

Milk Carbohydrates of Marsupials II.* Quantitative and Qualitative Changes in Milk Carbohydrates during Lactation in the Tammar Wallaby (*Macropus eugenii*)

M. Messer^A and B. Green^B

^ADepartment of Biochemistry, University of Sydney, N.S.W. 2006.

^BDivision of Wildlife Research, CSIRO, P.O. Box 84, Lyneham, A.C.T. 2602.

Abstract

Milk was collected at various stages of lactation from a group of tammar wallabies, *M. eugenii*, in which parturition had been synchronized.

The milk carbohydrate was determined by a phenol-sulfuric acid method which had been modified to give equal colour yields for galactose and glucose. The mean carbohydrate content increased gradually during the first 6 months of lactation to a peak of 13 g hexose/100 ml of milk, but then fell rapidly to much lower values, over the following 2 months.

Throughout lactation, galactose was the predominant monosaccharide constituent of acid hydrolysates of the milk carbohydrate. Glucose, glucosamine, galactosamine and sialic acid were the only other monosaccharides present.

Qualitative changes were investigated by gel filtration and thin-layer chromatography. During the first 6 months *post partum* the milk carbohydrate was composed of a variety of oligosaccharides including lactose, but from 8 months onwards it consisted mainly of free monosaccharides. Between 6 and 8 months an intermediate pattern was observed, i.e. a mixture of lower oligosaccharides and free monosaccharides. In two animals which suckled both a new-born pouch young and a young at foot, the mammary gland supplying the new-born secreted milk which was rich in oligosaccharides, whereas that supplying the young at foot produced milk in which the carbohydrates were mainly free monosaccharides, and which had a much lower carbohydrate content.

Introduction

The carbohydrate composition of marsupial milk is known to be rather unusual. Unlike the milk of eutherians, in which a disaccharide, lactose, is the principal carbohydrate, that of marsupials contains a variety of saccharides of which lactose, when present, is a relatively minor constituent (Bolliger and Pascoe 1953; Gross and Bolliger 1958, 1959; Jenness *et al.* 1964; Bergman and Housley 1968). Although the detailed structures of these saccharides are still unknown, the principal carbohydrates of milk from the grey kangaroo, *Macropus giganteus*, were recently found to be neutral tri- to hexasaccharides, in each of which galactose was the major monosaccharide (Messer and Mossop 1977).

Limited data led Jenness *et al.* (1964) to suggest that the stage of lactation might be a major factor influencing the composition of the carbohydrates of marsupial milk.

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In this paper we describe marked quantitative as well as qualitative changes in milk carbohydrates at different stages of lactation in the tammar wallaby, *Macropus eugenii*.

Materials and Methods

Milk Samples

The milk was obtained from a group of animals in which parturition had been synchronously induced in May 1977 by simultaneous removal of their pouch young during the previous month. The animals were kept in large enclosures of about 250 m² and at regular intervals lactating females were caught and placed in hessian sacks. The pouch young were removed from the teat by gentle but firm pressure on the sides of the mouth and placed in a humid incubator at 35°C. Larger pouch young were placed in small calico bags and wrapped in towelling. The young were kept from their mothers for about 6 h to allow milk to accumulate in the mammary glands. The mothers were then anaesthetized by intravenous injection of sodium methohexital (12 mg/kg) into the lateral tail vein. The teats were cleaned with water and tissue paper, after which the animals were given an intramuscular injection of oxytocin (0.7 i.u./kg). Milk started to appear within 1 min but the flow was greatly assisted by the slight pressure of fingers along the teat. The milk was collected in small plastic vials and stored at -20°C prior to analysis. After milking, the pouch young were assisted in taking the teat into the mouth. In the earliest stages of development it was necessary to force the end of the teat into the mouth by means of a tapered matchstick.

Determination of Milk Carbohydrate

The carbohydrate content of the milk samples was determined as total hexose using a modification of the phenol-sulfuric acid method of Dubois *et al.* (1956). Barnett and Tawab (1957) showed that this method can be used for the direct determination of lactose in diluted cow's milk, but in the original method equal amounts of glucose and galactose do not give the same absorbances (Dubois *et al.* 1956). This is a potential source of error in the analysis of milk, such as that of marsupials, which contains unequal amounts of galactose and glucose. One is faced with the same problem with at least three other methods for total hexose; with the orcinol-sulfuric acid method, galactose gives a higher colour yield than glucose (Francois *et al.* 1962), whereas with both the anthrone and the cysteine-sulfuric acid methods, it gives a lower colour yield (Morris 1948; Dische 1962).

