Greenbaum, A. L. & Greenwood, F. C. (1954). *Biochem. J.* **56**, 625.
Greenbaum, A. L. & Slater, T. F. (1957*a*). *Biochem. J.* **66**, 155.
Greenbaum, A. L. & Slater, T. F. (1957*b*). *Biochem. J.* **66**, 161.
Hoffman, J. G. (1953). In *The Size and Growth of Tissue Cells*. Springfield, U.S.A.: Charles C. Thomas.
Jeffers, K. R. (1935). *Amer. J. Anat.* **56**, 257.
Kirkham, W. R. & Turner, C. W. (1953). *Proc. Soc. exp. Biol., N.Y.*, **83**, 123.
Kleiber, M., Smith, A. H. & Levy, P. (1943). *Proc. Soc. exp. Biol., N.Y.*, **53**, 94.
Moore, R. O. & Nelson, W. L. (1952). *Arch. Biochem. Biophys.* **36**, 178.
Popják, G. & Tietz, A. (1954). *Biochem. J.* **56**, 46.
Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
Smith, T. C. (1956). *Arch. Biochem. Biophys.* **60**, 485.
Sternberg, M. J. (1950). *C.R. Soc. Biol., Paris*, **230**, 2341.
Tuba, J., Orr, P. F. & Wiberg, G. S. (1955). *Canad. J. Biochem. Physiol.* **33**, 904.
Vendrely, R. (1955). In *The Nucleic Acids*. Ed. by Chargaff, E. and Davidson, J. N. New York: Academic Press Inc.
Weatherford, H. L. (1929). *Amer. J. Anat.* **44**, 199.
Woodman, H. E. & Hammond, J. (1923). *J. agric. Sci.* **13**, 180.

Studies on the Particulate Components of Rat Mammary Gland

2. CHANGES IN THE LEVELS OF THE NUCLEIC ACIDS OF THE MAMMARY GLANDS OF RATS DURING PREGNANCY, LACTATION AND MAMMARY INVOLUTION

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The deoxyribonucleic acid (DNA) content of any particular type of cell appears to be constant (see Vendrely, 1955). This constancy of content has been repeatedly demonstrated and deviates only in cells displaying polyploidy, or in multinucleate varieties. Furthermore, isotope-turnover studies indicate that DNA, except in mitotically active cells, is metabolized to a very limited degree (Hevesy & Ottesen, 1943). DNA has, therefore, come to be used as a reference standard directly related to the number of cells present (see Davidson, 1953; Davidson & Leslie, 1950). The value of such a reference standard in studies of the mammary gland, where total nitrogen is not necessarily related to the amount of tissue present, is obvious.

Ribonucleic acid (RNA) is considered to be of importance in the synthesis of protein (see Brachet, 1955). Although the intracellular site of protein synthesis is not fully established, many workers believe it to be in the small-particle fractions of the cytoplasm, the microsomes and submicrosomes (see Brachet, 1955). RNA, however, is widely distributed in the cell and the rapid turnover rate of nuclear RNA (Smellie, McIndoe, Logan, Davidson & Dawson, 1953) indicates that the RNA of the nucleus is probably of considerable importance in protein synthesis.

The mammary gland at parturition undergoes a rapid and intense change in the rate of protein synthesis, lactation being well established within a few hours *post partum*. The amount of casein synthesized/day by an active rat mammary gland is comparable in amount with the parent tissue present. The mammary gland has, therefore, advantages to offer over other tissues, where the rate of protein synthesis is constant, in studies relating RNA to protein synthesis.

METHODS

Animals. Rats were killed by cervical dislocation and the entire lower abdominal mammary glands quickly removed and placed in ice-cold 0·25 M-sucrose.

Seven groups of female albino rats were used: (*a*) adult virgin rats aged 4–6 months; (*b*) adult rats, about 200 g. body wt. and 20 days pregnant; (*c*) adult rats 3, 10 and 18 days *post partum*; (*d*) adult rats in the second and fourth days of mammary involution. Litters, which in all cases were reduced to 6–8 pups, were weaned 22 days *post partum*.

