Time and Dose Relationships in Estriol-Estradiol Interaction.* (22729)

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Biologic effects of estradiol can be modified by concurrent administration of a second estrogenic substance. Uterotrophic effect of estradiol in castrate female rats can be diminished by injecting estriol(1). Similarly, estradiol-induced pituitary enlargement can be prevented by simultaneous treatment with weaker synthetic estrogens(2). The former effect is reflected not only by organ weights but also by diminished activity of particular enzyme systems in uterine tissue(3). For interpretation of these surprising effects it should be remembered that the dose-response curve of estradiol, after having reached a maximum, declines to a lower plateau. Thus it remains to decide whether combination of two estrogens might only produce the plateau effect of more than optimal estradiol dosages, before other mechanisms are drawn into consideration. Preceding an increase in tissue dry weight, uterine tissue accumulation of fluid results from estrogenic stimulation. The onset of this fluid uptake phase is more rapid with estriol administration than with estradiol(4). In concurrent injections of both hormones, rapidly absorbed and faster acting estriol may diminish the observed response to estradiol by causing a precocious development of maximal uterine response which would pass unnoticed in a standard 3 day assay.

It is the purpose of this study to investigate the possibility of masked-summation effects of estradiol and estriol. Evidently temporal and qualitative differences in uterine response produced by various hormones may also affect the outcome of combination treatments. An analysis has been made of weight changes of uteri in immature female mice following administration of estradiol, estriol and combinations of both.

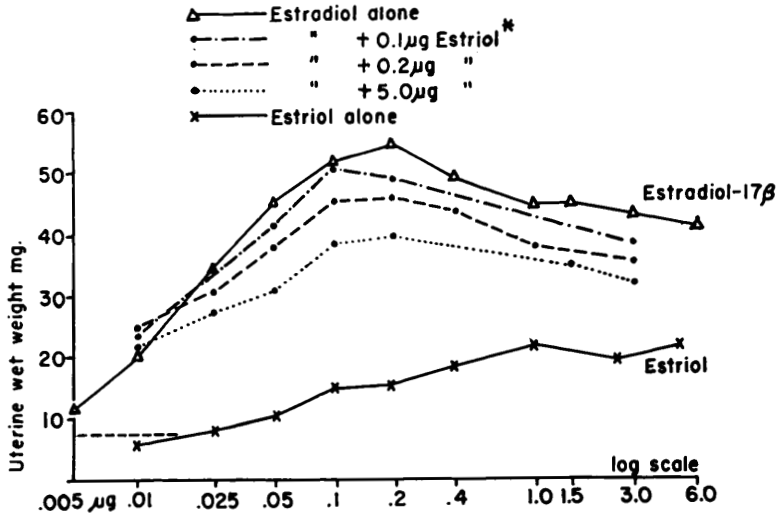

FIG. 1. Dose-response curves of estradiol-17β estriol and combinations of both. The dose of estriol is constant in each of the combination curves, as indicated in the key. Statistical computations appear in Table I.

Methods. Estrogen assays were performed according to the method of Evans, Varney and Koch(5). Dose groups of 5 to 20 immature female mice (Hamilton Laboratory strain) were injected with estradiol-17β, estriol or both, b.i.d. for 3 days. Each total

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TABLE I. Effects of Estradiol-Estriol Combinations on Uterine Wet Weight. 3-day assay.

Estradiol (μg)	Estriol (μg)	No. of animals	Mean	ϵ*	t†	P value
.005	—	14	11.5	.45	—	—
.01	—	24	20.6	.77	—	—
	.01-.1	10	23.7	1.10	2.31	>.02
	.2	5	23.9	1.70	1.77	>.05
	5.0	10	21.3	.74	.66	"
.025	—	10	34.6	1.24	—	—
	.2	10	30.3	1.29	2.40	>.02
	5.0	15	27.7	1.54	3.50	<.001
.05	—	61	45.3	1.14	—	—
	.01-.1	30	41.1	1.23	2.50	>.01
	.2	28	37.4	1.40	4.39	<.001
	5.0	53	30.3	.78	10.87	"
.1	—	42	51.4	1.76	—	—
	.01-.1	30	50.7	1.80	.28	>.1
	.2	20	45.5	2.46	1.95	.05
	5.0	29	38.7	1.90	4.90	<.001
.2	—	22	54.7	2.74	—	—
	.01-.1	30	48.4	1.32	2.07	>.02
	.2	20	45.7	2.14	2.59	>.01
	5.0	39	39.6	1.37	4.92	<.001
.4	—	29	48.5	2.10	—	—
	.2	10	43.4	2.94	1.41	>.05
1.0	—	39	44.5	2.23	—	—
	.2	9	37.1	3.05	1.96	>.05
1.5	—	40	44.9	1.79	—	—
	5.0	5	34.3	3.42	2.75	<.01
3.0	—	40	42.9	1.23	—	—
	.01-.1	10	38.0	2.60	1.70	>.05
	5.0	5	31.4	3.09	3.46	<.01
6.0	—	20	40.7	2.02		

