Milk composition of rats feeding restricted litters

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1. Milk samples were taken from rats feeding ten pups and from both the suckled and non-suckled glands of rats feeding two pups. 2. The lipid, protein and lactose concentrations were similar in the milks from the secreting glands, but the fluid from the non-suckled glands contained less lactose and lipid but significantly higher total protein and transferrin concentrations. 3. The fatty acid compositions of the milk from the three sources were very similar. 4. The mammary tissue from the rats feeding ten pups had a higher DNA content/g wet wt. than either the suckled or non-suckled mammary tissue of the rats feeding two pups. 5. The specific activities of several lipogenic enzymes were significantly lower in the non-suckled mammary tissue.

INTRODUCTION

Previous studies from our laboratory and others have shown that lactating rats have the ability to adjust the milk supply to the demand for milk. In the rat this demand can be manipulated simply by altering the number of pups to be fed. Thus when litter sizes are reduced from ten to two, either within 24 h of parturition or at mid-lactation, there appears to be an equivalent reduction in the milk supply as determined from the growth rates of the pups (Grigor *et al.*, 1982, 1984). Only one report described changes in milk composition brought about by reducing the litter size of rats (Yagil *et al.*, 1976), and this emphasized the electrolyte concentrations. The present study was performed to extend these observations to measurements of the concentrations of the macronutrients in milk obtained from rats suckling ten pups or two pups. In the course of the study it was observed that rats suckling two pups secreted milk from a limited number of nipples. However, after injection of large doses of oxytocin, it was possible to express a milk-like fluid from the non-suckled nipples. The composition of this fluid has also been examined.

EXPERIMENTAL

Animals

Rats of the Wistar strain were purchased from the University of Otago Animal Breeding Station and mated at an age of 3–4 months. Litter sizes were adjusted to ten or two within 48 h of parturition. Between days 12 and 16 of lactation, milk samples were taken from the lactating rats. For this, rats were anaesthetized with diethyl ether, injected with oxytocin (1 unit of Syntocinon; Sandoz, Basel, Switzerland) and milk was expressed manually from the nipples on one side only. Between 0.5 and 1.0 ml of milk was obtained from each animal and care was taken to ensure milk samples were taken within 1 h of the removal of the pups. After milking, the rats were killed and the mammary tissue from the side that had not been milked was removed, homogenized, and the 100 000 g/60 min supernatants were prepared as previously described (Grigor *et al.*, 1982). Samples of the mammary homogenates were retained for DNA analysis (Brunk *et al.*, 1979). Throughout the experiment,

rats were fed a commercial pelleted diet (A. E. Reeves Ltd., Dunedin, New Zealand) and maintained on a 12 h light/12 h dark cycle such that animals were milked and killed at the mid-dark period.

Analysis of milk

Total milk lipids were extracted and analysed as previously described (Grigor & Warren, 1980). Lactose was measured enzymically (Kuhn & Lowenstein, 1969) and total protein by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. α -Lactalbumin was measured immunochemically by single radial immunodiffusion (Mancini *et al.*, 1965). For this, α -lactalbumin was purified as described by Nicholas *et al.* (1981) and antisera were raised to it in rabbits. The purified protein migrated as a single band when examined by electrophoresis on 15%-polyacrylamide gels containing SDS (Laemmli, 1970). Its identity was determined by partial sequence analysis on a Beckman 890C (updated) sequencer using the 0.1 M-Quadrol program described by Brauer *et al.* (1975). The sequence of the first 48 residues was identical in 38 positions with a previously published partial amino acid sequence (Prasad *et al.*, 1979) and identical in all positions with the predicted sequence from analysis of the DNA sequence (Dandekar & Qasba, 1981; Qasba & Safaya, 1984). The antiserum raised against the purified protein gave a single precipitin line with whole rat milk proteins in either a double-diffusion assay or the radial-diffusion assay. The serum-albumin and transferrin concentrations of the milk were determined after pooled samples of whole milk were separated by electrophoresis on 15%-polyacrylamide gels containing SDS. The gels were stained with Coomassie Blue and the relative distribution of the major protein components was determined by cutting and weighing peaks after densitometry of the electrophoretograms. Care was taken to use concentrations of the milk where the peak areas were proportional to the amount of milk applied.

