Nucleic Acid Content of Rat Mammary Gland Nuclei during Pregnancy, Lactation, and Involution (34349)

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The relationship of the number of cells in an organ to the productive capability of that organ remains unclear due to imperfect measures of the cell numbers. The DNA content of a tissue has been widely used as an index of cell numbers (6–8), but its validity is uncertain. The use of DNA quantity per tissue as an index of cell numbers is valid only if the average DNA content per cell is constant during all physiological conditions. Diploid somatic cells of animals of one species reputedly have a constant deoxyribonucleic acid (DNA) content (1, 2), however, new evidence is making this hypothesis untenable (3–5).

From chemical measurements on rat mammary gland tissue, it has been reported that the DNA quantity per cell varies from pregnancy to lactation (9); however, the DNA quantity per nucleus has been reported to be constant from day 12 of pregnancy to day 21 of lactation (10). Microspectrophotometric measurements on individual nuclei in rabbit mammary tissue indicate that the DNA content is variable during gland growth, secretion, and involution (5). The initial postulation of metabolic DNA related to protein synthesis in the mammary gland was made from these data (5).

Significant differences in the DNA content of cells of several tissues depending on their functional status have been reviewed (4) and the evidence for metabolic DNA connected with protein synthesis has been discussed (3). Pelc (3) concludes that nuclear metabolic DNA is a reality in probably all cells and postulates that metabolic DNA of a differentiated cell consists of extra copies of genes that regulate and perform the transcription to RNA in that cell at that particular point in time.

Ribonucleic acid (RNA) content of a tissue (8) and the RNA, DNA ratio (8) of that tissue are used as indices of protein synthesis and metabolic activity, respectively. The relationship of RNA to protein synthesis is well established, but information on the turnover rate of RNA in mammary gland cells during various physiological stages remains scarce. The use of RNA, DNA ratio as an index of metabolic activity in the cell is based on the above assumption that DNA content per cell is constant.

The objectives of this experiment were to determine: (a) the average DNA and RNA contents and the RNA, DNA ratios of rat mammary cell nuclei during pregnancy, lactation, and involution, (b) if these parameters indicate differences among glands, and (c) the relationship of these parameters to production and maternal weight.

Materials and Methods. Primiparous Sprague-Dawley rats were used. Presence of a vaginal plug indicated day 1 of pregnancy. Litter size was adjusted to 8 pups on the second day of lactation. Approximately 10 rats were sacrificed on each: day 18 of pregnancy, day 10 of lactation, and day 7 of involution. The 6 abdominal-inguinal and 2 posterior thoracic mammary glands were quickly removed, carefully separated, trimmed and identified. The posterior thoracic, abdominal, anterior and posterior inguinal glands on the right side were numbered 1, 2, 3, and 4, respectively. The corresponding glands on the left side were numbered 5 to 8. The glands were placed in cold 0.5 *M* sucrose–1% citric acid media for homogenization in the Waring Blendor and subsequently treated to isolate nuclei and determine nucleic acid contents as previously outlined (10). Alterations to the procedure were

as follows: (a) approximately 100 ml of media were used for homogenization and each washing; (b) initial centrifugation at 1200*g* was continued for 1.5 hr, subsequent washings were centrifuged for 20 min at 1000*g*, and (c) the nuclei were resuspended in 15 ml of media, 0.2 ml of which was removed and diluted with 1.6 ml of 0.1% Tween 80 in media and 0.2 ml of 0.1% crystal violet in media before counting of nuclei in a hemocytometer. The remainder of the sample was used for chemical analysis. Average nucleic acid per nucleus was obtained by dividing the total acid by the number of nuclei in comparable volumes of media. The RNA, DNA ratio was calculated for each determination and treated as a separate variable. Highly polymerized calf thymus DNA (General Biochemicals) and Reagent grade RNA (Nutritional Biochemicals). were used as standards. The data were analyzed statistically using physiological stages of sampling and glands as fixed effects in a linear additive model with a random error term.

Results. There was a slight but not significant increase in the average DNA content of nuclei from day 18 of pregnancy to day 10 of lactation, after which a significant decrease ($p<.01$) occurred to day 7 of involution (Table I). The rat, like the rabbit, (5) has significant differences ($p<.05$) among glands in the amount of DNA per nucleus (Table II). The differences among glands were most pronounced during pregnancy and lactation. No apparent relationship exists between the level of DNA per nucleus during pregnancy and the level during lactation in a particular gland. By day 7 of involution there was only one gland significantly different from the rest in DNA content per nucleus (Table II).