Homogenizing. After homogenizing by the procedure outlined in the previous paper (Greenbaum & Slater, 1957*a*) the homogenate was diluted with 0·25 M-sucrose to give a 1:10 tissue suspension. Portions of this suspension were used for total-nitrogen determination, nucleic acid determinations and lactose estimation to determine the milk content of the suspensions.

Centrifuging. The mammary gland suspension (30 ml.) was centrifuged to obtain a large-particle (mitochondrial) fraction. The procedure was as follows: after two initial centrifugings at 600 *g* for 10 min. in a refrigerated centrifuge to remove cellular debris and nuclei, the suspension was centrifuged at 8000 rev./min. for 15 min. at plateau speed in a Servall refrigerated centrifuge (Model SS 2). The centrifuge constants for this stage were (Duve & Berthet, 1953): $r_{(min.)} = 11$ cm.; $r_{(max.)} = 15$ cm.; $g_{(plateau)} = 10\,900$; temp., 0°. These values give a time integral of about 230 000 *g* min.

Separation of sediment and supernatant suspension was achieved by decantation. The fatty layer, which was solid at the temperature used in centrifuging, was removed in

the process of decanting. The mitochondrial fraction was resuspended in the original volume of ice-cold 0·25 M-sucrose.

Chemical methods

Total nitrogen. This was estimated by a micro-Kjeldahl method.

Lactose. This was determined in the suspensions of mammary gland by the method of Folley & Greenbaum (1947). The percentage of retained fluid in the mammary gland was calculated by the method of Greenbaum & Slater (1957*a*).

Extraction of nucleic acids. Nucleic acids were separated from the tissue fractions by the procedure of Schneider (1945) in preference to that of Schmidt & Thannhauser (1945). An objection to the Schmidt & Thannhauser procedure is that phosphorus-containing compounds other than nucleic acids come through the extraction procedure and give high ribonucleic acid phosphorus (RNA-P) values compared with those from pentose determinations on the same samples (see Davidson, 1953). Further, as both DNA and RNA are estimated by phosphorus content in the Schmidt & Thannhauser method, the hydrolysis stage is critical and the accuracy of the final estimations will be a reflexion of the resolution of this step.

Schneider (1945) has observed that in many tissues the phospholipid extraction may be omitted without affecting the nucleic acid estimation, and we have confirmed that this applies to the mammary gland. As a routine practice we have therefore left out the phospholipid extraction. The standard procedure adopted was to precipitate the nucleoproteins of 2 ml. of a 1:10 tissue suspension or of 2 ml. of a mitochondrial fraction with 2 ml. of 10% (w/v) trichloroacetic acid (TCA). After standing in ice for several hours the precipitate was centrifuged off, washed twice with 5% TCA, and then heated for 15 min. at 90° with 5 ml. of 5% TCA. The residue was centrifuged off and reheated with a further 5 ml. of 5% TCA for the same time. The combined supernatants were filtered and used for the estimation of nucleic acids by determination of the pentose or deoxypentose content, which were assumed to be related to the RNA and DNA content respectively.

DNA was measured by the method of Dische (1955) at 600 mμ on a Unicam SP. 500 spectrophotometer. RNA was measured by the modification of the method of Mejbaum (1939) described by Albaum & Umbreit (1947), with 45 min. as the heating time for colour development.

The RNA value was corrected for the interference due to the DNA present, which also gives a colour under the conditions of the RNA estimation. Interference from the sucrose of the suspending medium and from the lactose of the milk retained in the gland was found to be negligible after two washes with cold 5% TCA before the nucleic acid extraction. Results for both types of nucleic acid were calculated in terms of nucleic acid phosphorus, i.e. deoxyribonucleic acid phosphorus (DNA-P) and RNA-P (see Leslie, 1955).

RESULTS

Nitrogen distribution

The results of the determination of nitrogen in the whole gland and in the mitochondrial fraction are shown in Table 1. The total nitrogen, when expressed as mg. of N/100 g. wet wt. of gland, increases throughout pregnancy and lactation and falls abruptly in mammary involution. The rise in pregnancy represents a doubling of the nitrogen content of the virgin gland, and a further doubling occurs over the period of parturition to the third day of lactation. During lactation the nitrogen increases further, so that by the eighteenth day of lactation it is 1·5 times as high as at the third day of lactation. The values at 10 days appear to be anomalous in that they show a decrease in magnitude compared to the values at 3 days. Observations of similar anomalous values for rats at mid-lactation have been reported by Kirkham & Turner (1953). The fall in nitrogen content at 10 days is not observed if the total nitrogen content of the entire abdominal glands is considered (Table 1, column 6) due to the increased weight of the glands (Table 1, column 3).

