Lactose Synthesis in the Rat, and the Effects of Litter Size and Malnutrition

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1. The rate of lactose synthesis per g of mammary tissue, measured in vivo by a radioisotopic technique, rose 13-fold between parturition and day 16 of lactation in the rat, but was unaffected by wide variation in litter size. 2. The increase reflected a greater tissue content of galactosyltransferase (EC 2.4.1.22), and was augmented by a rise in the total weight of mammary tissue. Superimposed on this were unpredictable changes in the functional efficiency of the enzyme. 3. Lactose synthesis in 14-day-lactating rats, permitted only 76% of the food intake of paired control rats over the previous 3 weeks, showed a pronounced diurnal variation at an overall rate markedly below that in control rats. 4. Such nutritional deficiency did not affect the tissue content of galactosyltransferase, but impaired its functional efficiency in a manner reversed by renewed feeding or by the preparation and incubation of acini in vitro. 5. Plasma insulin concentrations decreased at parturition and with increasing litter size, and remained relatively unchanged during lactation and malnutrition.

A radioisotopic method was recently introduced to measure rates of lactose synthesis in anaesthetized, lactating rats. The rates of whole-milk production calculated from these values agreed closely with rates measured by other methods (Carrick & Kuhn, 1978). The new method served to show a decrease in lactose synthesis during the afternoon, and a severe inhibition after food had been withdrawn for 6 h during the afternoon or evening. It thus appeared that lactose synthesis was responding sensitively to short-term nutritional changes in the animal. Unexpectedly, changes in the concentration of plasma insulin did not appear to be responsible.

In the present paper we examine how lactose synthesis is affected by longer-term nutritional deficiency, rates in control rats fed ad lib. being compared with those in rats receiving only 75% of the control diet over several weeks. Concentrations of plasma insulin, activities of galactosyltransferase (EC 2.4.1.22) and other parameters are measured in these rats and in rats with different litter sizes. The results point to the control of lactose synthesis by some readily reversible mechanism that does not appear to include insulin. Some of these results have been briefly reported (Wilde & Kuhn, 1977; Kuhn, 1977).

Methods

Measurement of lactose synthesis in vivo

Priming lactating rats were maintained with food and water ad lib. under a 14h-on-10h-off lighting schedule. Except during the feeding experiments described below, measurement of rates of lactose synthesis (Carrick & Kuhn, 1978) and collection of plasma and mammary-tissue samples were carried out at about mid-day in order to avoid complications caused by diurnal fluctuations (Carrick & Kuhn, 1978). Litters were adjusted from a natural size of 8–12 pups to leave 9–11 pups each, except where the effect of varying the litter size was examined; in this case we used rats with unadjusted litter sizes of 3–5 and 14–15 pups. Rats that lost pups during lactation were discarded.

Plasma glucose and insulin

For the measurements reported in Fig. 1, rats were killed by instant decapitation. Blood was collected in heparinized beakers, centrifuged immediately for 10 min at 2000g, and the plasma was stored at -20°C until assayed for glucose by the enzymic method of Bergmeyer & Bernt (1965) and for insulin by using a kit supplied by The Radiochemical Centre, Amersham, Bucks., U.K. A standard curve was prepared with rat insulin (Novo, Copenhagen, Denmark). Rats anaesthetized with halothane were used to provide blood for the insulin measurements reported in Tables 2 and 3.

Galactosyltransferase and lactose synthetase assays

Galactosyltransferase was assayed with N-acetylglucosamine as galactosyl acceptor, and with Triton X-100 to release the full activity, as previously described (Kuhn & White, 1977). Lactose synthetase was assayed in the same manner, but with glucose
replacing N-acetylglucosamine and with omission of Triton X-100.