The nuclear RNA content increased ($p<.01$) from pregnancy to lactation and decreased ($p<.01$) from lactation to involution (Table I). On day 7 of involution, the RNA content per nucleus had declined to approximately one half of the amount present at day 10 of lactation. Significant differences ($p<.05$) among glands in RNA content per nucleus were present at all stages of development (Table II). The differences were most pronounced during lactation. The ranking of glands on their RNA content per nucleus during one stage bears little resemblance to their rank in any other stage. The rank of glands on their RNA content per nucleus at day 10 of lactation is similar to their rank on DNA content per nucleus at day 18 of pregnancy.

A significant increase ($p<.01$) in the RNA, DNA ratio from pregnancy to lactation was followed by a significant decline ($p<.01$) to day 7 of involution (Table I). Both the DNA and the RNA quantity per nucleus had declined to day 7 of involution, resulting in an RNA, DNA ratio at day 7 of involution which is significantly higher ($p<.01$) than the ratio during pregnancy. Significant differences ($p<.05$) among glands in the RNA, DNA ratio were noted during lactation and involution, but there are no differences during pregnancy (Table II).

The correlation of RNA and DNA contents per nucleus is positive, increasing from 0.34 during pregnancy to 0.59 in lactation and declining to 0.45 at day 7 of involution (Table III). The DNA content per nucleus is positively related to litter weight at day 10

TABLE I. Average Nucleic Acid Content of Mammary Gland Cell Nuclei.[d]

	($\mu\mu$g/nucleus)		
	DNA	RNA	RNA/DNA
Pregnancy	11.16 ± 0.17^a (75)[e]	1.58 ± 0.16^b (36)	0.123 ± 0.005^c (36)
Lactation	11.43 ± 0.16^a (80)	2.71 ± 0.04^a (63)	0.234 ± 0.004^a (63)
Involution	9.54 ± 0.17^b (78)	1.38 ± 0.04^c (80)	0.158 ± 0.003^b (78)

[d] For a particular parameter $a > b > c$ ($p < .01$). Comparison wise error rate LSD $= t_\alpha \, d \cdot f_\alpha \, Sy \, [(1/r_1) + (1/r_2)]^{1/2}$.

[e] Number in parentheses = the number of observations in the mean.

TABLE II. Nucleic Acid Content of Average Nuclei from Individual Glands within Treatments.[e]

	Nucleic acid ($\mu\mu$g/nucleus)							
Gland:	1	2	3	4	5	6	7	8
				Pregnancy				
DNA	9.87 (9)^{af}	10.08 (8)^{b}	12.80 (10)^{mn}	11.68 (10)	10.85 (10)	11.62 (9)	10.13 (9)^{b}	12.22 (10)^{m}
RNA	1.27 (5)^{a}	1.79 (3)	1.56 (5)	1.60 (5)	1.32 (5)^{a}	2.14 (3)^{m}	1.31 (5)^{a}	1.66 (5)
RNA/DNA	0.11 (5)	0.14 (3)	0.11 (5)	0.11 (5)	0.11 (5)	0.17 (3)	0.11 (5)	0.12 (5)
				Lactation				
DNA	11.31 (10)	12.14 (10)^{m}	12.29 (10)^{m}	10.56 (10)^{b}	11.59 (10)^{m}	11.16 (10)	12.85 (10)^{mn}	9.55 (10)^{a}
RNA	1.65 (8)^{a}	1.86 (8)^{b}	3.49 (8)^{cmn}	2.30 (8)^{bm}	2.23 (8)^{bm}	2.18 (8)^{b}	4.34 (7)^{mnr}	3.63 (8)^{cmn}
RNA/DNA	0.16 (8)^{a}	0.16 (8)^{a}	0.27 (8)	0.21 (8)^{bm}	0.20 (8)^{b}	0.18 (8)^{b}	0.32 (7)^{dmnr}	0.38 (8)^{mnrs}
				Involution				
DNA	10.49 (10)^{m}	9.07 (10)	10.80 (9)^{m}	9.54 (10)	9.54 (10)	7.67 (10)^{a}	9.20 (9)	9.97 (10)^{m}
RNA	1.34 (10)	1.73 (10)^{m}	1.28 (10)	1.36 (10)	1.27 (10)	1.66 (10)^{m}	1.03 (10)^{a}	1.37 (10)
RNA/DNA	0.14 (10)^{a}	0.19 (10)^{m}	0.15 (9)^{a}	0.15 (10)^{a}	0.14 (10)^{a}	0.21 (10)^{m}	0.13 (9)^{a}	0.15 (10)^{a}

[e] For any parameter at any particular stage $m>a$, $n>b$, $r>c$, $s>d$, $p<.05$. Experiment wise error rate (HSD $= Q_{\alpha} d \cdot f \cdot s\bar{y}$). Standard errors DNA $\pm$ 0.49 when $n = 9$; RNA $\pm$ 0.16 when $n = 5$; RNA/DNA $\pm$ 0.01 when $n = 4$.

