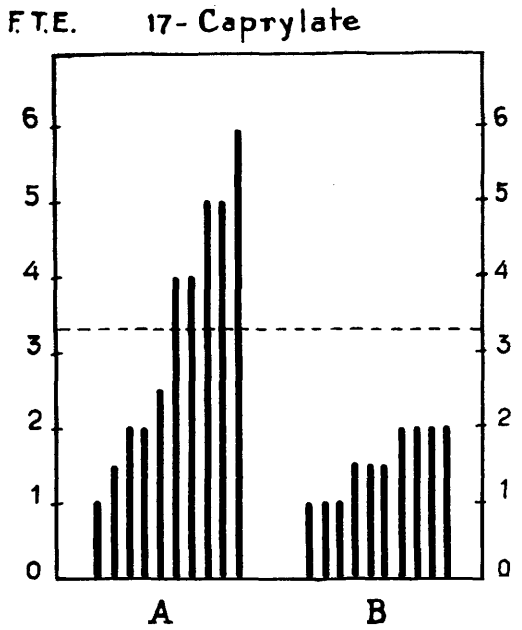

FIG. 3.—F.T.E. of 10 animals receiving the 17-caprylic ester of estradiol (A) and of 10 simultaneously receiving the 17-caprylic ester and progesterone (B). See Table I. In the B group there was not a single animal that would have reached the average F.T.E. of the A group (broken line).

transitional zone seems to have been reached; this zone coincides with that of similar quantities of progesterone acting against free estradiol. See the antifibromatogenic diagram of progesterone against free estradiol in the preceding paper (12). The antifibromatogenic threshold of progesterone, acting against the dipropionic ester of estradiol, was supposedly between 11 and 28 μgm. daily in the present experiments. This makes it very likely that the antifibromatogenic action of progesterone against the dipropionic ester occurs under similar quantitative conditions as its action against the free estrogenic hormone.

The preventive action of progesterone in the C group, with 28 to 49 μgm. daily, and even in the D group, with only 6 to 11 μgm. daily, also was obvious with reference to the uterine weight, as seen from Table I.

DISCUSSION

Our results with the preventive action of progesterone against the fibromatogenic action of esterified estradiol give no support to the assumption that esterification protects against the antifibromatogenic action of progesterone. Accordingly, when referring to the antagonizing of the estrogen by another steroid such as progesterone and desoxycorticosterone on the one hand, and to the metabolic fate of the estrogen in the

