for purines to be bunched in calf-thymus DNA and essentially no tendency in the other five types of DNA which were examined. Since these five sources include *M. lysodeikticus*, their results are evidence for the validity of the correction of 15% which has been applied to the results for this DNA in Figs. 1 and 2.

SUMMARY

1. Preparations of deoxyribonucleic acid (DNA) from eight sources (four bacterial species and four animal species) have been degraded with diphenylamine in formic acid. The amounts of inorganic phosphate and of several pyrimidine nucleotides have been measured.

2. The results have been compared with those expected for randomly arranged polynucleotides of the same base composition. Each DNA differs from the others and from the randomly arranged polynucleotide but there is no obviously consistent pattern. There appears to be a tendency for T_2p and CT_2p_2 to be obtained in amounts which are less than the random value.

3. There is no general tendency for purines or pyrimidines to be bunched together in a non-random fashion.

I wish to thank Professor Sir Hans Krebs, F.R.S., for his encouragement and interest, Miss Doreen B. Poole for her valuable technical assistance, Lord Rothschild, F.R.S., for a gift of sea-urchin sperm and Dr I. E. Bush for obtaining prostate glands.

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Levels and Distribution of the Acid-Soluble Ribonucleotides in the Mammary Glands of Pregnant and Lactating Rats

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The striking increases in the rate of metabolic and synthetic activity which are known to occur in the mammary gland in the transition from the relative quiescence of late pregnancy to the fully active state obtaining in late lactation could be expected to be reflected in similar changes occurring in either the level or turnover (or both) of the acid-soluble ribonucleotides.

Some studies on the distribution of the acid-soluble ribonucleotides in the mammary gland have already been reported. For instance, Forrest, Wilken & Hansen (1960) have examined the nucleotide levels in rat mammary tissue at late lactation and Manson (1956) has made similar studies in the cow and goat. Both of these studies found a pattern of distribution essentially similar to that found in rat liver by Hurlbert, Schmitz, Brumm & Potter (1954). Unfortunately, these investigations covered only one stage of lactation and did not attempt to correlate the level of ribonucleotides with lactational activity. Further Denamur, Fauconneau & Guntz (1958) have

studied the distribution of the nucleotides in ewe colostrum and milk at different stages of the lactation cycle and reported large-scale changes. There are no data on the changes in the glandular tissue itself although the metabolic changes mentioned above and the large increases in both ribonucleic acid and deoxyribonucleic acid (Kirkham & Turner, 1953; Greenbaum & Slater, 1957*b*; but see also McLean, 1958*a*) of rat mammary gland would suggest that increases in the nucleotides might be expected.

The present study on the changes in the level of acid-soluble ribonucleotides of rat mammary gland in response to the increase in lactational activity shows that the mammary gland contains most of the nucleotides present in rat liver in approximately the same ratios and further that there is a general increase in most of the ribonucleotides to a value approximately three times that found in late pregnancy.

METHODS

Animals. Adult female albino rats undergoing their first pregnancy or lactation were used in these investigations. The number of pups was restricted to eight and all rats with litters of less than six were rejected. The length of pregnancy was ascertained from the mating date and in this colony averages 22 days.

Chromatography of ribonucleotides. Each experiment was performed on the combined abdominal mammary glands of one rat (weight approx. 4–5 g.) or, where larger quantities of material were required for the rechromatography of the peaks, the pooled abdominal glands of three animals. The rats were killed by dislocation of the cervical vertebrae and the lower abdominal glands were quickly removed and placed into liquid nitrogen. The frozen tissue was then weighed and ground to a powder in the frozen state and extracted with ice-cold perchloric acid as described by Hurlbert *et al.* (1954). The extracts were neutralized with 5N-KOH and the potassium perchlorate was removed by centrifuging. Analysis of the neutralized extracts by anion-exchange chromatography on Dowex-1 (formate form) columns was then carried out by the gradient-elution procedure of Hurlbert *et al.* (1954) with three changes of solvent. The mixing vessel (initially containing water) was of 500 ml. volume and the resin column was 10 cm. $\times$ 0·7 cm.^{2} in size. When pooled tissue samples were used the column length was increased to 15 cm. for better resolution. The effluent from the columns was collected in 5 ml. fractions and the extinction was measured at 260 mμ. The development of the chromatogram generally took 3 days and was carried out without interruption of the flow, as it was found that any interruption of the elution procedure produced spurious peaks and distortion of the chromatogram. When rechromatography of the peaks was required the appropriate fractions were freeze-dried to remove the water and formic acid. When the fractions contained ammonium formate this was removed by exposing the freeze-dried fractions to a higher vacuum, attained by the use of an oil-diffusion pump, and the flasks were cautiously warmed with infrared lamps as described by Hurlbert *et al.* (1954). Rechromatography was carried out by gradient elution with ammonium formate (Hurlbert *et al.* 1954). The mixing flask was of 250 ml. volume and the resin column was 10 cm. $\times$ 0·7 cm.^{2} With careful drying 80% of the u.v.-light-absorbing material could be recovered after rechromatography.