Enzyme analyses

Fatty acid synthase, 'malic' enzyme, lactate dehydrogenase and malate dehydrogenase were all assayed spectrophotometrically as described previously (Grigor *et al.*, 1982). Glucose-6-phosphate dehydrogenase and

6-phosphogluconate dehydrogenase were assayed similarly by using the two-substrate method. Concentrations of 1 mM-glucose 6-phosphate and 2.5 mM-6-phosphogluconate and 65 μ M-NADP⁺ were used. One unit of enzyme activity is defined as 1 μ mol of NADP⁺ reduced or 1 μ mol of NADH oxidized/min. Substrates for the assays were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Statistics

All the results presented are means $\pm$ S.E.M. for between five and eight samples in each group. The significance of differences was determined by using Student's *t* test.

RESULTS AND DISCUSSION

All twelve nipples of the rats suckling ten pups were distended, indicating that all were secreting milk. By contrast, in the rats feeding only two pups, the number

of nipples suckled varied from four to six, and the size of these nipples was considerably smaller than those of the rats feeding ten pups. The non-suckled nipples of the rats feeding two pups were, however, no larger than those of late-pregnant animals. Yet it was possible to express a milk-like fluid from these nipples after injection of oxytocin, and up to 0.2 ml of this was obtained from each rat.

Table 1 lists the concentrations of the major components of the mammary secretions. The milk obtained from the suckled nipples of the rats feeding two pups was not different from that of rats feeding ten pups in any of the parameters examined, apart from the concentration of α -lactalbumin, which was significantly higher. However, the fluid from the non-suckled nipples of the rats feeding two pups contained significantly less lactose and total lipid. By contrast, the protein content was significantly higher than that for the rats feeding ten

Table 1. Milk composition of rats suckling ten and two pups

Values are means $\pm$ S.E.M. for samples from the numbers of rats indicated, except for those for transferrin and serum albumin, which were determined on pooled samples as described in text. Values where difference is statistically significant ($P < 0.01$) from that for the ten-pup sample are marked *, and from the two-pup suckled sample, †.

Component	Suckling conditions...	Composition (mg/ml of whole milk)		
		Ten pups	Two pups	
	No. of rats...	9	Suckled glands 6	Non-suckled glands 6
Lactose		38 $\pm$ 2	34 $\pm$ 2	11 $\pm$ 4*†
Lipid		168 $\pm$ 9	207 $\pm$ 22	98 $\pm$ 10*†
Total protein		80 $\pm$ 4	98 $\pm$ 5	105 $\pm$ 5*
α -Lactalbumin		3.3 $\pm$ 0.3	4.4 $\pm$ 0.2*	3.0 $\pm$ 0.3†
Percentage of Coomassie Blue-staining protein				
Transferrin		5	3	13
Serum albumin		11	10	11

Table 2. Milk fatty acid composition of rats suckling ten and two pups

Values are means $\pm$ S.E.M. for samples from the numbers of rats indicated, and where difference is statistically significant ($P < 0.05$) from that for the ten-pup sample the value is marked *.

Fatty acid	Suckling conditions...	Composition (mol/100 mol of total fatty acid)		
		Ten pups	Two pups	
	No. of rats...	5	Suckled glands 6	Non-suckled glands 5
C _{8:0}		8.8 $\pm$ 1.1	9.4 $\pm$ 0.4	10.5 $\pm$ 0.8
C _{10:0}		21.9 $\pm$ 1.2	21.3 $\pm$ 0.9	22.7 $\pm$ 0.6
C _{12:0}		13.8 $\pm$ 0.7	10.7 $\pm$ 0.7*	10.7 $\pm$ 0.6*
C _{14:0}		10.2 $\pm$ 1.3	6.6 $\pm$ 1.5*	6.3 $\pm$ 0.6*
C _{16:0}		17.1 $\pm$ 1.4	15.0 $\pm$ 0.5	15.3 $\pm$ 0.6
C _{16:1}		1.3 $\pm$ 0.1	1.8 $\pm$ 0.4	1.6 $\pm$ 0.7
C _{18:0}		2.7 $\pm$ 0.2	4.8 $\pm$ 0.3*	5.0 $\pm$ 0.6*
C _{18:1}		12.9 $\pm$ 1.3	16.5 $\pm$ 1.1	16.1 $\pm$ 0.6
C _{18:2}		6.7 $\pm$ 0.3	9.1 $\pm$ 0.3	7.9 $\pm$ 0.5
Others		4.5 $\pm$ 0.5	4.6 $\pm$ 2.6	3.9 $\pm$ 0.6

