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Changes in Milk Carbohydrates and Electrolytes during Early Lactation in the Tammar Wallaby, *Macropus eugenii*

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Abstract

Milk samples were collected from 13 tammar wallabies immediately post partum and from a further 10 animals at various times up to 3 weeks after birth.

Changes in milk carbohydrates were assessed by thin-layer chromatography and by determination of the concentrations of total hexose and free lactose. The only carbohydrate found in the milk on day 1 post partum was lactose. Higher oligosaccharides began to appear on day 4; their concentration increased gradually thereafter, while that of lactose decreased. There was a steady increase in the total hexose concentration from a mean of 3·2% (w/v) on day 1 to 7·0% on day 21. No differences with respect to carbohydrates were observed between milk samples obtained from suckled and unsuckled glands.

There was a significant decrease in the sodium concentration of the milk during the first week post partum, but the osmotic concentration remained unchanged.

Extra keywords: lactose, marsupials, oligosaccharides

Introduction

Several studies have shown that there are marked changes in milk composition during the course of lactation in marsupials (Gross and Bolliger 1959; Lemon and Barker 1967; Griffiths *et al.* 1972; Messer and Green 1979; Green *et al.* 1980; Green 1983; McKenzie *et al.* 1983). These studies, however, have been concerned mostly with changes which occur from mid to late lactation, mainly because of the difficulties involved in obtaining sufficient quantities of milk from a limited number of animals during the first few days after birth.

Recent work by Findlay *et al.* (1983) showed that at parturition, mammary gland tissue of the tammar wallaby contains only lactose and glucose, whereas a previous study had shown that tammar wallaby milk contains various higher oligosaccharides from 2 weeks post partum (Messer and Green 1979). Findlay *et al.* (1983) therefore suggested that synthesis of the higher oligosaccharides is delayed.

The present investigation was designed primarily to determine the stage of lactation at which higher oligosaccharides first appear in the milk of the tammar wallaby, but also includes quantitative data on carbohydrates, sodium, potassium and osmoconcentration of the early milk.

Material and Methods

Milk was collected as described previously (Messer and Green 1979; Green *et al.* 1980) except that the milk was taken into microhaematocrit tubes. During the first few days post partum only 10–20 μl of milk could be collected from each of the suckled glands, and less from the unsuckled

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glands. Milk from the unsuckled glands of an animal was pooled in each case. Two groups of animals were used:

(1) 13 animals which were milked on day 1*, in which the young were not replaced, and the samples were pooled, and
(2) 10 animals which were milked from day 2 onwards and in which attempts were made to replace the young, though not always with success. Thus one animal (5581) provided milk on four separate occasions, three (5698, 5476 and 5646) on three occasions, but the other six animals each provided milk only once.

For the analysis of carbohydrates, 10 μl of milk were diluted to 100 μl with water. Of the diluted milk, 1-μl samples were applied directly to thin-layer chromatography plates to monitor qualitative changes in the pattern of milk oligosaccharides (Hansen 1975). Some of the diluted milk was further diluted (to 100-fold of the original milk) for assay of total hexose by a modified phenol-sulfuric acid method (Messer and Green 1979). The remainder was subjected to serial dilutions (20-fold, 40-fold and 80-fold), 2 μl of each of which were used for the semiquantitative assay of free lactose by thin-layer chromatography, using 2-μl samples of various concentrations of lactose (0·05–0·40% w/v) as standards. After chromatography, the intensities of the lactose spots of the four diluted milk samples were compared visually with those of the lactose standards to obtain a mean value for lactose. Control experiments showed that differences between duplicate samples were no greater than 20%. Because of the small volume of milk available, no other assay which was specific for lactose as distinct from other β-galactosides, such as 3′-galactosyllactose (Messer *et al.* 1980) and its homologues (Collins *et al.* 1981), was readily applicable.

For the assay of sodium and potassium, 10 μl of milk were taken up in a Microcap micropipette and delivered into 3 ml of de-ionized water. Sodium and potassium concentrations were then determined with a Varian 1000 atomic absorption spectrophotometer using an acetylene–air gas mixture. The osmoconcentration of milk was measured with a Wescor 5100C vapour pressure osmometer using undiluted 8-μl milk samples.

Results

The milk obtained on the first day post partum was quite transparent, but from day 2 onwards it developed an opaline appearance. By day 7 the milk always had a white appearance.

Fig. 1 shows qualitative changes in carbohydrates of the milk observed during the first 21 days after birth. Up to day 3 post partum the only carbohydrate found was lactose. Higher oligosaccharades first appeared on day 4 and increased gradually thereafter while the lactose content decreased. The first higher oligosaccharides to appear migrated as tri- and pentasaccharides. There were no obvious differences in the carbohydrate patterns between milk from suckled and unsuckled glands of the same animal (cf. Figs 1*A* and 1*B*).

