2. Sjoerdsma, A., Gillespie, L., Udenfriend, S., *Lancet,* 1958, v2, 159.
3. Garber, E. D., Hauth, F. C., *J. Heredity,* 1950, v41, 122.
4. Udenfriend, S., Weissbach, H., Clark, C. T., *J. Biol. Chem.,* 1955, v215, 337.
5. Weissbach, H., Redfield, B. G., Udenfriend, S., *Fed. Proc.,* 1958, v17, 418.
6. Lewis, G. P., *5-Hydroxytryptamine,* Pergamon Press, 1958.
7. Kamil, I. A., Smith, N. N., Williams, R. T., *Biochem. J.,* 1953, v54, 390.

Received October 8, 1959. P.S.E.B.M., 1960, v103.

Thyroid Hormone and Mammary Gland Growth in the Rat.* (25441)

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

The beneficial effect of thyroid hormone and thyroid active substances upon lactation is well established(1), however, little is known concerning the role of this hormone in influencing growth of mammary gland. Lactation studies have shown that if hormones influencing lactation were administered in optimal amounts, increased milk yields occurred with marked reduction in variability of response among members of the population(2,3). Similarly, any hormone, if present in suboptimal amounts, may also limit mammary gland growth or, at least, contribute to variability in growth which occurs during normal pregnancy or to that of glands developed experimentally with optimal levels of estrogen and progesterone(4). In the present study we determined the effect of mild hyperthyroidism upon experimental development of rat mammary gland using desoxyribosenucleic acid (DNA) as an index of growth.

Materials and methods. Groups of ovariectomized rats of the Sprague-Dawley-Rolfsmeyer strain with an initial weight of 225-275 g were used. Body weight was recorded daily. Fifteen ovariectomized rats killed 34 days post-castration served as controls. Remaining ovariectomized rats received daily subc. injections for 19 days commencing 14 day post-castration as follows: 1) 18 received either 1 or 2 μg estradiol benzoate (EB); 2) 29 were injected with 1 μg EB and 3 or 6 mg progesterone (P); 3) 13 received 2 μg EB and 6 mg P; 4) 31 were treated with 1 μg EB, 3 mg P and 3 or 6 μg l-thyroxine; and 5) 49 received 2 μg EB, 6 mg P and 3 or 6 μg thyroxine. Rats were killed one day after last injection, skinned rapidly and abdominal-inguinal glands removed and immediately frozen for 4 days. Tissues were then thawed and fat extracted in boiling 95% ethanol for 6 hours followed by ether extraction for another 6 hours. DNA was extracted from 25 mg aliquots of finely ground, dry, fat-free tissue (DFFT) by the method of Schneider(5) with exclusion of the fat extraction and cold trichloroacetic acid (TCA) extraction steps. DNA was determined by the method of Webb and Levy(6). The product of the quantity DNA/mg DFFT and total DFFT, expressed as unit body weight, was estimated as the total DNA/posterior 6 glands. Visual observations were made for presence of lobule-alveolar development but no attempt was made to visually quantitate extent of growth.

Results. Total DNA of glands of ovariectomized rats receiving 2 μg EB for 19 days did not differ significantly from those of animals receiving 1 μg EB (Table I). Injections of 1 μg EB and 3 mg P increased mammary gland DNA above that of rats receiving EB alone, but upon increasing EB and P levels to 2 μg and 6 mg, respectively, no significant increase in growth occurred. Addition of 3 or

* Contribution from Mo. Agr. Exp. Sta., Journal Series No. 2073. Approved by Director.

† Postdoctoral Fellow of N.I.H. This investigation supported in part by grants from P.H.S. and Am. Cancer Soc. Present address: Dept. of Physiology, University of Tennessee, Memphis.

TABLE I. Mammary Gland DNA of Adult Rat Ovariectomized for 14 Days Followed by Estradiol Benzoate (EB), Progesterone (P) and Thyroxine (T_4) for 19 Days.

Treatment (amt/day)	No. of rats	Mean: Body wt (g) Initial	Mean: Body wt (g) Terminal	Mean: DFFT* (mg)	Mean: DNA/mg DFFT (μg)	Mean: Total DNA, posterior 6 glands (μg/100 g)
Ovariectomized controls†	15	263.0	273.7	359.2	23.20 ± .54‡	3.05 ± .14[1]
1 μg EB†	5	239.6	246.8	485.6	22.86 ± 1.27	4.48 ± .15[2]
+ 3 mg P†	19	250.0	269.0	590.1	35.65 ± 1.09	7.72 ± .25[3]
+ 6 "	10	253.6	278.4	628.1	35.50 ± 1.53	7.95 ± .34
+ 3 " + 3 μg T_4	19	268.2	270.0	558.6	39.77 ± 2.52	8.03 ± .28
+ 3 " + 6 "	12	238.5	248.1	384.7	47.05 ± .72	7.26 ± .27
2 μg EB	13	269.8	267.8	462.9	24.35 ± .94	4.11 ± .22[4]
+ 6 mg P	12	270.3	272.0	592.6	36.63 ± 1.51	8.13 ± .42[5]
+ " + 3 μg T_4	35	251.6	253.0	662.9	38.47 ± 1.18	9.87 ± .27[6]
+ " + 6 "	14	253.8	256.7	592.1	44.46 ± 3.01	9.95 ± .41[7]

* Dry, fat-free tissue.
† Data from Moon *et al.*(4)
‡ Stand. error of mean.