Fig. 1 shows that with the phenol-sulfuric acid method, either galactose or glucose may give the greater colour yield, depending on the final concentration of phenol employed. The method could therefore be modified by adjusting the phenol concentration to a value, 0.85% (w/v), at which equal amounts of galactose and glucose gave equal absorbances.

In the modified procedure, a 0.20-ml sample of diluted milk, containing up to 100 µg of hexose, was mixed with 1.0 ml of 3.55% (w/v) phenol solution in a 160 by 13 mm test tube. Concentrated sulfuric acid, 3.0 ml, was then added rapidly from a dispenser, the stream of acid being directed against the liquid surface to obtain good mixing and maximum generation of heat. After further mixing, the solution was allowed to cool over 30 min, and its absorbance was then measured at 490 nm. Lactose was used as the standard.

Comparison of this method with the anthrone procedure (Roe 1955), showed that the two methods gave the same values for the carbohydrate content of the milk of eutherians (cow, rat, human) but the present method gave higher values (by 9-12%) for that of the tammar wallaby and the red kangaroo (*Macropus rufus*). The discrepancy can be ascribed to the fact that galactose predominates over glucose in marsupial milk, so that the anthrone method gives spuriously low values.

Jenness and Sloan (1970) determined the milk carbohydrate content of several species of marsupials, using a modified phenol-sulfuric acid method (Marier and Boulet 1959) in which the final phenol concentration is 1.1% and the acid is added slowly down the side of the tube. We found that when the acid was added slowly, lower absorbances were obtained, possibly because less heat was generated (Fig. 1). With the method of Marier and Boulet (1959), the absorbance given by galactose was lower than that given by glucose (Fig. 1), and consequently the values obtained by Jenness and Sloan (1970) were probably underestimates.

Since the estimations of milk carbohydrate were done on diluted whole milk, the results could include contributions made by hexose bound to milk proteins or lipids. Control experiments were therefore done in which the hexose content of tammar wallaby milk, obtained 4 months *post partum*, was compared before and after removal of the protein and lipid by two-phase extraction with chloroform-methanol (Öhman and Hygstedt 1968). The results showed that any contributions made by such bound hexoses were below the limit of detectability of the method.

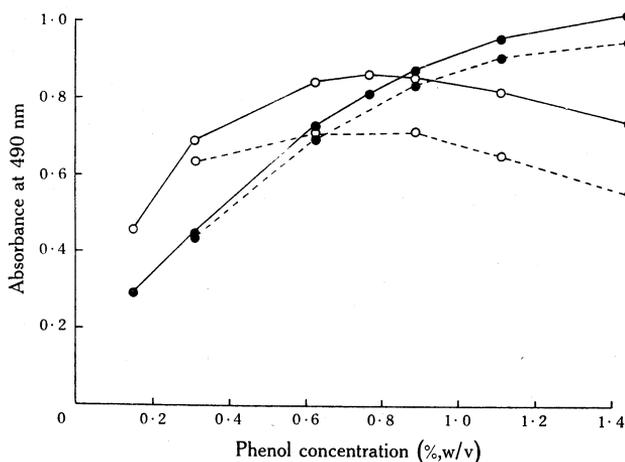


Fig. 1. Effect of phenol concentration and mode of addition of acid in the phenol-sulfuric acid method. The procedures were as described in Materials and Methods, except that the concentration of the phenol solution used was varied to give the final concentrations indicated. Solid lines: rapid addition of acid. Broken lines: slow addition of acid. ● Glucose, 50 μg . ○ Galactose, 50 μg .

Gel Filtration

A weighed amount (usually 20 mg) of the carbohydrate fraction of the milk, extracted as described previously (Messer and Mossop 1977) was dissolved in 0.6 ml of water, and the solution passed through two columns of Sephadex G25, superfine grade (Pharmacia Fine Chemicals) connected in series, each 150 cm long and 1.1 cm in diameter. Elution was done with water (saturated with toluene) at a flow rate of 5 ml/h, and fractions of 1.5 ml were collected.

