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Pituitary Stimulating Property of Stilbestrol as Compared with That of Estrone.

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The synthetic compound, 4,4'-dihydroxy-α,β-diethylstilbene (stilbestrol), appears to have all of the biological properties of the naturally-occurring estrogens. It is a potent estrogen, considerably less of it than of estrone being required to produce estrus in spayed female rats. It is known to cause the stimulation and release of pituitary adrenotropic and gonadotropic hormones when administered to rats over short intervals of time, as reflected by ovarian, adrenal, and pituitary hypertrophy and a decrease in the transplantable gonadotropic activity of the hypophysis.[1,2] No work has been reported, however, revealing whether more or less of it than of the naturally-occurring estrogens is required for these pituitary stimulating effects.

In a recent publication[3] results were presented indicating that estrone elicits these pituitary responses in mature male rats indirectly through the formation of inactivation products. It was shown that the presence of the testes or the administration of progesterone, both of which depress estrogen inactivation, completely inhibited the hypophyseal reactions to an otherwise effective dose of estrone. It was also demonstrated that Westerfeld's lactone,[4] an oxidative inactivation product of estrone, exerted these same influences upon the pituitary gland and in much smaller dosages than estrone, and, furthermore, that the responses to the lactone were uninfluenced by the presence of the testes or the administration of progesterone. Inasmuch as a compound chemically similar to Westerfeld's lactone could not possibly be formed in the metabolic inactivation of stilbestrol, it was considered of interest to investigate further the pituitary stimulating property of this estrogen.

Exactly the same procedure as previously described[3] was followed, the stilbestrol being given in 10 injections over 5 days to mature (70- to 100-day-old) male rats whose adrenals and pituitaries were weighed on the sixth day and the pituitaries transplanted into immature female rats in order to measure their gonadotropic activity. For each test donor an uninjected littermate was simultaneously autopsied and the results expressed in terms of percentage difference between the weights of the adrenals and pituitary of the injected animal and those of its control littermate. The pituitaries of 2 test donors were injected into an immature female rat, a control littermate of which received 2 pituitaries from uninjected donors. The gonadotropic activity was gauged by the percentage difference between the weight of the ovaries of the test animal and that of its control littermate. From the results of the previously published control experiments,[3] no increase of less than approximately 10% in the adrenal or pituitary weights or decrease of less than 10% in ovarian weights is significant.

In the earlier investigation[3] it was found that whereas a total dose of 10 γ of estrone to *castrated* rats caused a significant adrenal enlargement, as compared with castrated controls, and decrease in the transplantable gonadotropic activity of the pituitary, 120 γ had no demonstrable effect upon the pituitaries or adrenals of *intact* animals. Fifty γ of estrone alone to castrates caused a significant increase in pituitary weights as well as a significant enlargement of the adrenals and decrease in transplantable gonadotropic activity of the pituitaries, but if a total dose of 1.0 mg of progesterone was simultaneously ad-

[1] Von Haam, E., Rardin, T. E., and Schoene, R. H., *Endocrinology*, 1941, **28**, 263.

[2] Wolfe, J. M., and Brown, A. D., *Endocrinology*, 1942, **31**, 467.

[3] Smith, O. W., *Endocrinology*, 1944, **35**, 146.

[4] Westerfeld, W. W., *J. Biol. Chem.*, 1942, **143**, 177.

TABLE I.
Adrenal and Pituitary Weights and Transplantable Gonadotropic Activity of the Hypophyses of Mature Male Rats After Estrone.

Total dose of estrone, γ	No. of exp.	% change in organ weights vs. controls: Donor's adrenals (avg)	Donor's pituitaries (avg)	Recipient's ovaries (avg)
	A. Estrone alone to intact animals.			
10	4	+ 5.7	+ 0.7	+ 1.5
50	6	+ 3.2	+ 2.4	— 3.7
100-120	4	— 1.1	— 3.2	+ 0.5
500	4	+30.3	+17.7	—24.2
	B. Estrone alone to castrated animals.			
5	4	+ 5.2	— 1.5	—10.7
10	4	+20.7	+10.3	—47.4
50	4	+29.9	+33.8	—39.5
100	2	+40.2	+27.1	—45.5
	C. Estrone plus progesterone (1.0 mg) to castrated animals.			
50	6	+ 5.7	— 0.9	— 2.9

TABLE II.
Adrenal and Pituitary Weights and Transplantable Gonadotropic Activity of the Hypophyses of Mature Male Rats After Stilbestrol.

