fractionation of serum make the serologic diagnosis of perinatal infection a practical procedure for diagnostic serologic laboratories already employing fluorescent microscopic techniques. Continuing studies will reveal whether the immunologic responsiveness of the fetus to rubella, toxoplasma, and cytomegalovirus is exceptional or is encountered with other infectious agents.

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Mammary Gland Growth during Pseudopregnancy and Pregnancy in the Rat* (32980)

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Pseudopregnancy in the rat results in an extension of the lifespan of the corpora lutea to approximately 12 days. Mammary glands differentiate from a ductal system characteristic of virgin cycling rats to a lobule–alveolar system characteristic of pregnancy (1). The extent of the lobule-alveolar development in normal pseudopregnancy has been estimated by whole-mount observations to be similar to that of 13 days of pregnancy in the rat (2). Since it has been demonstrated that hysterectomy and uterine deciduomata prolong pseudopregnancy in the rat (3), one would expect that mammary gland growth continues during the more prolonged periods of diestrum which result from such manipulations. A quantitative assessment of mammary gland growth and function is available based on the chemical determination of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (4). The present investigation was undertaken to determine by quantitative measurements the extent of mammary gland growth in the intact or normal pseudopregnant rat, the pseudopregnant rat that was previously hysterectomized, the pseudopregnant rat having concomitant deciduomata, and the normal pregnant rat.

Materials and Methods. Two hundred and four female albino rats of the Sprague-Dawley-Rolfsmeyer strain were maintained at a constant temperature of 78 ± 2°F on Purina lab pellets under an artificial lighting regimen of 14 hours light and 10 hours darkness. Estrous cycle patterns were obtained by the vaginal lavage technique. At least two normal cycles were followed before pseudopregnancy or pregnancy was initiated.

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In order to establish guidelines for the duration of pseudopregnancy in a population, 10 animals in each of the pseudopregnancy groups (intact or normal, hysterectomized and deciduomata) were smeared daily until normal estrous cycles were reestablished. The first day of estrus was counted as day 0 and the day when a positive proestrus smear occurred was considered as the last day of pseudopregnancy.

Induction of pseudopregnancy was accomplished by stimulation of the cervix with a glass rod on the day of estrus. Initiation of pregnancy was diagnosed on the basis of sperm detection and/or absence of normal cycles during sequential daily smearing.

Hysterectomy was performed on 48 rats prior to the initiation of vaginal smearing. It should be pointed out that this operation by itself has no effect upon the normal estrous cycling pattern of a rat. It is only when hysterectomy is accompanied by cervical stimulation to initiate pseudopregnancy that the lengthening of the corpora lutea lifespan is demonstrated. After two normal estrous cycles followed the operation, the pseudopregnant condition was induced by cervical stimulation.

Deciduomata were produced by traumatization of both horns of the properly sensitized uterine endometrium (5). Thus, on day 5 of pseudopregnancy the sensitized uterus was approached by laparotomy and traumatized with a sharp needle along the antimesometrial aspect of the endometrium.

Six abdominal–inguinal mammary glands were removed, frozen and analyzed for DNA as described previously (6). A duplicate sample of 25 mg was treated with KOH, HCl, and perchloric acid as described by Tucker and Reece (7), except that the digestion in KOH was performed for 5 hours. The supernatant was analyzed for RNA by measurement of its purines and pyrimidines at an absorption maximum of 260 mμ with a Beckman DU spectrophotometer (8).

The data were analyzed by the multiple range test of Duncan (9). The Student's *t* test was considered inappropriate when more than two group means were being compared. The measurements included were: body weight (bw), μg of DNA/mg of dried fat-free tissue (DFFT), total mg of DFFT, mg of DNA/100 gm of bw, mg of RNA/100 gm of bw, and ratio of RNA to DNA.

Results. The lengths of pseudopregnancy to establish guidelines for the population were as follows: Normal pseudopregnancy was 12.0 ± 0.5 days, hysterectomized–pseudopregnancy was 18.1 ± 0.6 days, and deciduoma–pseudopregnancy was 20.6 ± 0.5 days.

Growth of the mammary gland, as measured by total mg of DNA or by mg of DNA/100 gm of bw, increased above the virgin control level in all experimental groups. The increase was significant when Student's *t* test was used in comparing each experimental group separately with the virgin control group. However, when the multiple range test was employed, the increase was not significant in normal pseudopregnant rats or in those which were hysterectomized and pseudopregnant except in the group that was hysterectomized and pseudopregnant for 15 days. Mean group values and standard deviations are presented in Table I. The DNA was significantly higher in all stages of pseudopregnancy with uterine deciduomata and in pregnancy than the DNA in the virgin control group. No significant differences were observed among any of the various pseudopregnant groups, however, and only days 15 and 18 of pregnancy showed DNA levels significantly higher than every pseudopregnant group. The DNA content of the mammary glands on day 18 of pseudopregnancy accompanied by deciduomata was 9.22 mg or 4.05 mg/100 gm of bw, somewhat less than that of day 9 of pregnancy. If the total growth of the glands from the virgin condition to day 18 of pregnancy was considered 100%, then growth during normal pseudopregnancy was 20% during pseudopregnancy in hysterectomized rats was 20%, and during pseudopregnancy with deciduomata was 25% of normal pregnancy. When day 12 of pregnancy was used as a 100% basis of growth, DNA reached only 44% of this value during normal pseudopregnancy, 42% during pseudopregnancy with hysterectomy, and 53% during pseudopregnancy accompanied by deciduomata. These percentages increased slightly