Identification of the ribonucleotides in the various peaks was based on the data of Hurlbert *et al.* (1954) and confirmed by the ratios of the extinction coefficients at 250, 260 and 280 mμ.

Chromatography of rat milk. Since the mammary glands as dissected from the animal contained a considerable volume of milk, the presence of high concentrations of ribonucleotides in this fluid could introduce an appreciable error into the estimation of the level of the nucleotides in the mammary tissue. A sample of milk was collected by milking out the glands of rats injected with 5 i.u. of Pitressin (Parke Davis and Co. Ltd.).

The acid-soluble ribonucleotides were determined in this milk and were found to be mainly uridine compounds. The total amount of nucleotide (based on a millimolar extinction coefficient at 260 mμ of 10) is of the order of 40 μmoles/100 ml. of milk. Assuming that 40% of the wet weight of the gland is due to the presence of retained milk in the tissue, an error of not more than 5% can be attributed to contamination of milk in the whole gland in the determination of total acid-soluble ribonucleotides.

RESULTS

Characterization of the individual fractions. Typical chromatograms for the nucleotides of the mammary glands from pregnant and lactating rats are shown in Figs. 1 and 2.

A series of fractions were isolated from each chromatogram as shown in these figures, and the compositions of these fractions were identified as follows.

I. This fraction consists of cytidine monophosphate and diphosphopyridine nucleotide (DPN). The cytidine monophosphate component was only about 10% in pregnancy, rising to 20% in lactation.

II. This fraction is composed solely of adenosine monophosphate (AMP). No other constituent was detectable.

III. This peak appears to consist almost entirely of guanosine monophosphate. No measureable triphosphopyridine nucleotide could be detected. This latter finding is in agreement with the results of McLean (1958*b*), who also failed to find any oxidized triphosphopyridine nucleotide in the mammary glands of rats.

IV. The fraction eluted at this position by Reid & Stevens (1957) with a liver extract was shown by them to contain cytidine diphosphate, inosine monophosphate and uridine monophosphate. Hurlbert *et al.* (1954) obtained these compounds as two peaks, one containing cytidine diphosphate and inosine monophosphate and the other uridine monophosphate. In the present study only one peak was observed and this seemed to be composed

entirely of uridine monophosphate both in pregnancy and lactation.

V. This fraction was pure adenosine diphosphate (ADP).

VI. This fraction was pure uridine diphosphate-*N*-acetylhexosamine, the $UDPX_1$ of Hurlbert *et al.* (1954). This substance was identified by the *p*-dimethylaminobenzaldehyde test of Aminoff, Morgan & Watkins (1952). This material has been tentatively identified as uridine diphosphate *N*-acetylglucosamine because it appeared in the same position in the chromatogram as an authentic sample of this compound. The tests, however, do not exclude the possibility of a greater or lesser contamination with the corresponding galactosamine compound.

VII. This fraction appears on the chromatogram and rechromatogram in the same position as uridine diphosphate glucose. On hydrolysis this peak was shown to contain a reducing sugar, as detected by the method of Park & Johnson (1949).

VIII. This fraction contained a single component identified as guanosine diphosphate.

IX. This fraction also contained only one component which corresponds to the compound ADX, described by Hurlbert *et al.* (1954).