Values for mitochondrial nitrogen, expressed as mg. of N/100 g. wet wt. of gland, also show the same type of rise throughout pregnancy and lactation, although in this case the rise during pregnancy (eightfold) is relatively much greater (double) than that found in whole-tissue nitrogen. At parturition there is a sharp rise in mitochondrial nitrogen, the value on the third day of lactation being double that found at the end of pregnancy. A similar pattern of increase up to the end of the lactation period, followed by a fall in the involution period, is shown when values for the mitochondrial nitrogen content of the entire gland are calculated.

Variations in the distribution of RNA-P and DNA-P

The results showing the changes in the RNA-P and DNA-P content of the whole tissue and of mitochondrial RNA-P over the secretory cycle are shown in Table 2. The pattern of change in the content of RNA-P is very similar to that found for the changes in nitrogen content, i.e. a general increase throughout pregnancy and lactation, followed by a sharp drop during involution. It should be noted that there is a very considerable rise of both tissue and mitochondrial RNA-P at parturition. It will be seen from Table 2, column 13, that, except at the third day of lactation, the mitochondrial RNA-P is a relatively constant fraction of the total tissue RNA-P (about 15–20%), and is within the range normally quoted for the RNA-P content of mitochondrial fractions (Hogeboom, Schneider & Palade, 1948; Schneider & Hogeboom, 1951).

The results of the determination of the DNA-P content of rat mammary gland are also shown in Table 2 (columns 3–5). The main point of interest in these results is the rapid rise in DNA-P concentration at parturition from 40·4 to 81·4 mg. of DNA-P/100 g. of milk-free gland (Table 2, column 4).

Table 1. *Distribution of nitrogen in rat mammary gland*

Status	Wt. of gland: No. of animals	Wt. of gland: (g.)	Total nitrogen: No. of animals	Total nitrogen: mg./100 g. wet wt. of gland	Total nitrogen: mg./total gland	Mitochondrial nitrogen: No. of animals	Mitochondrial nitrogen: mg./100 g. wet wt. of gland	Mitochondrial nitrogen: mg./total gland	$\frac{\text{Mitochondrial nitrogen}^*}{\text{Total nitrogen}} \times 100$
Virgin	6†	2·6±0·3‡	6	266±20	6·8±1·6	6	8·2±1·2	0·19±0·02	3·3±0·7
Pregnant (20 days)	11	4·6±0·3	10	504±37	22·9±1·9	7	65·7±14·7	2·9±0·6	14·4±2·1
Lactating (3 days)	10	4·5±0·3	9	1083±85	48·1±3·7	6	139·2±20·8	6·8±1·3	16·2±1·9
Lactating (10 days)	6	6·1±0·4	6	921±76	57·7±8·9	6	213·0±21·6	13·4±2·4	23·1±1·2
Lactating (18 days)	10	5·6±0·3	8	1513±59	81·5±4·7	8	271·8±28·3	13·8±1·8	20·0±1·9
Involuting (2 days)	6	6·6±1·0	6	1207±117	78·4±18·6	3	183·0±1·0	12·0±1·3	19·3±0·8
Involuting (4 days)	4	3·2±0·4	4	661±68	22·2±4·2	4	157·0±18·0	5·3±1·2	23·4±0·9

* Units: $\frac{\text{mg./100 g. wet wt. of gland}}{\text{mg./100 g. wet wt. of gland}}$.

† Analyses on two pooled samples; three animals were used per sample.

‡ In all tables the values given are group means±standard error of the mean.