Maintenance of rats during controlled feeding

Rats were maintained singly in wire-bottomed cages over removable trays on which uneaten, shredded, food was collected. Pregnant rats weighing about 250 g were allowed food and water ad lib. until day 15 of pregnancy, after which at 09:00 h or 17:00 h the control rats were fed more than sufficient food for the next 24 h, and the test animals were given an amount equivalent to 75% of that day's expected normal food consumption, with allowance made for food lost during feeding. At the end of each 24-h period, uneaten food in the hopper and on the tray was weighed, to give an accurate value for the daily food intake of the two groups. The average food intake of rats on the restricted diet was 76% of that of control rats.

Nesting material was supplied before parturition, and 2 days post partum the litter size was adjusted where necessary. At 09:00 h or 15:00 h on day 12 of lactation rats were placed under light halothane anaesthesia and a blood sample was taken from the tail for the assay of plasma insulin. Then, after intraperitoneal injection of oxytocin (1.0 i.u.), a sample of milk was taken from pectoral mammary glands for the measurement of lactose and N-acetyleneuraminyl-lactose (Kuhn, 1972). Two days later the rate of lactose synthesis in vivo was measured, and mammary tissue was taken for the assay of galactosyltransferase.

Preparation of mammary acini

Rats were used at 09:00 h on day 14 of lactation for the preparation of mammary acini by a modification of the method of Katz et al. (1974). The inguinal glands were removed and chopped at room temperature at 2-mm intervals with a mechanical chopper (Mcllwain & Buddle, 1953). The mince was rinsed in medium 199 (see under 'Materials') and transferred to a cylindrical silicone-treated glass vessel (4 cm x 11 cm) with medium 199 (40 ml) containing collagenase (1 mg/ml) and glucose (10 mM), which was then shaken at 200 oscillations/min at 37°C for 40–45 min. The vessel was designed with two recessed openings that permitted continuous gassing with O2/CO2 (19:1, v/v) during the digestion. The resulting suspension was then filtered through two layers of nylon gauze (1 mm mesh) on to a layer of medium 199 containing Ficoll (1%) and centrifuged at 500 g for 3 min at 4°C. The pellet was washed twice with the same medium and finally resuspended in medium 199 containing glucose (5 mM). The whole preparation generally took 75–90 min and yielded acini capable of utilizing glucose and synthesizing lactose at constant rates for at least 90 min; 1 mg of fat-free dry acini contained 0.9 mg of protein.

Incubation of acini

Samples (2.7 ml) of acini suspension containing about 3 mg of protein were incubated at 37°C in silicone-treated stoppered 25 ml conical flasks with gentle shaking and with oxygenation at 20-min intervals. [U-14C]Glucose (1 µCi in 0.3 ml of medium 199) was added after 15 min and a sample (0.25 ml) of suspension was immediately transferred to 0.1 ml of 5 m-HCl for subsequent determination of glucose. Then 1 h later the incubation was stopped by heating the flask at 100°C for 5 min.

Glucose in the medium was assayed manually, or by autoanalyzer, by the method of Bergmeyer & Bernt (1965). [14C]Lactose was measured by the method of Kuhn & White (1975). Protein was determined, after dissolution in 2 M-NaOH, by the method of Lowry et al. (1951), with bovine serum albumin as standard. A portion of the fresh acinar preparation was homogenized in 0.25 M sucrose and the homogenate was assayed for galactosyltransferase.

Materials

Lactating rats were of a Wistar-derived strain bred in the Department, fed on modified rat/mouse breeding diet, from Heygates Ltd., Brook Mill, Northampton, U.K.

Collagenase (type 1) was from Sigma (London) Chemical Co., London S.W.6, U.K., and medium 199 was from Wellcome Reagents, Beckenham, Kent BR3 3BS, U.K. Enzymes and nucleotides were from Sigma (London) Chemical Co. or from Boehringer Corp. (London) Ltd., Lewes, East Sussex, U.K. Radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. 14C was determined with a Philips liquid-scintillation analyser by using a xylene/Triton X-100 scintillator fluid (Kuhn & White, 1975). 125I radioactivity was counted with an ICN Gammaset-500 radiation analyser. The autoanalyzer was from Technicon Instruments Co., Chertsey, Surrey, U.K.