X. The composition of this fraction was variable, dependent on the stage of lactation. In late pregnancy this fraction contained 75% of adenosine triphosphate (ATP), 25% of uridine diphosphate and no detectable uridine diphosphate hexuronic acid. In late lactation the composition approxi-

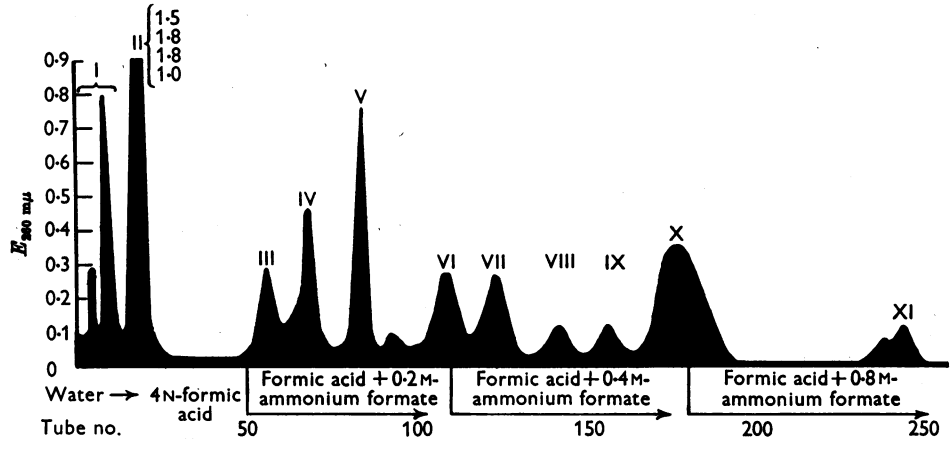

Fig. 1. Chromatogram of the acid-soluble ribonucleotides from the mammary tissue of one animal in late lactation. The method used Dowex-1 (formate form) ion-exchange resin and gradient elution with formic acid and ammonium formate as described in the Methods section. The number of 5 ml. fractions collected and the point of solvent change are indicated along the abscissae.

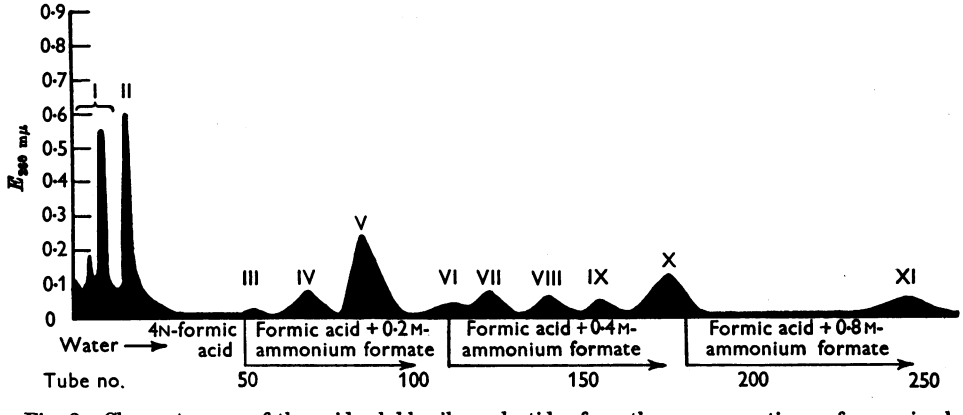

Fig. 2. Chromatogram of the acid-soluble ribonucleotides from the mammary tissue of one animal in late pregnancy. Other details are as given for Fig. 1.

mated to 65 % of ATP, 25 % of uridine diphosphate and 10 % of uridine diphosphate hexuronic acid. This last-named compound has been identified as uridine diphosphate hexuronic acid by virtue of its position on the rechromatogram and the positive test given for hexuronic acid when tested by the carbazole method of Dische (1947).

XI. On rechromatography this fraction could be resolved into three components in late lactation: uridine triphosphate (60 %), guanosine triphosphate (20 %) and a third component which appeared in the chromatogram in the same position as the unknown component cited by Hurlbert *et al.* (1954), and believed by them to be a pyrimidine nucleoside pyrophosphate. In pregnancy the amount of nucleotide was too low to permit a detection of this third component, if it were present, or even the amount of uridine triphosphate or guanosine triphosphate.