Quantitative changes in total hexose and free lactose in milk are shown in Fig. 2. There was a steady increase in total hexose from just over 3% (w/v) on day 1 to 7% on day 21. From day 4 onwards there was a concomitant decrease in the lactose concentration to less than 1% of whole milk by day 21, so that lactose, which represented over 90% of the total hexose on days 1 and 2, constituted only about 50% on days 7–9 and 10% on day 21. No clear differences in total hexose between milk from suckled and unsuckled glands of the same animal could be detected.

Fig. 3 shows how the results for total hexose relate to previous data (Messer and Green 1979) for the period from 1 week to 44 weeks post partum. It is seen that the increase in carbohydrate content during the first 3 weeks is quite steep when compared with the more gradual increase previously observed between weeks 5 and 26.

The osmotic concentration and sodium and potassium ion levels in milk from suckled glands are presented in Table 1. During the first 3 days of lactation the mean sodium concentration in milk was significantly higher than that found during days 4–7 post partum

*Day 1 was taken to be the first day on which a pouch young was found.

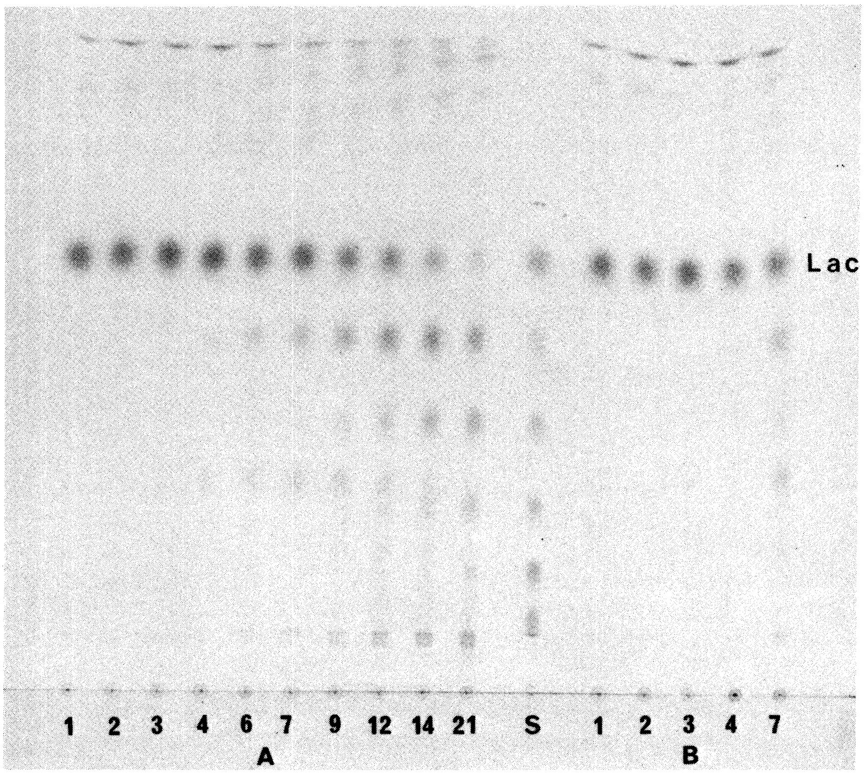

Fig. 1. Photograph of thin-layer chromatogram of carbohydrates of milk from (*A*) suckled and (*B*) unsuckled glands of the same animals. The numbers refer to days post partum. Milk from the following animals was used (code numbers in parentheses): day 2 (5661), day 3 (5581), day 4 (5698), days 7, 14 and 21 (5581), days 9 and 12 (5646). Milk obtained on day 1 was pooled from a separate set of 10 animals. S = standard mixture of lactose (*Lac*), galactosyllactose, digalactosyllactose, trigalactosyllactose, tetragalactosyllactose and pentagalactosyllactose (Collins *et al.* 1981). The material which migrated close to the solvent front could not be identified.

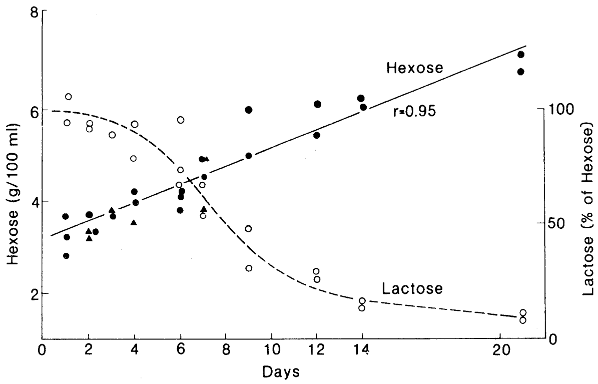

Fig. 2. Total hexose and relative proportions of lactose in milk in the early stages of lactation. ● Suckled glands. ▲ Unsuckled glands. The line for hexose was determined by least-squares linear regression. The curve for lactose was fitted by eye.

