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Lactogenesis in the Rat

CHANGES IN METABOLIC PARAMETERS AT PARTURITION

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1. Tissue concentrations of nucleic acids, protein, fat, water, metabolites and lactose, and the activities of seven enzymes concerned in milk biosynthesis, were measured in the rat mammary gland at closely spaced times before, at and after parturition. 2. Changes are seen in the tissue concentrations of most substances, and several changes are initiated at least during the day preceding parturition. 3. Lactose, which is absent 1 day before parturition, is found in amounts of 12 μmoles/g. fresh wt. of tissue at parturition. 4. From the tissue activities before parturition of three enzymes on the biosynthetic pathway of lactose, and, from the small changes observed in their activities at parturition itself, it is concluded that the factors responsible for the appearance of lactose at parturition remain to be demonstrated.

As an example of tissue differentiation under the influence of hormones, the parturient mammary gland offers several advantages for investigation over other tissues, in that a relatively well-established complex of hormones (Cowie, 1961) impinges on a quiescent tissue to initiate the secretion of a characteristic product. In the present study the appearance of milk in the tissue was followed by measurement of lactose, which is unique to this organ.

Previous biochemical studies of mammary gland have been largely concerned with elucidating metabolic pathways of fat, carbohydrate and protein synthesis, and the increase in tissue activity of a given enzyme that accompanies the increased milk production after parturition has been used as evidence for a role of the enzyme in milk biosynthesis (for recent reviews see Kon & Cowie, 1961). However, little detailed attention has been paid to changes at the time of parturition itself, which is of greatest interest from the point of view of tissue differentiation. This paper presents the results of a survey of some metabolic parameters in the mammary gland of the parturient rat. While this work was in progress a similar study was published by Baldwin & Milligan (1966).

METHODS

All studies were performed on the inguinal mammary glands of Sprague–Dawley rats carrying their first litters (about nine pups each). The animals were purchased, with the date of mating supplied, from the Charles River Breeding Laboratories, Wilmington, Mass., U.S.A. Litters were born after 22$_{\frac{1}{2}}$ days of pregnancy. For rats killed before parturition, the time at which they would have given litters was taken as equal to the mean time at which the remaining rats of the group gave birth.

Removal of tissue. Rats were exsanguinated under light ether anaesthesia, and each mammary gland was dissected free from adjacent fatty and connective tissue. Tissue to be used for the extraction of metabolites was at once frozen in liquid N_2. It was then stored in liquid N_2 or at $-20°$ until used. Tissues taken for glucose measurement were first dissected free from the living anaesthetized animal while retaining an intact supply of well-oxygenated blood, and were then removed after being frozen *in situ* with aluminium tongs cooled in liquid N_2 (Wollenberger, Ristau & Schoffa, 1960). Tissues to be used for enzyme assays were removed to ice-cold 0·25 M-sucrose before homogenization.

Extraction with perchloric acid. Each frozen gland was weighed, and powdered in a mortar and pestle cooled by liquid N_2. About 0·8 g. of powder was added to a previously weighed glass centrifuge tube containing 5 ml. of ice-cold 5% (w/v) $HClO_4$ and a glass stirring rod, and was rapidly ground into a slurry. The tube was then reweighed to obtain the weight of powder used (Hohorst, Kreutz & Bücher, 1959). After centrifugation in a clinical centrifuge at 2° for 20 min. at about 5000*g*, the supernatant fluid was transferred with a Pasteur pipette to a conical centrifuge tube surrounded with crushed ice. The acid was neutralized to pH 7 with approx. 0·4 ml. of 5 N-KOH and the precipitated $KClO_4$ was removed by centrifugation. The volume of the clear supernatant fluid was recorded. This solution was used for determinations of glucose, lactose, CoA and glycerol.

The solid residue remaining from the $HClO_4$ extraction was extracted once more with 5% (w/v) $HClO_4$, being kept carefully chilled throughout. It was then defatted by two extractions with acetone (10 ml. each) and one with ether

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(10ml.), and dried briefly under a current of air. Nucleic acids were then extracted with two successive 4ml. volumes of 5% (w/v) $HClO_4$ at 70° for 15min. each (Burton, 1956). The extracts were combined and used for determinations of RNA and DNA. Alkaline hydrolysis of the remaining residue showed that 95% of the RNA had been extracted with the hot acid.

Determination of tissue water. About 60mg. of tissue was weighed out immediately after the tissue had been powdered, and dried to constant weight at 50°. Water content was taken as the difference in weight before and after drying.

Determination of tissue fat and fat-free dry weight. About 200–300mg. of frozen powdered tissue was weighed out and extracted with 5ml. of acetone followed by 5ml. of ether. This removed essentially all fat. The fat-free residue was dried and weighed. The combined lipid extracts were evaporated to dryness in a weighed tube and the tube was reweighed.