Results are expressed as means ± S.E.M. with the numbers of determinations in parentheses. S.E.M. values are denoted by error bars in the Figures. Student's t test was used to evaluate significance of differences.

Results

Rates of lactose synthesis and galactosyltransferase activity during lactation

With a radioisotopic method developed in this laboratory (Carrick & Kuhn, 1978) it was possible to measure rates of lactose synthesis in intact rats throughout normal lactation (Fig. 1). A significant rate (0.6 µmol/h per g fresh wt. of tissue) was observed during parturition, rising to a peak value of 8.1 µmol/h per g on day 16 of lactation and then
Fig. 1. Changes of lactose synthesis and related parameters during normal lactation and late pregnancy

The rate of milk production is calculated from the measured rate of lactose synthesis and the milk content of lactose and N-acetylmuraminyl-lactose (Kuhn, 1972). Rats were used on day 12 of lactation.

Table 1. Effect of litter size on lactose synthesis, plasma insulin and weight of mammary tissue

The rate of milk production is calculated from the measured rate of lactose synthesis and the milk content of lactose and N-acetylmuraminyl-lactose (Kuhn, 1972). Rats were used on day 12 of lactation.

<table>
<thead>
<tr>
<th>Litter size</th>
<th>3-5 pups</th>
<th>9-11 pups</th>
<th>14-15 pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Litter wt. (g)</td>
<td>126 ± 10</td>
<td>—</td>
<td>316 ± 26</td>
</tr>
<tr>
<td>Mammary fresh tissue wt. (g)</td>
<td>13.6 + 1.5 (5)</td>
<td>18.3 ± 0.7 (5)</td>
<td>22.4 ± 1.1 (6)</td>
</tr>
<tr>
<td>Conc. plasma insulin (ng/ml)</td>
<td>3.54 ± 0.70 (8)</td>
<td>2.25 ± 0.50 (6)</td>
<td>1.60 ± 0.33 (5)</td>
</tr>
<tr>
<td>Rate of lactose synthesis (µmol/h per g fresh wt. of tissue)</td>
<td>6.22 ± 0.36 (5)</td>
<td>7.09 ± 0.75 (6)</td>
<td>5.77 ± 0.61 (6)</td>
</tr>
<tr>
<td>Rate of lactose synthesis (µmol/h per rat)</td>
<td>88.1 ± 11.5 (5)</td>
<td>121 ± 15 (5)</td>
<td>127 ± 12 (6)</td>
</tr>
<tr>
<td>Milk production (ml/day per rat)</td>
<td>21.1 ± 2.8 (5)</td>
<td>29.1 ± 3.7 (5)</td>
<td>30.5 ± 2.9 (6)</td>
</tr>
</tbody>
</table>

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Effects of litter size on lactose synthesis and plasma insulin

Table 1 shows that wide variation in the size of the litter being suckled caused no significant change in the rate of lactose synthesis expressed per g fresh wt. of tissue. However, rats bearing larger litters did have more mammary tissue, although not proportionately so, and this accounted entirely for their greater milk production. Our findings do not wholly support those of Morag et al. (1975), who apparently found a larger variation of milk yield with litter size. An unexpected finding was that plasma insulin concentrations tended to fall with increase in litter size. Neither the mechanism of this effect nor its significance, if any, is obvious, but the destruction of insulin by mammary tissue itself is an interesting possibility.