Table 3. Mammary enzyme content of rats suckling ten and two pups

Values are means $\pm$ S.E.M. for samples from the numbers of rats indicated. Values where difference is statistically significant ($P < 0.01$) from that for the ten-pup sample are marked * and from the two-pup suckled sample, †.

Enzyme	Suckling conditions...	Enzyme content (units/g)		
		Ten pups	Two pups	
	No. of rats...		Suckled glands	Non-suckled glands
		8	6	6
Glucose-6-phosphate dehydrogenase		16.2 $\pm$ 2.7	14.7 $\pm$ 2.9	1.6 $\pm$ 0.3*†
6-Phosphogluconate dehydrogenase		5.1 $\pm$ 0.5	4.3 $\pm$ 0.7	0.6 $\pm$ 0.1*†
'Malic' enzyme		5.1 $\pm$ 1.1	3.1 $\pm$ 0.8	0.5 $\pm$ 0.1*†
Lactate dehydrogenase		54 $\pm$ 4	59 $\pm$ 9	38 $\pm$ 5
Malate dehydrogenase		94 $\pm$ 6	78 $\pm$ 4	42 $\pm$ 3*†

pups. Of the milk proteins, the transferrin content was much higher in the fluid from the non-suckled nipples (Table 1), whereas no differences in the pattern of other proteins were apparent after densitometric scanning of the electrophoretograms.

The fatty acid analyses of the mammary secretions are listed in Table 2. In the rat the fatty acids made in the mammary gland can be distinguished from those derived from the circulation by their medium chain length (Dils & Popjak, 1962; Smith & Ryan, 1979; Grigor & Warren, 1980). All samples analysed had similar proportions of the total medium-chain-length acids, amounting to some 50 mol/100 mol of total fatty acids, although there were significant differences in the proportions of dodecanoic (lauric) acid and myristic acid, which were higher in the milk from the rats feeding ten pups than in either sample from the rats feeding two pups.

The results show that, whereas the suckled glands of the rats feeding ten or two pups produced milk of a very similar composition, the fluid expressed from the non-suckled glands contains all the major milk components, but at different concentrations. Clearly, then, these glands are capable of producing milk, even though they are obviously not secreting milk. Further, it is of interest that these glands can also produce a lipid that has retained the same proportion of fatty acids synthesized *de novo* to those taken up from the circulation as found in the milk from the secreting glands.

Recently, we reported on changes in the content of several soluble enzymes in the mammary tissue of rats after changes in litter size from ten to two, or two to ten, at mid-lactation (Grigor *et al.*, 1984). That study showed that, after the increase in litter size, milk production increased over the next 72 h and the content of several lipogenic enzymes also increased. We also suggested that the mammary tissue in the rats feeding two pups was at a uniform state of development. On closer examination this has been shown not to be the case. Therefore we have re-examined the enzyme levels and DNA content of the suckled glands from rats feeding ten or two pups and the non-suckled glands from rats feeding two pups.

Highly significant differences in the DNA content per g wet wt. were observed. The values obtained ranged from 688 ± 65 μ g/g ($n = 6$) for the glands of the rats feeding ten pups to 270 ± 40 ($n = 6$) and 109 ± 20 ($n = 6$) μ g/g for the

suckled and non-suckled glands of the rats feeding two pups. This suggests that the proliferation of the epithelial tissue in the rats with large litters is much greater than in the suckled glands of the rats with just two pups, which in turn is greater than that of the non-suckled glands.

The activities of several enzymes expressed per g wet wt. were not significantly different in the suckled glands of the rats feeding ten or two pups (Table 3). By contrast, the activities of all the enzymes, particularly the lipogenic enzymes, were much lower in the non-suckled glands when expressed on either a per-mass basis or a per-DNA basis. This shows clearly that these cells have not fully differentiated and resemble the cells found in the glands of late-pregnant rats.

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