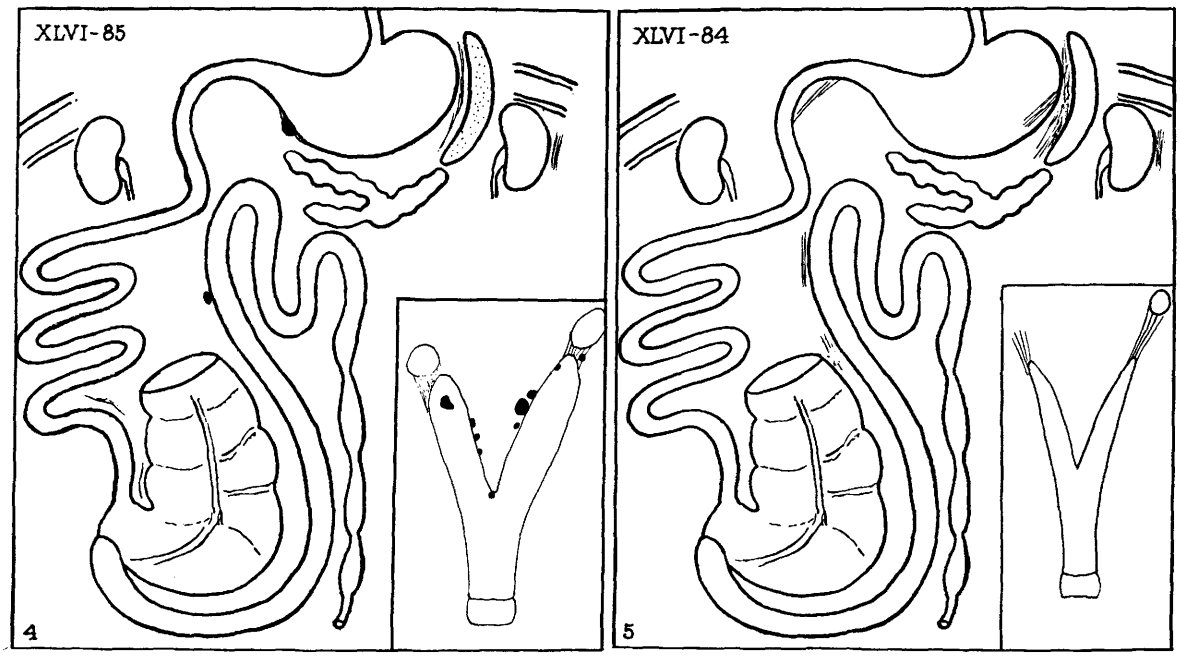

FIG. 4.—Dipropionic ester of estradiol, 67 days (XLVI-85). Estradiol daily, 14 μgm. Parametric and subserous tumors of the uterus = 2; apical tum. = 0.5; tum. of the epiploon and mesocolon = 1; small nodules on the surface of the spleen = 0.5. F.T.E. = 4. Weight of uterus, 10 gm.

FIG. 5.—Dipropionic ester of estradiol and progesterone, 68 days (XLVI-84). Estradiol daily, 14 μgm.; progesterone daily, 32 μgm. No tumors, only fibrous strands. F.T.E. = 1.5. *Maximal reaction of this series.* Weight of uterus, 1.6 gm. Diagrams of Figs. 3 and 4, including the scale in Fig. 1 (above) have been reduced to half the natural size.

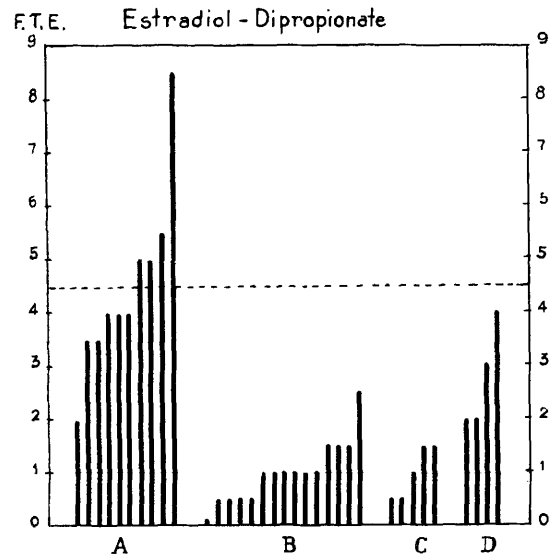

FIG. 6.—F.T.E. of 10 animals receiving the dipropionic ester of estradiol (A) and of 24 simultaneously receiving the dipropionic ester and progesterone (B, C, D). See Table I. No animal in B and C (55 to 140 μgm. and 28 to 49 μgm. of progesterone) approaches the average of the A group (broken line). In the D group (6 to 11 μgm.) the transitional zone is seemingly reached as with similar quantities of progesterone acting against free estradiol.

liver on the other, we are dealing with two *different* aspects of the antitumorous autodefense of the body (9, 10). We are dealing also with two different *sites* at which the toxic or tumor-producing agent is counteracted. Our actual results make it highly probable that the antagonistic steroid acts as a desensitizer, *i.e.*, renders the tissue incapable of responding to the estrogenic stimulus, as was supposed many years ago on the basis of our findings with experimental hermaphroditism produced by transplantation of the ovary into partially castrated male guinea pigs (6, 16).

SUMMARY

The fibromatogenic action of subcutaneously implanted tablets of the 17-caprylic ester and of the dipropionic ester of α-estradiol was prevented with quantities of progesterone not far from, or identical with, those that are necessary to antagonize the fibromatogenic action of free α-estradiol (antifibromatogenic threshold).

