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Initiation of Fatty Acid Synthesis in Rat Mammary Glands

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The rate of fatty acid synthesis from [6-^{14}C]glucose in mammary tissue remained low until parturition at 22 days of gestation and increased 10-fold at 1 day *post partum*. Administration of progesterone on days 20 and 21 or removal of pups at parturition abolished this increase. In the latter case, administration of prolactin, corticosterone or oxytocin had no stimulatory effect; tissue from suckled glands in which the ducts had been ligated at parturition also showed no increased rate in 24h. Foetoplacentectomy on day 18 did not stimulate fatty acid synthesis but subsequent suckling by foster pups did. Whereas lactose synthesis is initiated by withdrawal of progesterone from the circulation, a further stimulus related to removal of milk by suckling is required to initiate fatty acid synthesis.

In the rat mammary gland that has developed in response to the hormones of pregnancy, lactogenesis is thought to be held in abeyance by the effects of progesterone. Before parturition the concentration of progesterone in plasma decreases; this decrease is followed by liberation of anterior-pituitary hormones, increased synthesis of RNA and casein and initiation of lactose synthesis in the gland (for reviews see Kuhn, 1971, 1977).

Tissue removed during subsequent lactation synthesizes fatty acids *de novo* (Bartley & Abraham, 1976); mainly C_8–C_{14} fatty acids are synthesized in either tissue slices (Abraham *et al.*, 1961; Bartley & Abraham, 1976) or dispersed cells (Kinsella, 1974). The precise time at which milk-specific fatty acid synthesis begins is not known, but increased activities of a number of lipogenic enzymes after parturition have often been reported (e.g. Howanitz & Levy, 1965; Gumaa *et al.*, 1973); it has also been shown that the increase in acetyl-CoA carboxylase activity is due to an increase in the concentration of enzyme (Mackall & Lane, 1977).

In this laboratory, lactose synthesis has been demonstrated in tissue taken 2 days before normal parturition (Nicholas & Hartmann, 1975) or as soon as 4h after foetoplacentectomy of 18 day-pregnant rats (P. E. Hartmann, personal communication). This paper presents evidence that the stimulus that initiates lactose synthesis is insufficient to initiate fatty acid synthesis; a further stimulus is required.

Materials and Methods

[6-^{14}C]Glucose (sp radioactivity 53.5Ci/mol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Ovine prolactin (NIH. PS 10, 30i.u./mg) was a gift from the Endocrinology Study Section, National Institutes of Health, Bethesda, MD, U.S.A. Corticosterone and progesterone were obtained from Sigma, St. Louis, MO, U.S.A., and oxytocin was from Parke–Davis, Sydney, N.S.W., Australia.

Female Wistar rats from our colony were mated overnight and fertilization was assumed if spermatozoa were present in vaginal smears early next day (day 0). They were housed at 22°C under a regular schedule of 14h artificial light/10h darkness and fed *ad lib.* on a standardized commercial diet (mainly mixed cereals, and containing 22% protein and 52% soluble carbohydrate by wt.) and tap water. Parturition in normal rats occurred on day 22, taken as day 0 of lactation. All injections and operations were performed after anaesthetizing the animals lightly with diethyl ether.

For measurement of fatty acid synthesis the rats, either pregnant or having delivered 9–13 pups, were killed by cervical dislocation. The inguinal mammary tissue from each side was prepared separately and incubated as described by Bartley & Abraham (1976), except that it was not chopped but finely minced with scissors. The substrate used throughout was 10μmol of [6-^{14}C]glucose (0.1μCi) in 1.0ml of bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932). Incorporation of [^{14}C]glucose was linear for at least 30min. After incubation, the tissue was washed thoroughly with water and the lipid was extracted by the method of Folch *et al.* (1957) and methanolysed with sodium methoxide (Glass, 1971). The methyl esters of fatty acids were extracted into hexane and their radioactivity was measured by

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liquid-scintillation spectrometry in 5ml of toluene containing 2,5-diphenyloxazole (4g/litre). Quenching was corrected for by the channels-ratio method of Bush (1963).

