Oestrogen Antagonisms: the Effects of Various Steroids on the Uterine Growth Produced in Mice by Oestriol

In a series of earlier papers we have surveyed the effects of various steroids on the uterine growth produced in mice by oestrone[1–3]. In extending the range of substances evaluated in the standard assay developed for those studies, a similar technique has been employed here to compare the effects of various steroids on the uterine weight increase produced by oestriol. Results from progesterone in this assay have already shown that it does not inhibit the uterine growth induced by 100 μg of oestriol, but that in contrast the response to oestrone at a dose of 0·3 μg can be diminished by progesterone[4].

The methods of this assay procedure were not remarkably different from those described in the papers cited above. Immature female Rockland mice, 22 to 25 days of age, received test compound in three daily, equal injections. 24 h after the final injection the mice were sacrificed, the uteri were removed, cleaned of adherent tissues, scored, and blotted to remove contained fluids and weighed wet on a torsion balance. All evaluations are based upon the mean weight of uteri from groups of 8–10 mice.

Oestriol, at a dose of 100 μg, was employed as a standard uterine growth stimulator. This dose of oestriol produced responses that varied between a low of approximately 30 and a high of about 45 mg. Compounds to be tested were either mixed in oil solution with the oestriol, or in the case of the glucocorticoids, were given in separate, microcrystalline suspensions.

Cortisone and cortisol, as the acetates, were each tested in a single assay (Table). Neither of these substances had any obvious effect. The absence of an inhibition of oestriol-induced uterine growth by these glucocorticoids is similar to the results obtained in our oestrone test in which the glucocorticoids were inactive[3].

Absence of effects of cortisone acetate and cortisol acetate on the uterine growth produced by 100 μg of oestriol

Dose (μg) Oestriol	Dose (μg) Cortisone Acetate	N	Uterine Weight (mg)
100	0	9	34·9
100	100	9	33·6
100	300	10	36·3
100	1000	10	34·2
0	0	9	11·2
Average Uterine weight = 34·3. Slope of least squares line = 0·54 ± 2·89, not significantly different from 0			
Dose (μg) Oestriol	Dose (μg) Cortisol Acetate	N	Uterine Weight (mg)
100	0	10	32·0
100	30	10	38·2
100	100	10	40·6
100	300	9	35·2
100	1000	10	34·9
0	0	9	15·3
Average Uterine weight = 36·2. Slope of least squares line = 2·99 ± 2·03, not significantly different from 0			

Desoxycorticosterone acetate was studied in a series of tests in which the uterine response to the 100 μg dose of oestriol averaged 36 mg (Fig. 1). Inspection of the data suggested no remarkable effect of DCA on oestriol-induced uterine growth. This was confirmed by the fact that the slope of the least-squares dose-response curve was not significantly different from zero. Like the progesterone data cited above[4], DCA inhibited oestrone-induced uterine growth[3] while having little or no effect upon the growth produced by oestriol.

Testosterone propionate proved to be a blocker of oestriol-stimulated uterine growth (Fig. 2). Doses of 300 and 1000 μg of TP produced inhibition of this growth.

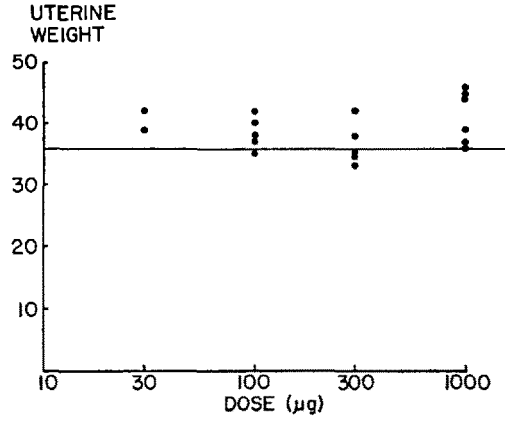

Fig. 1: Lack of effect of desoxycorticosterone on the uterine response to 100 μg of oestriol. Response of controls to 100 μg oestriol alone is shown by the horizontal line. In the five experiments summarized here, the average uterine weight of simultaneous, oil-treated controls was 15·44 mg. Average uterine weight = 38·33 mg. Slope of least squares line = 0·48 ± 1·72, not significantly different from 0