Effects of decreased diet on lactose synthesis and plasma insulin

The rats used in this study had been fed ad lib., or at 76% of this consumed amount of food, for about 3 weeks. The design of these experiments was dictated by the different pattern of 24 h food intake shown by rats on the decreased diet as compared with rats fed ad lib. Being hungrier, they consumed their (smaller) daily ration within 12 h, and then were without food for about 12 h. Control rats, however, fed throughout the 24 h, especially at night. Since Carrick & Kuhn (1978) showed that lactose synthesis was greatly impaired by 6 h of food deprivation, it was important to compare lactose synthesis in the two groups of rats at comparable times in the daily feeding cycle, so that short-term nutritional changes should not be reflected to the exclusion of long-term changes. The rats were therefore fed at 09:00 h or 17:00 h and measurements were made at 6, 16 or 24 h thereafter.

Table 2 shows that 6 h after food had been given the rate of lactose synthesis was similar in both groups of rats. The absolute rate was lower than that in control rats at 09:00 h, but similar to that noted by Carrick & Kuhn (1978) in control rats during the afternoon. At 16 h after feeding, rats on the decreased diet showed only 45% of the rate of lactose synthesis seen in the controls, and by 24 h the rate had fallen to only 11% of that in controls. It is clear, then, that rats maintained on a decreased diet show a rate of lactose synthesis that fluctuates during the day according to the food intake, but is overall markedly lower than that in rats fed ad lib. The difference in daily lactose production per rat must be even greater, since rats on a decreased diet had a lower total weight of mammary tissue.
By contrast with the above changes, the decreased diet affected neither the total activity of galactosyltransferase per g of tissue, nor the lactose and N-acetylneuraminyl-lactose content of the milk. It follows that the functional efficiency of the galactosyltransferase varied strongly during the day (Table 2). It may be noted that plasma insulin concentrations did not decrease with malnutrition, as might have been expected, and that values obtained from guillotined rats (Table 3) appeared lower than those from rats sampled under anaesthesia (Table 2). But apart from the considerable variation in individual values, indicated by S.E.M. values in the Tables and noted also by Carrick & Kuhn (1978), the effects of glucocorticoid and of the anticipation of feeding that have been recorded on insulin secretion may make predictions unwise in experiments of the present design (Curry & Bennett, 1973; Bellinger et al., 1975).

The lactose synthetase activity of whole-tissue homogenates was also unaffected by the decreased food intake (results not shown). To the extent that this assay monitors the retention of α-lactalbumin by Golgi vesicles found during homogenization, there is no sign that variation in α-lactalbumin concentration is responsible for the altered efficiency of galactosyltransferase.

### Table 3. Rates of glucose uptake and lactose synthesis in mammary acini in vitro

Table 3 shows that the rates of glucose uptake and lactose synthesis, both of which were similar to rates reported by others (Katz et al., 1974; Robinson & Williamson, 1977a), were not significantly different between the two groups of rats. Because the preparation of acini represents a selection of cell type, absolute rates of lactose synthesis and total galactosyltransferase activities cannot be compared on a protein basis with those in whole tissue. In order to overcome this problem we have expressed the actual rate of lactose synthesis by the acini relative to the galactosyltransferase activity as measured on homogenates of the acini. This ratio, which measures the functional efficiency of the transferase, was not significantly impaired by the decreased food intake, and remained similar to the ratio observed in vivo with rats fed ad lib. (Table 2). That is, the preparation and incubation of acini from rats on a decreased diet appeared to relieve the inhibition that was being exerted on the galactosyltransferase in vivo. This same series of rats served to provide plasma for the measurement of insulin and glucose concentrations, neither of which was significantly affected by the decreased diet (Table 3).
Discussion

The present study represents a part of our quest for the natural mechanisms by which the synthesis of lactose is regulated, and was guided by two considerations. Firstly, it seemed important simply to determine how lactose synthesis actually varies under different conditions. This aspect was initiated by a study of diurnal variation and the effect of very-short-term food withdrawal (Carrick & Kuhn, 1978), and is here extended to examine different stages of lactation, the variation of litter size and the effect of decreased food intake over the longer term. Secondly, it was considered essential that absolute rates of lactose synthesis should be measured, not in vitro, but in vivo under conditions as close to physiological as possible, together with such other relevant measurements as could be conveniently made in the same animals. This belief was vindicated by the finding that a decreased food intake was not reflected by an impaired rate of lactose synthesis in vitro as it was in vivo.

