Interaction of Estrogens on the Vaginal Smear of Spayed Rats

RICHARD A. EDGREN AND DAVID W. CALHOUN

From the Division of Biological Research, G. D. Searle and Company, Chicago, Illinois

ABSTRACT

EDGREN, RICHARD A. AND DAVID W. CALHOUN. (G. D. Searle and Co., Chicago, Ill.) *Interaction of estrogens on the vaginal smear of spayed rats.* Am. J. Physiol. 189(2): 355–357. 1957.—Employing the vaginal smear as an index of effect, combinations of various estrogenic substances were tested for interaction. Studies were concentrated at the approximate 50% response level. In a single experiment ED_{50} doses of the two estrogens in pure form were administered to two groups of spayed rats and a third group was injected with a mixture of one-half of each ED_{50}; in one experiment three intermediate doses were employed. In all of the studies a given proportion of the ED_{50} of one compound was replaced by the same proportion of the ED_{50} of the other without mensurable change in biological response. These data are interpreted as indicating simple additive relationships among the compounds tested. Pairs studied were: estradiol-17β with estriol; and estrone in combination with estradiol-17β, estriol, methallenestril (Vallestril) and stilbestrol. The additive relationships described here are of particular interest in view of literature reports that indicate an interference between certain pairs of these compounds when uterine growth rather than vaginal cornification was used as an index of activity. This target organ differentiation suggests that various end points of estrogenic action differ in their responses to varying hormonal balances.

RECENTLY Hisaw and Velardo and their collaborators (1) discovered that estriol and estrone inhibited the action of estradiol on the uterus of spayed rats, and that estriol depressed the uterine response to estrone. Huggins and Jensen (2) confirmed the estrone-estriol interaction using the uteri of hypophysectomized rats as an index. They also listed a series of estrogenic substances with similar action. Histological and gross studies on the vagina by these latter workers disclosed no remarkable relationships. These previous studies utilized estrone- or estradiol-induced uterine growth as an index of interaction or as the principal index in the case of Huggins and Jensen. In an attempt to extend our knowledge of estrogen interactions we have studied the effects of combinations of estrogens on the vagina, employing the vaginal smear for assay.

MATERIALS AND METHODS

Our assay technique was modified from Emmens (3). Female rats, 60 days of age, were spayed and allowed to recover for 30 days. Priming with two equal doses of estrone spaced 24 hours apart began when the rats were 90 days of age. Each injection of 2.5 μg of estrone was administered subcutaneously in 0.1 ml of corn oil. Smears were taken 56 and 72 hours after the first injection. New rats were integrated into the colony only after responding positively to two courses of priming. Rats in the assay colony were primed in the same way on alternate weeks. Those responding positively, generally about 90%, were used for testing purposes the following week.

Fresh smears were scored according to the method of Biggers and Claringbold (4): i.e., the absence of leucocytes in the smear was considered a positive response to the test drug and all other smears were considered negative.

In preliminary tests we attempted to compare the response to combinations of a fixed dose of one substance with varying doses of another to a previously established dose-response curve for the first substance. The quantal nature of our assay together with the high between-test variability in this laboratory made such a direct approach impractical. However, as the within-test variability was found to be satisfactorily low, each experiment was concentrated on a single response level, as close to 50% as possible. The two compounds to be compared were tested individually at the approximate 50% effective dose. A third group, ordinarily, received a mixture of both compounds, con-

Received for publication November 15, 1956.

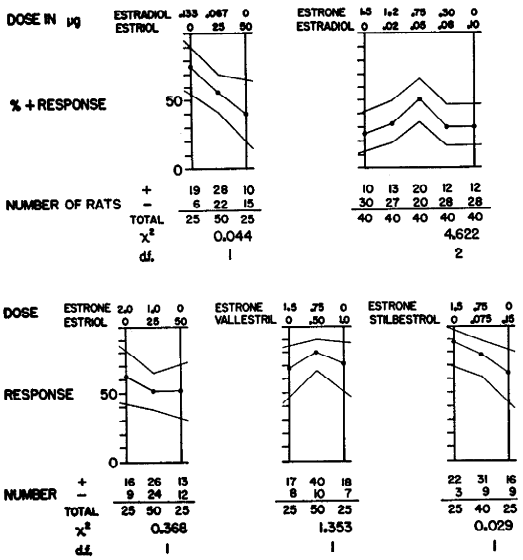

FIG. 1. Effects of estrogen combinations on vaginal cornification in spayed rats. Observed percentage positive responses are graphed at each dose or combination. Percentages are bracketed by their 95% confidence limits. Chi-square tests are described in the text.

taining half of each of these amounts. In the case of estrone-estradiol comparisons three intermediate combinations were employed; actual dose levels are shown in figure 1. Test materials were administered in a total of 0.4 ml of corn oil, which was injected subcutaneously in two equal parts at 0 and 24 hours. Smears were taken at 56 and 72 hours.