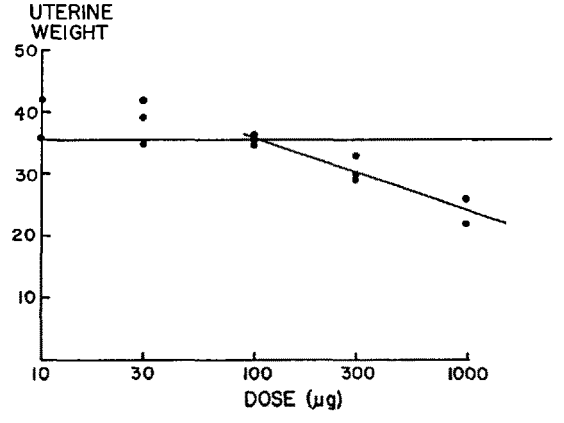

Fig. 2: Effect of testosterone propionate on the uterine response to 100 μg of oetriol. The horizontal line shows the control response to oestriol alone. In the three experiments summarized here, the average uterine weight of simultaneous, oil-treated controls was 9·84 mg. Uterine weight = 36·5–11·6 (log dose – 2·00) for doses of TP from 100 to 1000 μg. $b = -11{\cdot}6 \pm 1{\cdot}53$

[1] R. A. EDGREN and D. W. CALHOUN, Proc. Soc. Exp. Biol. Med., *94*, 537 (1957).
[2] R. A. EDGREN, D. W. CALHOUN, R. L. ELTON, and F. B. COLTON, Endocrinology *65*, 265 (1959).
[3] R. A. EDGREN and D. W. CALHOUN, in preparation.
[4] R. A. EDGREN, R. L. ELTON, and D. W. CALHOUN, in preparation.

On the other hand, doses of 10, 30, and 100 μg of TP diminished the growth produced by 0·3 μg of oestrone[1]. Thus a ratio of 3:1 to 10:1 testosterone to oestriol was inhibitory whereas a ratio of at least 33:1 testosterone to oestrone was required to produce inhibition. Testosterone would appear to be at least three times more effective as an oestriol antagonist than as an oestrone antagonist.

Thus the uterine growth produced by oestriol at a single dose of 100 μg was blocked by the simultaneous administration of testosterone propionate. On the other hand, this growth was not significantly affected by cortisone, cortisol, desoxycorticosterone, or progesterone. Oestrone-induced uterine growth (0·3 μg) in a test of this type was inhibited by DCA and aldosterone, testosterone propionate, and progesterone – only the glucocorticoids were inactive among naturally occurring, non-oestrogenic types of steroids. This difference in pattern of blockage led us to suggest that there were two receptor sites for steroids in the uterus[5]. According to this hypothesis one site (A) would accept oestrone, DCA, progesterone, and testosterone propionate and the other site (B) oestriol and testosterone. Further experiments (unpublished) have forced a modification of this hypothesis since some of the oestriol appears to attach to site A. Additional studies are in progress in an attempt to complete this dual receptor site hypothesis and to clarify the mechanisms involved in uterine growth.

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Division of Biological Research, G. D. Searle & Co., Chicago (Illinois), October 1, 1959.

Zusammenfassung

Das in juvenilen Mäusen durch Oestriol (100 μg) stimulierte Uteruswachstum wurde durch Testosteronpropionat blockiert. Mit Cortisonacetat, Cortisolacetat, Deoxycorticosteronacetat und Progesteron wurde keine solche Wirkung erzielt.

[5] R. A. Edgren and D. W. Calhoun, Anat. Rec. *134*, 558 (1959).

Stimulation of Plant Development by Some Uracil Analogues

Some time ago we were able to show that the organs of germinating seeds are the site of intensive nucleic acid synthesis[1]. For this reason, the first stages of germination appear to be particularly suitable for studying the effects of biologically active analogues of nucleic acid components. In pursuance of this line of investigation, we had earlier studied[2] the effect of 5-bromouracil, a compound known to inhibit the growth of certain microorganisms[3,4], on germinating plants, and found that at certain concentrations it not only failed to inhibit growth, but markedly stimulated development of the plants. A number of further derivatives of uracil were examined for analogous effects, and a number of them were found to stimulate the development of certain plants not only during germination, but also during the later vegetative period[5].

