vitamin administered is retained in serum, and a numerically lower percentage is excreted/day in urine, bile, and feces of patients with B_{12}-avitaminosis than in controls. These facts suggest that more B_{12} is retained in states of deficiency.

1. Willigan, D. A., Cronkite, E. P., Meyer, L. M., Noto, S. L., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 81.

2. Okuda, K., Gräsbeck, R., Chow, B. F., *J. Lab. Clin. Med.,* 1958, v51, 17.

3. Gräsbeck, R., Nyberg, W., Perman, G., Reizenstein, P., *Acta physiol. Scand.,* 1957, v42, suppl. 145.

4. Gräsbeck, R., Nyberg, W., Reizenstein, P., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 780.

5. Reizenstein, P., *Nord. Med.,* 1959, v61, 65, 79.

6. Lagerlöf, H., *Pancreatic function and pancreatic disease,* 1942, Nordstedt, Stockholm.

7. Reizenstein, P., *Acta Med. Scand.,* 1959, in press.

8. Sjöstrand, T., *Acta physiol. Scand.,* 1952, v26, 328, 338.

9. ———, *ibid.,* 1948, v16, 201, 211.

10. Engstedt, L., *Acta Med. Scand.,* 1957, v159, suppl. 332.

11. Glass, G. B. J., *J. Lab. Clin. Med.,* 1958, v52, 860.

12. Meyer, L. M., Arkun, S. N., Jiminez Casado, M., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 515.

13. Mollin, D. L., Pitney, W. R., Baker, S. J., Bradley, J. E., *Blood,* 1956, v11, 31.

14. Estrada, S. C., Lang, C. A., Chow, B. F., *J. Lab. Clin. Med.,* 1954, v43, 406.

15. Miller, A., *J. Clin. Invest.,* 1958, v37, 556.

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Effect of Relaxin on Mammary Gland Growth in Female Mice.* (25068)

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

In a previous paper it was reported that relaxin in synergism with estrogen stimulated lobule-alveolar growth of mammary gland of intact and castrate male mice pretreated with diethylstilbestrol to stimulate extensive duct growth(1). The extent of gland development induced experimentally was checked by whole mounts of the glands and by determination of total desoxyribosenucleic acid (DNA) content of mammary glands. The present report concerns the synergistic role of relaxin and estrogen in stimulating growth of the lobule-alveolar system of intact female, ovariectomized, and ovariectomized-hysterectomized mice. For comparison, the type of growth and DNA content of mammary gland of comparable mice injected with estrogen and progesterone are presented.

Procedure. Female virgin albino mice weighing approximately 28 g were fed a commercial mouse feed. The single or concomitant doses of relaxin, estradiol benzoate, and progesterone were injected subcutaneously on the back in 0.1 ml of olive oil daily for 10 days. On 11th day mammary glands were removed. Seven glands (4 posterior and 3 anterior) were analyzed for DNA by Webb and Levy method(2), and 3 anterior glands were prepared for whole mounts for morphological examination. Relaxin‡ was washed with anhydrous ether to remove possible progesterone contamination, dissolved in small amount of distilled water, lyophilized and suspended in olive oil by homogenization.

In one group the ovaries were removed whereas in a second group both ovaries and uteri were removed. Experiments were started 12 days after surgery.

Results. Intact female mice injected with estradiol benzoate 0.75 μg daily had mammary glands containing a mean of 2.541 mg

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† Research Scholar, Ministry of Education, Japanese Govt. and Medical Fellow of Population Council. This investigation supported in part by grant from Am. Cancer Soc.

‡ Relaxin preparations, W1164A-Lot 53, kindly supplied by Dr. R. L. Kroc, Chilcott Lab., Morris Plains, N. J. (assay 30 GPU/mg).

TABLE I. DNA Content of Mammary Gland of Female Mice Treated with Ovarian Hormones.

Group	Dose of hormones*	No. of mice	Dry, fat-free tissue wt (mg) Mean ± S.E.		DNA (μg)/dry, fat-free tissue (mg) Mean ± S.E.		Total DNA (mg) Mean ± S.E.	
Intact mice	E.B., .75 μg	19	63.6	2.2	40.2	1.2	2.541	.093
	Idem + R, .62 GPU	8	72.3	4.1	37.2	1.2	2.599	.202
	" + " 1.25 "	9	69.9	4.4	38.1	2.2	2.659	.246
	" + " 2.5 "	8	76.4	3.3	37.2	1.5	2.838	.150
	" + " 5.0 "	17	68.1	7.1	42.9	1.5	2.886†	.102
	R, 5.0 GPU	9	56.8	4.2	44.4	2.0	2.477	.161
	E.B., .75 μg + P, .75 mg	9	58.2	2.9	45.4	2.6	2.648	.202
	Idem + " 1.5 "	8	60.1	5.0	44.3	1.2	2.658	.213
Ovariectomized mice	E.B., .75 μg	9	61.8	4.9	37.6	1.1	2.343	.224
	Idem + R, 5.0 GPU	10	69.4	2.6	35.4	.7	2.449	.077
	" + P, .5 mg	10	68.6	2.7	35.6	1.1	2.430	.096
	" + " .75 "	10	66.4	1.0	37.9	3.1	2.510	.131
Ovariectomized-hysterectomized mice	E.B., .75 μg	10	63.0	2.8	38.5	.9	2.434	.124
	Idem + R, 5.0 GPU	10	60.1	4.7	42.8	2.4	2.605	.244
	" + P, .75 mg	9	66.9	3.2	39.3	1.5	2.645	.195
	" + " 1.0 "	10	65.0	3.0	40.5	1.1	2.620	.106

* E.B. = estradiol benzoate; R = relaxin; P = progesterone.
† Significant at 2% level from group inj. estradiol benzoate alone.

of DNA per 7 glands. This amount of estrogen concomitant with graded levels of relaxin stimulated mammary glands containing a mean maximum level of 2.886 mg of DNA which is significantly greater than the estrogen control. In comparison, mammary glands of mice on 2 levels of progesterone contained amounts of DNA comparable to the lower levels of relaxin (Table I). The group receiving relaxin alone were not different from the group on estrogen alone.