Milk for lipid analysis was manually expressed after subcutaneous injection of 2i.u. of oxytocin. Lipids were extracted with diethyl ether/ethanol (1:3, v/v) and methanolysed as described above, and the mixture of methyl esters was analysed by g.l.c. (Hansen *et al.*, 1969).

Statistical significance in this work was gauged by Student's *t* test.

Results

Inhibition of lactogenesis by progesterone

Under normal conditions the rate of incorporation of [6-^{14}C]glucose into fatty acids was very low until parturition but increased rapidly during the next few days (Fig. 1). The highest rates were obtained at mid-lactation and at this time the mean chain length of the synthesized acids, calculated by measuring the radioactivity of the individual acids separated by g.l.c., was 13–14.The slight decrease in the rate on day 4 was observed consistently and has also been noticed with lactose synthesis, but the explanation is unknown.

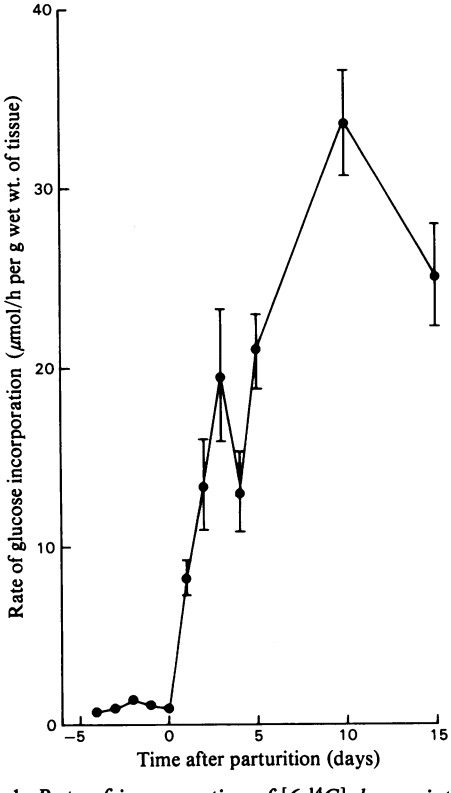

Fig. 1. *Rate of incorporation of [6-^{14}C]glucose into fatty acids by mammary tissue from rats before and after parturition*

About 100mg of minced tissue (accurately weighed) was incubated for 30min with 10μmol (0.1μCi) of [6-^{14}C]glucose in 1.0ml of Krebs–Henseleit bicarbonate buffer. Each value plotted represents the mean ± S.E.M. for seven to 14 individual observations (three to five animals).

Near the end of gestation in these rats the concentration of progesterone in plasma is about 40ng/ml. On day 20, when lactose synthesis begins, it falls to 30ng/ml and 24h before parturition is about 5ng/ml (Nicholas & Hartmann, 1975; Bartholomeusz *et al.*, 1976). In an attempt to delay the increase in fatty acid synthesis, rats were injected subcutaneously on day 20, and again on day 21, with 8mg of progesterone in peanut oil; control rats received oil only. At parturition, which was delayed to days 23–24 in treated rats, the size of each litter was adjusted to 12. On day 1 after parturition the rate of glucose incorporation into fatty acids by tissue from controls was 6.8 ± 1.5 (12) μmol/h per g; that from rats given progesterone was 1.07 ± 0.17 (10) μmol/h per g [means ± S.E.M.(*n*); $P < 0.01$], which is similar to the rate before parturition.

Effects of premature withdrawal of progesterone

When 18 day-pregnant rats are foetoplacentectomized, the concentration of circulating progesterone decreases to 30ng/ml in 4h and lactose synthesis begins. By 12h the progesterone concentration is 5–10ng/ml (Nicholas & Hartmann, 1975). However, as shown in Table 1, we found no stimulation of fatty acid synthesis within 96h of the operation.

One obvious difference between the glands from prematurely delivered and normal rats *post partum* was that the former were unsuckled. When prematurely delivered rats were given 12 foster pups (aged 1–2 days) between 2 and 4h after surgery, the rate of fatty acid synthesis increased progressively over 96h and was significantly greater ($P < 0.001$) than in unsuckled glands at all times more than 24h (Table 1). Suckling of 18 day-pregnant rats for 72h had no stimulatory effect on fatty acid synthesis and the rates of synthesis were similar to those in unsuckled glands after foetoplacentectomy (Table 1).