Six experiments tested the following five pairs of estrogens:

Exp. No.	Combination	No. of Animals
1, 2	Estrone: estradiol-17β	100, 100
3	Estrone: estriol	100
4	Estrone: diethylstilbestrol	90
5	Estrone: Vallestril[1]	100
6	Estradiol-17β: estriol	100

Figure 1 shows the doses employed.

RESULTS

Percentage positive responses with their 95% confidence limits are shown in figure 1. Actual numbers of animals responding positively and negatively are tabulated below the graph for each experiment.

The object of the experiments was simply to reveal if any of the intermediate mixed doses elicited a nonintermediate response. The response levels to the pure estrogens were not a primary concern. A refinement of the chi-square test of contingency tables such as described recently by Cochran (5), seemed an appropriate statistical test for our purpose. We assumed that responses to a given compound were consistent within a single experiment. Thus if the responses to one compound were high in a particular experiment while responses to the second were low we presumed that the constituents of the mixture would show similar fluctuations. The dose levels employed can be translated to a 1, 0.5 and 0 scale, which represent proportions of the ED_{50}, in the three point tests (a 1, 0.8, 0.5, 0.2, 0 scale in the 5-point test). If our assumptions be valid, percentage responses plotted against such a scale should approximate a straight line. The value of chi-square in figure 1 for each experiment is a test for simple curvature of this line. For example in a three-point test an observed mixture response of less than 30% or more than 70%, while end responses averaged 50%, would yield a just significant chi-square value of 3.84. Significant curvature was not found with any estrogen pair.

The estrone-estradiol combination comprises two independent experiments, each using 20 animals/dose level. The first experiment by itself strongly suggested the presence of curvature, but this was not confirmed on repeat. Chi-square was calculated for each experiment separately; the sum of these values is shown in figure 1.

DISCUSSION

Simple additivity, the condition in which one compound acts as a simple dilution of another, requires that the individual substances have similar dose-response curves. Unpublished data from this laboratory suggested that the compounds employed in this experiment had essentially similar dose-response curves when tested on vaginal smears. Estriol, with a shallow slope, was an exception, however, this slope difference was not as marked as has been obtained in uterine growth studies (2, 6). We considered that the slope differences were not of sufficient magnitude to have affected our results appreciably. Thus the intermediate responses obtained with combined administration implied that the compounds studied here could be considered as simple dilutions of each other, that is their interac-

[1] Searle brand of methallenestril.

tions were additive in the simplest sense of the word.

Curiously then, estrogens that showed inhibitory interrelationships when tested on uterine growth (1, 2) had simple additive interactions when tested on the vaginal smears. Slight dose and procedural differences between vaginal smear and uterine growth experiments preclude direct comparisons. Nevertheless, it seems reasonable to postulate that a given hormone combination may evoke differing levels of response in different target organs, and particularly, that increase of one component may increase response at one site while decreasing it at another. Many steroids, estrogens, androgens, progestins and gluco- and mineralocorticoids are present in the mammalian circulation during various phases of the sex cycle and are known to modify the effects of any given estrogen. This hormonal multiplicity apparently constitutes an estrogen-buffering system and supports the hypothesis that sexual responses depend "... upon a rather precise hormonal homeostasis." (7).

We are indebted to Miss Nancy Hanway and Mrs. Grace C. Perkins for technical assistance.

REFERENCES

1. HISAW, F. L., J. T. VELARDO AND C. M. GOOLSBY. *J. Clin. Endocrinol. & Metab.* 14: 1134, 1954.
2. HUGGINS, C. AND E. V. JENSEN. *J. Exper. Med.* 102: 335, 1955.
3. EMMENS, C. W. *Hormone Assay.* New York: Acad. Press, 1950, p. 396.
4. BIGGERS, J. D. AND P. J. CLARINGBOLD. *J. Endocrinol.* 11: 277, 1954.
5. COCHRAN, W. G. *Biometrics* 10: 417, 1954, p. 434.
6. EDGREN, R. A. *Proc. Soc. Exper. Biol. & Med.* 92: 569, 1956.
7. ROBERTS, S. AND C. M. SZEGO. *Physiol. Rev.* 33: 593, 1953.