The combined weights of water, fat and fat-free dry residue were equal to the weight of fresh tissue powder used within 2%.

Determinations of metabolites. Glucose was determined on 0·2ml. volumes of extract by the glucose oxidase method (Bergmeyer & Bernt, 1963). Lactose was determined by incubating 0·3ml. volumes of extract for 2hr. at 30° with 0·1ml. of 0·1M-sodium phosphate buffer, pH7·4, containing 0·7mg. of yeast β-galactosidase (Mann Research Laboratories Inc., New York, N.Y., U.S.A.). The glucose released was then measured by the glucose oxidase method with 0·1ml. of the incubation mixture. A correction was made for the glucose present endogenously. A standard lactose curve was run with each batch of unknown samples. Lactose is quantitatively cleaved to glucose in this procedure, and the assay is linear up to at least 250μg. of lactose. The glucose oxidase used (type III; Sigma Chemical Co., St Louis, Mo., U.S.A.) did not attack lactose under these conditions.

CoA and glycerol were determined by the methods of Stadtman (1955) and Garland & Randle (1962) respectively.

RNA was determined by the orcinol method (Schneider, 1957), and a correction was applied for the slight reaction of DNA in this determination. This amounted to about 10% for each sample. DNA was determined by the diphenylamine method (Burton, 1956). Determinations of protein were made on high-speed supernatant fluids by the method of Lowry, Rosebrough, Farr & Randall (1951), with bovine serum albumin (Cohn fraction V; Armour Pharmaceutical Co., Kankakee, Ill., U.S.A.) as a standard.

Enzyme assays. Chilled mammary tissue was weighed, chopped in a mechanical chopper (McIlwain & Buddle, 1953) and homogenized in about 3vol. of ice-cold 0·25M-sucrose solution in a ground-glass conical homogenizer. A portion of the homogenate was centrifuged at 105000*g* for 30min. in a Spinco model L preparative ultracentrifuge and, after removal of the fat at the surface, a measured part of the clear supernatant fluid was removed by Pasteur pipette. The remaining supernatant fluid was taken for volume measurement, and the combined volumes were recorded. The particulate residue was drained briefly and kept in a deep-freeze. It was subsequently defatted and extracted for the determination of DNA as described above.

All enzyme determinations were performed on fresh high-speed supernatant fluid, except for glucose 6-phosphate dehydrogenase, which was assayed after storage in the deep-freeze for about 12 days. Values for ATP citrate lyase are likely to be somewhat low, since this enzyme lost about 50% activity in 5hr. in extracts from mammary tissue kept at 0°. All enzymes were assayed under conditions in which the reaction rate was linearly related to incubation time and to the amount of enzyme added.

Phosphoglucomutase (*α-D-glucose 1,6-diphosphate–α-D-glucose 1-phosphate phosphotransferase, EC* 2.7.5.1). Formation of glucose 6-phosphate from glucose 1-phosphate was followed by the increase in E_{340} in the presence of added NADP and glucose 6-phosphate dehydrogenase. Reaction mixtures contained: glucose 1-phosphate (5μmoles), $MgCl_2$ (2·5μmoles), cysteine (25μmoles), NADP (0·2μmole), glycylglycine–NaOH buffer, pH 7·4 (37 μmoles), glucose 6-phosphate dehydrogenase (10μg., approx. 1·3i.u.) and high-speed-supernatant (25–50μg. of protein) in a final volume of 1·0ml. The reaction was followed for 10min. at room temperature (about 22°) in a quartz cuvette of 1cm. light-path. Glucose 1-phosphate was omitted from the blank cuvette.

Glucose 6-phosphate dehydrogenase (*D-glucose 6-phosphate–NADP oxidoreductase, EC* 1.1.1.49). The spectrophotometric method of Glock & McLean (1954) was used. Reaction mixtures contained: glucose 6-phosphate (2μmoles), $MgCl_2$ (20μmoles), NADP (0·2μmole), glycylglycine–NaOH buffer, pH7·4 (50μmoles), and high-speed supernatant (12–50μg. of protein) in a final volume of 1·0ml. Glucose 6-phosphate was omitted from the blank cuvette.

