Guinea Pig Mammary Gland Growth Changes in Weight, Nitrogen and Nucleic Acids.* (27207)

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Several workers(1-5) have reported data on rat mammary gland growth during pregnancy, at parturition and during lactation. Changes in deoxyribonucleic acid (DNA) content have been used to indicate the period of rapid cellular proliferation (hyperplasia), and ribonucleic acid (RNA) changes have been used to indicate both cellular enlargement (hypertrophy) and the periods of increased protein synthesis. The data reported for rat mammary gland tissue show general agreement in that the greatest increase in DNA occurs during pregnancy. The exact time at which rapid proliferative growth starts, as measured by DNA content of the tissue is not exactly clear from the data presented. Reports by Kirkham and Turner(1) and Shimizu(2) indicate that the increase in DNA occurs early in pregnancy, reaching a plateau after 10 days and increasing only slightly during lactation. Significant increases in DNA content, however, have been reported after parturition by Greenbaum and Slater (4) and Smith and Richterich(5). In those cases(2,3,4) where RNA content has been measured the greatest change has been shown to occur after parturition.

We wish to report on some of our observations concerned with changes in guinea pig mammary gland during pregnancy, at parturition and during lactation. We have used the total weight of the gland, total nitrogen content and the nucleic acids (DNA and RNA) as an index of mammary growth.

Materials and methods. Multiparous guinea pigs from our stock colony were used in this study. Timed pregnancy was determined by 2 methods. In some cases the average nose to toe length of the extended fetuses was used. In most of our work the postpartum estrous period was used. After a sow gave birth, she was left with the male for 2 days and then was transferred, with her young, away from the male to an individual cage. About 70% of these females were found to be pregnant and this pregnancy could be timed within a day. For our colony the average nose to toe length of 4, 5 and 6 inches for the fetuses corresponded very closely to gestation times of 40, 50 and 60 days as measured by the postpartum estrous breeding method.

Mammary glands were rapidly excised, chilled in cracked ice and then trimmed free of adhering tissue. They were then blotted, weighed, minced with scissors and homogenized in cold 0.25 M sucrose solution in an all glass homogenizer. Cellular particulates were isolated by differential centrifugation, essentially according to the method of Hogeboom, Schneider and Palade(6).

Total nitrogen was determined by the Conway microdiffusion technic(7) on 0.05 to 0.1 ml aliquots of the homogenate after digestion with 0.4 ml of a digestion mixture containing concentrated sulphuric acid, 0.1% selenium dioxide and 0.1% copper sulphate.

Total nucleic acids and deoxyribonucleic acid were determined by the method of Ceriotti(8). Ribonucleic acid was then estimated by difference.

Results and discussion. Fig. 1 represents the growth and involution of the mammary gland in terms of the parameter's fresh weight, total nitrogen content, DNA content and the RNA/DNA ratio. Inspection reveals a close similarity in general form of these curves, particularly in the growth phase. The similarity in behavior of gland weight and total nitrogen content during growth indicates that nitrogen retention, hence presum-

* This investigation was supported in part by a research grant from Nat. Inst. of Arthritis and Metab. Dis., Public Health Service.

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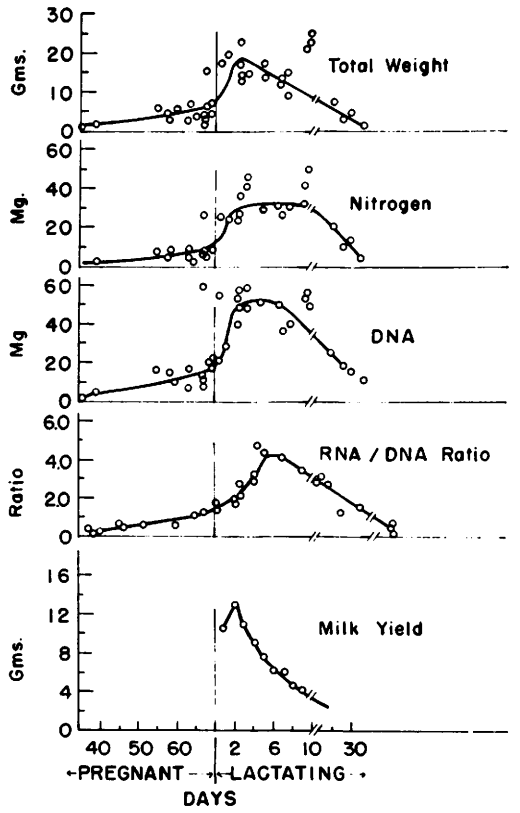

FIG. 1. Growth of guinea pig mammary tissue as measured by total weight, total nitrogen, total DNA and RNA/DNA ratios.

ably protein synthesis, is principally involved and suggests that no major change in composition (*e.g.*, fluid retention or fat infiltration) is responsible for the rapid gain in weight. There is, in fact, a modest but statistically significant increase in the percent nitrogen on a fresh weight basis (pregnancy 1.54%, lactation 1.88%). This may be interpreted as favoring the deposition of protein over the generally less metabolically active non-nitrogenous constituents and may reflect the relative increase in secretory tissue over the fatty supportive tissue which surrounds and permeates the gland. This is in excellent agreement with the data on nitrogen, lipide and water changes reported in rat mammary glands by Smith and Richterich(5).

