

cells in GMK and RMK cultures exhibited the most brilliant fluorescence, those in HFK and SV40 transformed HFK less, and those in SHK and SV40 transformed SHK least. The proportion of fluorescing cells diminished in the same order. It seems likely that the number of cells with fluorescence approximates the per cent of cells actively producing virus, and that the brilliance of fluorescence is proportional to quantity of virus produced. Evidence supporting these correlations is afforded by results previously reported of a comparative titration of SV40 production in GMK and HFK cultures (5). The end point as determined by back titration in GMK cultures was consistently 2 logs lower in HFK cultures. It is curious that *in vitro* transformation has been observed so far only in the 2 cell systems, HFK and SHK, where the virus appears to propagate less easily. A similar inverse relation between ease of propagation and ease of transformation has been generally observed with polyoma virus in mouse embryo and hamster cultures. Indeed as noted by Fraser and Gharpure in the hamster cell line of Stoker, using polyoma virus at infection multiplicities of 10^8 pfu per cell, a ratio producing a transformation rate of 1-4%, only 0.5% of cells show nuclear multiplication of virus by immunofluorescence although, at 24 hours or less, all cells show cytoplasmic uptake of the agent (9).

Conclusions. In SV40 infected grivet monkey, rhesus monkey, primary fetal human, SV40 transformed fetal human and primary

newborn Syrian hamster kidney tissue cultures viral antigen is found in the nucleus. Because of aggregation of antigen around what appears to be altered nucleoli in all 4 tissues it is suggested that viral synthesis and assembly may occur in the nucleolus. In the 4 tissues studied there is an inverse correlation between proportion of cells exhibiting specific nuclear fluorescence, brilliance of fluorescence and ease of induction of transformation *in vitro*.

No specific nuclear fluorescence was observed in SV40 transformed Syrian hamster renal cultures even when superinfected with SV40 virus.

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Nucleic Acid Content of Mammary Glands of Lactating Rats.*† (28060)

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In the rat, histological observations (1) suggested that mammary hyperplasia did not occur after mid-pregnancy, whereas deoxyribonucleic acid (DNA) content, which estimates cell numbers (2), increased throughout pregnancy (3,4,5). Histological studies using colchicine technic (6) revealed little mi-

totic activity during lactation but later work showed that DNA content (3,4) approxi-

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mately doubled between end of pregnancy and third day of lactation. Ovariectomy on the second day of lactation had no effect on glandular proliferation which followed parturition(7), but it has been reported(3) that a wave of mitoses occurred 30 hours after parturition and was of short duration. Epithelial cells have been observed in lumina of alveoli and ducts of mammary glands of lactating rats(8), but DNA has not been found in milk(9,10). Griffith and Turner(7) reported that DNA content was maintained until the 21st day of lactation, although other investigators(11,12) observed a reduced DNA content during later stages of lactation. Ribonucleic acid (RNA) values and RNA/DNA ratios reported for rat mammary tissue suggested that protein synthesis was most abundant at mid-lactation(3) or end of lactation (13).

The present study reports rat mammary gland DNA and RNA content and RNA/DNA ratios at 9 stages of lactation. In addition, the effect of ovariectomy immediately after parturition on nucleic acid content was determined.

Methods. Normal development and protein synthetic activity patterns were obtained from DNA and RNA content of 6 abdominal-inguinal mammary glands of hooded Norway rats lactating 1, 4, 8, 12, 14, 18, 21, 24, and 28 days. Day 1 of lactation was day of parturition. Litter size was adjusted to 8 pups per mother on day 3 of lactation. Another group of lactating rats was ovariectomized 1 to 4 hours after parturition and sacrificed on day 8 of lactation; a sham operation was carried out on controls. Nucleic acids were determined as previously described(2).

Results. Six abdominal-inguinal mammary glands contained 8.66, 10.90, 11.51, 11.07, 10.73, 11.15, 11.03, 10.62, and 9.67 mg of DNA per 100 g of body weight as lactation advanced from 1 to 28 days. Mammary DNA content per 100 g of body weight was highly significantly less ($P<0.01$) on day 1 of lactation than that in glands from all other stages of lactation except glands from rats lactating 28 days where it was significantly less ($P<0.05$). Mammary glands of rats lactating 28 days contained

less DNA per 100 g of body weight than those of all other groups, except day 1, differences being significant ($P<0.05$) in rats lactating 14 to 24 days and highly significant ($P<0.01$) in the remaining groups. All other mean comparisons were not significantly different one from another (Table I).