ATP citrate lyase [*ATP–citrate oxaloacetate-lyase (CoA-acetylating and ATP-dephosphorylating), EC* 4.1.3.8]. This was assayed by the optical method of Srere (1959), in which the oxaloacetate that is formed oxidizes $NADH_2$ in the presence of malate dehydrogenase. Reaction mixtures contained: ATP (5μmoles), $MgCl_2$ (5μmoles), CoA (0·6μmole), GSH (10μmoles), sodium citrate (20μmoles), tris–HCl buffer, pH7·5 (50μmoles), $NADH_2$ (0·2μmole) and high-speed supernatant (about 400μg. of protein) in a final volume of 1·0ml. Addition of pure malate dehydrogenase to several preparations did not increase reaction rates, showing that endogenous amounts of this enzyme were present in excess. Citrate was omitted from the blank cuvette.

UDP-glucose pyrophosphorylase (*UTP–α-D-glucose 1-phosphate uridylyltransferase, EC* 2.7.7.9). This was measured in the direction of UDP-glucose formation by coupling with excess of UDP-glucose dehydrogenase (Shatton, Gruenstein, Shay & Weinhouse, 1965). Reaction mixtures contained: $MgCl_2$ (6μmoles), EDTA (3μmoles), NAD (3μmoles), UTP (1·5μmoles), glucose 1-phosphate (10μmoles), glycine–NaOH buffer, pH8·7 (120μmoles), UDP-glucose dehydrogenase (0·01 i.u.) and high-speed supernatant (30–120μg. of protein) in a final volume of 1·2ml. UTP was omitted from the blank cuvette.

UDP-glucose 4-epimerase (*EC* 5.1.3.2). Formation of UDP-glucose from UDP-galactose was measured in a coupled assay similar to that for UDP-glucose pyrophosphorylase (Shatton *et al.* 1965). Reaction mixtures contained: UDP-galactose (0·150μmole), NAD (3μmoles), glycine–NaOH buffer, pH8·7 (100μmoles), UDP-glucose dehydrogenase (0·01 i.u.) and high-speed supernatant (about 70μg. of protein) in a final volume of 1·0ml. UDP-galactose was omitted from the blank cuvette.

Glycerophosphate dehydrogenase (L-*glycerol* 3-*phosphate–NAD oxidoreductase, EC* 1.1.1.8). This was assayed in the direction of glycerophosphate formation by the method of Beisenherz, Bücher & Garbade (1955). Dihydroxyacetone phosphate was freshly prepared from its dimethyl ketal cyclohexylammonium salt (Ballou, 1960). Reaction mixtures contained: dihydroxyacetone phosphate (10 μ-moles), $NADH_2$ (0·18 μmole), EDTA (10 μmoles), tris–HCl buffer, pH 7·4 (50 μmoles), and high-speed supernatant (about 14 μg. of protein) in a final volume of 1·0 ml. Reactions were started with enzyme and the decrease in E_{340} was measured at 30 sec. intervals for 3 min. against a water blank.

Acetyl-CoA carboxylase [*acetyl-CoA–carbon dioxide ligase* (*ADP*), *EC* 6.4.1.2]. Fixation of [^{14}C]bicarbonate was measured in the presence of acetyl-CoA. Extracts of mammary tissue, unlike those of liver, show essentially no fixation of CO_2 in the absence of added acetyl-CoA. Reaction mixtures lacking acetyl-CoA were therefore omitted from routine measurements. High-speed supernatant (70–160 μg. of protein) was activated by prior incubation at 37° for 30 min. with potassium citrate, pH 7·4 (5 μmoles), β-mercaptoethanol (0·25 μmole), $MnCl_2$ (1 μ-mole), tris–HCl buffer, pH 7·4 (10 μmoles), acetyl-CoA (0·07 μmole) and serum albumin (0·2 mg.) in a final volume of 0·25 ml. The reaction was then started by the addition of 0·25 ml. of a solution containing the above quantities of tris–HCl buffer, pH 7·4, potassium citrate, β-mercaptoethanol, $MnCl_2$ and acetyl-CoA, as well as ATP (2 μmoles) and $NaH^{14}CO_3$ (20 μmoles, $4·6 \times 10^4$ counts/min./μmole), and was run at 37° for 10 to 30 min. The reaction was stopped by addition of 0·1 ml. of N-HCl, and a 0·1 ml. sample was dried on an aluminium planchet and counted in a Geiger–Müller gas-flow counter (Tracerlab model TGC14).

Expression of results. Most of the results are expressed/mg. of DNA. This is a more fundamental reference quantity than fresh weight or protein. For the sake of comparison with values in the literature, some results are also quoted on a fresh-weight basis.

Glucose 6-phosphate dehydrogenase and phosphoglucomutase activities are expressed in terms of the amounts of $NADPH_2$ formed, and no corrections have been made for a possible contribution to these activities by 6-phosphogluconate dehydrogenase.

