and in lactating, pregnant rats carrying 6 or fewer fetuses. It would appear, however, that the mammary glands of the lactating, pregnant rat do not respond to the stimulus of pregnancy, and may begin to involute as nutrient requirements increase, due either to fetal size or numbers.

Summary. Concurrent pregnancy significantly enhanced mammary DNA and RNA in rats lactating 18 days when compared with non-pregnant rats lactating 18 days, but the RNA/DNA ratio was not changed. Concurrent pregnancy in rats lactating 21, 24, and 28 days produced, respectively, no change, significant decrease, and no change in nucleic acid content when compared with lactating, non-pregnant rats. Within days 24 and 28 of lactation, nucleic acid content decreased markedly with advancing pregnancy. Standardization of gestation length showed that glands of rats with 7 or more fetuses contained highly significantly less DNA and RNA than glands of lactating, non-pregnant rats.

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Nucleic Acid Content of Suckled and Non-Suckled Mammary Glands Within Lactating Rats.* (29066)

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Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) content have been used to estimate mammary gland cell numbers and protein synthetic activity, respectively(1,2, 3). Kuramitsu and Loeb(4) and Hesselberg and Loeb(5) observed in the guinea pig that ligation of one teat, with continued suckling of the contralateral, intact teat, resulted in rapid involution of the ligated gland, but the suckled gland continued to secrete milk. Selye(6) and Selye and McKeown(7), however, were of the opinion that the cause of mammary involution following litter removal in rats and mice was the absence of the suckling stimulus rather than the accumulation of milk. Griffith and Turner(8) found significantly less DNA in suckled glands than in non-suckled glands of the same rat, and suggested that some component of DNA was present in milk.

The present experiments were designed to estimate, in lactating rats, the effects of an increasing non-suckling interval on the DNA and RNA content and RNA/DNA ratio of suckled and non-suckled mammary glands.

Methods. Litters were separated from mothers on 13th to 14th day of lactation for 0, 3, 6, 9, or 12 hours (non-suckling interval). At the end of this separation mothers were anesthetized with sodium pentobarbital (Nembutal-30 mg/kg body wt), and all teats, except 3 abdominal-inguinal teats, were taped to prevent suckling. One international unit oxytocin (Pitocin-Parke, Davis and Co.) was injected 3 times at intervals of 20 minutes, and the litter replaced each time. The first 2 oxytocin injections were administered subcutaneously and the last in-

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jection intraperitoneally. Twenty minutes after the last oxytocin injection mothers were sacrificed. The 3 suckled and 3 non-suckled abdominal-inguinal glands were removed for nucleic acid analyses. The 60-minute suckling interval, allowed litters to remove milk from suckled glands, was constant in all groups and, therefore, was not included as part of the non-suckling interval in the statistical analysis. Long-term effects of a 12-hour non-suckling interval on nucleic acid content were estimated in 11 rats whose litters were removed for 12 hours on the evening of day 13 of lactation and replaced on the morning of day 14. Mothers were sacrificed on the 18th day of lactation. Nucleic acid content of mammary glands was determined as previously outlined(9).

Results. In every instance non-suckled glands were of greater weight than contralateral, suckled glands, and this difference increased with each increase in suckling interval (Table I). These observations confirmed the success of the experimental procedures in causing more milk to accumulate with advancing non-suckling intervals.

The difference in total mammary DNA content between suckled and non-suckled glands increased with the length of time litters were separated from mothers prior to replacement for a 60-minute suckling interval. The DNA content in non-suckled glands was highly significantly greater ($P<0.01$) than that in suckled glands and the linear regression of differences in total mammary DNA between suckled and non-suckled glands on the non-suckling interval was significant ($P<0.05$). There were no obvious trends in RNA content between suckled and non-suckled glands with increasing non-suckling intervals (Table I).