Variations in the concentration of the acid-soluble ribonucleotides during the lactation cycle. Because of the composite nature of some of the fractions in the chromatogram no attempt has been made to express the absolute amounts in Figs. 1 and 2 and the ordinate is the extinction at 260 mμ. For the results given in Table 1, which shows the tissue content of nucleotide of the mammary gland at times from late pregnancy to the end of lactation, molar units/100 g. of wet tissue are used. These figures were obtained from the known molar extinction coefficients and the determined composition of the complex fractions. There is a general increase in the concentration of the acid-soluble ribonucleotides during the passage from late pregnancy to late lactation (see also Table 1).

Variations in the concentration of the total ribonucleotide content are shown in Fig. 3 (*a*). It will be seen that there is a sharp rise in concentration immediately after parturition followed by a slow increase up to about the tenth day of lactation, where the level reaches a plateau value at about three times that obtaining in pregnancy.

Adenosine phosphates. Figs. 3 (*b*) and 3 (*c*) illustrate the changes in concentration of the adenosine phosphates AMP and ADP. (Changes in the ATP concentration are not given in this figure as

Table 1. *Distribution and variation of acid-soluble nucleotides at different stages of lactation*

Amounts are shown in μmoles/100 g. of tissue. Abbreviations used: DPN, diphosphopyridine nucleotide; CMP, cytidine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UMP, uridine monophosphate; ADP, adenosine diphosphate; $UDPX_1$ and $UDPX_2$, *N*-acetylhexosamine and hexose derivatives of uridine diphosphate respectively; GDP, guanosine diphosphate; ADX, compound described by Hurlbert *et al.* (1954); ATP, adenosine triphosphate; GTP, guanosine triphosphate; UTP, uridine triphosphate.

	Molar absorption $\times 10^{-3}$ conversion factor											
Lactational state	18 DPN CMP	14·5 AMP	11·6 GMP	9·9 UMP	14·5 ADP	9·9 $UDPX_1$	9·9 $UDPX_2$	11·8 GDP	14·5 ADX	13* ATP etc.	10·9* GTP UTP	Total
Day of pregnancy												
17	5·6	11·0	Trace	10·1	13·8	Trace	Trace	Trace	Trace	10	Trace	50·5
17	7·8	13·1	Trace	18·2	13·1	4·0	6·1	5·1	4·1	23·1	4·6	94·6
17	7·8	13·1	—	—	—	—	—	—	—	—	—	—
18	5·6	11·7	Trace	10·1	15·9	Trace	Trace	Trace	Trace	13·9	3·7	40·9
18	6·2	12·1	Trace	10·5	14·3	Trace	Trace	Trace	Trace	15·4	6·3	68·1
19	9·5	12·4	Trace	11·1	19·3	Trace	Trace	7·6	6·2	33·1	11·0	110·2
Day of lactation												
0	9·5	14·5	Trace	20·2	21·4	Trace	Trace	Trace	Trace	21·6	8·3	95·5
0	7·7	13·1	5·2	10·1	9·0	Trace	Trace	Trace	Trace	13·9	7·4	65·9
1	11·2	23·4	12·9	28·2	37·9	14·1	12·1	2·5	2·8	27·7	10·5	183·3
5	27·8	29·7	7·8	21·2	34·5	—	—	—	—	48·5	17·4	—
7	10·6	13·1	6·9	14·1	20·7	12·1	13·1	4·2	3·4	33·1	11·9	143
8	13·3	60·0	13·8	49·5	39·3	21·2	27·3	7·6	6·9	28·5	10·1	278
11	8·9	35·2	14·7	34·3	34·5	—	—	—	—	—	—	—
11	8·3	40·0	17·2	31·3	31·1	23·2	33·3	10·1	13·1	32·3	12·8	253
14	12·8	57·3	20·7	39·4	36·6	—	—	—	—	—	—	—
14	13·9	49·7	24·1	48·5	35·9	19·2	39·4	13·5	11·1	30·7	30·2	316
17	10·0	23·5	25·8	41·4	38·6	21·2	33·3	9·3	12·4	35·4	43·1	293
18	13·9	52·4	11·2	30·3	24·2	23·2	20·2	7·6	5·5	19·2	6·4	214
18	15·6	69·0	19·8	54·5	59·3	40·4	45·5	14·4	11·1	41·5	13·8	385
19	13·9	46·9	15·5	42·4	42·8	28·3	34·3	11·9	9·0	23·1	9·2	277
19	10·6	49·0	19·8	38·4	42·8	26·3	27·3	6·8	6·2	23·8	9·2	260
20	7·8	46·9	14·7	31·3	35·2	26·3	37·4	10·2	8·3	40·8	12·8	272

* Calculated from the known molar extinction coefficients and the observed composition of the fraction.

