The Incorporation of [2-¹⁴C]Glycine and [6-¹⁴C]Orotic Acid into the Acid-Soluble Ribonucleotides and Ribonucleic Acids of the Rat Mammary Gland

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It has been reported (Wang & Greenbaum, 1960) that there is an increase in both the total amount and the concentration of the acid-soluble ribonucleotides of the rat mammary gland over the period from pregnancy to late lactation and it was thought that these changes probably reflected the increase of metabolic activity associated with the onset of lactation. Over the same period the RNA of the gland also increases, particularly in the microsome fraction (Slater & Planterose, 1958). It was suggested that this increase was related to the greater rate of protein synthesis consequent on the greater rate of milk production. However, there are, as yet, no data available on the turnover rates of the RNA and acid-soluble ribonucleotides of the gland. The data presented here indicate that, over the period studied, there is an increased synthesis of the acid-soluble purine and uracil nucleotides, and also an initial increase in the total incorporation of the two precursors used into RNA at parturition, but later there follows a decrease as lactation advances.

METHODS

Animals. Adult female albino rats were used and the conditions for pregnancy and lactation were as described by Wang & Greenbaum (1960). They were fed *ad lib.* on stock diet (M.R.C. no. 41B; Bruce & Parkes, 1946).

The animals were injected intraperitoneally with 5 μ C of either [2-¹⁴C]glycine or [6-¹⁴C]orotic acid. Preliminary experiments indicated different time-courses for the incorporation of glycine and orotic acid into mammary-gland RNA. Glycine exhibited a peak of incorporation at 24 hr. after injection (see Fig. 3) whereas orotic acid incorporation appears to increase sharply up to 24 hr. but from then on increases less sharply up to at least 72 hr. after injection (see Fig. 4). On the basis of these experiments 24 hr. was used as the incorporation time for glycine, and for orotic acid 72 hr. was used for most experiments, but some were also carried out at 24 hr. for comparison with the data obtained for the incorporation of glycine.

In the experiments on the incorporation of these same two substrates into acid-soluble ribonucleotides, the animals were killed 4 hr. after the injection. The litters were left with these rats until killing as it has been shown that even short periods of non-suckling produce changes in the acid-soluble nucleotides of the gland (Wang, 1960).

Reagents. Radioactive substrates were obtained from The Radiochemical Centre, Amersham, Bucks. The

[2-¹⁴C]glycine (100 μ C in 2.46 mg.) was dissolved in water to yield a solution containing 10 μ C/ml. The [6-¹⁴C]orotic acid (100 μ C in 1.7 mg.) was similarly diluted to give 10 μ C/ml.

Chromatography of the acid-soluble ribonucleotides. Animals were killed and the acid-soluble ribonucleotides of the combined lower abdominal glands extracted and chromatographed on anion-exchange resin, a gradient elution of ammonium formate and formic acid being used as described by Wang & Greenbaum (1960), with the following additional step to remove contaminating radioactive glycine, which appeared to be adsorbed on the anion-exchange resin. Unlabelled glycine was added to the perchloric acid extract (1 g. to the extract from a single animal). After loading the column it was washed with 500 ml. of 2% unlabelled glycine solution, and then with 500 ml. of glass-distilled water. In the experiments involving the incorporation of [6-¹⁴C]orotic acid into acid-soluble ribonucleotides, about 1 mg. of unlabelled orotic acid was added to the perchloric acid extract to act as a marker for the radioactive orotic acid that is adsorbed on the column, and is eluted by the system used.

Extraction and hydrolysis of RNA. The abdominal mammary glands of either one or two rats were rapidly removed and homogenized in ice-cold water (40 ml. for each rat used) for 2 min. in a top-drive MSE blender. The homogenate was strained through muslin to remove intractable connective tissue, and the RNA was then prepared by the phenol method of Kirby (1958). The RNA was hydrolysed to mononucleotides by heating with 0.1 N-KOH at 80° for 40 min.

Under these conditions there is no appearance of inorganic phosphate and a recovery of material absorbing at 260 m μ of about 98%. The KOH was neutralized with perchloric acid and, after removal of the KClO₄, the nucleotides were separated on a Dowex 1 (formate form) anion-exchange resin column (5 cm. $\times$ 0.72 cm.²) by a discontinuous elution utilizing four concentrations of formic acid and ammonium formate (Herbert, Potter & Hecht, 1957). The flow rate was adjusted to approx. 30 ml./hr. The concentration of nucleotide present was determined from the known molar extinctions.