Since total RNA was constant and total DNA was greater in non-suckled than in suckled glands, regardless of the length of the non-suckling interval, the RNA/DNA ratio was lower, except at the zero-hour interval, in non-suckled glands than in suckled glands. There were no apparent trends in RNA/DNA ratios of suckled glands as the non-suckling interval increased. RNA/DNA

TABLE I. Nucleic Acid Content and RNA/DNA Ratio of Suckled and Non-suckled Mammary Glands Within Lactating Rats After Various Non-suckling Intervals.

Non-suckling interval* (hours)	No. animals	Body wt (g, mean)	DEET†			Total DNA			Total RNA			RNA/DNA	
			Suckled glands (mg, mean)	Non-suckled glands (mg, mean)	Difference	Suckled glands (mg) ‡§	Non-suckled glands (mg) ‡§	Difference‖	Suckled glands (mg) ‡	Non-suckled glands (mg) ‡		Suckled glands (ratio) ‡	Non-suckled glands (ratio) ‡
0	12	238	481.1	578.0	96.9	12.90 ±.30	13.37 ±.37	.47	32.71 ±1.50	34.06 ±1.58		2.54 ±.08	2.55 ±.08
3	12	240	465.1	651.2	186.1	12.45 ±.27	12.97 ±.25	.52	32.58 ± .78	32.24 ± .79		2.62 ±.08	2.49 ±.08
6	12	238	458.0	695.7	237.7	12.34 ±.25	12.96 ±.30	.62	32.60 ±.89	32.76 ±.91		2.64 ±.05	2.53 ±.05
9	12	240	471.3	772.1	300.8	12.89 ±.21	14.10 ±.28	1.21	32.31 ±1.28	32.28 ±1.06		2.51 ±.09	2.29 ±.07
12	12	242	478.0	847.2	369.2	13.12 ±.40	14.43 ±.38	1.31	33.35 ±1.57	32.94 ± .99		2.54 ±.07	2.28 ±.05

* Length of time litters were separated from mothers prior to replacement for 60-min. suckling period.
† Dried, ethanol-ether extracted tissue.
‡ Mean and standard error of mean.
§ Suckled glands highly significantly less ($P<0.01$) than non-suckled glands.
‖ Linear regression of differences on non-suckling interval was significant ($P<.05$).

ratios, however, of non-suckled glands were decreased with increasing lengths of the non-suckling interval.

Mammary glands of rats separated from their litters for 12 hours, followed by replacing the litters and then sacrificing the mothers 4 days later, contained 10.67 mg of DNA per 100 g of body weight, 25.81 mg of total DNA, 69.69 mg of total RNA, and a RNA/DNA ratio of 2.70. These averages were not significantly different ($P>0.05$) from the nucleic acid content of glands of normal rats lactating 18 days(3).

Discussion. The differences in DNA content between suckled and non-suckled glands within lactating rats increased in a linear manner as the non-suckling interval increased from 0 to 12 hours. RNA was not influenced by the presence or absence of milk; therefore, the RNA/DNA ratios inversely reflected the trends and changes of the DNA content. The number of mammary epithelial cells *per se* was not influenced by a prolonged non-suckling interval, because separation of litters followed by replacing litters and sacrificing the mothers 4 days later caused no changes in DNA content when compared to glands of lactating rats whose litters were not removed.

It is suggested that the difference in DNA content between suckled and non-suckled glands was probably due to leucocytes in milk which were removed from suckled glands. Increased mammary DNA occurred during the first 48 hours following weaning, when glands contain very large amounts of milk, and this increase has been at least partially explained by increased numbers of leucocytes(10). Furthermore, leucopenia has been observed during active suckling, and the passage of leucocytes into the mammary gland was well correlated with blood changes (11). Leucocyte numbers doubled in the blood of mice 24 hours after removal of litters, and the number decreased to its former level when the young were returned(12).

Leucocytes in mouse mammary lumina contained abundant amounts of RNA shortly after parturition, but no RNA at post-weaning(13). These results indicated that older degenerating cells, such as one might find in milk of mid-lactation, contained little RNA, which would explain why in the present study the RNA content was constant in suckled and non-suckled glands with increasing non-suckling intervals.