The data in this paper are drawn from three series of experiments. Each series involved 45–58 rats mated at the same time, and killed in groups at known times before, at or after parturition. Separate measurements were made on material from each rat, and the results are expressed as mean values $\pm$S.E.M. Vertical bars are used in graphs to indicate the S.E.M. values. Where no such bars are drawn, they lie within the area of the symbol marking the given point. In some cases the same type of data is presented for more than one series, to show the variation from one series to another.

Chromatography of sugars. Paper chromatography of sugars was carried out by the ascending technique with Whatman no. 1 filter paper and either propan-1-ol–ethyl acetate–water (7:1:2, by vol.) or ethyl acetate–pyridine–water (2:1:2, by vol., upper phase). Sugars were located by spraying with a mixture of 4% aniline in ethanol–4% diphenylamine in ethanol–syrupy H_3PO_4 (5:5:1, by vol.) followed by heating for 10 min. at 80°.

Materials. Purified lactate dehydrogenase was kindyl provided by Mr F. Stolzenbach. CoA was purchased from P-L Biochemicals, Milwaukee, Wis., U.S.A. Malate dehydrogenase and pyruvate kinase were supplied by C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. All other nucleotides, enzymes and phosphate derivatives were from Sigma Chemical Co., St Louis, Mo., U.S.A. $NaH^{14}CO_3$ was obtained from New England Nuclear Corp., Boston, Mass., U.S.A.

RESULTS

Gross tissue composition. Parturition is accompanied by a moderate increase in the fresh weight of the mammary gland, which varies somewhat from one series of experiments to another. Results from two series are given in Fig. 1. Changes in fat and water content are considerably larger, however. Fig. 2 shows that the fat, which forms 47% of the weight of the gland in rats at late pregnancy, falls to around 22% within 24 hr. *post partum*. The percentage weight of water increases from 42% to nearly 65% in this time. The fat-free weight increases by about one-third from a level of 10% in the gland *pre-partum*. All three changes are highly significant at the time of parturition itself. Very similar results are obtained when expressed on a basis of tissue DNA.

Nucleic acids. Determinations of DNA were made to obtain a measure of the number of cells in the tissue, and to provide a useful basis on which to express specific activities of other variables. Fig. 3 shows the total DNA/gland over the period of parturition; each curve is the result of one series

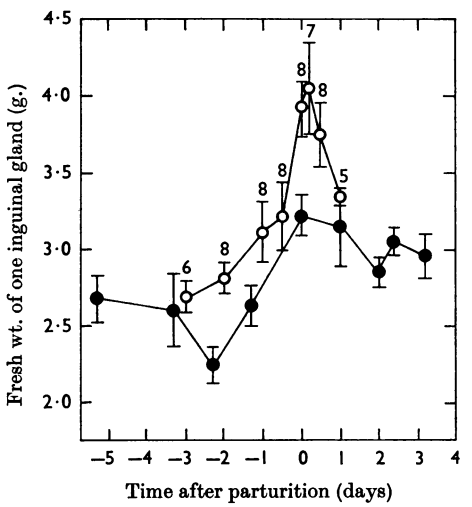

Fig. 1. Change in fresh weight of mammary gland. Each curve represents one series of rats. The number of rats used per point was five (●) or as indicated (○).

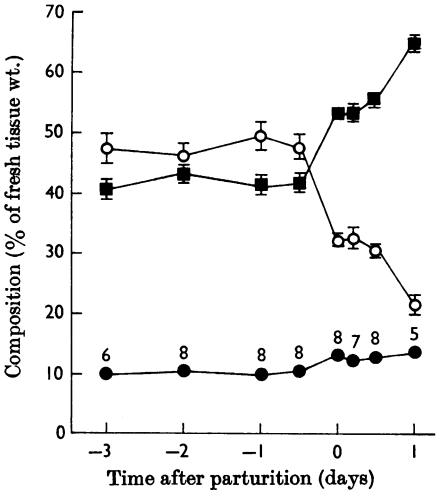

Fig. 2. Change in percentage composition by weight of mammary gland. ■, Water; ○, fat; ●, fat-free dry tissue. Numbers above the lowest curve show the number of rats used to obtain each point, and apply to all three curves.

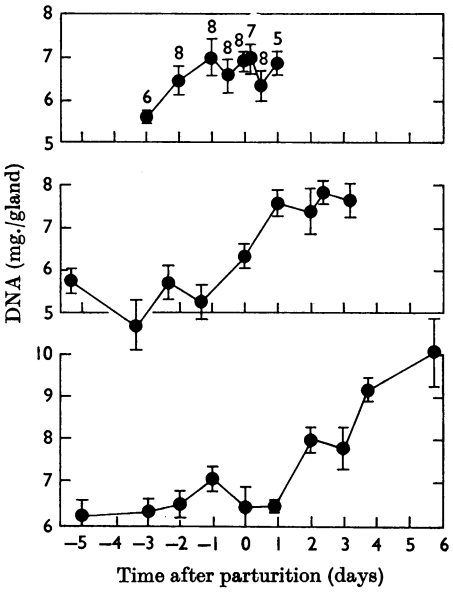

Fig. 3. Amount of total DNA/inguinal gland. Each curve represents one series of rats. Each point represents the mean of values obtained with five rats, except as shown in the upper curve.

of experiments. It is seen that a constant concentration of about 5–7 mg./gland before parturition is followed by a small and variable increase at about the time of parturition. One curve suggests that the total DNA continues to increase gradually after this time.