[f] Number in parentheses = the number of observations in the mean.

of lactation (correlation 0.47, Table III). The RNA content per nucleus is not related to litter weight and RNA, DNA ratio is inversely related (Table III).

An inverse correlation (—0.43) exists between the dam weight and their nuclear DNA content at day 18 of pregnancy, but there is no relationship during lactation and involution (Table III). There is a very low correlation of RNA content per nucleus with dam weight at any stage (Table III). At day 10 of lactation a positive correlation of 0.64 exists between dam weight and litter weight.

Discussion. Variation in DNA content per cell could be due to variation in DNA content of subcellular organelles. Since the nucleus has the greatest amount of cellular DNA, it has been accepted that the DNA content per cell is constant if the nuclear DNA content is constant. Hence the nucleus has received the greatest attention.

The absolute DNA quantities per nucleus (Table I) are higher than those previously reported (9, 10). The difference may be due to the quality of standard used.

The decline in DNA content per nucleus from pregnancy and lactation to involution supports the theory that metabolic DNA exists. It similarly opens to question the validity of the hypothesis that DNA content per cell is constant. Theoretically, the DNA content of the metabolically quiescent nuclei during involution approaches the diploid quantity (5). Using the involuted amount as the diploid quantity of DNA, the DNA at day 10 of lactation is 20% higher than the diploid quantity. Metabolic DNA was estimated to be 37% of the diploid amount in rabbit mammary gland nuclei during lactation (5) and increases of 45–75% in DNA content in other tissues have been reported (3). As previously hypothesized (3, 5) the high level of metabolic DNA is present at the time of a high rate of protein synthesis. If the litter size had not been limited, the suckling stimulus would have been greater; possibly higher metabolic DNA values would have resulted since total tissue DNA is positively related to suckling stimulus (13). The slightly lower level of DNA during late pregnancy is expected of an asynchronous population of cells that are undergoing mitotic activity and synthesizing metabolic DNA (3).

The differences in DNA content per nucleus among glands during pregnancy indicate that they develop differently. The glands with higher amounts of DNA per nucleus theoretically have more template available for copying and thereby have a greater productive capability at that time. This in no way indicates, however, that the productive capability developed in pregnancy will be realized.

At day 10 of lactation, differences among glands in DNA content per nucleus indicate that some glands may have a greater productive capability than others. These differences may reflect differential suckling stimuli of the individual glands.

If weaning is considered an external environmental control, then all glands should respond synchronously with resultant loss of metabolic DNA to leave only a quantity that approaches the stable chromosomal 2n complement. The data support this concept as there is only one gland that is significantly different to the rest in amount of DNA per nucleus by day 7 of involution.

The fact that glands rank differently on DNA content per nucleus during different

TABLE III. Relationship of Nucleic Acid per Nucleus to Dam Weight and Litter Weight.

	Correlations				
	of DNA/nucleus with			of RNA/nucleus with	
	Dam wt	Litter wt	RNA/nucleus	Dam wt	Litter wt
Pregnancy	—0.43	—	0.34	0.17	—
Lactation	—0.03	0.47	0.59	—0.20	0.02
Involution	0.08	—	0.45	0.02	—

stages of development further suggests that a portion of the DNA is metabolic and can change under environmental influence. From pregnancy to lactation, some glands lose DNA from their nuclei while others increase in DNA content (Table II). One environmental influence resulting in differential gland production capability and nuclear DNA quantity may be the efficiency of removal of milk from the gland by the pup, since milk protein accumulation inhibits its own synthesis (14).

The average nuclear RNA quantity increased 72% from pregnancy to day 10 of lactation (Table I) and it is likely the rate of turnover of RNA also increased. The RNA content in the nucleus declined from day 10 of lactation when protein synthesis was high to approximately one half the amount by day 7 of involution. The RNA in the nucleus would comprise approximately 5–10% of the total RNA in the cell. This is very similar to values reported for liver (12).

