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Relation of Relaxin to Steroid Ovarian Hormones on Production of Mammary Spreading Factor.* (20076)

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Hamolsky and Sparrow(1) reported that daily treatment for 13 days with a combination of 0.83 μ g of estradiol benzoate, 1 mg of progesterone and 25 guinea pig units of relaxin produced greater mammary gland growth in immature ovariectomized female rats than treatment with any 2 of these hormones. Since it has been well established that estrogen and progesterone in sufficient quantities produce good mammary gland growth (see review by Folley and Malpress(2)) it was concluded by Garrett and Talmage(3) that relaxin must act as a potentiator of estrogen rather than producing specific changes attributable to relaxin. A recent investigation by Trentin(4) on the effect of relaxin on mammary gland growth in the mouse has shown that there was little or no increase in the percentage of positive mammary alveolar responses in the ovariectomized mice treated with estrogen, progesterone and relaxin as compared to those treated with estrogen and progesterone only. Earlier observations by Elliott and Turner (5) showed that the injection of estradiol benzoate and progesterone into castrate albino rats in the proper proportions caused maxi-

mum elaboration or activation of a spreading factor which could be extracted from the mammary gland.

It has been suggested that the spreading factor which can be extracted from the growing mammary gland makes possible the forward extension of the mammary duct system into the fatty pad. It was thought possible that relaxin might directly or indirectly influence the elaboration or activation of the spreading factor and thus contribute to the greater mammary gland extension observed by Hamolsky and Sparrow(1).

Methods and materials. Castrate female albino rats weighing from 150 to 250 g were employed starting on the eleventh day post-operatively. The estradiol benzoate and progesterone used was dissolved in olive oil while relaxin was prepared as an aqueous extract of swine ovaries.[†] Daily subcutaneous injections were continued for 10 days. On the eleventh day the animals were killed, the mammary glands removed, extracted, and the amount of spreading factor determined by a method previously described(6).

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[†] The relaxin used was generously provided by Dr. R. L. Kroc, Chilcott Laboratories, Morris Plains, N. J.

TABLE I. Effects of Hormone Treatment on Amount of Spreading Factor.

Controls and treatments	No. of animals	§	— Assay of spreading factors† —	
			Avg final bleb, mm	Area of spread, mm ² , mean ± S.D.
Aqueous buffer solution, pH 6	8		16 × 17	59.7 ± 13
Castrate ♀	5†		16 × 16	47.1 ± 12.6
10 day pregnant ♀ *	"		21 × 21	192.5 ± 16.5
1 µg estradiol benzoate *	"		16 × 18	72.3 ± 13.3
5 " " *	"		20 × 21	176 ± 16.4
5 mg progesterone *	"		18 × 19	114.7 ± 14.5
1 µg E. B. + 5 mg progesterone *	"		21 × 22	209 ± 16.9
1 " + 25 GPU relaxin	"		17 × 18	86.4 ± 14.1
5 " + " " "	"		20 × 22	191.7 ± 16.5
5 mg progesterone + 25 GPU relaxin	"		18 × 19	114.7 ± 14.5
1 µg E. B. + 5 mg progesterone + 25 GPU relaxin	"		21 × 22	209 ± 16.9

* Inserted from previous exp. for comparison (5,6).

† Castrate female rats.

‡ .2 cc extract + .1 cc Evans blue dye.

§ Avg initial bleb 14 × 14 mm.

Groups of 5 rats were treated as follows:

- a) 1 µg estradiol benzoate and 25 guinea pig units of relaxin; b) 5 µg estradiol benzoate and 25 guinea pig units of relaxin; c) 5 mg progesterone and 25 guinea pig units of relaxin; and d) 1 µg estradiol benzoate, 5 mg progesterone and 25 guinea pig units of relaxin.

Results. The extracts from both the 1 µg and 5 µg levels of estradiol benzoate plus relaxin showed only slightly more mammary spreading factor than the same levels of estradiol benzoate alone. The addition of relaxin to the 5 mg of progesterone failed to increase the amount of spreading factor in the mammary gland extracts. Similarly the injection of 1 µg estradiol benzoate, 5 mg progesterone and relaxin produced no increase in the amount of spreading factor in comparison with a group administered estradiol benzoate and progesterone alone (Table I).

Summary. The injection of 25 guinea pig units of relaxin daily for 10 days to castrate female albino rats as an addition to estradiol benzoate and to progesterone failed to increase, significantly, the elaboration or activation of the mammary gland spreading factor in comparison with the steroid hormone action.

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Effect of Age on Local Action of Adrenocortical Hormones.* (20077)

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The direct application to rat skin of adreno-

cortical extract or alcoholic solutions of several

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