Table 2. *Distribution of nucleic acids in rat mammary gland*

Status	DNA-P in tissue suspension: No. of animals	DNA-P in tissue suspension: mg./100 g. wet wt. of gland	DNA-P in tissue suspension: mg./100 g. of gland (milk-free)	DNA-P in tissue suspension: mg./total gland	RNA-P in tissue suspension: No. of animals	RNA-P in tissue suspension: mg./100 g. wet wt. of gland	RNA-P in tissue suspension: mg./100 g. of gland (milk-free)	RNA-P in tissue suspension: mg./total gland
Virgin	6‡	4·8±0·1	—	0·12±0·04	6	2·7	—	0·06±0·01
Pregnant (20 days)	11	14·7±2·2	40·4±3·1	0·71±0·15	11	17·7±2·1	55·2±8·9	0·85±0·14
Lactating (3 days)	10	22·9±2·3	81·4±2·9	1·02±0·10	8	37·9±3·9	156·0±14·4	1·73±0·14
Lactating (10 days)	6	20·7±1·9	80·5±6·7	1·28±0·18	6	55·6±5·1	219·8±24·5	3·45±0·46
Lactating (18 days)	9	25·2±2·6	82·2±6·5	1·46±0·17	11	64·5±3·8	225·2±20·1	3·48±0·27
Involuting (2 days)	9	17·5±3·1	30·9±3·8	1·38±0·32	6	22·9±1·8	84·0±24·0	1·70±0·06
Involuting (4 days)	5	16·4±1·2	—	0·53±0·07	5	18·4±3·1	—	0·61±0·13

Status	RNA-P in mitochondrial suspension: No. of animals	RNA-P in mitochondrial suspension: mg./100 g. wet wt. of gland	RNA-P in mitochondrial suspension: mg./total gland	$\frac{\text{Mitochondrial RNA-P}^*}{\text{Homogenate RNA-P}} \times 100$	$\frac{\text{Homogenate RNA-P}^\dagger}{\text{DNA-P}}$
Virgin	3	0·54	0·01	15	0·70
Pregnant (20 days)	9	3·8±0·9	0·17±0·05	20·1±2·9	1·22±0·15
Lactating (3 days)	7	9·9±1·2	0·49±0·06	27·9±4·3	1·86±0·13
Lactating (10 days)	6	11·8±2·8	0·77±0·23	16·7±2·0	2·72±0·19
Lactating (18 days)	9	11·9±1·1	0·64±0·06	21·1±2·2	2·69±0·23
Involuting (2 days)	4	4·1±1·1	0·32±0·11	18·4±5·7	1·63±0·43
Involuting (4 days)	4	4·1±1·5	0·15±0·06	21·7±4·8	1·08±0·13

* Units: $\frac{\text{mg./100 g. wet wt. of gland}}{\text{mg./100 g. wet wt. of gland}}$.

† Units: $\frac{\text{mg./total gland}}{\text{mg./total gland}}$.

‡ Analyses on two pooled samples; three animals were used per sample.

Throughout lactation the DNA-P concentration is remarkably constant, although the total DNA-P/gland increases slowly.

The ratio RNA-P:DNA-P is assumed to be a measure of RNA-P/cell, and this has been evaluated at the various stages considered (Table 2, column 14). It can be seen that the RNA-P content/cell increases during pregnancy and the first half of lactation, after which it levels off, finally falling during mammary involution.

Table 3 shows the variations with the secretory period of several other ratios, derived from the results listed in Tables 1 and 2.

The tissue-nitrogen values given were calculated by the intercept method previously published (Greenbaum & Slater, 1957*a*). The percentage of the tissue nitrogen which is recoverable as mitochondrial nitrogen is shown in column 3. As the tissue-nitrogen figure for each stage is a calculated mean value, no standard errors of the mean of ratios involving tissue nitrogen are available. The highest value for this ratio is found in the late pregnant gland and is about 35%, a figure which compares with values of about 25% found by other investigators for the nitrogen content of rat-liver mitochondria (see Schneider & Hogeboom, 1951). Since at all stages of the lactation cycle investigated here the mammary gland is a mitotically quiescent tissue, the DNA-P level is proportional to the number of cells present (Greenbaum & Slater, 1957*a*). The results for the mitochondrial nitrogen have therefore been related to amount per cell by considering the ratio of mitochondrial nitrogen to DNA-P (Table 3, column 4); and similarly the RNA-P content is compared to the mitochondrial nitrogen in columns 5 and 6.