(P<0·01). The latter mean sodium concentration is not significantly different from that described previously for 1-week milk (Green *et al.* 1980). The concentration of potassium

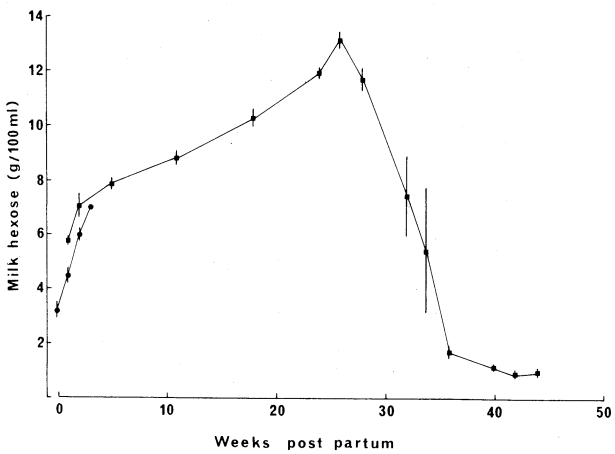

Fig. 3. Carbohydrate content (total hexose, means ± s.e.m.) of tammar wallaby milk during early, mid and late lactation. ● Data from present study: the values for weeks 0, 1, 2 and 3 are the means of the results (suckled and unsuckled glands, Fig. 2) for days 1, 7, 12 plus 14 and 21 respectively. ■ Data from a previous study (Table 1; Messer and Green 1979). Note that in the previous study, s.d. values were erroneously labelled s.e.m., and that the values for s.e.m. shown in Fig. 3 have been recalculated from the previous data.

did not appear to change during the first week of lactation. A gradual increase of potassium concentration in the milk is nevertheless discernible, the mean potassium concentrations up to 3 days post partum being significantly lower (P<0·05) than those found at 2 weeks by Green *et al.* (1980).

Table 1. Sodium and potassium ion concentrations and osmolarity of tammar wallaby milk during the first 2 weeks post partum

All values are means ± s.d. for milk from suckled glands. Levels of significance are placed between the means being compared

Days post partum	No. of samples	Na (mmol)	K (mmol)	Osmoconcn (mOsmol/1)[A]
1–3	6	62·8±10·0	27·0±7·4	292±10
		P<0·01	n.s.	n.s.
4–7	7	44·4± 3·6	29·1±7·8	285± 5
		n.s.	P<0·05	n.s.
7–14[B]	6	46·5± 2·4	33·2±3·8	278± 2[C]

[A] Value for plasma = 284±7 (Green *et al.* 1980).
[B] Values from Green *et al.* (1980).
[C] At day 14.

The sodium and potassium concentrations in the milk obtained from unsuckled glands were higher and lower respectively than those found in milk from suckled glands from day 2 onwards, maintaining similar Na/K ratios as exhibited in 1-day milk (mean Na/K = 3·3±1·5). The mean Na/K ratio in milk from suckled glands was 1·5±0·6, significantly lower than that of milk from unsuckled glands ($P<0\cdot001$) but similar to that found in tammar milk in later stages of lactation (Green *et al.* 1980).

The osmotic concentrations of milk from suckled and unsuckled glands did not differ significantly and were similar to those previously described by Green *et al.* (1980) for milk and maternal plasma from tammar wallabies.

Discussion

Although there are numerous studies on the composition of colostrum and early milk in various species of eutherian mammals (e.g. Luckey *et al.* 1954; Ling *et al.* 1961; Kulski and Hartmann 1981) the only previous data on marsupials are those of Griffiths *et al.* (1972) concerning the lipids of milk of the red kangaroo.

This study has clarified the overall pattern of changes in the carbohydrate fraction of tammar wallaby milk during the course of lactation. At parturition the milk carbohydrate consists only of the disaccharide lactose, but tri- and pentasaccharides appear after about 4 days post partum and there is an increase in the concentration of total hexose. Over the next 26 weeks the hexose concentration continues to increase and progressively larger neutral oligosaccharides appear in the milk in place of lactose until tri- to heptasaccharides represent the bulk of the hexose, along with the sialyl saccharides (Messer and Green 1979). Between 26 and 32 weeks post partum the larger oligosaccharides decline in abundance while shorter saccharides again predominate and there is a major decline in total hexose content in the milk, after which all oligosaccharides disappear leaving only monosaccharides at low concentrations until the end of lactation (Messer and Green 1979).

Our finding that lactose is the only carbohydrate present in the milk until day 4 post partum is in line with the observations of Findlay *et al.* (1983) with respect to the carbohydrate content of the mammary gland at parturition. It is apparent that the enzymatic machinery required for the synthesis of higher oligosaccharides by the mammary gland is absent or non-functional at parturition and develops only gradually during the first 2 weeks after birth.

Although unsuckled mammary glands usually cease milk secretion by the fourth day post partum it was possible to obtain milk from some unsuckled glands for up to 7 days. The composition of the milk from unsuckled glands was basically similar to that from the suckled glands except that the Na/K ratios remained high in unsuckled glands. The reason for this is not known.

Acknowledgments

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Corrigendum

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Qualitative and quantitative changes in milk fat during lactation in the tammar wallaby (*Macropus eugenii*)

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p. 459, para. 4, line 5: *for* $C_{18:0}$–$C_{20:0}$ *read* $C_{8:0}$–$C_{10:0}$