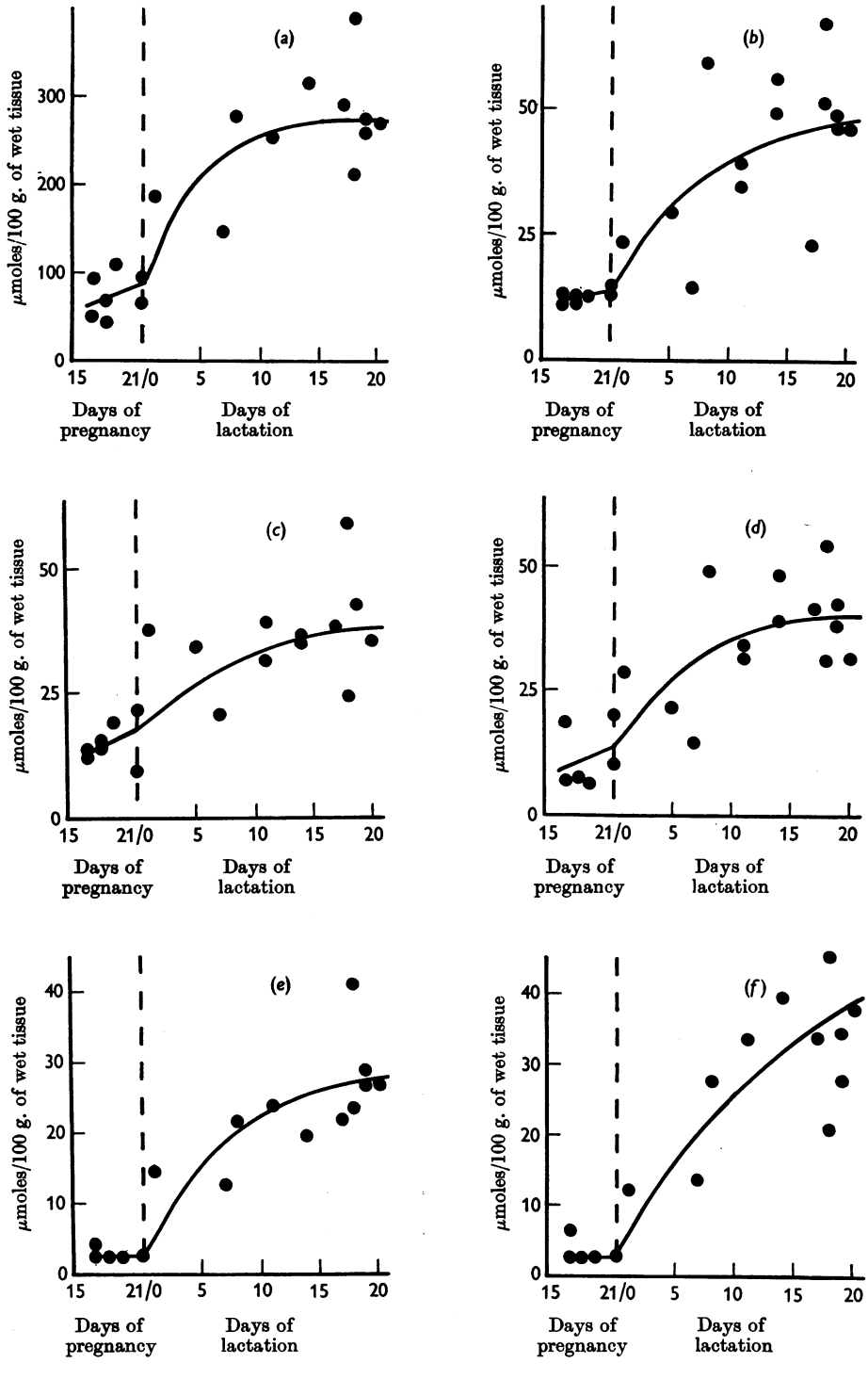

Fig. 3. Time curve of: (*a*) total acid-soluble nucleotides; (*b*) AMP; (*c*) ADP; (*d*) uridine monophosphate; (*e*) uridine diphosphate *N*-acetylhexosamine; (*f*) uridine diphosphate hexose.