The whole mounts of glands of mice stimulated with estrogen in some cases showed slight lobule-alveolar growth. Mice stimulated with estrogen and graded doses of relaxin presented a progressive lobule-alveolar development up to the highest dosage. The type of gland development in these groups was essentially the same as that stimulated with estrogen and progesterone.

Mammary glands from ovariectomized and hysterectomized mice injected with estrogen contained less mean total DNA than intact mice. Further, the groups on estrogen and relaxin had less mean total DNA than corresponding intact groups. The groups on estrogen and varying levels of progesterone showed that 0.50 mg per day is less effective than 0.75 or 1.00 mg in promoting mammary gland growth.

In the above groups, removal of the ovaries caused involution of the glands. Injection of estrogen alone stimulated only duct growth, whereas the combinations of estrogen and relaxin or progesterone produced lobule-alveolar growth. The latter development was usually related to the total mean DNA.

Discussion. These data confirm and extend our previous observations(1) with male mice indicating that estrogen and relaxin in suitable amounts has the capacity of stimulating growth of the lobule-alveolar system of the mammary glands of intact, ovariectomized and ovariectomized-hysterectomized female mice comparable to that obtained by estrogen and progesterone as indicated by total mean DNA and histological examination of whole mounts of glands. Neither estrogen nor relaxin alone, in the amounts injected, stimulated lobule-alveolar growth.

In comparison with the male study, it will be noted the extent of total duct development was much greater in females based upon mean total DNA. Thus, in intact male mice total DNA after estrogen administration was 1.572 mg compared to 2.541 mg in intact females. Comparable data on males and females after estrogen and 5 GPU of relaxin are 2.019 and 2.886 mg respectively.

Primiparous mice at 6, 12, and 18 days of

pregnancy exhibit mean total DNA of mammary glands of 2.853, 4.118 and 5.969 mg. Thus, estrogen and relaxin stimulated lobule-alveolar growth as indicated by DNA equalled that of 6 days of pregnancy(3).

In the 2 groups ovariectomized, a period of involution of 12 days was allowed. Restimulation with estrogen did not restore the total DNA level to that shown by the intact animals. Neither was estrogen and relaxin able to stimulate total DNA equal to that of the intact groups. This difference is not considered as related to the lack of ovaries or uteri, *per se*, but rather to the effect of gland involution before hormones were begun. Since hysterectomized mice showed slightly greater total DNA than did the corresponding ovariectomized groups, the uterus has no inhibitory action on the effect of relaxin on mammary gland growth.

Summary. Intact, ovariectomized, and ovariectomized-hysterectomized mice were treated with estrogen, estrogen and relaxin, and relaxin alone for periods of 10 days. It was shown that estrogen and higher levels of relaxin synergize in the promotion of lobule-alveolar growth as indicated by total DNA of the glands and by histological examination of whole mounts. Estrogen or relaxin alone did not stimulate such growth. Mean total DNA at optimal levels of relaxin was equal to that observed in mice pregnant 6 days. In the operated groups, reduced total DNA may be due to gland involution of 12 days.

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

2. Webb, J. M., Levy, H. B., *J. Biol. Chem.*, 1955, v213, 107.

3. Wada, H., Turner, C. W., *J. Dairy Sci.*, 1959.

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Method for Determination of 17,21-Dihydroxy-20-Ketosteroids in Urine and Plasma.* (25069)

EDWARD L. RONGONE (Introduced by Albert Segaloff)
(With technical assistance of Luxurie Sorenson)
Dept. of Biochemistry, Tulane University School of Medicine; Endocrine Research Lab., Alton Ochsner Medical Fn., New Orleans, La.

The adrenal cortex secretes several steroids directly associated with human metabolism. Metabolism, separation, measurement and identification of these steroids have been the subject of much research. A quick, accurate method of measurement of these steroids is of great value to the clinician for correct diagnosis and treatment. Porter and Silber(1) reported that a solution of phenylhydrazine in sulfuric acid formed a colored product when mixed with 17,21-dihydroxy-20-ketosteroids. Using this color reaction Nelson and Samuels (2) formulated the first practical method for routine quantitative determination of 17-hydroxycorticosteroids in blood. By their procedure the blood extract was purified by solvent partition and chromatography on a synthetic magnesium silicate (Florisil) column. Several other methods for determination of free plasma 17-hydroxycorticosteroids have since been developed. Sweat(3) described a procedure involving chromatographic analysis of pure mixtures of several steroids on a silica gel micro column. Quantitative recovery of steroids was determined by technics employing both fluorescence and reaction with phenylhydrazine. A simple, accurate method for determination of the 17,21-dihydroxy-20-ketosteroids in plasma and urine has now been devised. It consists in a method of extraction similar to that of Paterson(4) and a procedure for chromatographic resolution on silica gel columns similar to the one reported by Sweat (3).

Materials. 1) A 1 ml serologic pipette is used as the column. 2) Glass wool (washed repeatedly with redistilled absolute ethanol)

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