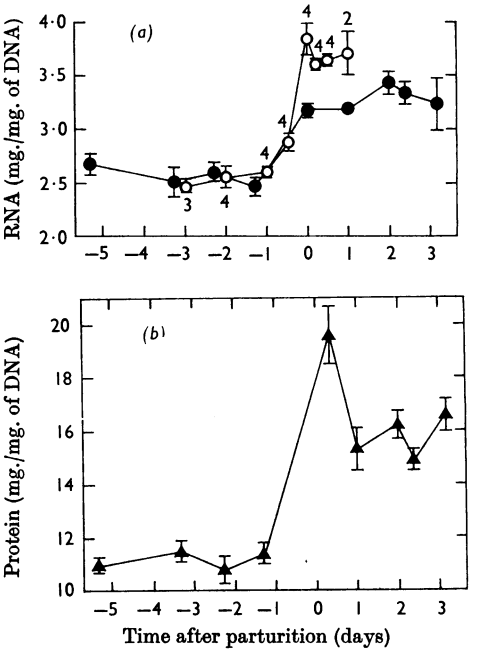

Fig. 4. (*a*) Change in amount of RNA/mg. of DNA (○ and ●). Each point represents the mean of values obtained with five rats, except as shown in the one curve (○). (*b*) Change in amount of protein of high-speed supernatant fluids of mammary tissue (▲). Each point represents the mean of values obtained with five rats.

There is a more pronounced increase in the total RNA content, which occurs just before parturition. The two series of experiments reported in Fig. 4 show a 40–50% increase in the amount of RNA/mg. of DNA. The very small standard errors of these values indicate a close relationship between RNA and DNA.

Protein. The high-speed-supernatant protein of sucrose homogenates of mammary glands increases 80% at parturition and then falls somewhat (Fig. 4). This increase parallels the change in fat-free dry weight, which probably also represents chiefly protein.

Sugars. Glucose concentrations average about 1·2 μmoles/g. fresh wt. before parturition (Table 1). The data show a transient, but not significant, increase at parturition. These concentrations fall to about one-third if a few minutes of anoxia precedes freezing of the tissue (data not shown). In contrast, lactose was not detected at all in the pregnant animal, but at parturition some 12 μmoles/g. fresh wt. were found, followed by a slight decrease in the next day or two. The same pattern of appearance has been found in other series of experiments. In the series shown in Table 1 small amounts of lactose were detected a few hours before

Table 1. *Tissue concentrations of glucose and lactose in the parturient rat mammary gland*

Results are expressed on the basis of DNA and of fresh wt. ± S.E.M.

Time after parturition (days)	Glucose (μmole/mg. of DNA)	Glucose (μmoles/g. fresh wt.)	Lactose (μmoles/mg. of DNA)	Lactose (μmoles/g. fresh wt.)	No. of rats/group
−3	0·55±0·05	1·15±0·12	0·00	0·0	6
−2	0·53±0·05	1·21±0·12	0·00	0·0	8
−1	0·56±0·03	1·29±0·09	0·00	0·0	8
−0·5	0·60±0·04	1·23±0·07	0·43±0·14	0·9±0·3	8
0	0·59±0·08	1·03±0·14	7·33±0·40	12·8±0·7	8
+0·2	0·78±0·10	1·36±0·17	6·65±0·22	11·5±0·4	7
+0·5	0·71±0·12	1·19±0·17	6·57±0·47	11·2±0·8	7
+1	0·62±0·12	1·29±0·25	4·40±0·81	8·7±1·6	5