these cannot be derived directly from the chromatograms, in which ATP appears as a composite peak.) Both of these nucleotides increase at parturition and at least the former continues to rise throughout lactation. The AMP curve shows a very sharp transition at parturition. It is worth noting that as lactation advances AMP increases in proportion to the other two adenine nucleotides (Table 1). The increase in ATP appears to be much less striking than that of AMP or ADP.

Uridine phosphates. Since the fractions containing uridine diphosphate, uridine triphosphate and uridine diphosphate hexuronic acid are composite peaks which are not easily resolved into the individual components the changes in these individual compounds with advancing lactation are not readily followed. Uridine diphosphate *N*-acetylhexosamine and uridine diphosphate hexose are clear-cut fractions and, as stated above, uridine monophosphate probably comprises the total nucleotides of the fraction in which it occurs. Thus figures for these three compounds are available and are shown in Figs. 3 (*d*), 3 (*e*) and 3 (*f*). There is considerable scatter in these figures of uridine diphosphate hexose (fraction VII) and uridine diphosphate *N*-acetylhexosamine (fraction VI), but both nevertheless show a pattern of a constant low value in pregnancy followed by a sharp rise at parturition and a slower rise during the course of lactation. Uridine monophosphate also has a low value in pregnancy and a sharp rise at parturition but there is only a small rise to a constant value which is attained about the tenth day of lactation.

Guanosine and cytidine phosphates. Both of these groups of phosphates increase over the lactation cycle (Table 1). Indeed, the guanosine and cytidine phosphates are present only in trace quantities during pregnancy. Although there is an increase in the concentrations of both these groups of compounds in late lactation the level attained is considerably lower than that of either the adenosine or uridine compounds.

DISCUSSION

The studies of Forrest *et al.* (1960) on rat, and Manson (1956) on cow and goat mammary-gland acid-soluble ribonucleotides relate to a single stage of lactation. The present study reports on changes in the level of these nucleotides at periods throughout the lactation cycle, during which there are considerable changes in the rate of protein and carbohydrate synthesis. It has been estimated (Brody & Nisbet, 1938) that the rate of protein synthesis increases tenfold over the period of lactation and it is probable that lactose synthesis increases by a similar amount. The observed threefold increase of the acid-soluble ribonucleotides may be correlated with two aspects of mammary-gland physiology: first the sevenfold rise in ribonucleic acid content of the gland (Slater & Planterose, 1958) and, secondly, the increase in the rate of synthetic activity. The ribonucleic acid content of the gland increases only up to the twelfth to fourteenth days of lactation and thereafter remains virtually constant (Greenbaum & Slater, 1957*b*, Shimizu, 1957), whereas the progress of synthetic activity, i.e. the rate of synthesis of milk, which may be deduced from the composition figures of rat milk (Cox & Mueller, 1937; Greenbaum & Slater, 1957*a*) and from the shape of the milk-yield curve given by Brody & Nisbet (1938), appears to increase linearly over the whole period of lactation. The curve showing the increase in total acid-soluble ribonucleotides (Fig. 3*a*) increases only up to about the tenth day of lactation and therefore shows a similar pattern of change to that found for the ribonucleic acid content of the gland.

Two rather surprising points emerge from the pattern of increase of the ribonucleotides. First there appears to be no correlation between the levels of nucleotide found and metabolic activity of the gland (as exemplified by the milk-yield curve or Q_{O_2} values of Folley & French, 1950). Secondly, it might have been expected that there would have been a preferential increase in adenosine phosphates, which would be associated with the increasing energy requirements of the gland and with the formation of the amino acid adenylates in protein synthesis, and of uridine compounds, which would be involved in the increased rate of carbohydrate transfer and in lactose synthesis. As can be seen from Table 1, there is no preferential increase in either of these two nucleotides. On the contrary all appear to increase together and to the same degree. It is probable that the activity of the nucleotides in terms of their coenzymic function would be better expressed as turnover rates and work is now in progress to measure these.

