TABLE I. Survival following Intravenous Infusion of 5 ml/100 g Body Weight following 4½ Hr Bilateral Hind Limb Tourniquet Application.

	Type infusion	24 hr Lived	24 hr Died	24 hr % survival	48 hr Lived	48 hr Died	48 hr % survival	pH at conclusion of infusion
Exp. 1	.9% NaCl	8	2		7	3		7.40, 7.43
	Saline bicarbonate	8	2		5	5		7.53, 7.43
	Untreated control	2	2		0	4		
2	.9% NaCl	9	8		5	12		
	Saline bicarbonate	8	10		4	14		
	Untreated control	0	4		0	4		
Pooled results	.9% NaCl	17	10	63.0	12	15	44.4	
	Saline bicarbonate	16	12	57.3	9	19	32.1	
	Untreated control	2	6	25	0	8	0	

was made of therapeutic effectiveness of isotonic NaCl and saline-bicarbonate solutions when used in treatment of an otherwise lethal tourniquet trauma. The method of treatment allowed slow constant intravenous infusion of therapeutic solutions over period of greatest loss of fluid from circulating volume into traumatized extremities. The solutions were equally effective in increasing survival rate over that of untreated, traumatized controls. 2. Both solutions produced the same alteration in serum electrolyte concentration, *i.e.*, elevation of serum Cl values. The lowered CO_2 combining power of untreated control was not appreciably altered in the treatment groups.

1. Serkes, K. D., Lang, S., Pareira, M. D., *Surgery,* 1959, v45, 623.

2. Rosenthal, S. M., Tabor, H., *A.M.A. Arch. Surg.,* 1945, v51, 244.

3. Allen, F. M., *ibid.,* 1957, v75, 210.

4. Moyer, C. A., Coller, F. A., Iob, V., Vaughan, H. H., *Ann. Surg.,* 1944, v120, 367.

5. Schales, O., Schales, S., *J. Biol. Chem.,* 1941, v140, 879.

6. Rosenthal, S. M., *Pub. Health Rep.,* 1942, v57, 1923.

Received July 13, 1959. P.S.E.B.M., 1959, v102.

Effect of Relaxin on Mammary Gland Growth in the Female Rat.* (25321)

HIROSHI WADA† AND CHARLES W. TURNER
(With technical assistance of Mary E. Powell)
Dept. of Dairy Husbandry, University of Missouri, Columbia

All previous studies concerning the role of relaxin in growth of mammary gland in the rat have suggested that relaxin acts as a potentiator (or synergist) of lobule-alveolar growth in conjunction with estrogen and progesterone(1,2,3,4). Recently, in studies using desoxyribosenucleic acid (DNA) as a measure of mammary gland growth in both intact and gonadectomized male and female mice, it was shown that estrogen and relaxin alone are capable of stimulating increases in DNA associated with morphological evidence of marked lobule-alveolar growth(5,6). These and previous studies suggested that estrogen and progesterone stimulate endogenous secretion of relaxin and that relaxin might be the agent which stimulates increased secretion of pituitary mammogen rather than progesterone, since it was shown that estrogen and relaxin were ineffective in hypophysectomized mice. The present study extends our studies on the role of estrogen and relaxin in stimulating lo-

* Contribution from Mo. Agr. Exp. Sta., Journal Series No. 2027. Approved by Director.

† Research Scholar, Ministry of Education of Japanese Government and Medical Fellow of Population Council. Present address: College of Agri., Univ. of Okayama, Japan. This investigation supported in part by grant from Am. Cancer Soc.

TABLE I. Effect of Relaxin on Mammary Gland Growth and Pituitary Weight in the Female Rat.