Measurement of radioactivity. After measurement of the extinction coefficient of the fractions from the chromatograms of either the acid-soluble nucleotides or the hydrolysed RNA the fractions corresponding to each peak were bulked and freeze-dried to remove water and formic acid. Ammonium formate was removed, if present, by a higher vacuum (Edwards' high-vacuum oil-diffusion pump: type 102) with cautious warming by infrared lamps (Hurlbert, Schmitz, Brumm & Potter, 1954). Fractions were then dissolved in 5 ml. of aqueous Triton X-100 solution (2 p.p.m.) and samples were plated on plastic planchets

with an area of 2 cm.² They were dried under infrared lamps in a warm-air draught from a hair-drier. The plated planchets were then counted in a Tracerlab SC 16 gas-flow windowless counter.

RESULTS

Incorporation of [2-¹⁴C]glycine into acid-soluble ribonucleotides. (a) Time-course of incorporation. Incorporation of [2-¹⁴C]glycine into the purine nucleotides was followed by studying the adenine compounds isolated from the acid-soluble fraction obtained from the glands of rats in late lactation. The animals were killed 1, 2, 4 or 8 hr. after injection of 5 μ C of the isotope and the extracted acid-soluble ribonucleotides were hydrolysed to mononucleotides by heating in 0.1N-KOH at 80° for 40 min. The extract was chromatographed and the

extinction of the fractions and their radioactivity were determined as described above. Results are shown in Fig. 1. Optimum time for incorporation in these experiments is about 4 hr. The peak of radioactivity does not coincide with the peak of extinction shown in Fig. 1 (b). The highly radioactive material could be separated from the AMP peak by the ammonium formate-elution procedure described by Hurlbert *et al.* (1954). There was still a maximum of incorporation into the AMP at 4 hr. The nature of the highly active contaminant is not known. It is present in acid-soluble extracts prepared in the normal way and not subjected to alkaline hydrolysis, and is not therefore a reaction product of the hydrolytic procedure.

(b) Incorporation into the acid-soluble purine nucleotides over the lactation cycle. In view of

Fig. 1. Time-course of the incorporation *in vivo* of [2-¹⁴C]glycine into the AMP fraction of the acid-soluble ribonucleotides of mammary gland at lactation. One animal was used in each experiment. After the intraperitoneal injection of 5 μ C of [2-¹⁴C]glycine the animals were killed at the stated time-intervals. (a) Radioactivity measurements of the AMP fractions; (b) extinctions of the corresponding AMP peaks, measured at 260 m μ .

Fig. 2. Time-course of the incorporation *in vivo* of [6-¹⁴C]orotic acid into the uracil nucleotides of the acid-soluble ribonucleotides of mammary gland at late lactation. One animal was used in each experiment. After the intraperitoneal injection of 5 μ C of [6-¹⁴C]orotic acid the animals were killed at the stated time intervals. ▲, UTP; ■, UDP; ●, UMP; ○, UDP-hexose; □, UDP-N-acetylhexosamine.

Table 1. Incorporation of [2-¹⁴C]glycine into the acid-soluble purine ribonucleotides

All results (means $\pm$ S.E.M.) are expressed as total counts in the total nucleotides of the combined abdominal glands from one animal per 100 sec., although in each experiment two animals were used (injected intraperitoneally with 5 μ C of [2-¹⁴C]glycine 4 hr. before killing).

Status	No. of expts.	Total counts/100 sec.					
		AMP	ADP	ATP	GMP	GDP	GTP
Early lactating (2-5 days)	5	321 (± 35)	374 (± 53)	322 (± 21)	223 (± 23)	288 (± 27)	74 (± 7)
Late lactating (16-18 days)	5	633 (± 53)	675 (± 46)	443 (± 14)	384 (± 18)	397 (± 21)	79 (± 7)

Table 2. *Incorporation of [6-¹⁴C]orotic acid into the acid-soluble pyrimidine nucleotides*

All results (means $\pm$ S.E.M.) are expressed as total counts in the combined abdominal glands from one animal per 100 sec. Each animal was injected with 5 μ C of [6-¹⁴C]orotic acid 4 hr. before killing. Single animals were used for all experiments except in the late-pregnant group, when two animals were used in each experiment.