Suckling as a stimulus to fatty acid synthesis after parturition

Normally, young rats suckle very soon after birth. When the whole litter was removed before suckling could take place, the rate of glucose incorporation into fatty acid by mammary tissue 24h after parturition was less than 0.5μmol/h per g (10 observations).

Table 2 shows the fatty acid composition of milk

Table 1. *Rate of fatty acid synthesis by mammary tissue from 18 day-pregnant rats after foetoplacentectomy, suckling or both*

At zero time animals were either foetoplacentectomized, foetoplacentectomized and given 12 foster pups (aged 1–2 days) about 2–4 h afterwards, or exposed to suckling by 12 foster pups aged 5 days. Fatty acid synthesis was measured as in Fig. 1. Each value represents the mean ± S.E.M. (*n*) for individual observations on tissue from two to seven animals.

Time after intervention (h)	Glucose incorporation (μmol/h per g wet wt. of tissue)		
	Foetoplacentectomized	Foetoplacentectomized and suckled	Pregnant and suckled
0	0.6 ± 0.1 (10)		
6	0.8 ± 0.1 (4)		
24	1.3 ± 0.2 (7)	1.1 ± 0.1 (12)	
48	0.4 ± 0.1 (15)	3.2 ± 0.3 (10)	0.7 ± 0.0 (4)
72	0.2 ± 0.1 (8)	10.2 ± 1.5 (8)	0.4 ± 0.0 (4)
96	0.0 (8)	13.4 ± 1.4 (7)	

Table 2. *Fatty acid composition of milk lipid after parturition*

Milk was collected by manual expression after injection of 2 i.u. of oxytocin. Unsuckled rats were those separated from their pups at parturition. Mean values are shown, with the numbers of rats given in parentheses. Acids comprising ≤1% of the total in every sample analysed have been omitted from the Table.

Fatty acid	Fatty acid composition (wt. %)						
	Unsuckled		Suckled				
	Day 0 (5)	Day 1 (4)	Day 1 (4)	Day 2 (2)	Day 3 (6)	Day 5 (3)	Day 15 (3)
$C_{8:0}$	1.3	1.7	3.8	3.6	2.9	2.6	4.9
$C_{10:0}$	2.5	2.8	7.0	7.8	11.6	10.2	17.7
$C_{12:0}$	1.4	1.2	3.3	6.4	8.4	9.5	13.3
$C_{14:0}$	3.0	2.2	3.0	4.9	7.0	9.1	12.1
$C_{16:0}$	29.3	27.9	24.7	22.2	21.2	23.1	20.9
$C_{16:1}$	7.5	6.0	5.7	4.9	4.4	3.3	2.5
$C_{18:0}$	4.9	5.2	4.5	3.0	4.6	3.8	4.3
$C_{18:1}$	34.1	34.4	31.4	27.9	26.9	22.3	13.6
$C_{18:2}$	11.0	12.9	11.9	14.2	9.7	12.7	9.1
$\Sigma(C_8–C_{14})$	8.2 ± 0.8	7.9 ± 1.4	17.1 ± 0.7*				

* Mean ± S.E.M., statistically significant when compared with day 0 ($P < 0.001$) and with day 1 'unsuckled' ($P < 0.01$).

lipids from parturition to day 15 and also on day 1 in unsuckled glands; the composition is shown as wt.%, as this directly reflects the number of acetyl units incorporated into synthesized acids. From day 1, the medium-chain fatty acids (C_8–C_{12}) and C_{14} fatty acid, which are synthesized in the mammary tissue, constitute a much greater proportion of the total than they do on day 0. However, the proportion in the secretion from unsuckled glands on day 1 was not significantly different from that on day 0. These results agree well with data on the rate of incorporation of glucose.