$$* \epsilon = \frac{\sqrt{\Sigma d^2}}{n(n-1)}. \qquad \dagger t = \frac{M_1 - M_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}.$$

dose was contained in 0.3 cc corn oil solution so that a single injection was a volume of 0.05 cc delivered from a 0.25 cc syringe. When 2 estrogens were administered each was given at a separate injection site. A control group injected with corn oil was included with each experiment. Small volumes used eliminated leakage from the needle puncture. On the fourth day, 18 hours after last injection, animals were sacrificed by cervical dislocation and uteri were freed at vagina and caudal ends of the oviducts. Uteri were blotted dry and weighed on a 50 mg Roller-Smith torsion balance. For each group receiving estradiol or mixed estrogens the mean and its standard error were calculated. Student's t test was applied using the formulae indicated in Table I, and employing the 1% confidence limit for determining significance. In some cases means represent the combined data of several experiments. Number of animals per group and pertinent statistical computations appear in Table I. In a separate set of experiments injection schedules were set up as above with 30 animals per group. At the end of 1 day, 2 days, and 3 days, 10 animals of each dosage group were sacrificed and uterine weights recorded (Table III). Dry weights of uteri were obtained by placing organs in preweighed aluminum foil pans and drying in an oven at 100°C. Usually constant final dry weight was achieved after 18 hours.

Results. Dose-response curves of estradiol and estriol (Fig. 1). A uterine weight increase of 100% above control value is produced with about .01 μg of estradiol (total 3 day dose). Maximal response occurs only with a total dose of .2 μg. At higher doses uterine weight increase remains below this maximal value. Estriol stimulates 100% increase in uterine weight at a dose of about .1 μg and an even greater increase with a total dose of 1.0 μg. Higher doses of estriol, up to 5.0 μg, do not enhance uterine stimulation. Both dose-response curves, therefore, ascend to maxima and then plateau. Slope of the ascending portion is steeper on the estradiol than on the estriol curve.

Dose-response curves of estradiol and estriol combinations (Fig. 1). Estriol inhibits the uterine response to estradiol at all dosage levels tested except .01 μg. At this point there is no significant difference between estradiol alone and any of the combination doses (Table I). Extent of inhibition increases in proportion to the estriol fraction. As a result there is a gradual decline in slopes of mixed dose-response curves; the greater amount of added estriol, the greater decline. In no case does the addition of estriol enhance the estradiol effect.

Effects of estrogen combination on uterine dry weights (Table II). The inhibiting effect described above, measured by weights of freshly dissected uteri, is not merely a matter

TABLE II. Effect of Estriol on Uterine Response to Estradiol Dry Tissue Weights—3 Day Assay.

Group*	Dose, μg	Dry wt uterus, mg
Control		1.64 ± .23†
Estriol	5.0	4.23 ± .31
Estradiol	.05	7.33 ± .34
Both	as above	5.46 ± .41‡

* 20 animals/group.
† ± stand. error.
‡ P value, vs. estradiol alone, .001.

of changed water balance but is also reflected by changes in dry weights. This is illustrated by combination of 5.0 μg estriol with .05 μg estradiol, which produces a significantly smaller gain than occurs following administration of .05 μg estradiol alone.

Daily increments in uterine weights with estrogen treatments (*Table III*). Daily uterine wet weight gain during administration of a total dose of .05 μg estradiol over a 3 day period is shown in Table III. Largest percentage gain occurs during the second day of treatment and a further uterine weight increase takes place during the third day. Uterine weight changes induced by a total dose of 5.0 μg estriol in 3 days follow a different course. After a slight initial gain during the first day, major portion of weight increase occurs during the second day and the third day's treatment fails to cause a further increase.

Uterine weight gain of each 24 hour period during the 3 day estradiol treatment is inhibited when estriol is given concurrently (doses of each as above). Uteri of doubly injected animals sacrificed after the first day show only 73% of weight gain resulting from estradiol alone (an inhibition of 27%). For a 2 day period expected estradiol stimulation is diminished by 42%. After completion of the 3 day injection schedule inhibition of weight gain is also 42% (Table III).

TABLE III. Daily Effects of Estrogens on Uterine Weight.