Group		Treatment, amt × days	No. of rats	Body wt (g)	Pituitary		Mammary gland			
					Wet wt (mg)	Wet wt (mg) / 100 g body wt	DFFT wt (mg)	DNA (μg) / mg of DFFT	Total DNA (mg)	Total DNA (mg) / 100 g body wt
Intact	(1)	None—Control	10	281 ± 4	12.8 ± .5	4.6 ± .2	303.2 ± 8.6	28.8 ± 1.0	8.769 ± .481	3.110 ± .161
	(2)	EB, 1 μg × 7	"	279 ± 6	13.3 ± .7	4.8 ± .2	329.4 ± 15.6	27.8 ± 1.5	9.220 ± .778	3.298 ± .243
	(3)	EB, 1 μg × 7 R, 15 GPU	"	286 ± 5	14.8 ± .5*	5.2 ± .1*	330.8 ± 17.5	32.8 ± .9*	10.885 ± .742*	3.793 ± .232*
	(4)	EB, 1 μg × 7 R, 45 GPU	"	262 ± 5	15.0 ± .8*	5.7 ± .3†	364.3 ± 9.9‡	29.8 ± .9	10.846 ± .316†	4.149 ± .166‡
Castrated	(5)	None—Control	10	279 ±11	12.4 ± .3	4.5 ± .3	356.1 ± 21.0	25.9 ± .7	9.261 ± .604	3.320 ± .183
		"	15	273.7				23.2 ± .54		3.05 ± .14 §
	(6)	EB, 1 μg × 9	10	281 ± 4	14.8 ± .5†	5.3 ± .2*	326.7 ± 15.6	26.6 ± 1.2	9.162 ± .255	3.109 ± .095
	(7)	EB, 1 μg × 9 R, 15 GPU	11	240 ± 3	14.0 ± .5*	5.9 ± .3†	342.9 ± 10.8	26.3 ± .5	9.006 ± .219	3.760 ± .097*
	(8)	EB, 1 μg × 9 R, 45 GPU	10	273 ± 4	15.3 ± .6†	5.6 ± .2†	273.1 ± 12.8	37.0 ± 1.16‡	10.545 ± .672	3.852 ± .222

EB = Estradiol benzoate. R = Relaxin. DFFT = Dry, fat-free tissue. Values are means ± S.E.
* Significant at 5% level to control. † Significant at 1% level to control. ‡ Significant at .1% level to control. § Data from Moon *et al.*(7).

bule-alveolar growth in the intact and ovariectomized rat.

Materials and methods. Adult female rats of Sprague-Dawley-Rolfsmeyer strain weighing approximately 230 to 250 g, kept in a room artificially illuminated during daylight hours, at a uniform temperature of 78 ± 1° F, were fed commercial rat feed. One lot was ovariectomized 2 weeks before start of experiment. Estradiol benzoate (E.B.) was injected daily in 1 μg amounts for 7 days to intact rats and for 9 days to ovariectomized rats concomitant with 30 and 90 GPU of relaxin suspended in 0.1 ml sesame oil plus 5% beeswax carrier every other day.‡ One day following final injection, animals were sacrificed, 6 abdominal-inguinal glands removed and frozen for 4 days. The 4 anterior glands were prepared for whole mount examination. Tissues were prepared for DNA determination by method previously described(7). The pituitaries were weighed and frozen until assayed for mammogenic activity. Mammogenic potency of rat pituitaries was assayed in 19 g ovariectomized mice. Each pituitary was ground in cool agate mortar and suspended in 0.6 ml normal saline. Each mouse received ½ rat pituitary/day subcutaneously for 10 days. On 11th day glands were removed for DNA determination (using 30 mg sample) and whole mount examination made of glands.

Results. Indices of mammary gland growth stimulated by the hormones involves increase in dry-fat-free tissue (DFFT), DNA/mg DFFT, total DNA/6 glands, and total DNA/100 g body weight (Table I). Increase in DFFT in intact animals was associated with hormone administration. The rats given estrogen and the higher level of relaxin had glands significantly heavier than the controls (at 0.1% level). It will be noted also that total DNA/6 glands increased significantly above controls as relaxin was increased (at 5 and 1% level respectively) and when compared on body weight basis, the difference at higher level of relaxin became significant at the 0.1% level.

