different age groups varied within a narrow range. Samples of tissue lost from 68-76% of wet weights following drying. Likewise, amount of collagen in tissue, calculated from hydroxyproline values, did not vary significantly for various age groups. Amount of collagen in tissue samples of all age groups studied was between 0.5 and 1.5%.

Discussion. The content of RNA in anterior pituitary tissue does not vary markedly from one age group to another. Higher values obtained for rats of Group III sacrificed by decapitation cannot be explained by any alteration in experimental procedure. For most age groups studied, there are no significant differences in RNA values obtained after sacrificing rats by either of the 2 methods. Stress produced by subjecting animals to ether is probably of too short a duration to influence nucleic acid changes. No explanation can be offered to account for the higher RNA values observed in Group II rats sacrificed by ether overdosage compared to the same age group sacrificed by decapitation.

Tissue content of RNA is not influenced by variation of degree of hydration or collagenous connective tissue infiltration from one age group to another. There are no differences in these 2 constituents among various age groups. Use of hydroxyproline determinations as index of amount of collagen present is reliable. Only collagenous connective tissue contains this amino acid in any appreciable amount(4).

Summary. A study was made of RNA, water, and collagen contents of adenohypophyses of rats of various age groups from 35 days to one year old, sacrificed by decapitation or an overdose of ether. There were no significant differences in level of RNA in tissue from one age group to another. Concentration of anterior pituitary nucleic acid was not influenced by manner of sacrifice for most age groups studied. Contents of water and collagen in the tissue were similar in young and old rats.

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Dihydrotachysterol (AT 10) and Mammogenesis in Ovary-Thyro-Parathyroidectomized Rats.* (25920)

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Continuing study of mammogenesis in ovary-thyro-parathyroidectomized female rats, influence of estradiol benzoate (EB) and progesterone (P) with thyroxine (T4) and dihydrotachysterol (AT 10) were compared.

Materials and methods. Sprague-Dawley-Rolfsmeyer rats were ovary-thyro-parathyroidectomized, injected, killed, and DNA of mammary glands determined under standardized conditions described(1). AT 10‡ was given every second day by stomach tube. Completeness of thyroidectomy checked by I^{131} uptake described(1). Group I received 1 μg EB plus 3 mg P. Group II received in addition 20 μg/100 g AT 10. Group III re-

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‡ Kindly supplied by Sterling-Winthrop Research Inst., Rensselaer, N. Y. The commercial solution of AT 10 or dihydrotachysterol (Hytakerol) contains about 1250 μg of active principle per ml.

TABLE I. Effect of Dihydrotachysterol (AT 10) on Mammogenesis in Ovary-Thyro-Parathyroidectomized Albino Rats.

Daily treatment from day 15-33	No. of rats	Body wt			Pituitary wet wt/100 g BW (mg)	48 hr uptake of I^{131}		6 posterior mammary glands		
		Day 1 (g)	Day 34 (g)	Gain (%)		Neck (%)	Body (%)	DFFT, total/100 g BW (mg)	DNA μg/mg DFFT (μg)	DNA Total/100 g BW (mg)
EB: 1 μg; P: 3 mg	14	244	277	14 ± 2.1	6.6 ± .13[1]	1.2 ± .10	3.3 ± .32[10]	227 ± 10[19]	35 ± .8[28]	7.9 ± .22[37]
Idem + AT 10: 20 μg/100 g	14	226	254	12 ± 1.5	7.6 ± .22[2]	2.3 ± .27	4.9 ± .54[11]	276 ± 6[29]	34 ± .6[29]	9.3 ± .17[38]
Idem + T4: 1.5 μg/100 g	13	237	261	10 ± 1.3	6.0 ± .14[3]	.4 ± .05	1.2 ± .17[12]	227 ± 7[21]	34 ± .7[30]	7.7 ± .23[39]
EB: 2 μg; P: 6 mg*	37	228	257	13 ± .8	7.7 ± .20[4]	1.1 ± .12	1.8 ± .20[13]	229 ± 7[22]	36 ± .9[31]	8.1 ± .21[40]
Idem + T4: 3 μg/100 g	33	228	254	11 ± .81	6.2 ± .18[5]	.2 ± .03	.4 ± .04[14]	204 ± 5[23]	41 ± 1.0[32]	8.3 ± .19[41]
EB: 2 μg; P: 6 mg; AT 10: 10 μg/100 g	16	235	261	11 ± 1.3	7.9 ± .27[6]	1.0 ± .09	3.0 ± .25[15]	238 ± 8[24]	33 ± .8[33]	7.9 ± .23[42]
Idem + T4: 3 μg/100 g	15	226	255	13 ± 2.1	6.7 ± .24[7]	.3 ± .05	.8 ± .10[16]	209 ± 6[25]	35 ± .5[34]	7.4 ± .16[43]
EB: 2 μg; P: 6 mg; AT 10: 20 μg/100 g	20	224	247	10 ± 1.7	8.5 ± .35[8]	2.0 ± .22	4.6 ± .58[17]	268 ± 7[26]	32 ± .6[35]	8.5 ± .22[44]
Idem + T4: 3 μg/100 g	16	247	267	8 ± 1.9	6.9 ± .23[9]	.4 ± .08	1.1 ± .13[18]	230 ± 6[27]	36 ± .7[36]	8.1 ± .20[45]