Lactose synthesis during normal lactation

The low rate of lactose synthesis observed in parturient rats (0.6 μmol/h per g of tissue) at once poses a problem, since, if sustained, it would have required some 20 h to accumulate the amount of lactose normally occurring at parturition (about 12 μmol/g; Kuhn & Lowenstein, 1967; Kuhn, 1972). Yet previous studies of lactogenesis have suggested that lactose synthesis is initiated within only about 12 h before parturition and that it actually accelerates from the time of its inception (Kuhn, 1969; Murphy et al., 1973). To resolve this paradox we suggest that the last half-day of pregnancy is characterized by an accelerating burst of lactose synthesis that, having charged the tissue with the appropriate reserve of lactose, subsides to the rate actually observed at parturition. One could envisage a negative feedback by accumulated milk in the tissue. Such an arrested surge of lactose synthesis might well have gone un-noticed during the experimentally awkward period just before parturition.

The increase in lactose synthesis during subsequent lactation is due partly to an increase in the amount of mammary tissue, but more to an increased rate per g of tissue. Much of this can be ascribed to the larger tissue content of galactosyltransferase, the functional efficiency of which, however, changes unpredictably.

Effect of decreased food intake on lactose synthesis

The main long-term effect of malnutrition that we noticed was a decreased total weight of mammary tissue. This, together with the exaggerated circadian variation imposed by the pattern of food intake of these rats, resulted in an impairment of overall lactose synthesis that was probably disproportionate to the actual shortfall in food consumed. Although differently designed experiments might elucidate some more profound deficiency in these rats, the rates of lactose synthesis that were observed at different times after feeding suggest that the overriding factors are in fact short-term and reversible, related to the rhythms of feeding, and perhaps of hormones, as is the case for rats fed ad lib. (Carrick & Kuhn, 1978). The reversible nature of the impairment is also indicated by its apparent lack of expression in vitro.

It is noteworthy that the tissue content of galactosyltransferase was unaffected by prolonged malnutrition (Table 2). From this one may infer that the increase of this enzyme that occurs during normal lactation is not a response to the increased food intake that accompanies it. At the same time studies on the hypophysectomized rabbit indicate that it is not acutely controlled by the pituitary, so that the cause of its induction remains something of a puzzle (Jones & Cowie, 1972).

The close dependence of lactose synthesis on food intake emphasizes the importance of mechanisms regulating feeding during lactation. Insofar as this is stimulated by suckling, operating as a feed-forward activation of lactation, one can envisage a purely neural control involving the afferent nerves of the teats and the hypothalamus (Cross, 1961; Ota & Yokoyama, 1967). Yet a part of the changes shown in Fig. 1 might be ascribed to progesterone, the plasma concentration of which declines before parturition and rises again thereafter (Pepe & Rothchild, 1974), and which does appear to stimulate food consumption by female rats (Hervey & Hervey, 1967). At the same time there is a widespread impression that feeding is controlled by some metabolic factor that reflects the carbohydrate or fat status of the body (see Eleftheriou & Sprott, 1975; Novin et al., 1976), but from Fig. 1 it seems unlikely that plasma glucose or insulin can be this factor.

Since the lactose and N-acetylneuraminyl-lactose content of the milk is unaffected by the decreased diet, the impairment of lactose synthesis must be accompanied by a similar change in whole milk production. It is surprising, then, that the litter weight is not correspondingly affected. Lactose makes only a small caloric contribution to rat milk, and possibly some increase in the fat content occurs by way of compensation. The maternal weights were 298 ± 4 g (26) and 244 ± 4 g (30) for rats fed on the normal and decreased diet respectively, implying a mobilization of body reserves to offset partially the effect of decreased food intake.