Status	No. of expts.	Total counts/100 sec.				
		UMP	UDP	UTP	UDP-hexosamine	UDP-hexose
Late pregnant (20-22 days)	4	210 (± 22)	214 (± 8)	89 (± 10)	133 (± 20)	159 (± 42)
Early lactating (2-5 days)	4	440 (± 110)	357 (± 46)	205 (± 29)	247 (± 47)	371 (± 89)
Late lactating (16-18 days)	5	492 (± 77)	392 (± 74)	218 (± 54)	462 (± 82)	461 (± 76)

the time-course described above, a time of 4 hr. was chosen for study of the rate of incorporation of [2-¹⁴C]glycine into the acid-soluble purine nucleotides over the lactation cycle (Table 1). The rate of incorporation into glands from pregnant rats was so low that the figures have not been included in the Table. Even in early lactation (2-5 days) there is an appreciable turnover of the nucleotides, and this is considerably increased in late lactation (16-18 days). The increase is a general one in which all the purine nucleotide phosphates, with the exception of GTP, participate. The counts for GTP are too low to be accurate, and no firm conclusions can be drawn from these figures.

Incorporation of [6-¹⁴C]orotic acid into the acid-soluble ribonucleotides. (a) Time-course of incorporation. A time-course of incorporation of [6-¹⁴C]orotic acid into the uracil nucleotides was followed by the same procedure as that described above for the incorporation of [2-¹⁴C]glycine, except that the time-intervals were 2, 3, 4 or 6 hr. after the injection of 5 μ C of the radioactive substrate (Fig. 2). There are two patterns of incorporation. The labelling of UDP-hexose and UDP-N-acetylhexosamine appears to increase in a linear manner throughout the whole period studied and has obviously not yet reached a maximum at the end of 6 hr. The other uridine compounds reach a maximum by 4 hr. and thereafter remain roughly constant in activity. In the experiments described below an incorporation time of 4 hr. was chosen.

(b) Incorporation into the acid-soluble pyrimidine nucleotides over the lactation cycle. Table 2 shows the incorporation of orotic acid into the uracil nucleotides and their sugar complexes over the lactation cycle, the results being expressed as total counts found in the abdominal glands of one rat. Although some incorporation of orotic acid into CMP was observed, the figures are not included in Table 2 since the values were too low to be significant. Figures for the other cytidine complexes are also omitted as these occur in composite peaks in

the chromatogram. The figures in Table 2 show a considerable scatter; nevertheless, for all the substances shown there is an increasing rate of turnover (about twofold) from pregnancy to lactation and a much slower rate of increase during lactation. The turnover of the UDP-N-acetylhexosamine and UDP-hexose is of a comparable order and follows the same course.

Incorporation of [2-¹⁴C]glycine into the purine nucleotides of RNA. (a) Time-course of incorporation. The time-course of incorporation of [2-¹⁴C]glycine into RNA has been followed by measuring incorporation into the purine nucleotides isolated from a RNA hydrolysate. The animals were killed 8, 16, 24 or 48 hr. after the intraperitoneal injection of the labelled glycine. The activity of the separated nucleotides at the different time-periods is shown in Fig. 3. The rate of incorporation is very slow: even after 24 hr. the count had risen only to about 100 counts/ μ mole in 400 sec., and this time-period was the time of maximum activity. There is a maximum in the specific activities at 24 hr. and this time was used in the incorporation experiments described below.

(b) Incorporation into the RNA of the mammary glands of pregnant and lactating rats. The incorporation of [2-¹⁴C]glycine into mammary-gland RNA has been measured in pregnant rats, and in lactating rats where the time of lactation varied from less than 1 day to 17 days. Results in Table 3 show the great increase in the rate of incorporation during the first 24 hr. *post partum*. Both for adenine and guanine the incorporation (expressed as total counts in the abdominal mammary glands) is increased by six- to ten-fold. A high rate of incorporation is still found in animals lactating for 24-48 hr. but thereafter the rate falls. The specific activities show a similar sharp increase immediately after parturition and a decline at the later time-intervals, but by mid-lactation the specific activity of both the adenine and guanine has fallen to a value below that obtaining in late pregnancy. The last column of the Table shows the

ratio of the specific activities at the different stages of the lactation cycle. In spite of the spread of these results it would seem that there is no marked

preferential synthesis of one of the nucleotides, although there is a suggestion that guanine may be synthesized more rapidly in early lactation.

Incorporation of [6-¹⁴C]orotic acid into the pyrimidine nucleotides of RNA. (a) Time-course of incorporation. The time-course of incorporation of [6-¹⁴C]orotic acid into uracil and cytosine nucleo-

Fig. 3. Time-course of the incorporation *in vivo* of [2-¹⁴C]-glycine into mammary-gland RNA at late lactation. One animal was used in each experiment. After the intraperitoneal injection of 5 μ C of [2-¹⁴C]glycine the animals were killed at the stated time-intervals and the RNA of the combined abdominal glands was extracted. The RNA was hydrolysed and the resulting mononucleotides were chromatographed and counted as described in the text. ●, GMP; ○, AMP.