TABLE I. Mammary Gland DNA and RNA in Pseudopregnant and Pregnant Rats.[a]

Group	Day	No. of rats	Parameter							
			Body wt. (gm)	μg of DNA/ mg of DFFT	DFFT (mg)	DNA (mg)	mg of DNA/ 100 gm of bw	RNA (mg)	mg of RNA/ 100 gm of bw	RNA/ DNA
Virgin control	—	12	218	18.0 ± 0.6[b]	327 ± 17	5.82 ± 0.26	2.67 ± 0.16	3.89 ± 0.28	1.78 ± 0.15	0.67
Normal pseudopregnancy	3	12	224	21.3 ± 1.1	358 ± 28	7.38 ± 0.32	3.30 ± 0.14	4.97 ± 0.35	2.22 ± 0.17	0.67
	6	12	226	22.4 ± 0.8	350 ± 24	7.70 ± 0.96	3.41 ± 0.13	5.36 ± 0.28	2.38 ± 0.11	0.70
	9	12	231	23.0 ± 0.5	311 ± 18	7.42 ± 0.38	3.22 ± 0.14	5.32 ± 0.32	2.31 ± 0.13	0.72
	12	12	246	23.9 ± 0.6	364 ± 29	8.60 ± 0.46	3.49 ± 0.17	6.36 ± 0.38	2.58 ± 0.12	0.74
Pseudopregnancy after hysterectomy	9	12	240	26.0 ± 1.0	302 ± 21	7.66 ± 0.39	3.20 ± 0.17	7.11 ± 0.68	2.96 ± 0.22	0.93
	12	12	240	26.3 ± 1.0	333 ± 38	8.34 ± 0.55	3.46 ± 0.16	7.67 ± 0.73	3.18 ± 0.25	0.91
	15	12	233	26.1 ± 0.6	325 ± 25	8.50 ± 0.69	3.63 ± 0.24	7.35 ± 0.74	3.13 ± 0.28	0.86
	18	12	235	25.8 ± 0.8	302 ± 17	7.72 ± 0.42	3.29 ± 0.12	6.94 ± 0.56	3.92 ± 0.19	0.90
Pseudopregnancy with deciduomata	9	12	209	29.5 ± 1.8	287 ± 25	8.21 ± 0.59	3.93 ± 0.28	5.80 ± 0.41	2.78 ± 0.19	0.72
	12	12	218	24.7 ± 0.9	341 ± 29	8.23 ± 0.51	3.82 ± 0.28	6.89 ± 0.43	3.18 ± 0.22	0.84
	15	12	230	25.3 ± 1.8	366 ± 35	8.83 ± 0.74	3.83 ± 0.31	6.66 ± 0.63	2.89 ± 0.27	0.76
	18	12	228	27.8 ± 1.4	344 ± 24	9.22 ± 0.34	4.05 ± 0.13	5.86 ± 0.32	2.58 ± 0.15	0.64
Normal pregnancy	9	12	262	25.7 ± 1.5	477 ± 60	11.44 ± 1.01	4.48 ± 0.38	7.73 ± 0.80	2.95 ± 0.31	0.66
	12	12	262	28.9 ± 1.7	426 ± 21	12.21 ± 0.78	4.70 ± 0.29	8.25 ± 0.56	3.17 ± 0.20	0.68
	15	12	284	27.4 ± 1.3	547 ± 55	15.25 ± 1.11	5.34 ± 0.35	13.16 ± 0.85	4.61 ± 0.27	0.88
	18	12	271	33.9 ± 2.0	578 ± 27	19.58 ± 1.41	7.27 ± 0.54	16.90 ± 1.13	6.26 ± 0.43	0.90

[a] Abbrev.: DNA = deoxyribonucleic acid; RNA = ribonucleic acid; and DFFT = dry fat-free tissue.
[b] Mean and SE.

when calculated on a unit body weight basis.

The RNA varied from 3.89 mg in the virgin controls to 16.90 mg on day 18 of pregnancy. Only days 15 and 18 of pregnancy had significantly higher RNA values than those in the various pseudopregnant groups. The ratio of RNA to DNA, an indirect estimate of protein synthetic activity, was significantly higher in the pseudopregnant groups that were hysterectomized than it was in the virgin control group. Days 15 and 18 of pregnancy also had significantly higher RNA-DNA ratios than the virgin control.