Mammary gland development from day 20 of pregnancy to day 1 of lactation increased only 9.8%, whereas the increase from day 1 of lactation to day 4 of lactation was 25.9%. Maximum mammary development during lactation occurred at day 8. Considering maximum development as 100%, it was found that 24.8% of total mammary development occurred during lactation. Mammary development remained essentially constant from day 4 through day 24 of lactation. Mammary DNA per 100 g of body weight at 28 days of lactation declined 16.0% from maximum.

Total mg of DNA revealed a trend in mammary development somewhat similar to that found when body weights were standardized. Mammary glands contained 17.67, 23.10, 24.98, 26.34, 25.01, 26.31, 25.47, 24.65, and 20.22 mg of total DNA as the lactation period progressed. Total DNA of mammary glands of rats on day 1 of lactation was highly significantly less ($P<0.01$) than that of all other groups of lactating rats. Development attained by day 4 of lactation was highly significantly less ($P<0.01$) than that attained on either day 12 or 18 of lactation and significantly less ($P<0.05$) than that attained on day 21 of lactation. The DNA content of glands on day 28 of lactation was highly significantly less ($P<0.01$) than that of all other groups except day 1. All other group comparisons were not statistically different. On the basis of total DNA, mammary growth increased during early lactation to a maximum on day 12, remained constant from day 8 through day 24 of lactation, then declined from day 24 to day 28 of lactation (Table I).

With the onset of lactation a striking increase in total mg of mammary RNA was observed. Means for lactating groups were 21.93, 35.69, 50.37, 64.96, 65.15, 74.93, 75.06, 64.23, and 39.61 mg. Therefore, total mammary RNA increased rapidly until day 21 of lactation, and thereafter declined

TABLE I. Nucleic Acid Content of Mammary Glands of Lactating Rats.

Days of lactation	No. animals	Body wt (g, mean)	DEEET* (mg, mean)	Total DNA/100g body wt (mg)†	Total DNA (mg)†	Total RNA (mg)†	RNA/DNA†
20‡	12	218	440.7	7.89 ± .27	17.19 ± .65	15.44 ± .56	.90 ± .02
1	12	204	564.7	8.66 ± .33§	17.67 ± .68¶	21.93 ± 1.25	1.24 ± .04
4	12	212	698.7	10.90 ± .26	23.10 ± .43**	35.69 ± .90	1.55 ± .03
8	12	217	875.6	11.51 ± .25	24.98 ± .66	50.37 ± 2.19	2.02 ± .05
12	12	238	1058.0	11.07 ± .20	26.34 ± .76	64.96 ± 2.14	2.47 ± .04
14	12	233	1028.8	10.73 ± .34	25.01 ± .94	65.15 ± 2.35	2.60 ± .03
18	12	236	1206.8	11.15 ± .21	26.31 ± .46	74.93 ± 2.44	2.85 ± .10
21	12	231	1162.6	11.03 ± .30	25.47 ± .64	75.06 ± 2.69	2.95 ± .08
24	12	232	1081.3	10.62 ± .28	24.65 ± .30	64.23 ± 2.58	2.61 ± .11
28	12	209	776.2	9.67 ± .34	20.22 ± .64††	39.61 ± 3.39	1.96 ± .12

* Dried, ethanol-ether extracted tissue.

† Mean and standard error of mean.

‡ Pregnant 20 days; data from Tucker and Reece(5).

§ Highly significantly less ($P < .01$) than all other lactation stages except 28th day where it was significantly less ($P < .05$).

|| Highly significantly less ($P < .01$) than all other lactation stages except 14th and 24th day where it was significantly less ($P < .05$) and 1st day where it was significantly greater ($P < .05$).

¶ Highly significantly less ($P < .01$) than all other lactation stages.

** Highly significantly less ($P < .01$) than 12th and 18th days and significantly less ($P < .05$) than 21st day of lactation.

†† Highly significantly less ($P < .01$) than all other lactation stages except 1st day where it was highly significantly greater ($P < .01$).

precipitously (Table I).