Fig. 4. Time-course of the incorporation *in vivo* of [6-¹⁴C]-orotic acid into mammary-gland RNA at late lactation. One animal was used for each experiment. After the intraperitoneal injection of 5 μ C of [6-¹⁴C]orotic acid the animals were killed at the stated time-intervals and the RNA of the combined abdominal glands was extracted. The RNA was hydrolysed and the resulting mononucleotides were chromatographed and counted as described in the text. ●, UMP; ○, CMP.

Table 3. *Incorporation of [2-¹⁴C]glycine into ribonucleic acid*

Specific activities are expressed as counts/400 sec./ μ mole of nucleotide and the total counts as those for the total RNA from the abdominal glands from one animal per 400 sec. Each animal was injected with 5 μ C of [2-¹⁴C]glycine 24 hr. before killing. Single animals were used for all experiments except for those in the late-pregnant group, when two animals were used in each experiment.

Status	Adenine		Guanine		Specific activity of guanine Specific activity of adenine
	Total counts/ 400 sec.	Specific activity	Total counts/ 400 sec.	Specific activity	
Late pregnant (18 days)	506	263	818	207	0.79
	590	285	738	197	0.69
	494	249	646	169	0.68
Lactating (0-24 hr.)	3920	613	7430	572	0.93
	4520	707	6880	530	0.75
Lactating (24-48 hr.)	3340	523	5950	460	0.88
	3010	428	4410	342	0.80
Mid-lactating (10 days)	1230	123	1600	80	0.65
	2210	216	3510	188	0.87
	1030	88	1560	63	0.72
Late lactating (17 days)	1610	104	1810	62	0.60
	1830	114	2310	75	0.66

Table 4. *Incorporation of [6-¹⁴C]orotic acid into ribonucleic acid*

Specific activities are expressed as counts/400 sec./ μ mole of nucleotide and the total counts as those for the total RNA from the abdominal glands from one animal per 400 sec. Each animal was injected with 5 μ C of [6-¹⁴C]orotic acid 24 or 72 hr. before killing. Single animals were used for all experiments except for those in the late-pregnant group, when two animals were used in each experiment.

Status	Cytosine		Uracil		Specific activity of uracil Specific activity of cytosine
	Total counts/ 400 sec.	Specific activity	Total counts/ 400 sec.	Specific activity	
Incorporation for 24 hr.					
Late pregnant (18 days)	3 640	1 200	884	552	0.46
Early lactating (2 days)	17 400	1 780	5 160	1 000	0.56
Mid-lactating (10 days)	13 160	828	4 180	492	0.59
Late lactating (18 days)	14 200	640	3 960	328	0.51
Incorporation for 72 hr.					
Late pregnant (18 days)	4 400	1 428	1 080	692	0.48
	4 840	1 444	1 160	696	0.48
Early lactating (2 days)	11 800	1 196	2 680	508	0.42
	13 200	1 360	3 800	768	0.57
Mid-lactating (10 days)	22 520	1 408	5 400	672	0.48
	24 960	1 580	5 680	664	0.42
Late lactating	27 720	1 256	8 440	696	0.55
	32 720	1 472	8 160	704	0.48

tides is shown in Fig. 4. The degree of labelling increases throughout the period of 72 hr. and, in contrast with the labelling of the purines with [2-¹⁴C]glycine, it appears to be still increasing at 72 hr. The curves for the two monophosphates are practically parallel, indicating a constant ratio of labelling for these two substances throughout the measured period.

The greater rate of incorporation into the CMP was rather surprising in view of the general finding in other tissues that UMP becomes labelled faster than CMP. It was thought therefore that a possible explanation might be that the hydrolysates of mammary-gland RNA contained a contaminating substance similar to that described by Weed (1958) as occurring in rat liver after incorporation of orotic acid *in vitro*. The possibility was eliminated because on elution of the CMP fraction with a slow gradient the specific activity was found to be constant throughout the peak, i.e. the peak appeared homogeneous. Further, when the eluted CMP was deaminated by heating with N-KOH for 1 hr. at 100° (Hurst & Kuksis, 1958), the resulting UMP, separated by rechromatography as described above, was found to have the same specific activity as the CMP from which it was derived. These data confirm that the greater labelling was, indeed, in the CMP fraction and that in mammary gland the CMP is labelled more rapidly than UMP.