In the last few years, we have carried out field trials on crop plants using 5-bromouracil and 5-nitrouracil, which had proved most effective in laboratory experiments. The seeds were treated as in earlier work, by soaking them in solutions containing 5 to 50 μg of the stimulators per ml for 24 h just prior to sowing. The most general result of such a single treatment with 5-bromouracil is an accelerated development of the plants which in some cases may persist up to the time of harvesting. For instance, treated tomatoes on the average ripen 10 to 14 days earlier than untreated controls, and in the case of lettuce the first harvest from treated seeds is about 20% higher than from controls. The effect of 5-nitrouracil on lettuce is similar. The same compound increases the yield of cucumbers to a statistically significant extent – about 20–40% above that of controls (seeds soaked in water for the same period). The cucumbers treated with 5-nitrouracil show certain morphological peculiarities in the course of development. The ovaries of such plants begin to elongate much earlier so that at the full development of the perianth they are 4–5 times longer than the ovaries of control plants at the same stage of development. The ovaries of the treated plants do not lie along the ground, as is usual with cucumbers but are characteristically erect.

In an effort to elucidate the mechanism of stimulation, we first studied the tissues of stimulated plants cytologically. In certain cases, the stimulators were found to cause a massive increase in the number of mitoses. Thus, in the roots of onions (*Allium cepa*) allowed to sprout in aqueous solutions of the stimulators, the number of mitoses after 4 to 8 days is markedly higher than in the controls sprouting in water; 5-bromouracil at 10 μg/ml and 100 μg/ml was found to increase the number of mitoses by 30% and 60% respectively on the fourth day, and by 70% and 35% respectively on the eighth day.

We further attempted to follow the metabolic fate of 5-bromouracil in plants. The experiments were carried out with germinating cucumbers grown under standard conditions and infiltrated with 0·001 *M* solutions of 5-bromouracil-2-^{14}C[6]. After 3 h and 18 h, no combined 5-bromouracil was found in the nucleotide fraction, nor in the ribonucleic or deoxyribonucleic acids obtained according to Ogur and Rosen[7]. This finding indicates that the fate of 5-bromouracil in the plant organism differs distinctly from its fate in microorganisms in which it is known to be able to replace a considerable proportion of the thymine in deoxyribonucleic acid[8]. This difference is presumably due to the fact that 5-bromouracil is rapidly degraded in the plant. When seedlings are incubated after infiltration with 5-bromouracil, this degradation may be followed by the evolution of $^{14}CO_2$, which reaches a maximum after only 1 h incubation. However, 5-bromouracil does have a distinct effect on the metabolism of the purine and pyrimidine bases, particularly of uracil. Plants infiltrated with uracil-2-^{14}C together with (unlabelled) 5-bromouracil show a distinctly decreased rate of degradation of uracil to $^{14}CO_2$ as against control plants infiltrated

[1] Z. Šormová and F. Šorm, Chem. listy *50*, 629 (1956); Coll. Czechosl. chem. Comm. *21*, 1043 (1956).

[2] Z. Šormová, F. Šorm, J. Bauerová, and M. Zelinková, Fiziologia rastenij *3*, 204 (1956).

[3] G. H. Hitchings, E. A. Falco, and M. B. Sherwood, Science *102*, 251 (1945).

[4] F. Weygand, A. Wacker, and H. Dellweg, Z. Naturf. *7b*, 19 (1952).

[5] Czechoslovak Patent 87672.

[6] The labeled 5-bromouracil was prepared by Dr. J. Morávek of the Institute for Research, Production, and Utilisation of Radioisotopes, Prague.

[7] M. Ogur and G. Rosen, Arch. Biochem. *25*, 262 (1950).

[8] S. Zamenhof, B. Reiner, R. de Giovanni, and K. Rich, J. biol. Chem. *219*, 165 (1956).