

estrogen and may have acquired enough estrogen by contact to elicit some mammary response. A moderately extensive mammary growth occurred in all mice receiving both estrogen and the same preparation of prolactin (Table I).

The rudimentary glands were not stimulated in the 10 operated animals receiving either of the 2 preparations of highly purified prolactin (SP and AW) at either of the 2 different dose levels. The 10 operated mice receiving estrogen in addition to the purified lactogenic preparations showed mammary growth rated as ++ in 8 animals and + in the other two. At the time they were removed the glands were growing rapidly as indicated by the enlarged end buds containing numerous mitotic figures. The extent of mammary proliferation averaged somewhat less than that obtained in unoperated mice receiving estrogen, and was comparable to that induced in operated mice receiving estrogen and progesterone.⁹

The failure of mammary growth to occur in hypophysectomized rats receiving lactogenic hormone has been demonstrated.¹¹ Mammary growth likewise failed to occur in hypophysectomized male mice when given the purified preparation of lactogenic hormone used in this investigation. The mammary growth occurring when estrogen was administered together with the purified lactogenic hormone indicates that prolactin may either sensitize the mammary tissue of hypophysectomized mice to estrogen or improve the general condition of these animals so that mammary proliferation may occur.

Summary. The mammary rudiments grew in hypophysectomized male mice receiving either of 2 purified preparations of prolactin and estrogen. The injection of these preparations of prolactin alone was not followed by mammary growth.

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Some Effects of Thyrotropic Hormone on the Pregnant Rat.

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Grumbrecht and Loeser¹ reported death and maceration of the embryos in rats treated with thyrotropic hormone, whereas the ovaries

¹¹ Evans, H. M., Simpson, M. E., and Lyons, W. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **46**, 586.

of their animals were found to be enlarged but otherwise normal. Rats are known to be relatively unresponsive to the thyrotropic hormone and the available preparations of this hormone apparently contain luteinizing as well as thyroid-stimulating principles.²⁻⁵ The following experiments, therefore, were undertaken on pregnant rats to determine whether (1) there would be death and resorption of the embryos; (2) luteinization of the unruptured Graafian follicles; and (3) histological evidence of stimulation of the maternal and embryonic thyroids.

Insemination of virgin albino rats (inbred Wistar strain) was determined by the vaginal smear method and on the 10th day of pregnancy the number of implantation sites ascertained by laparotomy. Daily intraperitoneal injections of thyrotropic hormone* were begun the next day and given in the amounts and for the periods indicated in Table I. The animals were killed on the 16th day and the number of living and dead embryos counted. One ovary, 1 lobe of the thyroid, and a portion of the embryonic sites were fixed in Bouin's solution, sectioned and stained with hematoxylin and eosin.

Results. 1. The percentage of living embryos was inversely proportional to the duration, rather than the size, of the dose; for a smaller number of living embryos was observed when a 0.5 cc dose was distributed over a period of 5 days than if a similar dose was given as a single injection† (Table I). 2. With the exception of the animal injected with 0.1 cc dose, whose ovarian histology was normal, the ovaries of all the other animals contained 3 to 20 unruptured Graafian follicles in the process of luteinization (pseudolutein bodies). No correlation was found between the number of pseudolutein bodies and the amount of hormone administered. 3. The maternal-thyroids showed histological evidence of stimulation when the animals were treated for 2 days or more; no response of the

1 Grumbrecht, P., and Loeser, A., *Klin. Woch.*, 1938, **17**, 233.

2 Loeb, L., and Friedman, H., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **29**, 172.

3 Starr, P., Patton, H., and Bruner, R. G., *Medical Papers Dedicated to Dr. H. A. Christian*, 1936, 329.

4 Stephens, D. J., and Allen, W. M., *Endocrinol.*, 1941, **28**, 580.

5 Tobin, C. E., in press.

* The thyrotropic hormone (Antuitrin T), which was assayed to contain 50 to 100 guinea pig units per cc, was generously supplied by Parke, Davis & Company.

† The average number of implantation sites was 10.2 (5-13) on the 10th day. Since the average litter size of our colony is 8 young, a limited number of embryonic deaths and resorptions normally occur before delivery. K. E. Mason (personal communication) found in 50 stock animals, which were autopsied on the 16th day of pregnancy, that an average of 89% of the implantation sites contained live embryos.