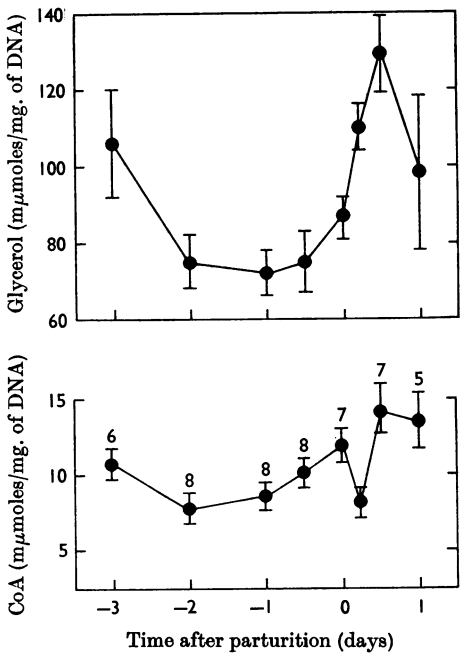

Fig. 5. Changes in concentrations of CoA and glycerol. Numbers above points on the lower curve show the numbers of rats used per point, and apply to both curves.

parturition. The identity of the lactose was confirmed by paper chromatography. This technique also revealed lactose in extracts taken at or after parturition, but not in those taken a few days before parturition.

Glycerol. Fig. 5 shows the results of glycerol analysis. Despite the large variations, which have also been noticed for guinea-pig mammary glands (Kuhn, 1967), there is a significant increase in tissue glycerol at parturition.

CoA. Mammary tissue of pregnant rats shows a small increase in CoA content at parturition (Fig. 5). The total amounts of CoA are extremely small (about 10–20 mμmoles/g. fresh wt.), but agree with those found at parturition by Lauryssens, Peeters & Donck (1956).

Changes in enzyme activities. Figs. 6, 7 and 8 show the changes of seven enzyme activities measured in high-speed supernatant fluids of sucrose homogenates. The most striking increases are shown by UDP-glucose pyrophosphorylase, acetyl-CoA carboxylase, glucose 6-phosphate dehydrogenase and ATP citrate lyase, and begin at about parturition or earlier. UDP-glucose 4-epimerase shows a much more gradual increase, the onset of which cannot be pinpointed from these data, and phosphoglucomutase shows a similar pattern. On the other hand, the activity of glycerophosphate dehydrogenase falls steadily from a time 2 days before parturition.

Apart from acetyl-CoA carboxylase, all enzymes were assayed at room temperature (approx. 22°). We have experimentally determined temperature correction factors (37°/22°) for glucose 6-phosphate dehydrogenase, phosphoglucomutase and ATP citrate lyase in mammary tissue taken before parturition. These were 2·8, 2·7 and 3·1 respectively. For UDP-glucose pyrophosphorylase and UDP-glucose 4-epimerase, also assayed before parturition, we have experimentally determined the factors 2·8 and 1·6 respectively to correct simultaneously for the same temperature change and for a pH change from 8·7 to 7·4. Application of these correction factors gives the following minimum enzyme activities shortly before parturition, at pH 7·4 and 37° (in μmoles of product/mg. of DNA/min.): glucose 6-phosphate dehydrogenase, 1·92; phosphoglucomutase, 3·52; UDP-glucose pyrophosphorylase, 0·140; UDP-glucose 4-epimerase, 0·120; ATP citrate lyase, 0·150.

An attempt was made to see whether the enzyme activities in the non-secreting glands of pregnant

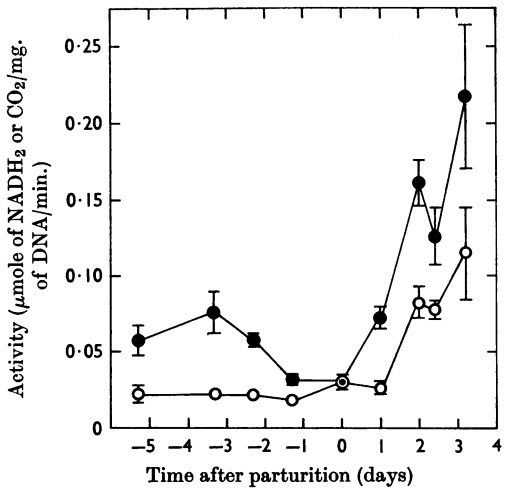

Fig. 6. Changes in activities of ATP citrate lyase (●) and of acetyl-CoA carboxylase (○) in high-speed supernatant fluid of mammary gland. Each point represents the mean of values obtained with five rats.

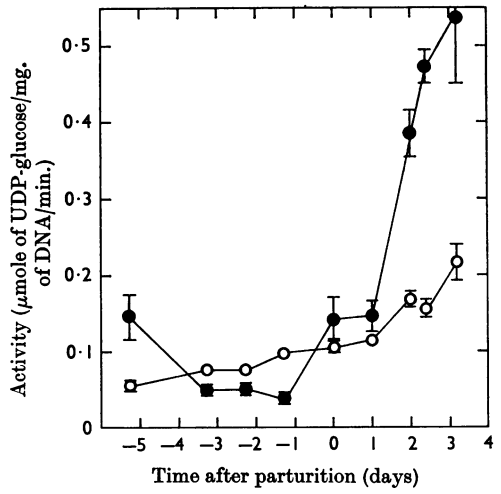

Fig. 7. Changes in activities of UDP-glucose pyrophosphorylase (●) and of UDP-glucose 4-epimerase (○) in high-speed supernatant fluid of mammary gland. Each point represents the mean of values obtained with five rats.