Students "t"	Probability
1–2	.001
2–3	"
3–6,7	"
5–6,7	.005

6 μg thyroxine to the hormone regime has no beneficial effect upon mammary gland growth at the 1 μg EB and 3 mg P level, whereas thyroxine administered concomitantly with 2 μg EB and 6 mg P results in a significant increase in mammary gland DNA. Body weight increased in all groups except those injected with 2 μg EB alone. Animals receiving 2 μg EB concomitantly with the other hormones maintained their body weight throughout the experimental period. Visual examination of mammary glands of rats treated with EB alone revealed a well developed duct system with endbuds and side branch formation. Animals receiving EB and P and/or thyroxine exhibited excellent lobule-alveolar development.

Discussion. It has been suggested that the observed variability in normal and experimental mammary gland growth may be due, in part, to endogenous secretion of sub-optimal amounts of thyroid hormone(4). If this is true, an increase in mean growth response might be expected upon administration of thyroid active substances. However, as thyroid hormones are general metabolic stimulants, it is important that the levels administered do not cause stress to the animal. In the present study, daily administration of thyroxine at levels slightly above the highest TSR (2.5 μg/100 g) previously observed in our rats(7) did not adversely affect body weight indicating that the levels employed did not produce severe hyperthyroidism. Concomitant administration of 3 or 6 μg/100 g thyroxine with 1 μg EB and 3 mg P had little beneficial effect upon mammary growth, although it had been shown these levels of ovarian steroids produce growth comparable to that of rats pregnant 18-20 days(4). Upon doubling the steroid levels, thyroxine resulted in a significant increase in total DNA which did not occur when the higher levels of EB and P were administered alone. The failure to observe enhanced growth at the low dosage of EB and P may be due to increased metabolic removal (half-life) of these steroids. It is known that removal rate of cortisol is markedly elevated in hyperthyroidism. The increased removal rate of cortisol has been attributed to an increase in metabolic activity of hepatic cells(8). Since the liver is also the principal site of estrogen (E) and P metabolism, it is likely that removal rates of these steroids are also elevated. Increased metabolic removal of E and P, which normally exhibit a very short half-life (9), would tend to reduce the effective circulating level of these steroids below that which is necessary for additional growth. The significant increase in total DNA resulting from injection of thyroxine and the higher level of ovarian steroids suggests that sufficient quantities of the hormones were available to meet the increased metabolic requirements of mam-

mary tissue. Griffith and Turner (unpublished results) have recently observed an increase in total DNA of pregnant rats injected daily with 2.5 or 3.5 μg/100 g thyroxine. It has been shown also that pregnant rats fed desiccated thyroid exhibit precocious mammary gland development(10). These data indicate that during pregnancy endogenous secretion of E and P may be sufficient to overcome the increased metabolic removal of the steroids during hyperthyroidism and as a result, mammary gland growth may be increased. Although the thyroid hormone is not essential for mammary gland growth(11), the present data suggest that variability in total mammary gland growth may be due to a deficiency in TSR or of E and P, or both. When the TSR is high, it is fully effective in stimulating the greatest mammary gland growth only when endogenous E and P secretion is adequate.

Summary. 1) The effect of mild hyperthyroidism upon mammary gland growth has been studied in adult ovariectomized rats treated with estradiol benzoate (EB) and progesterone (P) for 19 days. 2) Using total DNA as index of mammary growth, glands of animals receiving daily injections of 1 μg EB, 3 mg P and 3 or 6 μg/100 g thyroxine did not differ from that of controls. Upon doubling EB and P levels, a significant increase in total DNA was observed. 3) It is suggested that thyroid hormone may be a limiting factor in mammary gland growth when E and P secretions are adequate, but when TSR is optimal, E and P secretion may limit growth.

1. Blaxter, K. L., *Vitamins and Hormones,* 1952, v10.
2. Grosvenor, C. E., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 158.
3. ———, *ibid.,* 162.
4. Moon, R. C., Griffith, D. R., Turner, C. W., *ibid.,* 1959, v101, 788.
5. Schneider, W. C., *J. Biol. Chem.,* 1945, v161, 293.
6. Webb, J. M., Levy, H. B., *ibid.,* 1955, v213, 107.
7. Grosvenor, C. E., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 517.
8. Samuels, L. T., *et al., Ciba Found. Coll. on Endocrinol.,* 1957, vXI, 208.
9. Pearlman, W. H., *ibid.,* 233.
10. Weichert, C., K., Boyd, R. W., *Anat. Rec.,* 1934, v59, 157.
11. Chen, T. T., *et al., Endocrinol.,* 1955, v57, 153.

Received October 9, 1959. P.S.E.B.M., 1960, v103.

Intradermal Vaccination in Healthy Adults With Type A Asian Strain Influenza Vaccine.* (25442)

MARGUERITE L. CANDLER, GERALD R. COOPER, U. PENTTI KOKKO, ROSLYN Q. ROBINSON AND MYRON J. WILLIS
Communicable Disease Center, Public Health Service, U.S.D.H.E.W., Atlanta, Ga.

The intradermal route for administration of Asian strain Type A influenza vaccine was employed widely by practicing physicians in the fall of 1957 when the demand for vaccine exceeded the supply. In view of widespread use of this method of vaccination, this study was undertaken to determine the relative efficiency of various doses of vaccine in producing measurable immune responses. In Sept. 1957 when the program was started, no clinical influenza had been reported in the study area. The vaccine used throughout was a single lot of commercially prepared monovalent Type A Asian strain influenza vaccine which contained 1000 chicken cell agglutination (CCA) units/ml. To administer different amounts of vaccine in the same volume the vaccine was diluted with pyrogen-free saline to make 4 concentrations containing 12.5, 25, 50, and 100 CCA units in 0.1 ml. Two hundred and forty-two civilian employees from

* We wish to acknowledge the cooperation of South Atlantic Corps of U. S. Engineers, who provided volunteers which made this study possible and Dr. Charles C. Rand, Supt. of Grafton State School, Grafton, N. D., who provided convalescent sera.