Table 3. *Interrelationships between nucleic acid content and nitrogen content of rat mammary gland*

Status	Tissue nitrogen* (mg./total gland)	Mitochondrial N / Tissue N × 100†	Mitochondrial N‡ / DNA-P	Total RNA-P† / Mitochondrial N	Mitochondrial RNA-P† / Mitochondrial N
Virgin	6·8	2·8	1·7	0·42	0·060
Pregnant (20 days)	8·4	34·5	6·1 ± 1·6	0·38 ± 0·13	0·033 ± 0·009
Lactating (3 days)	30·2	22·5	7·9 ± 1·2	0·28 ± 0·05	0·077 ± 0·021
Lactating (10 days)	37·2	36·0	10·7 ± 1·3	0·27 ± 0·03	0·052 ± 0·007
Lactating (18 days)	39·7	34·8	16·3 ± 1·4	0·22 ± 0·01	0·044 ± 0·006
Involuting (2 days)	76·7	15·6	12·3 ± 2·5	0·14 ± 0·004	0·017 ± 0·004
Involuting (4 days)	—	—	9·6 ± 0·5	0·10 ± 0·005	0·023 ± 0·006

* From Greenbaum & Slater (1957*a*).

† Units: $\frac{\text{mg./total gland}}{\text{mg./total gland}}$.

‡ Units: $\frac{\text{mg./100 g. of fresh gland}}{\text{mg./100 g. of fresh gland}}$.

DISCUSSION

Ribonucleic acid

Two phases of protein synthesis may be recognized in the mammary gland. The first occurs during pregnancy and is associated with the growth of the gland itself; the second occurs after parturition, when protein synthesis is required not only for the maintenance of the existing mammary tissue but also for the elaboration of the protein constituents which are secreted in the milk. In view of current ideas linking RNA with protein synthesis it is of interest to consider variations in the level of RNA over the lactation cycle. The only previous work of this nature is, to our knowledge, that of Kirkham & Turner (1953), who described a gradual increase in the RNA content of the gland from pregnancy throughout lactation. They did not find the sharp increase in RNA content at parturition indicated by our results. The probable reason for this discrepancy is that Kirkham & Turner did not group

their animals but rather used animals scattered in all stages of the mammary cycle.

The results reported here are consistent with the idea of an RNA increase associated with the build up of the glandular material in early pregnancy and with the synthesis of colostrum proteins late in pregnancy. Comparison of the increases in the levels of tissue nitrogen, RNA-P and DNA-P indicates that the growth of the gland is hyperplastic and is in agreement with the many cytological studies of the gland during pregnancy (Roberts, 1921; Weatherford, 1929). There is a gradual increase in RNA throughout lactation, when expressed as RNA-P/100 g. wet tissue, concurrent with an increase in total nitrogen, but the DNA-P/100 g. wet tissue remains constant during this time. Thus the two main phases of protein synthesis are reflected by increases in the RNA-P. Although it is not possible to say whether the RNA is directly or indirectly related to protein synthesis, these results are further evidence for the corresponding times of appearance of both protein and RNA. It should be noted here that the transition at parturition is not from a tissue deficient in RNA-P to one rich in nucleic acid, but rather a transition from a tissue already rich to one extremely rich in RNA-P. Thus the values obtained from rats late in pregnancy and from those late in lactation were 55 and 225 mg. of RNA-P/100 g. wet wt. of gland (milk-free), respectively, compared with values of 72–99 mg. of RNA-P/100 g. of normal rat liver (Fukuda & Sibatani, 1953; Thomson, Heagy, Hutchison & Davidson, 1953) and of 198 mg. of RNA-P/100 g. of pancreas (Schneider, 1946).

Mitochondrial RNA

Mitochondrial RNA-P shows a similar pattern of change over the lactation cycle to that found for total RNA-P. The rapid increase in mitochondrial RNA-P at parturition, together with the similar increase in mitochondrial nitrogen (Tables 1 and 2) and succinic oxidase activity (Greenbaum & Slater, 1957*b*), lead to the view that the particles in the mitochondrial size range increase rapidly in numbers or size or both over this period. The conclusion of increased numbers has already been suggested by cytologists and confirmed by Howe, Richardson & Birbeck (1956), who found an increase in mitochondrial numbers per cell over parturition, but no significant change in the number of mitochondria per unit volume of cytoplasm.