Since, with increasing intervals, total RNA in suckled and non-suckled glands did not change it would seem that the mammary gland has the potential to secrete milk proteins at a constant rate up to 12 hours. It is suggested that this potential decreased between a non-suckling interval of 12 and 24 hours, since total RNA declined significantly in 1-day post-lactational glands when compared to glands of 21-day lactating rats(14). It should be recognized, however, that two different stages of lactation were compared, and it is not yet known whether the mammary glands of rats secrete milk proteins in the same manner at different stages of lactation.

Summary. Within rats lactating 13 to 14 days, non-suckled mammary glands contained significantly more DNA than suckled glands, and this difference increased in a linear manner as the non-suckling interval increased from 0 to 12 hours. It is suggested that increased numbers of leucocytes account for this difference. The presence or absence of milk did not affect RNA content, and it is suggested that the rat mammary gland has the potential to secrete milk proteins at a constant rate up to 12 hours.

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Effect of Methionine, Vitamin B_{12} and Antibiotics Supplementation on Protein Nutritive Value of Navy Beans.* (29067)

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The effectiveness of methionine, Vitamin B_{12} and antibiotics supplementation in improving the nutritive value of soybean protein has been reported by a number of investigators(1,2,3,4,5). Borchers(6) observed an apparent increased requirement for methionine in rats fed raw soybean meal. Barnes *et al*(7) suggested that the growth inhibitor in raw soybean specifically interferes with tissue utilization of methionine. This would explain why supplementary methionine is necessary for proper utilization of raw soybean protein. The role of Vit. B_{12} may be attributed to its methionine sparing action as suggested by Liener and Schultze(8). However, the mode of action of antibiotics in overcoming the growth depression of rats fed raw soybean meal is not known. Since raw navy beans fed to rats have a low protein quality, similar to raw soybean, this study was undertaken to determine the effectiveness of methionine, Vit. B_{12} and antibiotics supplementation in overcoming growth inhibition of rats fed raw navy beans(9).

Experimental. Navy beans of the Sanilac variety† were ground into fine flour. Heated samples were prepared by autoclaving the finely ground beans in shallow pans at a thickness not exceeding 1.0 cm at 121°C for 5 minutes after this temperature was reached. After autoclaving the samples were placed before a fan and allowed to dry at room temperature.

Preparation of diet. The percentage composition of the basal diet was as follows: sucrose, 30; corn oil, 6; Hegsted salt mixture,‡ 4; vitamin diet fortification mixture,§ 2. Raw or autoclaved navy beans or vitamin free casein‡ was incorporated into the basal diet to provide a level of 10% protein (N × 6.25). The chemical composition of navy beans is presented in Table I. The essential amino acid values for navy beans are given elsewhere(10). Corn starch‡ was added to make the total to 100.

TABLE I. Chemical Composition of Navy Beans.*

(expressed in g per 100 g of beans)	
Moisture	8.96
Ash	3.71
Crude fiber	4.18
Carbohydrate	58.07
Protein (N × 6.25)	24.00
Fat (ether extractable)	1.02
Calcium	.15
Phosphorus	.46
Iron	.007

* Determined according to standard procedures recommended by A.O.A.C.(21).

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† Courtesy of Dr. M. Wayne Adams, Farm Crops Dept., Mich. State Univ.

‡ Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

§ Purchased from Nutritional Biochemicals Corp., Cleveland,Ohio. Supplies the following vitamins in mg/100 g of diet: Vit. A concentrate (200,000 units per g), 9.0; vit. D concentrate (400,000 units per g), 0.5; alpha tocopherol, 10.0; ascorbic acid, 90.0; inositol, 10.0; choline Cl, 150.0; menadione, 4.5; P-aminobenzoic acid, 10.0; niacin, 9.0; riboflavin, 2.0; pyridoxine HCl, 2.0; thiamine HCl, 2.0; Ca pantothenate, 6.0; biotin, 0.04; folic acid 0.18; vit, B_{12}, .0027.