* Data from (1).

BW = body wt
DFFT = dry, fat-free tissue
EB = estradiol benzoate
P = progesterone
T4 = thyroxine
AT 10 = dihydrotachysterol (Hytakerol)

Student's "t" probability

1–2		19–20		6–7		4–9		5–7	.05 < p < .1		
2–3		20–21		13–15	.001 < p < .005	5–9		22–25			
4–5		22–26		38–40		10–11		31–33			
8–9		26–27		4–7		15–17	.01 < p < .025	40–41	.1 < p < .2		
11–12	< .001	31–32	< .001	22–23	.005 < p < .01	24–25		42–43			
13–14,17		32–34,36		24–26		25–27		44–45			
14–16,18		35–36		31–35		38–44					
15–16		37–38				4–8	.025 < p < .05	40–41	.2 < p < .4		
17–18		38–39						22–24	.4 < p < .5		

ceived in addition 1.5 μg T4/100 g. Group IV received 2 μg EB plus 6 mg P and 10 μg AT 10/100 g. Group V received in addition 3 μg T4/100 g. Group VI received 2 μg EB, 6 mg P plus 20 μg AT 10/100 g. Group VIII received in addition 3 μg T4/100 g.

Results. In OTPEC rats 1 μg EB plus 3 mg P without either T4 or parathyroid hormone replacement therapy stimulated mammary gland growth (as measured by DNA) equal to that stimulated in OE animals (Table I)(2). When 20 μg AT 10/100 g was added, DNA was increased significantly to 9.3 mg/100 g due to a significant increase in DFFT. Addition of 1.5 μg/100 g T4 depressed favorable effect of AT 10 resulting in DFFT and DNA comparable to effect of EB and P alone.

Same significant increase of DFFT was observed when 20 μg AT 10 was added to 2 μg EB plus 6 mg P. However, increase in total DNA did not occur due to a significant reduction in DNA/mg DFFT. Addition of 3 μg/100 g T4 again reduced DFFT but DNA/mg DFFT was increased resulting in total DNA comparable to EB and P alone.

Total DNA of groups receiving 10 μg AT 10/100 g alone and with 3 μg T4/100 g were not significantly influenced in comparison to 2 μg EB plus 6 mg P, although again T4 depressed DFFT slightly.

Wet weight of pituitaries of EB plus P injected rats are increased significantly in comparison to normal controls(1) but were further significantly increased with 20 μg AT 10/100 g. Addition of T4 reduced this hyperplasia markedly.

Completeness of thyroidectomy was proven by I^{131} uptake at 48 hours on day 33 or 34. Intact control animals showed 9.87 ± .84% in neck region and .91 ± .05% in body, whereas operated animals showed highly significant lower uptake in neck region (Table I). All animals treated with AT 10, with or without T4, showed significantly increased I^{131} uptake in comparison with other groups.

Discussion. Data presented confirm previous observation that normal mammogenesis can be induced experimentally with adequate levels of ovarian hormones in OTPEC rats (1). Effectiveness was not changed when 1 μg EB plus 3 mg P instead of 2 μg EB plus 6 mg P were given. 20 μg AT 10/100 g/day, a level far below "toxic borderline dose" of 100 μg AT 10/100 g/day(4) exerted significant positive effect on mammogenesis.

In previous study(5) T4 was shown to cause a significant increase in total DNA when 3 μg and 6 μg/100 g were injected with 2 μg EB plus 6 mg P in OE rats. DFFT was maintained and an increase in DNA/mg DFFT was observed.