From these findings the conclusion can be drawn that esterification, though relatively protective against the inactivating action of the liver, does not confer protection against the antagonizing action of progesterone.

Our results suggest that the antagonistic action of this steroid against estradiol is effected not by inter-

fering in the inactivation of estrogens in the liver but by rendering the reacting tissue unable to respond to the action of estrogens.

REFERENCES

1. Acuña, L. Injerto intraesplénico comparativo del estradiol y de sus ésteres. Tesis Universidad de Chile, 1-20. 1942. (Public. Med. Exp., No. 12)
2. Biskind, G. R. The Inactivation of Estradiol and Estradiol Benzoate in Castrate Female Rats. Endocrinology, **28**: 894-896. 1941.
3. Carrasco, R. Influencias hepáticas en la tumorigénesis inducida por estrógenos. Tesis Universidad de Chile, 1-34. 1943. (Public. Med. Exp., No. 18)
4. González, S. Estudios cuantitativos sobre la acción antitumorígena preventiva de la progesterona sintética. Tesis Universidad de Chile. 1-32. 1943. (Public. Med. Exp., No. 19)
5. Grismali, J. Acción protectora de la progesterona en la tumorigénsis provocada por los esteres de estradiol. Tesis Universidad de Chile, 1-26. 1942. (Public. Med. Exp., No. 17)
6. Lipschütz, A. Experimenteller Hermaphroditismus und der Antagonismus der Geschlechtsdrüsen. XII. Mitt. Pflügers Arch., **211**:745-760. 1926.
7. Lipschütz, A. Induction and Prevention of Abdominal Fibroids by Steroid Hormones, and Their Bearing on Growth and Development. Cold Spring Harbor Symposium, **10**:79-90. 1942.
8. Lipschütz, A. Experimental Fibroids and the Antifibromatogenic Action of Steroid Hormones. J. A. M. A., **120**:171-175. 1942.
9. Lipschütz, A. Endocrine Aspects of Antitumoral Autodefense. Cancer Research, **2**:727 (Abstract). 1942.
10. Lipschütz, A. Endocrine Aspects of Antitumoral Autodefense. Rev. Canad. de Biol., **2**:92-104. 1943.
11. Lipschütz, A., Bellolio, P., Chaume, J., and Vargas, L., Jr. Comparative Conjunctive Tumorigenic Action of Three Different Esters of Estradiol. Proc. Soc. Exper. Biol. & Med., **46**:164-167. 1941.
12. Lipschütz, A., Bruzzone, S., and Fuenzalida, F. Comparative Antifibromatogenic Activity of Progesterone and Related Artificial Steroids. Cancer Research, **4**:179-185. 1944.
13. Lipschütz, A., Murillo, R., and Vargas, L., Jr. Antitumorigenic Action of Progesterone. Lancet, **2**:420-421. 1939.
14. Lipschütz, A., and Vargas, L., Jr. Prevention of Experimental Uterine and Extrauterine Fibroids by Testosterone and Progesterone. Endocrinology, **28**:669-675. 1941.
15. Lipschütz, A., and Vargas, L., Jr. Absorption of Estradiol and Its Esters from Subcutaneously Implanted Tablets in the Guinea Pig. Proc. Soc. Exper. Biol. & Med., **48**:464-467. 1941.
16. Lipschütz, A., and Voss, H. E. Experimenteller Hermaphroditismus und der Antagonismus der Geschlechtsdrüsen. II. Mitt. Pflügers Arch., **207**:563-582. 1925.
17. Murillo, R. Acción protectora del ovario en la tumorigénesis conjuntiva experimental y el papel de la progesterona en esta acción. Tesis Universidad de Chile, 1-44. 1940. (Public. Med. Exp., No. 4)
18. Segaloff, A., and Nelson, W. O. Absorption and Metabolism of Estrogens. I. Estrogen Assay by Intrasplenic Injection. Proc. Soc. Exper. Biol. & Med., **48**:33-34. 1941.