20. Miya, F., Marcus, S., *ibid.*, 1961, v86, 652.
21. Muschel, L. H., *Ann. N. Y. Acad. Sci.*, 1960, v88, 1265.
22. Landy, M., Michael, J. G., Whitby, J. L., *J. Bact.*, 1962, v83, 631.
23. Muschel, L. H., Treffers, H. P., *J. Immunol.*, 1956, v76, 1.
24. Litchfield, J. T., Jr., *J. Pharm. Exp. Ther.*, 1949, v97, 399.

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Nucleic Acid Content of Mammary Glands of Pregnant Rats.*† (28048)

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Deoxyribonucleic acid (DNA) content as a measure of cell numbers and ribonucleic acid (RNA) content as an estimate of protein synthesis were first applied to mammary tissue by Kirkham and Turner(1). Since DNA content per mammary cell nucleus was constant in glands of pregnant and of lactating rats(2), DNA content of mammary tissue may be used directly as an index of development. Histological studies of the mammary gland of the rat have indicated that the gland reached its maximum growth, so far as the number of epithelial cells was concerned, by the thirteenth day of pregnancy(3) and that evidence of cellular proliferation was extremely rare in the second half of pregnancy (4). Results of a cytological study(5) and experiments based on colchicine technic(6) and DNA content (7,8) however, have indicated that mitosis continued throughout pregnancy. A very limited number of RNA values have been reported for rat mammary glands during pregnancy(1,7).

DNA and RNA content of mammary glands of the hooded Norway rat was determined at 7 stages of pregnancy. Protein synthetic activity per cell was estimated from the RNA/DNA ratio.

Methods. Six abdominal-inguinal mammary glands were obtained from rats pregnant 0, 4, 8, 12, 14, 18, and 20 days (12 animals per stage). Adult virgin female rats, sacrificed at a weight (approximately 170 g) corresponding to that of other female rats when first placed in breeding cages, represented day zero of pregnancy. Day one of pregnancy was the day sperm were noted in vaginal smears. DNA and RNA content was determined as previously described(2). The pattern of mammary gland development was obtained from mg of DNA per 100 g of body weight(9) and from total mg of DNA in the 6 glands. Amount of protein synthesis was estimated from total mg of RNA and the ratio of total RNA and total DNA estimated protein syntheic activity per cell.

Results and discussion. Six abdominal-inguinal mammary glands contained 2.77, 2.80, 3.33, 4.47, 5.69, 7.36, and 7.89 mg of DNA per 100 g of body weight as stage of pregnancy advanced. Thus, little mammary gland growth occurred during the first 4 days of pregnancy; by the eighth day, however, mammary development increased 20.2% over virgin controls (day zero). Glands continued to grow throughout pregnancy and at the 20th day the number of cells had increased 184.8% over virgin controls. Rate of cellular proliferation was greatest between days 12 and 14. Total mg of DNA showed a somewhat similar pattern. Averages obtained for the 7 stages of pregnancy were 4.76, 4.85, 6.03, 8.86, 11.50, 15.97, and 17.19 mg. Total DNA on days 8 and 20 increased 26.7 and 261.1%, respectively, over virgin controls. These values are greater than those observed when body weights were standardized (Table I). These results indicate that mammary hyperplasia occurs throughout pregnancy.

Advancing pregnancy caused total RNA to increase, as follows: 3.06, 3.08, 4.04, 7.00, 8.87, 13.94, and 15.44 mg. On the eighth

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TABLE I. Deoxyribonucleic Acid Content of 6 Abdominal-Inguinal Mammary Glands of Pregnant Rats.

Days of pregnancy	No. animals	Body wt (g, mean)	DEEET* (mg, mean)	Total DNA/100 g body wt — mg†	Total DNA/100 g body wt — % increase over day 0 of pregnancy	Total DNA — mg†	Total DNA — % increase over day 0 of pregnancy
0‡	12	172	204.3	2.77 ± .08	—	4.76 ± .16	—
4	12	173	197.6	2.80 ± .12	1.1	4.85 ± .26	1.9
8	12	181	218.3	3.33 ± .12	20.2	6.03 ± .28	26.7
12	12	198§	278.0	4.47 ± .22	61.4	8.86 ± .42	86.1
14	12	202§	329.9	5.69 ± .19	105.4	11.50 ± .43	141.6
18	12	217§	406.7	7.36 ± .21	165.7	15.97 ± .67	235.5
20	12	218§	440.7	7.89 ± .27	184.8	17.19 ± .65	261.1

* Dried, ethanol-ether extracted tissue.
† Mean and standard error of mean.
‡ Sexually mature virgins.
§ Body weight corrected for fetal weight.

TABLE II. Ribonucleic Acid Content and Ribonucleic Acid/Deoxyribonucleic Acid Ratio of 6 Abdominal-Inguinal Mammary Glands of Pregnant Rats.

Days of pregnancy	No. animals	Body wt (g, mean)	DEEET* (mg, mean)	Total RNA — mg†	Total RNA — % increase over day 0 of pregnancy	RNA/DNA — Ratio†	RNA/DNA — % increase over day 0 of pregnancy
0‡	12	172	204.3	3.06 ± .13	—	.64 ± .02	—
4	12	173	197.6	3.08 ± .14	.7	.64 ± .02	.0
8	12	181	218.3	4.04 ± .20	32.0	.67 ± .02	4.7
12	12	198§	278.0	7.00 ± .30	128.8	.79 ± .02	23.4
14	12	202§	329.9	8.87 ± .47	189.9	.77 ± .02	20.3
18	12	217§	406.7	13.94 ± .61	355.6	.87 ± .02	35.9
20	12	218§	440.7	15.44 ± .56	404.6	.90 ± .02	40.6

* Dried, ethanol-ether extracted tissue.
† Mean and standard error of mean.
‡ Sexually mature virgins.
§ Body weight corrected for fetal weight.

day of pregnancy the per cent increase was 32.0 and at the 20th day it was 404.6 over virgin control (Table II). Average calculated differences between total DNA and total RNA with advancing pregnancy were 1.70, 1.77, 1.99, 1.86, 2.63, 2.03 and 1.75 mg. These data suggested that the increase in protein synthesis and mammary growth paralleled one another during pregnancy.