The percentage of the total RNA-P found in the large-particle fraction isolated at 230 000 *g* min. is relatively constant at 15–20 % (Table 2, column 13). This is the same order as that found in rat-liver mitochondrial fractions, although the centrifugal field used here is much higher than is usual for the sedimentation of mitochondria.

The major part of the RNA-P of mammary tissue is in the particles smaller than the mitochondria: the mitochondria account for some 15–20 % of the total RNA-P and the nuclei for a further 10 % (Greenbaum & Slater, unpublished observations). Centrifuging a mammary-tissue suspension from lactating rats for $1{\cdot}5 \times 10^6$ *g* min. yields a supernatant which is virtually devoid of RNA-P, and it thus follows that some 70 % of the total RNA-P is to be found in those particles which fall in the microsomal and submicrosomal range. The changes in RNA-P content of the mammary gland at parturition seem to occur in these two fractions, a conclusion which is in agreement with other views linking protein synthesis and the microsomal fractions (see Brachet, 1955). The conclusion that the microsomal RNA-P/gland increases can be derived by subtracting the mitochondrial RNA-P/gland (Table 2, column 12) from the total RNA-P in the gland (Table 2, column 9), the difference being mainly the microsomal RNA-P/gland. The difference between these two columns increases from 0·05 to 0·68 mg. of RNA-P/gland during pregnancy, and to 2·84 mg./gland by the end of lactation.

Deoxyribonucleic acid

The increase in the DNA-P content of the gland found during pregnancy indicates a considerable degree of cellular proliferation. The DNA-P level at the end of pregnancy was six times that of the virgin gland, which, in the adult female rats used, already had some mammary development after its passage through several oestrous cycles.

The constancy of the DNA-P/100 g. wet wt. of gland (milk-free) (Table 2, column 4) throughout lactation appears to be in accord with such cytological evidence as is available and which claims that growth of the gland is complete by mid-pregnancy and that no mitotic figures are found in the gland after this time (Maeder, 1922; Weatherford, 1929). When, however, the constant DNA-P/100 g. wet wt. of gland (milk-free) found during lactation is considered in conjunction with the increase in total glandular DNA-P, a slow growth and expansion of the gland is indicated. The relatively slow nature of this increase in DNA-P/gland (1·4 times in the 15 days between the third and eighteenth days of lactation) might account for the failure of cytologists to observe cell division in the lactating gland. The sharp drop in DNA-P level in mammary involution is consistent with existing views of a breakdown of cellular material during this stage.

The period covering parturition requires special mention. Between the end of pregnancy and the third day of lactation the DNA-P level is doubled. It is almost certain that some of this increase may be accounted for by an increased leucocyte content

consequent upon a greater degree of vascularization, but this can hardly account for more than a small part of the rise. It seems more probable that the greater part of the rise is due to a hyperplasia of existing cellular tissue and, although this view is in conflict with older views, it has been confirmed by independent methods: (i) the observation that the number of cells per alveolus increases by some 70% from late pregnancy to early lactation (Jeffers, 1935); (ii) Lewin & Lewin (personal communication) have found a rise in the number of nuclei/mg. wet tissue of mouse mammary gland from 136 000 to 260 000 over the period from parturition to the second day of lactation. The increase in DNA-P reported here, and of nuclei found by Lewin & Lewin, suggests that a wave of mitosis occurs in the gland about the time of parturition. Such a wave could easily be completed in a few hours, and it is therefore not surprising that mitosis has not been observed in the few glands examined cytologically. Richardson, Slater & Greenbaum (unpublished results) have, however, made some preliminary observations (in which the mitoses were arrested by colchicine) which show that a wave of cell division occurs some 30 hr. after parturition and is of relatively short duration.

In the absence of significant mitosis, the ratio RNA-P:DNA-P is a measure of the RNA-P/cell. Since the stages of the lactation cycle studied in this investigation included neither the growth period of pregnancy nor the short period after parturition when mitosis occurs, the ratio can be used to follow the changes in the cellular content of RNA-P. It can be seen from Table 2 that there is a rapid rise in RNA-P/cell at parturition, but that the value increases only slowly as lactation advances. The ratio 2·72 for the mammary gland at the tenth day of lactation is surprisingly small for so active a tissue when compared with rat liver or pancreas, which have ratios of 4·38 and 4·1 respectively (Thomson *et al.* 1953; Schneider, 1946).