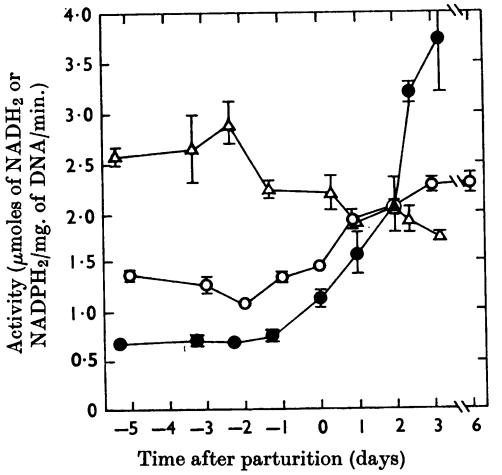

Fig. 8. Changes in activities of glucose 6-phosphate dehydrogenase (●), phosphoglucomutase (○) and glycerophosphate dehydrogenase (△) in high-speed supernatant fluid of mammary gland. Each point represents the mean of values obtained with five rats.

animals could be due to the adipose tissue, which forms a large part of the mammary tissue (Fig. 2). Four enzymes were assayed in both the mammary tissue and the juxtaposed adipose tissue of each of two rats 2 days before parturition. From these enzyme activities (calculated per g. fresh wt. of tissue), and taking a value of 50% as the proportion of fresh mammary tissue that is made up of adipose tissue (Fig. 2), the percentage contribution made by adipose tissue to the enzyme activities of mammary tissue was calculated. It is assumed that the adipose tissue that is assayed is representative of that actually in the gland. It is seen in Table 2 that the contribution is different for each enzyme, but is not more than about 10% in the extreme case.

Table 2. *Percentages of four enzyme activities in whole mammary-gland preparations that are accounted for by adipose tissue within the gland*

Enzyme	% of total gland activity due to adipose tissue: Rat 1	Rat 2
Phosphoglucomutase	9·5	11·5
UDP-glucose 4-epimerase	1·8	2·0
UDP-glucose pyrophosphorylase	5·3	4·1
Glucose 6-phosphate dehydrogenase	0·7	0·8

DISCUSSION

Of several metabolic changes that occur at parturition, the appearance of lactose is the most striking. Its absence up to the last day of pregnancy shows that the 12–13 μmoles of lactose/g. fresh wt. that are found at parturition have been accumulated during the previous day. Since no suckling has occurred at this time, it is clear that such a stimulus is not responsible for the initiation of lactation (lactogenesis), though it may promote

its continuation (galactopoiesis). The apparent lactose found in mammary tissue before parturition by other workers (Folley & Greenbaum, 1947; Greenbaum & Slater, 1957*a*; Baldwin & Milligan, 1966) can probably be ascribed to the non-specific chemical method used for determination. For this reason, and because we have noted a complete lack of correlation between the presence of lactose and of visible milk in the glands of parturient rats, it does not seem justifiable to correct tissue weights for 'retained fluid' calculated from lactose measurements, as has previously been done (Greenbaum & Slater, 1957*a*).

Despite the small changes in the fresh weight of the tissue, there are large changes in its gross composition. The percentage gains of water and fat-free dry weight, and the loss of fat, represent actual gains and losses of each material. These changes are already in evidence at parturition and must therefore have been initiated during the previous day. The significance of these changes is not apparent at the present time. The uptake of water is also an early event in the action of oestradiol on the immature uterus (Astwood, 1938).

Despite the abundant fat of mammary tissue, its contribution to the measured tissue metabolism is probably small, even in pregnant animals. Thus the data in Table 2 suggest a contribution of 10% or less towards several overall enzyme activities, and the calculations of Rees (1960) show a similar contribution towards tissue respiration. Assuming the dry adipose tissue of mammary gland to contain 1·5% of protein (Rees, 1960), it may be calculated from our data that, before parturition, it contributes only 7% to the total protein. Although the signal for the onset of fat disappearance at parturition is not known, it may be noted that facilitation of lipolysis has been demonstrated for growth hormone, thyroid-stimulating hormone, adrenocorticotrophic hormone and glucocorticoids, all of which are known to be concerned in the processes of milk formation (Folley, 1952; Cowie, 1961). That the disappearance of fat in this case also involves lipolysis is indicated by the increase of tissue glycerol that occurs. It must be noted, however, that glycerol could also arise from the hydrolysis of plasma triglyceride (McBride & Korn, 1963, 1964; Robinson, 1963; Barry, Bartley, Linzell & Robinson, 1963).