From the data given in Fig. 4 it was decided to use a period of 72 hr. for the experiments on the incorporation of orotic acid into mammary-gland RNA. Also, in order to provide figures to compare

with those obtained with [2-¹⁴C]glycine, some animals were killed after a period of only 24 hr.

(b) Incorporation into the RNA of mammary gland during the lactation cycle. Results showing the incorporation of [6-¹⁴C]orotic acid into RNA of the mammary gland during the lactation cycle are given in Table 4 for two time-intervals, 24 and 72 hr. At 72 hr. a steady level of incorporation has been reached, in which there is no difference in any of the four stages of lactation examined. At the shorter time-interval a pattern of incorporation is found that closely resembles the results with [2-¹⁴C]glycine and may be said to confirm the pattern of a sharp rise immediately *post partum* and a decline from this high level as lactation advances.

Thus all four nucleotides conform to this pattern if measured at 24 hr. Cytidine is labelled to a greater extent than uridine at all stages measured and the ratio of the specific activities, shown in the last column of Table 4, indicates that there is no variation in the proportion of radioactive orotic acid being incorporated into the two nucleotides.

DISCUSSION

It might be expected that the heavy stress of lactation would result in striking changes in the nucleotide and nucleic acid metabolism of the mammary gland. Results already published show an increase in the concentration of the acid-soluble nucleotides (Wang & Greenbaum, 1960) and nucleic acids (Greenbaum & Slater, 1957) with the advance of lactation. The present results extend

these observations and show that there is also an increased rate of biosynthesis of both these groups of compounds in response to the increased metabolic activity of the lactating mammary gland.

Incorporation into the acid-soluble ribonucleotides

Tables 1 and 2 show an increase in the rate of incorporation of both glycine and orotic acid into the acid-soluble ribonucleotides in the transition from pregnancy to lactation and, probably, a further but slower increase during lactation. There are two exceptions to this general pattern. First, although GTP synthesis increases from pregnancy to lactation, it remains constant as lactation advances (Table 1). The constancy of the GTP value in lactation contrasts with the rises found for GDP and GMP as well as for the phosphates of adenine, and no explanation of this anomaly can be advanced at present. Secondly, CMP remains almost unlabelled over the whole period. The small extent of labelling in this compound, and presumably in CDP and CTP, is in keeping with the results of Hurlbert & Potter (1954) and Hurlbert *et al.* (1954), who found only small amounts of CMP, with low rates of orotic acid incorporation, in rat liver. The low levels in the mammary gland are difficult to reconcile with the requirement of CTP for phospholipid synthesis, which is quite active in the mammary gland (Garton & Popják, 1952; Garton, 1955), and for protein synthesis, the high rate of which is one of the characteristics of the lactating gland. The CMP of RNA is labelled to a much greater extent than the acid-soluble CMP (Table 4). The results could be explained either in terms of the rapid depletion of the acid-soluble CMP pool for RNA or soluble-RNA synthesis or by assuming separate CMP pools. This point is discussed more fully below.

The time-courses of incorporation of glycine and orotic acid into acid-soluble ribonucleotides over the periods studied show differences (Figs. 1 and 2) in that the former exhibits a maximum, but the second does not. The linear labelling of the UDP complexes (Fig. 2) contrasts sharply with both of these patterns and appears to preclude the possibility that mammary-gland UTP acts as a precursor of the uridine-sugar complexes. However, Reid (1959) has also reported anomalous labelling of the UDP complexes in rat liver when orotic acid was the precursor.

The existence of a maximum in the glycine-incorporation curve permits the calculation of the half-life of the acid-soluble adenine. On the same assumptions as those of Haydar, Chernigoy, Khatchadourian & Kerr (1957) for rat liver, a half-life of approx. 2 hr. can be calculated for adenine, a figure almost identical with the half-life of adenine in the rat liver (Haydar *et al.* 1957).

Incorporation into the ribonucleic acids

Since the metabolic behaviour of the gland changes both in intensity and direction at parturition, changes might be expected in the composition of RNA synthesized at the onset of lactation. In the event, although a greater uptake of the labelled substrates was observed at parturition, no change was found in the relative incorporation of these substances into the four bases of mammary-gland RNA, a result in accord with previous work on the base composition of whole-cell RNA over the lactation cycle (Wang, Greenbaum & Slater, 1960). Further work on the fractionation of glandular RNA is required before it can be decided whether there is a synthesis of an RNA characteristic of lactation.

