Relaxin and Mammary Growth in the Mouse. (18956)

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Daily administration of .83 μg of estradiol benzoate, 1 mg of progesterone, and 25 guinea pig units of relaxin for 13 days produced an advanced degree of mammary alveolar development in ovariectomized immature rats. Combinations of any two of these hormones caused only a slight growth of the mammary gland(1). Since an advanced degree of development of the mammary gland of the castrated male or female rat may be obtained with larger dosages of estrogen and progesterone(2,3), exogenous relaxin, while enhancing the response, is not essential for the mammary alveolar response to estrogen and progesterone. In the guinea pig, in which estrogen alone is capable of producing full alveolar development, a quantitative increase in the degree of development was achieved by the addition of relaxin to the estrogen(4). In the rabbit, addition of relaxin to estrogen transferred the emphasis of growth from the primary ducts to the entire ramifying duct system without inducing significant alveolar growth. These authors conclude that the action of relaxin in respect to mammary growth is in the role of a potentiator of estrogen rather than producing specific changes attributable to relaxin *per se*(4).

The present report represents an attempt to determine the effect of relaxin on mammary growth in the mouse. Mixner and Turner (5,6) developed a method for the assay of materials which stimulate mammary alveolar development in the ovariectomized virgin female mouse. The assay mice receive 10 daily injections of a low level of estrogen, in itself insufficient to cause an alveolar response. When graded doses of progesterone were simultaneously administered, the percentage of mice showing a minimum but definite alveolar response was directly proportional to the dose of progesterone. A total dose of from .75 to 1 mg of progesterone gave a positive response in approximately 50 per cent of the mice. The level of estrogen could be varied over a fairly wide range without significantly altering the response obtained.

Methods and materials. Using mice of different strains than those used by Mixner and Turner, a lower order of response to estrogen and progesterone was observed (Table I). Mixner and Turner (personal communication) have observed strain differences in the mammary response of mice to estrogen and progesterone, 3 additional sources of mice showing a lower response than those originally reported on.

In an attempt to carry the extent of alveolar development beyong the minimal or initial stage, 3 ovariectomized C_{57} mice were given .3 μg of estradiol benzoate plus 1 mg of progesterone daily for 21 days (6.3 μg plus 21 mg total). Each mouse showed only a slight alveolar development. It was felt that perhaps the addition of relaxin might result in a more advanced degree of alveolar response to estrogen and progesterone, as well as in a higher percentage of positive responses. Ovariectomized virgin female mice received either 10 or 20 daily injections of estradiol benzoate, progesterone, and relaxin,† in various combinations, in the dosages indicated in Tables II and III. A mammary biopsy was taken at

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2. Trentin, J. J., unpublished.

3. Curtiss, C., *Endocrinology,* 1949, v45, 284.

4. Garrett, F. A., and Talmage, R. V., *Anat. Rec.,* 1950, v108, 523. (Abstract)

5. Mixner, J. P., and Turner, C. W., *Endocrinology,* 1941, v29, 324.

6. Mixner, J. P., and Turner, C. W., *Endocrinology,* 1942, v30, 591.

† The relaxin, generously provided by Dr. R. L. Kroc, Chilcott Laboratories, Morris Plains, N. J., was prepared from sow ovaries and assayed 100 GPU per cc.

TABLE I. Mammary Alveolar Response of Ovariectomized Virgin Female Mice to 10 Daily Injections of Estrogen and Progesterone.

Strain	Total dose: Estrone (I.U.)	Total dose: Progesterone (mg)	No. of mice	Response: Pos.	Response: Neg.
CBA × A Hybrids (F_1)	75	1.	6	0	6
"	75	2.	6	1	5
C_3H	75	2.	9	3	6

TABLE II. Mammary Alveolar Response of Ovariectomized Virgin Female Mice to 10 Daily Injections of 1 μg Estradiol Benzoate, 1 mg Progesterone, and 25 Guinea Pig Units of Relaxin in Various Combinations.

Strain	Treatment	No. of mice	Response: Pos.	Response: Neg.	Avg interpubic gap (mm)
C_3H	E*	5	1	4	.5
	E + R	4	1	3	.9
	E + P	4	1	3	0
	E + P + R	4	3	1	.07
C_3H × A Hybrids (F_1)	E + P	12	2	10	.03
" "	E + P + R	12	4	8	.14

* E = estrogen. R = relaxin. P = progesterone.

TABLE III. Mammary Alveolar Response of Ovariectomized Virgin Female Mice to 20 Daily Injections of 1 μg Estradiol Benzoate, .25 mg Progesterone, and 25 Guinea Pig Units of Relaxin in Various Combinations.