Total dose of stilbestrol, γ	No. of exp.	% change in organ weights vs. controls: Donor's adrenals (avg)	Donor's pituitaries (avg)	Recipient's ovaries (avg)
	A. Stilbestrol alone to intact animals.			
1	4	+ 5.3	+ 8.4	+ 3.3
5	4	+33.7	+12.7	—35.6
10	4	+40.1	+17.9	—31.5
	B. Stilbestrol alone to castrated animals.			
1	4	— 1.5	+ 6.3	+ 3.1
5	4	+32.1	+31.0	—29.5
10	4	+27.1	+26.5	—21.5
25	2	+31.3	+26.9	—34.5
50	2	+32.0	+24.7	—29.0
	C. Stilbestrol plus progesterone (1.0 mg) to castrated animals.			
10	4	+22.0	+32.6	—25.0
50	4	+43.2	+25.3	—28.4

ministered, the pituitary responses of castrated male rats to 50 γ of estrone were completely nullified. In Table I these reported results are summarized and 10 additional experiments are included demonstrating that 500 γ of estrone are required to elicit significant changes in intact rats and that 10 γ is the minimal effective dose in castrates.

The results of 36 experiments with stilbestrol are summarized in Table II. The minimal effective dose of this synthetic estrogen to both castrated and intact rats was 5 γ, the only difference being that the pituitary enlargement was less marked in intact animals. The simultaneous administration of a total dose of 1.0 mg of progesterone had no demonstrable effect upon the pituitary responses of castrated males to 10 or 50 γ of stilbestrol.

Stilbestrol, therefore, appears to be about 100 times as active as estrone in eliciting the pituitary responses of intact rats, although only about twice as active in castrated animals. The fact that its effectiveness is uninfluenced by the presence of the testes or the administration of progesterone indicates, as would be expected, that its metabolism is not regulated by the same mechanism as the naturally-occurring estrogens. Possibly its effect upon the pituitary gland is direct rather than through the formation of some metabolic inactivation product, as appears to be the case with estrone. The results reported shed no

light upon the mechanism of its action but suggest that, as a therapeutic agent in women with hypo-pituitary-ovarian disorders, it should be very much more effective than the naturally-occurring estrogens. The clinical experience of one of us (G.V.S.) corroborates this.

Summary. Stilbestrol is about 100 times as active as estrone in causing the stimulation and release of adrenotropic and gonadotropic factors from the pituitaries of intact mature male rats. In castrated animals it is only about twice as active as estrone. Unlike estrone, the pituitary responses to stilbestrol are not influenced by the presence of the testes or the administration of progesterone, both of which depress the rate of inactivation of estrone as well as its pituitary stimulating activity. It would appear that the mechanism of stilbestrol's action upon the pituitary differs from that of the naturally-occurring estrogens.

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Antigenic Types of *Shigella alkalescens.*

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Until 1939, *Shigella alkalescens,* a member of the *Shigella* group, was considered to be an antigenically homogeneous species. In that year Assis[1] described as *S. alkalescens* type II a strain (No. 648) which differed both in biochemical characteristics and antigenic structure from the hitherto known strains comprising type I. In 1943, Stuart, Rustigian, Zimmerman, and Corrigan[2] demonstrated that type I strains contain 5 antigenic components, which they designated A, B, C, D and E. The major antigens A and B and the minor antigen C are present in all typical strains. The antigenic fractions D and E were found either singly or in combination. In May, 1944, Captain William H. Ewing, Sanitary Corps, United States Army, sent several strains, identified as *Shigella,* to the author to determine whether some of them may be identical with type II of *S. alkalescens.* Serological studies carried out in this laboratory revealed that Ewing's strain No. 2-193 differed from both types I and II of *S. alkalescens.* While these studies were in progress a *S. alkalescens* strain (No. 9731) was isolated from the feces of a healthy infant, which was not agglutinated to titer by antisera to types I and II as well as strain No. 2-193. The experiments reported in this communication reveal that strains No. 9731 and No. 2-193 represent hitherto undescribed types of *S. alkalescens.*

Material and Methods. The following strains were employed: (1) Strain No. 1-2, *S. alkalescens* type I, received from Captain Ewing; (2) Strain No. 648, *S. alkalescens* type II, obtained through the courtesy of Dr. Arlindo de Assis; (3) No. 2-193 sent by Captain Ewing; (4) No. 9731 isolated at this laboratory from the feces of a healthy infant.

Antisera were prepared in rabbits. Two rabbits were injected intravenously with 3 ml of each bacterial suspension. Two injections, one week apart, were given. Ten days after the last injection the animals were bled and the serum was collected.

In addition to these sera, 2 anti-*S. alkalescens* type I sera were employed: (1) No. 842 prepared at this laboratory and (2) a serum obtained through the courtesy of Dr. E. G. E. Murray, McGill University, Montreal.

Sera used in the slide agglutination test for the determination of the various types belonging to the genus *Shigella* were procured

[1] Assis, A., *O Hospital,* 1939, **15**, 447 and 655.

[2] Stuart, C. A., Rustigian, R., Zimmerman, A., and Corrigan, F. V., *J. Immunol.,* 1943, **47**, 425.