SUMMARY

1. The acid-soluble ribonucleotides of rat mammary gland have been measured at different stages of the lactation cycle. There is a general increase of these nucleotides so that the level of the total acid-soluble ribonucleotide is raised about threefold between late pregnancy and late lactation.

2. All the ribonucleotides appear to participate in this increase and all increase roughly to the same extent. Most of the nucleotides measured showed an abrupt increase at parturition and thereafter increased until about the fifteenth day of lactation, at which time they reached a plateau value.

3. The pattern of increase appears to be more closely related to the rate of increase of ribonucleic

acid in the gland than to the metabolic and synthetic activities of the tissue.

We wish to express our thanks to the Medical Research Council for the grant of a studentship to one of us (D.Y.W.) during the tenure of which this work was done.

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Synthesis of Protein in a Purine-Requiring *Escherichia coli* Infected with Bacteriophage T2

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Strain B-96 is a mutant of *Escherichia coli* which requires adenine, guanine, hypoxanthine or xanthine for growth (Gots, 1950, 1953). Infection with T2r bacteriophage does not appear to alter the defect, since in the absence of adenine the deoxyribonucleic acid content of the culture remains constant and few bacteriophages are formed (Burton, 1955). This paper presents evidence that *E. coli* B-96 infected with T2r bacteriophage can, in the absence of exogenous adenine, synthesize bacteriophage-specific proteins and the enzymes required for the synthesis of bacteriophage deoxyribonucleic acid. The addition of adenine is, however, required for net synthesis of deoxyribonucleic acid.

METHODS AND MATERIALS

Bacteria. E. coli B-96 was obtained from Dr J. S. Gots. The bacteria were grown at 37° with aeration in the following growth medium (amounts/l.): 3·4 g. of Na_2HPO_4, 1·5 g. of KH_2PO_4, 1·0 g. of NH_4Cl, 6·0 g. of NaCl, 10 mg. of $MgSO_4$, 0·16 mg. of $FeCl_3$, 10 g. of mannitol, 30 mg. of adenine and 15 mg. of L-histidine hydrochloride. These concentrations of adenine and histidine are in the optimum range for growth.

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Bacteriophage. The T2r strain was prepared and assayed as described by Adams (1950) and Burton (1955).

iso*Citric dehydrogenase.* A partially purified preparation was kindly given by Dr H. L. Kornberg.

Nucleotides. Deoxyribonucleotides were obtained from the California Corporation for Biochemical Research (Los Angeles 63, Calif., U.S.A.) and other nucleotides from Sigma Chemical Co. (St Louis 18, Mo., U.S.A.).

Conditions of bacterial growth and bacteriophage infection. E. coli B-96 was grown on agar slants overnight at 37°. The growth medium, described above, was inoculated from this culture, aerated overnight at 37° and then added to 7 vol. of fresh growth medium. Aeration was continued until the concentration of cells was about 5×10^8/ml. (2–2½ hr.). The bacteria were centrifuged, washed at 4° with the original volume of buffered saline (Burton, 1955) and resuspended in growth medium at 37° in the absence of adenine. The bacteria were infected with an average of five infective T2r particles per cell and aeration was continued. Thereafter adenine was added as indicated.

The medium used to suspend the bacteria in the experiment of Table 3 had a low concentration of phosphate and contained (per l.) 10 g. of mannitol, 3 g. of NaCl, 27 mg. of KH_2PO_4, 10 mg. of $MgSO_4$, 0·16 mg. of $FeCl_3$ and 15 mg. of histidine hydrochloride and was buffered with 0·05 M-triethanolamine (adjusted to pH 7·4 with HCl) and 0·01 M-succinic acid (adjusted to pH 7·4 with NH_3).

Some difficulty was caused by unexplained variations in the growth rate of the bacteria. For the experiments which are described in detail, the cultures reached the required