As the effect of suckling may be mediated through hormones, unsuckled dams were given one of the following treatments: three injections of 50 i.u. of prolactin in 0.15 M-NaCl, either 0.5 ml subcutaneously or 1.0 ml intramuscularly; or three injections of 1 mg of corticosterone in 0.5 ml of peanut oil subcutaneously [these hormones (or vehicle only) were injected at parturition and again at 10 and 20 h afterwards]; or one injection of oxytocin (2 i.u.) subcutaneously 24 h after parturition.

The rats were killed 4 h after the last injection of prolactin or corticosterone or 5 min after injection of oxytocin, and incorporation of [6-^{14}C]glucose was measured in the usual way. The rate of incorporation was again less than 0.5 μmol/h per g of tissue (8–14 observations in each case); this indicates that suckling may stimulate fatty acid synthesis by some means other than mere release of these hormones.

To test the effects of suckling without milk removal, the ducts of the right-hand-side abdominal and inguinal mammary glands of four unsuckled

parturient rats were ligated through an incision in the adjacent skin, and the contralateral glands were sham-operated. The rats were then allowed to suckle 12–14 pups for 24h. The rates of incorporation of [6-^{14}C]glucose into fatty acids were 0.5 ± 0.01 and 7.3 ± 0.9 μmol/h per g of tissue (means ± S.E.M., 12 observations) in the suckled ligated glands and the sham-operated glands respectively.

Discussion

Lactogenesis in the rat is generally held to occur shortly before parturition. The main reason for this is that a number of morphological and metabolic changes, and synthesis of casein and lactose, can be observed at this time. That lactose synthesis occurs in response to a decreased concentration of progesterone in plasma seems to be confirmed by experiments in which the concentration was artificially altered (Kuhn, 1971, 1977).

The present work shows clearly that fatty acid synthesis is not stimulated before parturition. Although electron microscopy has shown fat droplets in the epithelial cells from day 6 of gestation (Murad, 1970) and in the alveolar lumen about 8h before parturition (Chatterton *et al.*, 1975), in our experiments the rate of fatty acid synthesis *in vitro* remained low right up to the time of parturition; 1 day later it had increased 10-fold. This increase was abolished by injecting small amounts of progesterone, which delayed parturition slightly but otherwise caused minimal disturbance of behaviour.

After normal parturition or foetoplacentectomy, although the plasma progesterone concentration is low, suckling with milk removal is necessary to stimulate fatty acid synthesis. This is also demonstrated by the large increase in the proportion of medium-chain fatty acids in milk that occurs on suckling the gland after parturition. Suckling is said to cause release of prolactin (Subramanian & Reece, 1975), oxytocin (Lincoln *et al.*, 1973), corticotropin and adrenocorticoids (Meites, 1959; Cowie & Folley, 1961) into the circulation, but administration of hormones does not seem to replace suckling as a stimulus for fatty acid synthesis. Glands that were obstructed, but suckled in the same way as sham-operated contralateral glands, should have been subject to the same neural and hormonal influences; their failure to respond in the same way suggests that milk removal is required. The lack of fatty acid synthesis in suckled glands in late pregnancy is not easily explained. On days 18–20 the progesterone concentration is high. On day 21 it is low enough to allow lactose synthesis, but perhaps the structural state of the glands does not allow adequate milk removal for the initiation of fatty acid synthesis. Alternatively, initiation by suckling may always lag behind the withdrawal of progesterone since, in both normal parturient and prematurely delivered rats that are suckled, fatty acid synthesis appears to begin around 24h after the concentration in plasma first reaches about 5ng/ml.

It appears that development of fatty acid synthesis in the rat differs radically from that in the rabbit, where it is biphasic, occurs before parturition (Strong & Dils, 1972) and parallels lactose synthesis (Mellenberger & Bauman, 1974). The inhibitory effects of progesterone are not clearly understood; although it suppresses release of pituitary hormones, it also acts directly on the mammary gland (Kuhn, 1977) and possibly does this by interacting with specific progesterone receptors rather than by competing for glucocorticoid receptors (Shyamala & McBlain, 1979). Whether suckling removes inhibitory substances or stimulates lipogenesis in some other way is unknown, but the factors involved are not necessarily the same as those that decrease fatty acid synthesis on weaning after established lactation (Abraham & Chaikoff, 1959; Levy, 1964; Agius *et al.*, 1979).

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