In normal pregnant animals T4 at levels of 2.5 and 3.5 μg/100 g increased total DNA by maintenance of DFFT and increase of DNA/mg DFFT (unpublished).

In present study T4 added to AT 10 at either 1.5 or 3 μg/100 g level depressed total DNA by significantly depressing DFFT without producing a corresponding increase of DNA/mg DFFT observed in ovariectomized and pregnant animals. It is suggested that if parathyroid hormone acts similar to AT 10 in stimulating total DNA and further that T4 increases parathyroid hormone secretion rate, then the results obtained with T4 in ovariectomized and pregnant animals could be harmonized with present observations.

Favorable effect of T4 in non-TPEC animals might be due, in part, to increased secretion of parathormone. Higher levels of AT 10 may show increased total DNA of mammary glands when administered with T4.

Increased uptake of I^{131} in OTPEC rats administered AT 10 raises question of its role in I^{131} uptake, release and T4 secretion rate in normal animals.

Summary. Daily administration of 1 μg EB plus 3 mg P to mature ovary-thyro-parathyroidectomized rats for 19 days stimulated mammary gland growth (DNA) comparable to ovariectomized rats receiving same treatment. Replacement therapy with 10 and 20 μg AT 10/100 g/day, respectively, in combination with 1 μg EB plus 3 mg P increased total DNA significantly. No benefit was observed by increasing ovarian hormones to 2 μg EB plus 6 mg P. Combined administration of AT 10 at above mentioned levels and 1.5 μg and 3 μg T4/100 g depressed DFFT significantly resulting in reduced total DNA.

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Production of Incomplete Vi Antibody in Mice. (25921)

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Landy(1) reported that Swiss Bagg mice respond to isolated Vi antigen by producing specific antibody demonstrable by the standard hemagglutination test(2), and that the passive protection afforded by this antibody was sufficient to account for the immunogenic activity of the antigen. These findings have been confirmed for BALB/c mice (an inbred variant of the Bagg), the strain employed most extensively in our laboratory. Recently, however, it was observed that specific antibody could not be demonstrated in the serum of Vi-immunized Cinnamon mice, a strain not previously employed by us for serological studies. Inasmuch as Cinnamon, as well as BALB/c mice, could be protected actively against challenge with virulent *Salmonella typhosa* by administration of Vi antigen, this observation was unexpected, and suggested that response to antigenic stimulation might differ with the strain of mouse employed. The present study was therefore designed to elucidate and compare responses of these 2 strains of mice to Vi antigen. Evidence was obtained that the type of antibody evoked by Vi antigen does indeed vary with the mouse strain. The antibody developed in Cinnamon mice immunized with Vi antigen was of the incomplete type, but was nonetheless protective against challenge with *S. typhosa*.

Materials and methods. Mice. The animals ranged in weight from 11-16 g, and were of mixed sex. BALB/c mice were obtained from Microbiological Associates, Inc., Bethesda, Md., and Cinnamon mice from Dept. of Lab. Animal Medicine, Walter Reed Army Inst. of Research. *Vi Antigen.* The purified Vi antigen was prepared from the Vi strain of *Escherichia coli* (5396/38) by the method of Webster *et al.*(3). Immediately prior to use, antigen was dissolved in 0.85% NaCl to give desired concentration. All inoculations were made intraperitoneally. *Sera.* Specimens for use in passive tests and for serological study were prepared as follows: mice were inoculated with 10 μg of Vi antigen, bled 6 days later, and the serums pooled. *Absorption of sera.* 0.3 ml of packed bacterial cells (killed by heating at 60°C for 1 hr) was added to each ml of serum, the suspension incubated at 37°C for 1 hr, at 4°C overnight, and clarified by high speed centrifugation. *Passive protection tests.* Tests for serum potency were based on use of graded immunizing doses and a constant challenge dose, and were performed in Cinnamon mice. Serum was given intraperitoneally 1 hour prior to challenge with a saline suspension of 3 x 10^7 *S. typhosa* Ty2V (fully virulent form) *via* the same route. ED_{50}s were determined by the method of Reed and Muench(4). *Serological procedures.* The hemagglutination test for complete (saline) Vi antibody was performed as described by Landy and Lamb(2). The procedure for demonstration of incomplete Vi antibody was the same except that serum dilutions were made in 15% bovine serum albumin (BSA) obtained from Armour Pharmaceutical Co.

Results. During our studies on the role of Vi antigen in virulence of *S. typhosa* for mice,