Samples, 0.20 ml, of each fraction were analysed for total hexose by the modified phenol-sulfuric acid method described above. Other samples (0.40 ml) were analysed for sialic acid by the thio-barbituric acid method of Aminoff (1961) after liberation of free sialic acid by hydrolysis with 0.05 M H_2SO_4 at 85°C for 45 min. Some fractions were also assayed for free *N*-acetylhexosamine by the following modification of the method of Reissig *et al.* (1955): 0.20 ml of the sample, containing up to 60 μg of *N*-acetylgalactosamine or 20 μg of *N*-acetylglucosamine, was mixed with 0.20 ml of 0.6 M potassium tetraborate, (pH 9.2; Levvy and McAllan 1959). The solution was heated at 100°C for 3 min, cooled, treated with 2.0 ml of *p*-dimethylaminobenzaldehyde reagent, and the mixture incubated at 37°C for 30 min; its absorbance was then measured at 585 nm. Both *N*-acetylgalactosamine and *N*-acetylglucosamine were used as standards.

Monosaccharide Composition

Approximately 2 mg of the milk carbohydrate fraction was weighed into a small culture tube and dissolved in 2 ml of 2 M HCl. The tube was flushed with nitrogen gas, sealed with a Teflon-lined screw cap, and placed in a boiling water bath for 1 h. Of the hydrolysate, 0.20 ml was transferred to a small vial and dried *in vacuo* over KOH; the dried material was dissolved in 0.05 ml of water, and the monosaccharide composition of the solution examined qualitatively by paper chromatography.

The remainder of the hydrolysate was adjusted to pH 7 with 1 M NaOH solution and diluted to 10 ml with water. Samples of this diluted hydrolysate were then analysed for glucose with glucose oxidase, for galactose with galactose dehydrogenase and for hexosamines with an amino acid analyser, each as described previously (Messer and Mossop 1977).

Sialic acid was determined on a separate sample of the carbohydrate fraction, as described above under 'gel filtration'.

Chromatography

Descending paper chromatography was done on Whatman No. 2 paper (42 cm long) with ethyl acetate-pyridine-water, 12.5:4 (v/v) as solvent for 24 h. Sugars were located with alkaline AgNO₃ reagent (Smith 1958).

Thin-layer chromatography was done on precoated silica gel 60 plates (E. Merck, Darmstadt, Art. 5553, 20 by 20 cm) with isopropanol-acetone-0.1 M lactic acid, 4:4:2 (v/v) as solvent (Hansen 1975). Aniline-diphenylamine was used as the location reagent.

Results

Changes in Milk Carbohydrate Content

Table 1 summarizes the results of carbohydrate determinations on the milk obtained, at various times *post partum*, from a group of up to 11 lactating tamar wallabies. The number of animals varied because not all were milked at each time selected. The milk carbohydrate increased slowly but steadily during the first 6 months, reaching a peak at 26 weeks; it then fell rapidly to 1.7 g/100 ml at 36 weeks, and to still lower values thereafter.

Table 1. Effect of time of lactation on the milk carbohydrate concentration in the tamar wallaby

Time of lactation (weeks <i>post partum</i>)	No. of animals	Mean carbohydrate concn \pm s.e.m. (g/100 ml) ^A	Time of lactation (weeks <i>post partum</i>)	No. of animals	Mean carbohydrate concn \pm s.e.m. (g/100 ml) ^A
1	3	5.8 \pm 0.14	30	2	10.1,7.4
2	9	7.1 \pm 1.1	32	6	7.4 \pm 3.3
5	11	7.9 \pm 0.57	34	3	5.4 \pm 3.3
11	9	8.8 \pm 0.47	36	6	1.7 \pm 0.45
18	7	10.3 \pm 0.77	40	6	1.1 \pm 0.18
24	6	11.9 \pm 0.48	42	6	0.83 \pm 0.21
26	6	13.1 \pm 0.58	44	5	0.86 \pm 0.18
28	6	11.7 \pm 0.84			

^A As total hexose.

The values for s.e.m. (Table 1) indicate that the results were considerably more variable during the period of rapid fall in carbohydrate concentration between 26 and 36 weeks *post partum*, than at other times. Fig. 2 illustrates the results with each of a group of six animals; it shows that this variability arose from the fact that the fall in carbohydrate occurred somewhat earlier, and more rapidly, in some animals than in others. Thus in some it had hardly begun at 32 weeks, whereas in others it had almost run its full course by this time.