Strain	Treatment	No. of mice	Response: Pos.	Response: Neg.	Avg interpubic gap (mm)
C_3H × A and CBA × A Hybrids (F_2 and F_3)	Untreated controls	3	0	3	0
	E*	4	0	4	1.5
	E + R	4	0	4	2.5
	E + P	4	4	0	.4
	E + P + R	4	4	0	1.5

* E = estrogen. R = relaxin. P = progesterone.

the time of ovariectomy to eliminate "false positive" animals. Those mice receiving all 3 hormones for 10 days showed a somewhat higher percentage of positive responses than those receiving only estrogen and progesterone (Table II). In neither case, however, was alveolar development very extensive. Of the mice receiving 20 injections, all those receiving estrogen and progesterone showed a positive response whether or not relaxin was also given (Table III). The extent of alveolar development was roughly comparable in the 20-day estrogen plus progesterone, and estrogen plus progesterone plus relaxin groups, with only one mouse in the latter group showing a more advanced development than in the former group. This animal showed a development equal to that of the 10th or 12th day of pregnancy. It is apparent, therefore, that even the most advanced degree of alveolar development obtained did not approach that of late pregnancy. The mouse appears to differ from the rat with respect to the rate and extent of mammary growth attainable with the ovarian steroids, and with respect to the effect of added relaxin. It is possible that higher levels of relaxin may be required in the mouse than in the rat, or that dividing the daily dose of relaxin, as was done by Hamolsky and Sparrow, may give a greater effect.

Measurements of the interpubic gap (Tables II and III) provide confirmation of the observation of Hall(7) that in the mouse, unlike the guinea pig, progesterone inhibits the pelvic relaxing effect of estrogen or of estrogen plus relaxin.

A composite x-ray photograph was taken of one femur from each of 16 of the 19 mice on the 20-day experiment. The expected increase in bone density of the estrogen-treated

7. Hall, K., *Jour. of Endocrinology*, 1950, v7, 54.

mice, as compared to the untreated controls, was observed(8). No significant modifying effect of either progesterone or relaxin was apparent.

Summary. Little or no increase in the percentage of positive mammary alveolar responses was obtained in ovariectomized mice treated with estrogen plus progesterone plus relaxin as compared to those treated with estrogen plus progesterone only. Progesterone inhibited the pelvic relaxing effect of estrogen or of estrogen plus relaxin. Neither progesterone nor relaxin exerted any significant modifying effect on the increased bone density resulting from estrogen administration.

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Protection of Mice Against Vaccinia Virus by Administration of Benzaldehyde Thiosemicarbazone.* (18957)

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Domagk and associates(1) reported that benzaldehyde thiosemicarbazone inhibited growth of the tuberculosis bacillus *in vitro*. Certain derivatives of this compound were found to protect experimental animals against tuberculosis(2). Hamre *et al.*(3,4) investigated the effect of substituted benzaldehyde thiosemicarbazones on other microorganisms. The *p*-amino analogue was observed to delay death and to protect a small percentage of chick embryos and mice infected with vaccinia virus. The *p*-acetamido analogue was less effective.

The present report deals with the effect of benzaldehyde thiosemicarbazone and of several *para* substituted derivatives on vaccinia virus. The compounds used in the study were obtained from Dr. A. F. Langlykke of E. R. Squibb and Sons.

Experimental. In *in vitro* experiments the CVII strain of vaccinia virus was cultivated in a medium containing minced chick embryonic tissues(5). The International Health Division (IHD) strain of virus was employed in *in vivo* tests(6). Mice, in groups of 6, were inoculated with viral dilutions of 10^{-2}, 10^{-3} and 10^{-4} by the cerebral route. Materials to be tested were fed to mice in a sucrose-casein diet.

Results. Benzaldehyde thiosemicarbazone (No. 1) was found to inhibit completely viral multiplication when present in a concentration of 1 μg per ml of culture medium (Table I). Substitutions in the *para* position of the benzene nucleus reduced activity in all instances (No. 2-8). The introduction of an isobutyl group in the 4-position of the thiosemicarbazone portion of the molecule (No. 9) likewise decreased *in vitro* activity. With the exception of compound No. 3, none of the substances was completely in solution when added to the culture medium.

The failure of vaccinia virus to multiply in the presence of thiosemicarbazones does not appear to be due to inactivation of the virus by these substances. Benzaldehyde thiosemicarbazone was added to Tyrode's solution containing 5% normal rabbit serum and a known strength of vaccinia virus to give a concentration of 0.05 mg of the compound per ml of

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2. Domagk, G., *Beitrage z. Klin. d. Tuber.*, 1948, v101, 365.

3. Hamre, D., Bernstein, J., and Donovick, R., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 275.

4. Brownlee, K. A., and Hamre, D., *J. Bact.*, 1951, v61, 127.

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6. Thompson, R. L., Price, M. L., Minton, S. A., Jr., Falco, E. A., and Hitchings, G. H., *J. Immunol.*, in press.