The great variation among glands in the amount of RNA per nucleus during lactation may indicate different rates of protein synthesis and may reflect a degree of control by the above-mentioned environmental influences. The changes in nuclear RNA could be due to any one or combination of the following changes, as measured at a point in time, (a) changes in the number of units of RNA actually being synthesized, (b) changes in the number of units of RNA in transport, (c) changes in the number of RNA molecules waiting to be transported, or a combination of these changes with (d) changes in the quantity of RNA which performs its function in the nucleus.

RNA, DNA ratio can be used as an index of metabolic activity in a cell or nucleus (8) only if two assumptions are accepted, (i) that DNA content per cell or per nucleus is constant, and (ii) the rate of turnover of RNA is unchanged among the stages of development for which comparisons are being made. Meaningful interpretation of the RNA, DNA ratio in relation to metabolic activity becomes impossible when both RNA and DNA content are variable. It is unknown whether a nucleus or cell with high amounts of RNA and DNA should be classified as having the same metabolic activity as a nucleus or cell that has the same RNA, DNA ratio but only a fraction of the total RNA and DNA content. An example of the problem is evident in Table I, the RNA, DNA ratio is significantly lower during pregnancy than during involution, primarily because DNA content per nucleus is high at day 18 of pregnancy, while both RNA and DNA decline significantly from pregnancy to involution.

Assuming the major part of the variation in nuclear RNA content to be due to (a) above, the RNA, DNA ratio can be readily interpreted as an indicator of the efficiency of utilization of the available DNA template. Efficiency is defined here as the number of units of RNA present per unit of DNA at any point in time. Even if there are large changes in (b) it seems logical that the changes would be positively correlated with those in (a), therefore only slightly decreasing the accuracy of the comparison of efficiencies. Lack of information prevents any comment on the importance of (c) and (d).

If the above hypothesis is accepted, metabolic DNA that was formed during pregnancy is relatively inefficiently utilized when the RNA, DNA ratio for pregnancy is compared to that in lactation. The change in ratio indicates a 90% increase in efficiency. If the meaning of efficiency is broadened to be defined as the number of units of RNA per unit time produced per unit DNA, the estimate of 90% is low due to a bias downwards if there is a decrease in time required from the initiation of synthesis of a RNA molecule until it leaves the nucleus. It is possible that differences in the time required from initiation of synthesis of a RNA molecule until it leaves the nucleus are sufficiently great between two stages of development as to make the narrower definition of efficiency above, relatively insensitive. Measurements on change of rate of RNA synthesis and transport per unit time for mammary gland were not found. Using the narrow definition of efficiency there is no difference among glands in the efficiency of utilization of the DNA template during pregnancy, but differences

among glands do exist during lactation and involution.

The positive correlation of DNA content per nucleus and litter weight at day 10 of lactation supports the hypothesis that metabolic DNA is directly associated with protein synthetic capability. The lack of correlation between nuclear RNA quantity and litter weight suggest there are factors in RNA metabolism and its relationship to rate of protein synthesis by the gland as measured by litter weight which are not fully understood.

The relationship between dam weight and litter weight (correlation + 0.64) on day 10 of lactation indicates that large dams produced large amounts of milk. Therefore the negative correlation between dam weight and nuclear DNA quantity on day 18 of pregnancy (Table III) apparently does not mean that large females would be at a disadvantage during lactation.

Rapid recognition of the fact that DNA exists in the cell in other than the basic 2n stable chromosome form will facilitate the resolution of the dichotomy between autoradiographic results and histological search for mitoses. The characteristics of metabolic DNA indicate that it can be renewed or repaired periodically, hence there are three periods during which nuclei can be labeled, premitotic synthesis, formation of metabolic DNA, and renewal or repair (3). In addition, cells can become labeled by DNA precursors whenever there is formation of new mitochondria.

Summary. The quantity of DNA per rat mammary gland nucleus was significantly lower during involution in comparison to pregnancy and lactation. Significant differences in both RNA and DNA contents per nucleus among glands occurred during pregnancy, lactation, and involution. Nuclear RNA contents and RNA, DNA ratios increased from pregnancy to lactation and then decreased during involution. A correlation of 0.47 was found between the litter weight at day 10 of lactation and the DNA content per nucleus. The presence of metabolic DNA and its relationship to protein synthesis in rat mammary gland is indicated. The efficiency of utilization of the available DNA template is suggested as the most meaningful interpretation of the RNA, DNA ratio. Sufficient evidence has been accumulated to reject the use of DNA per mammary gland as an index of the number of cells in the gland.

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