Morphological observations of whole

‡ Relaxin preparation kindly supplied by Dr. R. L. Kroc, Chilcott Lab., Morris Plains, N. J., assayed 30 GPU/mg.

mounts of glands indicated chiefly duct development in the controls and those receiving estrogen alone, whereas those on estrogen and relaxin showed increased lobule-alveolar growth.§ In general, the extent of lobule development was related to the mean total DNA in the 2 groups.

In the ovariectomized groups similar but less significant differences were noted in comparison to intact rats. This may be due in part to the relatively high total DNA values for the castrate-control rats (3.32 mg/100 g body wt.) in comparison to the intact control rats (3.11 mg) and to a control group of 15 similar animals in a separate but concurrent experiment(7) with mean total DNA/100 g body wt. of 3.05 mg. Based on total DNA/100 g body weight of control rats, only the increase in DNA of rats on 15 GPU relaxin/day was significant (at 5% level). However, if the control value for ovariectomized rats reported previously is used (3.05 mg), then the 15 GPU rats are significantly increased at the 1% level and the 45 GPU rats are significant at the 5% level. Less lobule-alveolar development was noted in these glands as compared to glands of the intact groups.

The less significant lobule-alveolar growth in the rats receiving estrogen and relaxin following ovariectomy in comparison to the corresponding intact rats is believed to be due, in part, to the time lag in restoring DNA of glands to normal after involution resulting from ovariectomy, but it may be due to possible hormonal contribution supplied by intact ovaries.

The influence of estrogen alone and concurrent relaxin on pituitary weight is shown (Table I). Relaxin and estrogen stimulated significant increases in weight in comparison with control values.

No evidence of mammogenic activity in the pituitaries of rats under test was shown by ½ pituitary/day for 10 days in mice. Total DNA of mice mammary glands was not increased significantly.

Discussion. Our study with female rats confirms our previous work in male and female mice concerning the influence of relaxin and estrogen in stimulating growth of the mammary lobule-alveolar system. It was previously shown(7) that 1 μg estradiol benzoate and 2 mg progesterone injected into mature ovariectomized rats for 19 days resulted in mammary gland growth (mean total DNA/100 g) equal to that of rats pregnant for a similar period. In unpublished work currently in progress, it has been shown that estradiol benzoate 1 μg and 15 GPU relaxin for 19 days had induced mean total DNA/100 g increase comparable to that induced by 1 mg progesterone. If estrogen and progesterone synergize to stimulate endogenous secretion of relaxin which, in turn, stimulates mammary gland growth, it would indicate that approximately 15 GPU of relaxin/day may be stimulated by 1 mg progesterone/day.

Summary. 1. Intact and ovariectomized rats injected with 1 μg estradiol benzoate and 15 and 45 GPU relaxin stimulated significant growth of lobule-alveolar system in about 10 days as indicated by total DNA/6 glands/100 g and morphological observations. Thus, relaxin need no longer be considered merely a synergist of estrogen and progesterone in mammary gland growth in rats as previously suggested.

§ It should be pointed out that the mature virgin rat duct system contains large numbers of branching ducts in contrast to the virgin mouse). It is far more difficult in the rat to differentiate between ducts and lobule-alveoli than in the mouse.

1. Hamolsky, M., Sparrow, R. C., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 8.

2. Garrett, F. A., Talmage, R. V., *J. Endocrinol.*, 1952, v8, 336.

3. Trentin, J. J., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 9.

4. Smith, T. C., *Endocrinology,* 1954, v54, 59.

5. Wada, H., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 194.

6. ———, *ibid.,* 1959,

7. Moon, R. C., Griffith, D. R., Turner, C. W., *ibid.,* 1959, v101, 788.

Received July 23, 1959. P.S.E.B.M., 1959, v102.