Mitochondrial nitrogen

The variations in mitochondrial nitrogen are shown in Table 1, and it now remains to correlate these changes with the changes in the tissue nitrogen of the mammary gland reported previously (Greenbaum & Slater, 1957*a*). A comparison of the tissue-nitrogen increase in pregnancy and lactation (Greenbaum & Slater, 1957*a*) with the increase in mitochondrial nitrogen reported here shows that in pregnancy and lactation the mitochondrial material builds up faster than the other fractions of the cell, whereas at parturition there is a more rapid increase of non-mitochondrial nitrogen.

The mitochondrial nitrogen/cell may be assessed by considering the ratio of mitochondrial nitrogen/DNA-P. This ratio (Table 3) rises throughout pregnancy and lactation, indicating a progressive rise in mitochondrial nitrogen/cell.

A further point of interest arises from columns 5 and 6 of Table 3. It can be seen that from late pregnancy to the end of lactation the ratio, whole-tissue suspension RNA-P:mitochondrial nitrogen, is sensibly constant; deviations from this constancy occur only in the virgin and involuting glands. However, the ratio mitochondrial RNA-P:mitochondrial nitrogen is by no means constant, indicating that the mitochondrial RNA-P and nitrogen are, to a certain extent, independent variables. The correspondence of total-suspension RNA-P and mitochondrial nitrogen leads, on the other hand, to the idea of a correspondence between mitochondrial material (represented by nitrogen) and the rate of protein synthesis (represented by the RNA-P of the whole suspension). It would be of particular interest in this connexion to study the changes in the microsomal and submicrosomal RNA-P throughout the lactation cycle.

The response of the mammary gland to the stress of lactation appears to occur as two distinct phases. The first, occurring soon after parturition, involves the sudden rise in the number of functional cells as a result of the wave of mitosis and may be regarded as the immediate response. The second is the slower change which occurs during lactation itself and is more probably related to the increasing demands on the gland by the growing pups.

SUMMARY

1. Changes in the total nitrogen, mitochondrial nitrogen, total ribonucleic acid (RNA), mitochondrial RNA and in the deoxyribonucleic acid (DNA) content of the rat mammary gland have been studied at intervals during the lactation cycle.

2. There is a rise in the total nitrogen of the gland through pregnancy and lactation; a similar rise is found in the mitochondrial nitrogen.

3. RNA increases progressively from early pregnancy to late lactation and declines again during mammary involution. Parturition is accompanied by a rapid rise in the RNA of the mammary gland. The mitochondrial RNA rises during pregnancy, but reaches a plateau value by the third day of lactation which is maintained until mammary involution sets in.

4. The DNA of the gland increases during pregnancy. There is a doubling of the DNA level at parturition after which it remains constant throughout lactation.

5. Changes in the level of RNA are discussed in relation to the rate of protein synthesis in the gland and evidence is adduced for an increase in the microsomal and submicrosomal fractions during lactation.

6. The significance of the changes in DNA content of the mammary gland are discussed. It is suggested that the changes in DNA content of the gland at parturition indicate that a wave of cell division occurs in the gland around that time.