RNA/DNA ratios obtained, 0.64, 0.64, 0.67, 0.79, 0.77, 0.87, and 0.90, showed that protein synthetic activity per cell (RNA/DNA) gradually increased throughout pregnancy, becoming slightly greater in later stages of pregnancy (Table II). This observation corresponds quite well with histological observations(5,10,11) that initiation of milk secretion is a gradual process.

Summary. Total DNA of rat mammary glands per 100 g of body weight increased from 2.77 mg to 7.89 mg during pregnancy, a 184.8% increase, and total DNA increased from 4.76 mg to 17.19 mg, a 261.1% increase. Total RNA increased from 3.06 mg to 15.44 mg during pregnancy and these increases paralleled those of total DNA. RNA/DNA ratio increased from 0.64 to 0.90 as gestation advanced.

1. Kirkham, W. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 123.
2. Tucker, H. A., Reece, R. P., *ibid.*, 1962, v111, 639.
3. Roberts, F. L., Papers from the Mayo Foundation for Medical Education and Research and the Medical School, W. B. Saunders Co., Philadelphia, 1921, v1.
4. Weatherford, H. L., *Am. J. Anat.*, 1929, v44, 199.
5. Jeffers, K. R., *ibid.*, 1935, v56, 257.
6. Reece, R. P., Warbritton, V., *J. Dairy Sci.*, 1953, v36, 589.
7. Greenbaum, A. L., Slater, T. F., *Biochem. J.*, 1957, v66, 155.
8. Griffith, D. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 448.

9. Harkness, M. L. R., Harkness, R. D., *J. Physiol.*, 1956, v132, 476.

10. Hammond, J., *The Physiology of Reproduction in the Cow*, Cambridge Univ. Press, London, 1927.

11. Speert, H., *Carnegie Inst. Wash. Pub. 575, Contrib. to Embryol.*, 1948, v32, 9.

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Plasma Lipids in Scurvy: Effect of Ascorbic Acid Supplement and Insulin Treatment.* (28049)

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Banerjee *et al.*(1) reported that total body cholesterol was increased in scorbutic guinea pigs, and that insulin treatment of the scorbutic animals reduced body cholesterol to normal. Body cholesterol was further increased when scorbutic guinea pigs were fed acetate(2). The clearance of fat from blood has been suggested to be dependent on normal carbohydrate utilization(3). The diminished glucose tolerance of scorbutic guinea pigs seemed to be due to the diminished insulin formation associated with the condition (4,5). Utilization of carbohydrate inhibits release of nonesterified fatty acids (NEFA) from fat depots(6). It was, therefore, of interest to estimate the different fractions of plasma lipids, *e.g.*, triglycerides, NEFA, cholesterol, phospholipids, lipoproteins and beta-lipoprotein cholesterol in scorbutic guinea pigs, and to study the effects of insulin treatment and ascorbic acid supplementation on these lipids.

Materials and methods. Male guinea pigs weighing about 300 g and maintained on a scorbutogenic diet(7), 5 mg ascorbic acid per day and 2 drops of a concentrate of Vit. A and D twice a week, were divided into groups of 3 animals, one normal, one scorbutic and one insulin-treated scorbutic. The amount of diet consumed per day by the scorbutic member of a group was fed to the other 2 members of the group on the subsequent day. The 'normal' animals were fed 5 mg ascorbic acid per animal per day. From the 10th day of the experiment the animals intended for insulin treatment were given a daily subcutaneous injection of protamine zinc insulin in a dose increasing from 0.1 unit per 100 g body weight to 0.3 unit throughout the experimental period. Another batch of guinea pigs was fed the scorbutogenic diet *ad libitum*. When symptoms of scurvy developed, they were fed a daily dose of 10 mg ascorbic acid for 4 weeks. By this time the animals had completely recovered from scurvy. In the fourth week of experiment when the animals deprived of ascorbic acid showed signs of scurvy, all the animals were fasted overnight and the next morning blood was collected by cardiac puncture without anesthesia. Total cholesterol(8), NEFA(9), phospholipid(10), lipoproteins(11) and triglycerides(12) of plasma were estimated. Triplicate unstained plasma samples were run parallel to stained plasma samples in the paper electrophoresis apparatus and the zones corresponding to the beta-lipoproteins were cut into small pieces. Cholesterol in the paper pieces was eluted with warm alcohol-acetone mixture (1:1) and the eluted samples were evaporated to dryness. The residue was used for estimation of cholesterol(8).

Results. The results are given in Table I. Total plasma cholesterol did not change appreciably in the scorbutic guinea pigs. Ascorbic acid supplementation reduced the cholesterol level below normal. Beta-lipoprotein cholesterol which was increased markedly in scurvy returned to normal on ascorbic acid supplementation but remained unaltered after insulin treatment. The altered lipoprotein pattern in scurvy became normal when the scorbutic animals recovered after the ascorbic acid treatment and did not change

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