Although increases in total DNA are observed (Fig. 3) the timing appears to vary from one series of rats to another. Moreover, these increases do not seem to precede changes in other parameters of the mammary gland. Similar measurements were made on the rat by Greenbaum & Slater (1957*b*), who referred to a 'burst of mitotic activity' 30hr. after parturition, represented by a 30% rise in total DNA. However, the possibility that a smaller percentage increase in DNA precedes lactogenesis cannot be determined by this type of data. The independence of lactogenesis from mitosis was indicated in Weatherford's (1929) detailed histological study of alveolar cells in the rat, where hyperplasia was reported to be very rare in the second half of pregnancy and in early lactation, and where major signs of cellular activity concerned the Golgi apparatus and mitochondria. On the other hand, frequent mitoses have been reported over this period by Jeffers (1935), and Baldwin & Milligan (1966) found a fourfold increase in total DNA, paralleled by a similar rise in total cell number, at parturition. Although these data are partly contradictory, it is likely that at least some of the increases in DNA or cell number can be accounted for by non-secretory cells such as leucocytes or capillary epithelium. The generally accepted presence of fully formed alveoli containing accumulated secretory granules within the lumen, during the days immediately preceding parturition, suggests that the first milk products in the rat may be formed in pre-existing cells.

With cultured mouse mammary-tissue explants, however, evidence has been presented for a relationship between DNA synthesis and the onset of casein formation (Stockdale & Topper, 1966).

The increase in the amount of RNA per cell, coinciding with the rise of fat-free dry weight and of soluble protein, is consistent with the relationship between RNA and protein synthesis established for other tissues (Brachet, 1955), and probably represents mainly an increase of endoplasmic reticulum. From the amount of lactose measured in the tissue at parturition, and from the data on the composition of rat milk (Nelson, Kaye, Moore, Williams & Herrington, 1951), about 14·4mg. of milk protein/g. fresh wt. of tissue might be expected at parturition, if it can be assumed that the ratio of lactose to milk protein at this time equals the ratio in rat milk. This amount of protein would account for about 90% of the increase in high-speed-supernatant protein that occurs at this time (Fig. 4) and about 50% of the increased fat-free dry tissue (Fig. 2).

The choice of enzymes for examination in this study was dictated by the ease of assay and by their established association with biosynthetic pathways of major milk products. Phosphoglucomutase, UDP-glucose pyrophosphorylase and UDP-glucose 4-epimerase represent the first three enzymes on the pathway of lactose biosynthesis (Leloir & Cardini, 1961), glucose 6-phosphate dehydrogenase, acetyl-CoA carboxylase and ATP citrate lyase are concerned with lipogenesis (Glock & McLean, 1954; Lowenstein, 1961; Spencer & Lowenstein, 1962; Howanitz & Levy, 1965), and glycerophosphate dehydrogenase is concerned with the provision of

glycerophosphate for the synthesis of glyceride (Kleiber *et al.* 1955; Dils & Clark, 1962; Kuhn, 1967).

The increases of activity for glucose 6-phosphate dehydrogenase, acetyl-CoA carboxylase and ATP citrate lyase, that we have shown, are in agreement with the findings of others (Howanitz & Levy, 1965; Shatton *et al.* 1965; Glock & McLean, 1954; Baldwin & Milligan, 1966), though detailed measurements have not generally been made immediately before parturition. Our data suggest the existence of a stimulus to enzyme synthesis occurring at, or within 2 days before, parturition. Phosphoglucomutase and UDP-glucose 4-epimerase also begin to increase at about this time, but the time of onset cannot be clearly pinpointed and the increases are much slower than for other enzymes. Glycerophosphate dehydrogenase actually falls from about 2 days before parturition, although remaining extremely active throughout. Others have reported an increase at this time (Baldwin & Milligan, 1966).

Although the data show some enzymes to have increased in activity already by parturition, the magnitude of different increases is variable and the increases do not suggest themselves as the cause of the abrupt appearance of lactose at this time. Further, all the enzymes assayed here are present at appreciable activities before parturition. Thus the measured UDP-glucose 4-epimerase activity, which is the lowest of the lactose-biosynthetic enzymes measured here, would enable the formation of 950 μmoles of lactose/day/mammary gland. Unless a very rapid removal of lactose is postulated, alternative reasons are required to explain its absence before parturition. It is possible that the measured enzyme activities do not derive from alveolar cells, but from other elements of the tissue, although adipose cells would appear to be excluded.

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