Monosaccharide Composition of the Milk Carbohydrate

The phenol-sulfuric acid method used for the estimation of milk carbohydrate determines hexoses only, and the results obtained with it do not include contributions made by amino sugars such as glucosamine and sialic acid, both of which are components of the milk carbohydrate of grey kangaroo (Messer and Mossop 1977). In

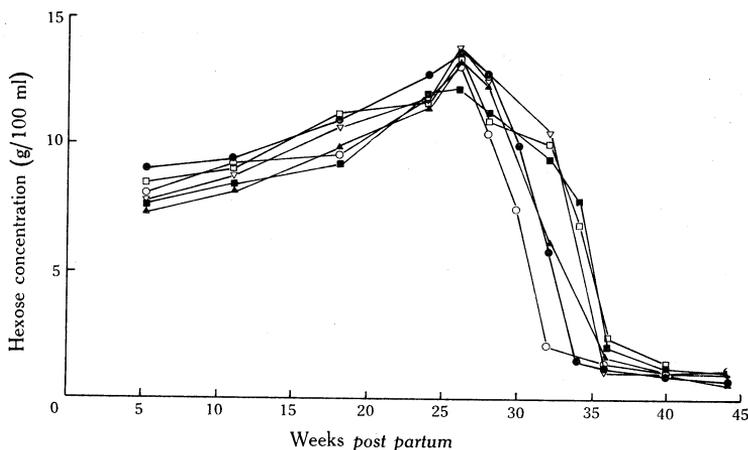


Fig. 2. Carbohydrate content of milk collected from six tammar wallabies, as a function of time of lactation. Each set of symbols refers to a single animal.

addition, the results provide no information on the identity of the hexoses. Acid hydrolysates of the carbohydrate fractions of milk obtained at five different times of lactation were therefore examined chromatographically to identify the monosaccharides present, and then analysed quantitatively (see Materials and Methods).

Table 2. Monosaccharide composition of the milk carbohydrate of tammar wallaby at various times of lactation

Estimations were done on acid hydrolysates, prepared as described in Materials and Methods. In each case the carbohydrate was from milk pooled from six animals, except for that at 32 weeks *post partum* which was from a milk sample, containing 5.8% hexose, taken from a single animal.

Results given as percentage of total (w/w)

Time of lactation ^A	Galactose	Glucose	Glucosamine	Galactosamine	Sialic acid
4	71	20	4.0	<1	5.5
18	72	19	3.8	<1	4.6
26	80	12	3.7	<1	4.0
32	68	20	7.4	<1	3.6
44	51	21	14	5.1	9.1

^A Weeks *post partum*

Paper chromatography indicated that the only hexoses present in the hydrolysates were galactose and glucose, while the only hexosamines were glucosamine and galactosamine. No other monosaccharides, such as fucose, were detected. The results of the quantitative analyses (Table 2) showed that galactose was the predominant monosaccharide throughout lactation, especially at 26 weeks *post partum*.

Non-hexose sugars contributed less than 10% of the total, except at the end of lactation. Glucosamine and sialic acid were present at all stages, but significant amounts of galactosamine were found only at the end of lactation.

Gel Filtration

Previous work (Messer and Mossop 1977) had shown that the milk carbohydrates of grey kangaroo could be separated into oligosaccharides of various sizes by means of gel filtration on Sephadex G25. The same technique was therefore used in the present work, the aim being to monitor changes in the pattern of oligosaccharides during the course of lactation.

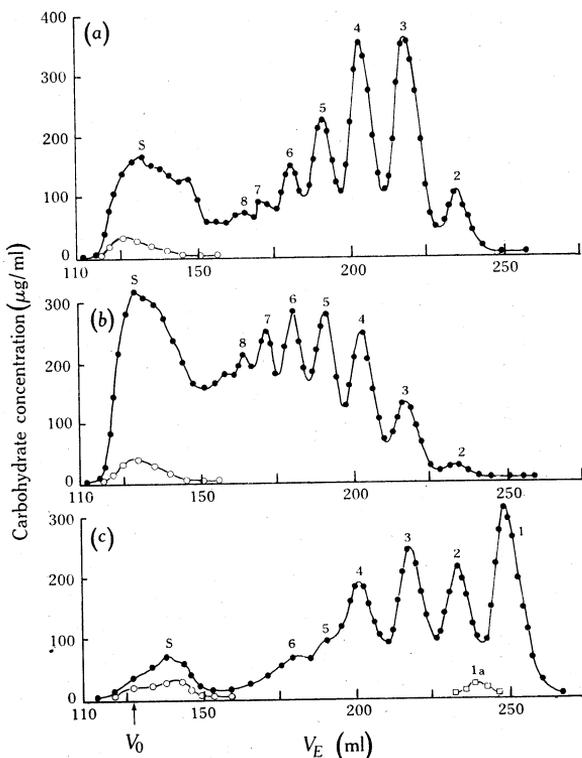


Fig. 3. Gel filtration of the carbohydrate of milk collected at (a) 5 weeks, (b) 26 weeks and (c) 32 weeks. In (a) and (b) the carbohydrate was from milk pooled from six animals. In (c) the carbohydrate was from a single animal (see text). ● Hexose. ○ Sialic acid. □ *N*-Acetylhexosamine (*N*-acetylgalactosamine equivalents).