The mammary RNA/DNA ratio increased very rapidly when lactation was initiated, indicating that total RNA increased much more rapidly than did total DNA. The mean ratios of these 2 parameters with increases in stage of lactation were 1.24, 1.55, 2.02, 2.47, 2.60, 2.85, 2.95, 2.61, and 1.96. Therefore, a continued increase in protein synthetic activity per cell was observed until 21 days of lactation, after which a marked decline occurred (Table I).

Mammary glands of ovariectomized and sham-operated rats contained 10.97 and 11.46 mg of DNA per 100 g of body weight; 24.03 and 25.22 mg of total DNA; and 43.15 and 46.62 mg of total RNA, respectively. The RNA/DNA ratio was 1.80 and 1.85 for ovariectomized and sham-operated rats, respectively. The DNA and RNA content of glands of ovariectomized or sham-operated rats was not significantly different ($P > 0.05$) from DNA and RNA content of glands from intact rats lactating 8 days. RNA/DNA ratios were highly significantly greater ($P < 0.01$) in intact rats lactating 8 days than in either ovariectomized or sham-operated rats lactating 8 days.

Discussion. Increase in mammary DNA content from day 20 of pregnancy to day 1 of lactation was only slight in comparison to

the increase between days 1 and 4 of lactation. This rapid increase in mammary growth during early lactation was not influenced by ovariectomy on day of parturition. Mammary DNA content increased to a maximum by day 8 of lactation (DNA per 100 g body weight) or by day 12 of lactation (total DNA), after which it remained essentially constant through day 24 of lactation. These findings indicate that lactational stimuli are sufficient to maintain mammary cells for 24 days.

With the onset of lactation a striking increase in total mammary RNA occurred which, it is assumed, reflected increased protein synthesis associated with initiation of lactation. This increase in total RNA was not affected by ovariectomy at parturition. Total RNA reached its maximum on day 21 of lactation, which agrees with the results of Kirkham and Turner(13), and thereafter declined precipitously despite continued suckling of litters.

Protein synthetic activity per mammary cell increased very rapidly when lactation was initiated. A continued increase was observed until day 21 of lactation, and thereafter a marked decline occurred. It is proposed that the plot of these data against stage of lactation represents the normal lactation curve of the rat, which previously has not

been adequately depicted. The RNA/DNA curve during lactation corresponded quite well with the limited RNA/DNA ratio data presented by Kirkham and Turner(13). The shape of the mammary gland RNA/DNA ratio curve reported in the present experiment is in almost perfect agreement with the shape of the mammary gland activity curves during lactation of glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase, 2 enzymes involved in the pentose phosphate pathway(14).

Summary. Total mammary DNA per 100 g of body weight increased 9.8% from day 20 of pregnancy to day 1 of lactation and 25.9% from day 1 to day 4 of lactation. Ovariectomy on day of parturition had no influence on mammary hyperplasia during early lactation. On the basis of total DNA per 100 g of body weight maximum mammary development occurred on day 8 of lactation (11.51 mg) and 24.8% of mammary growth occurred during lactation. No significant decrease occurred in mammary DNA until after day 24 of lactation. With the onset of lactation total mammary RNA increased much more rapidly than did total mammary DNA. Maximum mammary RNA content was observed on day 21 of lactation (75.06 mg) after which it declined precipitously to day 28 of lactation (39.61 mg). Mammary gland RNA/DNA ratio increased rapidly when lactation was initiated (1.24),

attained its maximum on day 21 of lactation (2.95) and then decreased to day 28 of lactation (1.96). It is suggested that RNA/DNA ratios, when plotted against stage of lactation, represent the normal lactation curve of the rat.

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Scleral Contact Filters for Domestic Birds.* (28061)

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The effect of the electromagnetic spectrum (4000 to 7000 Å) on growth and development of avian gonadal tissue has been reviewed thoroughly by Withrow(1). However, the wave length-intensity relationships,

as well as the efficiency of various wave lengths, require further investigation. More intensive work is also required on birds receiving no ocular illumination. The purpose of this research was to develop an inexpensive and relatively simple method for controlling wave length and light intensity for photoperiodic experiments with birds.

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