Group*	Dose, μg	Hr	Wet wt uterus, mg	Inhibition of daily wt gain, %†
Estriol	1.66	24	10.1	
Estradiol	.16	"	15.4	
Both	as above	"	13.2	27
Estriol	3.33	48	17.6	
Estradiol	.033	"	34.1	
Both	as above	"	21.9	42
Estriol	5.0	72	18.9	
Estradiol	.05	"	49.2	
Both	as above	"	30.0	42
Control		"	7.3	

* 10 animals/group.
† Estradiol-induced wt gains above control, considered 100%.

Discussion. Impeding effect of estriol occurs along the ascending segment of the estradiol dose-response curve as well as with higher doses. Extent of inhibition, using a conventional 3 day assay, is proportional to amount of estriol administered. There is no instance of an additive effect. From these observations it may be concluded that diminution of uterine weight increase is not a masked-summation effect in which two estrogens exceed the maximum estrogenic response dose. Among the dosages of estradiol employed, only .01 μg gives a response comparable to that obtained with estriol. It is of particular interest, therefore, to compare this dose of estradiol alone with a combination using an amount of estriol which causes the same uterine response (5.0 μg). In this case responses to single estrogens or to the combination are the same. These facts concerning dose relationships of estriol-estradiol interaction suggest that a competitive inhibition results in the attenuated response. Day by day analysis of uterine changes in doubly injected animals supports this view. There is no indication that combination of estriol and estradiol shifts time of maximal effect since inhibition occurs as early as the first day of treatment. Hence doubly injected animals never equal or exceed uterine response resulting from only estradiol.

In addition to estriol, several other steroids have been found to exert a limiting effect on estradiol-induced uterine stimulation in rats (6.7). Particular emphasis has been placed on the ability of adrenal cortical steroids to impede uterine response to estradiol(8). The possibility remains to be tested that estriol and other non-adrenal steroids known to inhibit estradiol stimulation of uterine growth and enzyme systems(3) act by causing release

of ACTH and secondarily adrenocorticosteroids with their modulating effect on uterine metabolism.

Summary. Uterine response to estradiol in immature female mice is inhibited significantly by concurrent injection of estriol. Extent of inhibition is proportional to amount of estriol added. Impeding interaction occurs along the ascending segment of the estradiol dose-response curve and with higher doses as well. In no case does a combination of estrogens have a positive additive action. These conclusions are based on both wet and dry uterine weight observations. Day by day analysis shows that uterine weight gain of each 24 hour period during 3 day estradiol treatment is inhibited when estriol is given concurrently. These studies negate the possibilities that estriol-estradiol interaction is a masked-summation effect in which time of maximum effect has shifted, or that an additive effect occurs in which two estrogens combined exceed maximum response dose. Rather, it appears that estriol competes actively at a physiologic site essential in development of usual estradiol stimulation of the uterus.

1. Hisaw, F. L., Velardo, J. T., and Goolsby, C. M., *J. Clin. Endocrin. and Metab.*, 1954, v14, 1134.

2. Segal, S. J., and Thompson, C. R., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 623.

3. Villee, C. A., *J. Clin. Endocrinol. and Metab.*, 1956, v16.

4. Astwood, E. B., *Endocrinology*, 1938, v23, 25.

5. Evans, J. S., Varney, R. F., and Koch, F. C., *Endocrinology*, 1941, v28, 747.

6. Velardo, J. T., Hisaw, F. L., and Bever, A. T., *Anat. Rec.*, 1953, v117, 552.

7. Szego, C. M., and Roberts, S., *Amer. Jour. Physiol.*, 1948, v152, 131.

8. Velardo, J. T., and Sturgis, S. H., *J. Clin. Endocrinol. and Metab.*, 1956, v16, 496.

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Factors Influencing Host-Virus Interactions. II. Alteration of Coxsackie Virus Infection in Adult Mice by Cold.* (22730)

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Coxsackie virus infections in infant and adult mice are remarkably dissimilar(1,2,3). Many tissues, such as brain, muscle, liver, and heart, develop extensive lesions in very young animals but become refractory to the virus as mice reach 1 to 3 weeks in age. In infections with Conn. 5 strain of Coxsackie virus in adult mice the pancreas appears to be the only regular site of virus multiplication and histopathology. Even though, as a result of initial viremia, virus is available to other tissues, viral multiplication and tissue damage remain localized and non-fatal. A single injection of cortisone alters the infection with Conn. 5 virus in adult mice in such a manner that the infection becomes lethal (4) with multiplication of the virus and histopathology occurring in tissues that are unaffected in untreated animals of the same age (3). This report presents data indicating that the natural resistance of adult mice to disseminated infection with this virus can be altered by another factor, namely a cold environment, to result in rapidly and uniformly fatal disease.

Materials. Virus. The Conn. 5 strain of Coxsackie virus was used and methods for

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