DNA content per cell, in a given tissue, is generally regarded as a constant. Hence changes in DNA content reflect a change in the number of cells in a tissue. The data shown in Fig. 1 thus provide an index of proliferation in the mammary gland. The correspondence of the form of this graph with that for total gland weight indicates that the observed growth is in all stages primarily a hyperplastic process rather than extensive hypertrophy of pre-existing cells. This situation is rather different from the early burst of proliferation followed by prolonged hypertrophy reported for rat mammary gland by Kirkham and Turner(1) but is in good agreement with the data for DNA for rat mammary gland reported by other workers (4,5).

The RNA/DNA ratio, which reflects cellular RNA content, has been closely associated with protein synthesis. In the case of a secretory tissue, such as is found in the mammary gland, it may be viewed as an index of the specialization of the cells. This view is consistent with the behavior of the RNA/DNA ratio shown in Fig. 1. During the quiescent stage of the gland, the ratio is low and relatively constant. Shortly before parturition and during onset of lactation, it rises abruptly to a high level, about several times the initial value, after which it declines at a relatively slow rate. Thus specialization toward synthesis appears to occur almost simultaneously with growth. The large increase in RNA during lactation agrees with observations reported for rat mammary tissue (2,3,4).

It is of interest that milk output in the guinea pig as measured with a miniature milking machine, rose rapidly immediately after parturition and then steadily decreased (9). As a basis of comparison a typical lactation curve taken from the publication of Nelson *et al.*(9) is included for comparison. Of the various measurements made, the decline in total weight of the gland more closely approximates the steady decrease in milk yield. The relative high nitrogen, DNA and RNA/DNA ratios persist even after milk yield has apparently declined. This would appear to confirm the quoted histological observations that "the scarcity of mitosis in the lactating gland indicates that in milk secretion there is little turnover of cells"(1).

The sharp rise in DNA following parturi-

tion confirms the histological observation of Kuramitsu and Loeb(10) on guinea pig mammary gland. These workers reported numerous mitoses 6 to 12 hours after labor and established lactation 2 days after labor. In contrast, they observed rare mitoses 6-12 hours after labor in the rat with "active mitotic proliferation" 2 days after labor.

Summary. The growth of guinea pig mammary glands during pregnancy, parturition, lactation and involution was measured using total weight, total nitrogen content, total DNA and RNA/DNA ratios as parameters of cellular changes. Only slight changes were found to occur during pregnancy. The rapid increase in total weight after parturition, correlated with the increase in total nitrogen and DNA, indicates that the observed growth is primarily a hyperplastic process. Extensive hypertrophy of pre-existing cells as measured by increased RNA/DNA ratios occurs after the period of rapid proliferative growth, thus the greatest cellular changes in guinea pig mammary glands occur immediately after parturition.

1. Kirkham, W. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v83, 123.
2. Shimizu, Hirokazu, *Tohoku J. Agr. Res.*, 1957, v7, 339.
3. Kuretani, Kazuo, *Kagaku Kenkyusho Hôkoku*, 1957, v33, 80.
4. Greenbaum, A. L., Slater, T. F., *Biochem. J.*, 1957, v66, 155.
5. Smith, T. C., Richterich, Britta, *Arch. Biochem. Biophys.*, 1958, v74, 398.
6. Hogeboom, G. H., Schneider, W. C., Palade, G. E., *J. Biol. Chem.*, 1948, v172, 619.
7. Conway, E. J., *Microdiffusion Analysis and Volumetric Error*, Van Nostrand, New York, 1950, 3rd Ed.
8. Ceriotti, G., *J. Biol. Chem.*, 1955, v214, 59.
9. Nelson, W. L., Kaye, A., Moore, M., Williams, H. H., Herrington, B. L., *J. Nutr.*, 1951, v44, 585.
10. Kuramitsu, C., Loeb, L., *Am. J. Physiol.*, 1921, v56, 40.

Received November 6, 1961. P.S.E.B.M., 1962, v109.

Investigations on Somatotropin Production of Human Anterior Pituitary Cells in Tissue Culture. (27208)

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The demonstration of species specific biological activity of growth hormones necessitates new sources for human growth hormone since only human and simian preparations are active in man(1,2). A logical approach to this problem is the study of *in vitro* cultivation of human or simian anterior pituitary cells.

The present communication describes isolation of anterior pituitary cell lines from human glands and investigations of somatotropin production using a highly sensitive and specific fluorescent antibody method.

Materials and methods. Isolation of cell lines. The cell lines used in these studies originated from human pituitary glands obtained at autopsy* which were designated Borg-11, Borg-12 (both from 7-month-old male fetuses) and Cook-3 (from 60-year-old male). Surrounding membranes and the posterior lobe of intact glands were removed aseptically. The anterior lobe was washed in sterile culture medium and minced with sterile scalpels. Tissue fragments were then treated for 15 minutes at 37°C in 0.01% collagenase solution(3). Single cells as well as the remaining tissue fragments were recovered by centrifugation and transferred into 100 × 20 mm plastic tissue culture petri dishes† containing 15 ml of culture medium. Prior to inoculation the bottom of each dish was lined with 10-13 sterile Corning 18 mm

* Courtesy of Drs. P. S. Rutherford and F. H. Cox, Borgess Hospital, Kalamazoo, Mich. and Dr. P. B. Santoz, Cook County Hospital, Chicago, Ill.
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