The higher rate of incorporation of [6-¹⁴C]orotic acid into the RNA cytidine than either the RNA uridine or the acid-soluble CMP requires comment. The labelling is anomalous if uridine compounds act as cytidine precursors and also it would seem that the mammary-gland acid-soluble CMP could not act as precursor for RNA cytidine. A possible explanation is that the RNA cytidine of the mammary gland had been synthesized in another tissue and transported to the gland before incorporation, the transported cytidine derivative having a higher specific activity than that of the gland. Such a possibility, already proposed by Hurlbert & Potter (1952) to explain the higher rate of labelling in the CMP than the UMP of the Flexner-Jobling tumour, may be imposed on active tissues like mammary gland by the high demand of RNA synthesis coupled to a low concentration and turnover of CMP in the gland.

The most striking feature of this study is the marked increase, at and around parturition, in the total counts incorporated and in the specific activity of the nucleic acid nucleotides when the precursor had been injected 24 hr. previously (Tables 3 and 4). There is now evidence, both biochemical (e.g. Greenbaum & Slater, 1957) and cytological (I. Lewin & S. R. Lewin, personal communication), for a wave of mitosis in the mammary gland some 12–36 hr. after parturition with a sustained, but lower, rate of cell division persisting throughout the period of lactation (Kirkham & Turner, 1953; Greenbaum & Slater, 1957; McLean, 1958). There is also evidence that the appearance of new cells is accompanied by increases in cellular RNA (Kirkham & Turner, 1953; Greenbaum & Slater, 1957; Slater & Planterose, 1958). The present results show that there is a considerable burst of RNA synthesis at a shorter time after parturition than has hitherto been found and, further, that it is probably maximal at some period less than 24 hr. Indeed, 48 hr. after parturition the rate of syn-

thesis has already declined by about 25 % and by mid-lactation the rate is only about 50 % of that found earlier. Although it may be surprising that the turnover of mammary RNA decreases at a time when milk production is increasing, the result is in keeping with that of Bucovaz & Davis (1961), who found that the activation of amino acids in rat mammary gland is faster in the first half of lactation than in the latter half.

The abrupt increases reported here are of interest physiologically, in that when the rate of RNA synthesis increases seven- to eight-fold, the demands of the suckling litter must be negligible and hence the changes noted can hardly be adaptive in response to the needs of suckling. A similar burst of RNA synthesis has been reported to occur in regenerating rat liver (Åqvist & Anderson, 1956) at a short time after partial hepatectomy, and this also precedes a wave of mitosis and the main regeneration period. There is thus a reasonable parallelism between these two tissues in the period before they each begin the phase of active protein synthesis. The changes that occur in the mammary gland in the first 24 hr. *post partum*, i.e. the increase in cell numbers and the changes in concentration and turnover of the nucleic acids and enzyme content, represent not an adaptive change to an immediate metabolic stimulus but a preparatory adaptation for the stress of lactation and the pattern of change may be laid down by the changes in hormonal balance coincident with parturition.

SUMMARY

1. The rate of incorporation of [2-¹⁴C]glycine and [6-¹⁴C]orotic acid into the acid-soluble ribonucleotides and ribonucleic acid of the mammary gland of the rat have been measured at intervals from late pregnancy to late lactation.

2. There is a marked increase in the rate of incorporation of both labelled compounds into the acid-soluble ribonucleotides over the period of parturition and a slower increase in the rate of incorporation thereafter. A possible exception to this trend is cytidine monophosphate, the labelling of which was too low to allow any trend to be detected.

3. There is a considerable increase in the rate of incorporation of the labelled substrates into mammary-gland ribonucleic acid at parturition and the first 24 hr. thereafter. The incorporation rate decreases as lactation advances so that at the time when the gland is lactating maximally it is less than that found in the immediate pre-partum period. The relationship between the maximum

incorporation and the known changes in cytological and biochemical behaviour during this same period is discussed.

4. These observed variations in the synthesis of ribonucleic acid over the lactation cycle apply to all four nucleotide bases of ribonucleic acid since there is a constancy in the proportion of labelling entering each nucleotide.

5. An anomalous labelling of the mammary-gland ribonucleic acid cytidylic acid after administration of [6-¹⁴C]orotic acid is reported. The ribonucleic acid cytidylic acid is labelled to a greater extent than either the ribonucleic acid uridylic acid or the acid-soluble cytidylic acid. A possible explanation for this is suggested.

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