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REFERENCES

Albaum, H. G. & Umbreit, W. W. (1947). *J. biol. Chem.* **167**, 369.
Brachet, J. (1955). In *The Nucleic Acids*. Ed. by Chargaff, E. and Davidson, J. N. New York: Academic Press Inc.
Davidson, J. N. (1953). *The Biochemistry of the Nucleic Acids*, 2nd ed. London: Methuen and Co.
Davidson, J. N. & Leslie, I. (1950). *Nature, Lond.*, **165**, 49.
Dische, Z. (1955). In *The Nucleic Acids*. Ed. by Chargaff, E. and Davidson, J. N. New York: Academic Press Inc.
Duve, C. de & Berthet, J. (1953). *Nature, Lond.*, **172**, 1142.
Folley, S. J. & Greenbaum, A. L. (1947). *Biochem. J.* **41**, 261.
Fukuda, M. & Sibatani, A. (1953). *J. Biochem., Tokyo*, **40**, 95.
Greenbaum, A. L. & Slater, T. F. (1957*a*). *Biochem. J.* **66**, 148.
Greenbaum, A. L. & Slater, T. F. (1957*b*). *Biochem. J.* **66**, 161.
Hevesy, G. & Ottesen, J. (1943). *Acta Physiol. scand.* **5**, 237.
Hogeboom, G. H., Schneider, W. C. & Palade, G. E. (1948). *J. biol. Chem.* **172**, 619.
Howe, A., Richardson, K. C. & Birbeck, M. S. C. (1956). *Expt. Cell Res.* **10**, 194.
Jeffers, K. R. (1935). *Amer. J. Anat.* **50**, 257.
Kirkham, W. R. & Turner, C. W. (1953). *Proc. Soc. exp. Biol., N.Y.*, **83**, 123.
Leslie, I. (1955). In *The Nucleic Acids*. Ed. by Chargaff, E. and Davidson, J. N. New York: Academic Press Inc.
Maeder, L. M. A. (1922). *Amer. J. Anat.* **31**, 1.
Mejbaum, W. (1939). *Hoppe-Seyl. Z.* **258**, 117.
Roberts, F. L. (1921). *Pap. Mayo Fdn med. Educ.* I. Philadelphia: W. B. Saunders and Co.
Schmidt, G. & Thannhauser, S. J. (1945). *J. biol. Chem.* **161**, 83.
Schneider, W. C. (1945). *J. biol. Chem.* **161**, 293.
Schneider, W. C. (1946). *J. biol. Chem.* **164**, 747.
Schneider, W. C. & Hogeboom, G. H. (1951). *Cancer Res.* **11**, 1.
Smellie, R. M. S., McIndoe, W. M., Logan, R., Davidson, J. N. & Dawson, I. M. (1953). *Biochem. J.* **54**, 280.
Thomson, R. Y., Heagy, F. C., Hutchison, W. C. & Davidson, J. N. (1953). *Biochem. J.* **53**, 460.
Vendrely, R. (1955). In *The Nucleic Acids*. Ed. by Chargaff, E. and Davidson, J. N. New York: Academic Press Inc.
Weatherford, H. L. (1929). *Amer. J. Anat.* **44**, 199.

Studies on the Particulate Components of Rat Mammary Gland

3. THE RELATIONSHIP BETWEEN ENZYME ACTIVITY AND PARTICLE COUNTS IN MAMMARY GLAND SUSPENSIONS

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The two preceding parts of this series (Greenbaum & Slater, 1957*a*, *b*) have reported the rapid changes in chemical content which occur in the rat mammary gland over the period of parturition. This paper describes changes in the enzymic activity of the gland throughout the lactation cycle.

Part 1 of this series showed that the increase in tissue nitrogen at parturition was both rapid and considerable, and Part 2 described the rapid increases in milk synthesis and nucleic acid content which occur over this same period. Thus the gland in late pregnancy contrasts vividly, both in secretory activity and in composition, with the gland a few days later when lactation is well established.

It is to be expected that the transition of the gland into the 'active' state would be accompanied by an increase in the activity of the oxidative-enzyme systems. That such an increase occurs is evidenced by the rapid rise in Q_{O_2} and R.Q. (Folley & French, 1949), the increases in succinic oxidase and cytochrome oxidase activity (Tuba, Orr & Wiberg, 1955) and in glucose 6-phosphate and 6-phosphogluconate dehydrogenases (Glock & McLean, 1954).

Recent investigations have localized the majority of the oxidative enzymes in the particles of mitochondrial size (see Schneider, 1956). It seemed of interest, therefore, to follow in mammary gland suspensions the alterations in the activity of an enzyme which, in other tissues, is known to be associated with the mitochondrial fraction. In particular, it was of interest to see whether the rapid increase in synthetic activity found in the gland *post partum* was associated with changes in the number of mitochondrial particles, an increase in size of the existing particles or an increasing efficiency of the enzyme systems.

It has repeatedly been shown, in diverse tissues, that succinic oxidase is almost completely localized in the mitochondrial fraction. Although this enzyme has been studied in mammary tissue (guinea pig,