Body weight per experimental group ranged from a mean of 209 gm in the group that was pseudopregnant for 9 days and having deciduomata to 284 gm in the group sacrificed on day 15 of pregnancy. All pregnant groups had significantly higher body weights than other experimental groups in spite of the fact that provision was made for fetal weight changes. Dried fat-free tissue (DFFT) weight, which is an estimate of gross mammary gland size, varied from 287 to 578 mg. There were no significant differences among any of the pseudopregnant groups. However, DFFT levels in all pregnant groups were significantly higher than all the pseudopregnant groups.

Discussion. Growth of the mammary gland in relation to the secretion of estrogens and progesterone by the ovaries has been studied in this laboratory by replacement therapy techniques. It has been shown that 2 μg of estradiol benzoate and 6 mg of progesterone per day for 19 days given subcutaneously in oil will result in mammary gland development which is almost equal to that at 18 days of pregnancy (10). Experiments of this nature attempt to mimic the circumstances of the natural situation and, thus, suggest that the normal secretion rates of the ovarian steroids are of a similar magnitude.

Since the lobule–alveolar system is so well differentiated during pseudopregnancy, the ratio of estrogens to progesterone must be favorable or the differentiation at such a low level of quantitative development would not be so developed qualitatively. The quantitative estimation by DNA determination has revealed that this growth is only 18% of that found in the normal pregnant rat on day 18, when body weight is standardized. Even when one considers a comparison of day 12 normal pregnancy to any stage of normal or experimental pseudopregnancy, the growth of the mammary gland reached only 45% in normal pseudopregnancy, 53% in hysterectomized–pseudopregnancy, and 76% in deciduomata–pseudopregnancy, when calculated on a unit body weight basis. It is suggested that the mammary gland growth observed in this study reflects the functional capacity of the ovaries to secrete sex steroids. On this basis the corpora lutea of pseudopregnancy function at one-fifth to one-third the level of corpora lutea of pregnancy. Support of this concept is found in the study involving the measurements of corpora lutea diameters in which growth during pseudopregnancy was approximately 28% of the growth of corpora lutea during pregnancy (11).

Fajer and Barraclough (12) have measured the ovarian secretion of progesterone and found that the secretion rate during pseudopregnancy is approximately one-third of that during pregnancy.

Wrenn *et al.* (13) have studied the extent of mammary development in pseudopregnant rats which were intact or traumatized to produce deciduomata. Based on histological whole-mount measurements they concluded that decidual tissue was responsible for elaborating a substance having mammotropic properties. The enhanced mammary gland growth as a result of deciduomata production was verified in the present study. However, the amount of luteotropic or mammotropic hormone secreted by the deciduomata is probably far less than that elaborated by the combined maternal and fetal membranes during the latter half of normal pregnancy.

Hysterectomy results in the prolongation of pseudopregnancy. One of the reasons for initiating this study was to determine if increased mammary gland development might reflect increased and prolonged ovarian secretion of steroid hormones. Such an effect was not found in this study. The significant increase in mammary gland RNA during pseudopregnancy of the hysterectomized rat was noted. This observation may prove to be

of significance but is difficult to explain on the basis of our present knowledge.

Summary. Growth of the mammary gland of the rat during normal pseudopregnancy was only 18% of the growth found at day 18 of normal pregnancy as measured by DNA. This suggests that the corpora lutea of pseudopregnancy function at approximately one-fifth the level of those in pregnancy. Hysterectomy, which resulted in a 6-day extension of pseudopregnancy, was without effect in the enhancement of mammary gland growth over normal pseudopregnancy. However, the ratio of RNA to DNA in the mammary glands of these animals was raised significantly. The production of deciduomata concomitant with pseudopregnancy resulted in a 9-day extension of pseudopregnancy and a slight increase in mammary gland growth over that of normal pseudopregnancy. It was only 30% of that at day 18 of pregnancy and 76% of that at day 12 of pregnancy when DNA was standardized to body weight.

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Mycobacterial Suppression of Delayed Hypersensitivity in Experimental Allergic Encephalomyelitis (32981)

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The theory that delayed hypersensitivity is involved in the induction of experimental allergic encephalomyelitis (EAE) is supported by the good correlation between delayed skin reactivity to the encephalitogenic protein 10 days after challenge and the subsequent development of disease (1–4). Shaw *et al.* (2) have suggested that the skin test is sufficiently reliable in its prediction of illness to provide a valuable "shortcut" in the testing of unknown fractions. In their analysis of delayed hypersensitivity in EAE, these authors also reported that both manifestations of delayed hypersensitivity (positive skin test and induction of disease) were suppressed by multiple injections of basic protein (BP) in Freund's incomplete adjuvant (whether the injections were made before challenge or in the interval between challenge and development of clinical signs).

The mechanism of disease suppression by injection of basic protein in incomplete adjuvant has not been clarified. It is possible that circulating antibody interferes somehow with the sensitization or proliferation of specific cells. This has been difficult to evaluate in the past because of the inadequacy of the methods available for detection of antibodies to encephalitogenic BP. Falk *et al.* (5) have recently shown that antibody to homologous