TABLE I.
Pregnant Rats Treated with Thyrotropic Hormone.*

Pregnant rats treated with thyroxine						
No. of animals	Dosage			Avg % embryos alive 16th day	Implantation sites and embryos alive in each animal	
	Total cc	Single inj., cc	Days			
1	.1	.1	1	90	10 (9)†	
5	2	.2	.1	2	100	13 (13); 11 (11)
	3	.2	.2	1	87	12 (10); 8 (7); 12 (11)
10	3	.5	.5	1	91	12 (11); 9 (8); 12 (11)
	7	.5	.1	5	17	12 (0); 10 (1); 12 (0); 10 (2); 10 (5); 5 (1); 10 (3)
4	1.0	.2	5	6	7 (0); 10 (1); 8 (0); 6 (1)	
4	0	0	0	89	10 (8); 7 (6); 12 (11); 10 (10)	

†Number in italics—number of implantation sites on 10th day.

Number in parenthesis—Number of embryos alive on 16th day.

thyroid could be elicited by injections given over a shorter period. The thyroids of the embryos, which had not undergone autolysis, did not show any histological signs of stimulation even though the maternal-thyroids were stimulated.

The embryonic deaths are attributed to the secretion from the stimulated maternal-thyroids rather than the abnormal luteinization of the ovaries. The incidence of embryonic death prior to the 16th day was much greater in those animals whose thyroids showed histological evidence of stimulation than in those not so stimulated, and bore no constant relation to the number of pseudolutein bodies present.

As a control for hyperthyroidism produced by the thyrotropic hormone injections, 10 animals (studied as in the previous experiment) were given daily intraperitoneal injections of crystalline thyroxin† in the dosage and for the periods shown in Table II. Animals Nos. 1-3 were killed on the 13th day; the others on the 16th day. In only one case (No. 5) was there an abnormal number of dead embryos. This treatment did not alter the histological structure of the thyroids from that usually found during pregnancy. The ovaries, however, contained many atretic follicles but no pseudolutein bodies.

† Crystalline thyroxin (E. R. Squibb & Sons) was injected in the form of 1 mg per cc solution of N/100th NaOH. First injection was given on the 11th day of pregnancy.

TABLE II.
Pregnant Rats Treated with Thyroxin.†

Animal No.	Dosage			% embryos alive 16th day	Implantation sites and embryos alive in each animal
	Total mg	Single inj., mg	Days		
1	.1	.1	1	100	9 (9)†
2	.4	.2	2	92	13 (12)
3	.6	.2	3	100	10 (10)
4	.8	.2	4	91	11 (10)
5	1.0	.2	5	60	10 (6)
6	1.0	.2	5	90	10 (9)
7	1.0	.2	5	100	6 (6)
8	1.0	.2	5	89	9 (8)
9	1.2	.4	1	90	10 (9)
10	1.4	.2	4	100	12 (12)
		.4	2		
		.2	3		

†Number in italics—number of implantation sites on 10th day.

Number in parenthesis—number of embryos alive on 16th day.

The dose of thyroxin used in this experiment was not large enough to produce any marked effects in the animals and since it was not given during the last quarter of pregnancy the survival of the embryos was not affected. Bodansky and Duff⁶ found that pregnant rats treated with 1 mg of thyroxin daily during the last 10-12 days of pregnancy showed only a moderate loss in body weight and delivered the same number of young, but a higher percentage of still births, than controls. Since the period of gestation was prolonged,⁶ crystalline thyroxin must interfere with the birth mechanism and cause embryonic deaths only during the latter part of gestation.

Conclusion. Rats injected with thyrotropic hormone, between the 10th and 16th days of pregnancy, showed a high percentage of dead embryos, stimulation of the maternal-thyroid and luteinization of unruptured Graafian follicles. The embryonic deaths are attributed to secretion from the stimulated maternal-thyroid. Injections of crystalline thyroxin produced no such effects.

⁶ Bodansky, M., and Duff, V. B., *Endocrinol.*, 1936, **20**, 537.