A drastic decrease in the lipogenic capacity of rat mammary tissue is seen at 48 h starvation (Coniglio & Culp, 1965) and may also account for the decreased consumption of glucose at 16–24 h starvation (Hawkins & Williamson, 1972; Robinson & Williamson, 1979).
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1977c). The possibility has been explored that the action of increased plasma ketone bodies might account, at least in part, for the altered glucose metabolism characteristic of starvation. Insulin can restore towards normal both the uptake of glucose and the synthesis of fatty acids, although it appears to have little effect on tissue taken from fed rats (Williamson et al., 1975; Robinson & Williamson, 1977a,b,c). So far, however, no clear link has emerged between the factors affecting glucose uptake and lipogenesis on the one hand and lactose synthesis on the other.

Effect of litter size

It was surprising that a 3–4-fold variation in natural litter size had no effect on the rate of lactose synthesis per g of tissue. Therefore, to the extent that the increased suckling of a large litter may elevate the plasma concentration of prolactin (Ford & Melampy, 1973), this hormone is not obviously regulatory in this capacity. The rather greater weight of mammary tissue seen in rats bearing larger litters may stem from a greater production of placental lactogen during pregnancy. The lower concentration of plasma insulin in these rats also awaits an explanation.

Insulin and lactose synthesis

The present observations, together with those of Carrick & Kuhn (1978), suggest that some factor circulates in the bloodstream, at a concentration that varies with the nutritional state of the body, and exerts a controlling effect on lactose synthesis in the mammary gland. Insulin may seem a natural candidate for this role, in view of its ability to stimulate the analogous process of hepatic glycogen formation (Nuttall, 1972; Miller et al., 1973) and of its long-recognized stimulation of milk fatty acid synthesis (Balmain et al., 1954; Abraham et al., 1957; Robinson & Williamson, 1977c). Nevertheless both in the present experiments and in those of Carrick & Kuhn (1978) there is no consistent relationship between changes in lactose synthesis and in the plasma concentration of insulin. Even injected insulin failed to restore lactose synthesis impaired by a short period of food withdrawal. On the basis of this evidence, therefore, we exclude insulin as being likely to regulate lactose synthesis in the short term, and a similar conclusion has been reached for the goat (Hove, 1978).

At first sight this conclusion seems at variance with the literature. Injected insulin has been reported to raise milk yields in late-lactating rats (Kumaresan & Turner, 1965a), and alloxan-diabetes was found grossly to impair pup growth (Walters & McLean, 1968; Martin & Baldwin, 1971a). However, lactose synthesis was not specifically measured by these authors, who in any case may have subjected the rats to an insulin status well beyond the physiological range. Insulin was reported to stimulate lactose synthesis directly by preparations of rat mammary gland in vitro (Martin & Baldwin, 1971a,b), but this was not observed by Hills & Stadie (1953) or by us (C. J. Wilde & N. J. Kuhn, unpublished work). In rats treated with alloxan during lactogenesis Kyriakou & Kuhn (1973) found no impairment of the tissue lactose content, despite a profoundly lowered concentration of plasma insulin. Further, Sud (1971) has challenged the importance of insulin for mammary growth in vivo, despite the stimulatory effect reported by Kumaresan & Turner (1965b) and the well-documented mitogenic effect of this hormone in vitro (see Forsyth, 1971). Insulin does stimulate glucose uptake by mammary tissue in vitro, but the effect is small, unless the uptake has been previously decreased by the addition of ketone bodies, and may be secondary to a stimulation of lipogenesis (Williamson et al., 1975; Robinson & Williamson, 1977c). The lack of any clear evidence for a role of insulin in lactose synthesis contrasts with its ability to stimulate lipogenesis in the same tissue. It becomes clear, therefore, that the synthesis of different milk components can no longer be assumed to enjoy a common hormonal control, and that future studies of lactational performance will require the specific measurement of the individual milk components in question.

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References

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