Figs 3a and 3b show the elution patterns observed with the carbohydrate of milk obtained 5 and 26 weeks *post partum*, respectively. In each case there were seven distinct peaks of neutral oligosaccharides (numbered 2–8 in reverse order of elution) which emerged from the column following a large, ill-defined peak of material (peak S) that contained sialic acid. The elution volumes of peaks 2–8 corresponded to those of di- to octasaccharides, respectively (cf. Messer and Mossop 1977). The contents of peak 2 co-chromatographed with lactose. The patterns were very similar

to the previous results with milk carbohydrate from grey kangaroo, the most notable difference being the absence, in Figs 3*a* and 3*b*, of a peak for monosaccharides.

The elution patterns of milk carbohydrate obtained at various other times up to 26 weeks *post partum* were all similar to those of Figs 3*a* and 3*b*, but there was a gradual increase, during this time, in the amounts of the higher saccharides (hexasaccharides and above) relative to those of the lower ones (di- to tetrasaccharides). Thus there was an increase in the mean molecular size of the milk carbohydrates. Calculations based on the areas under each peak showed that lactose contributed 5.9% of the total hexose at 4 weeks *post partum* (Fig. 3*a*) compared with only 1.1% at 26 weeks (Fig. 3*b*). Peak S, on the other hand, constituted 28% at 4 weeks, and 45% at 26 weeks. Peak S has been shown to contain a mixture of sialyl saccharides and neutral polysaccharides (Messer and Mossop 1977); since the amount of sialic acid remained unchanged, the increase in peak S indicated an increase in the neutral polysaccharides.

Fig. 3*c* shows the elution pattern of the carbohydrates of a milk sample, obtained at 32 weeks *post partum*, from a single animal. The hexose content of the sample was 6.5 g/100 ml, indicating that it was obtained about half-way through the period of rapid decline in milk hexose (cf. Table 1). Compared with the previous patterns, there was an obvious increase in the amount of carbohydrate eluted after the tetrasaccharide peak (peak 4), at the expense of that eluted prior to it. Lactose (peak 2) now constituted 17% of the total hexose, and free monosaccharides (peak 1, containing galactose and glucose), which had not been present before, constituted 24%. In addition there was a small peak (1*a*) which contained free *N*-acetylgalactosamine.

An elution pattern very similar to that of Fig. 3*c* was observed with a milk sample, containing 5.8 g hexose/100 ml, obtained from another animal at 32 weeks *post partum*. With milk samples from two further animals, however, the elution patterns at 32 weeks resembled that of Fig. 3*b* rather than 3*c*, but the total hexose content of each of these samples was greater than 10 g/100 ml, indicating that they were obtained prior to the fall in carbohydrate content. The pattern of Fig. 3*c* was therefore characteristic of milk obtained at the stage of lactation during which there was a pronounced fall in its carbohydrate content, and was not related to the exact time after birth.

At 36 weeks, by which time the fall in carbohydrate had taken place in all animals the milk contained no neutral saccharides larger than trisaccharides, and there was a very marked increase in the amount of monosaccharides (peaks 1 and 1*a*, Fig. 4*a*). By 40 weeks the milk contained almost only monosaccharides (Fig. 4*b*). Quantitative analysis indicated that peak 1 of Fig. 4*b* consisted of galactose and glucose in a ratio of 3.3:1, while peak 1*a* consisted of *N*-acetylglucosamine and *N*-acetylgalactosamine in a ratio of 2.2:1.

The results shown in Figs 3*c*, 4*a* and 4*b* indicate that there was a progressive replacement of higher by lower saccharides, i.e. a reduction in the mean molecular size of the milk carbohydrates, late in lactation.

Thin-layer Chromatography

When the milk carbohydrate obtained at various stages of lactation was examined by t.l.c. (Fig. 5*a*), it was found that up to week 26 *post partum* the chromatographic patterns all showed lactose and a series of spots with lower R_F values which were

presumably other oligosaccharides. With carbohydrate from milk obtained after 36 weeks, almost only free monosaccharides (galactose, glucose, *N*-acetylglucosamine and *N*-acetylgalactosamine) were observed, very little lactose and other oligosaccharides being detected. Milk carbohydrate obtained between 26 and 36 weeks showed intermediate patterns, i.e. both oligosaccharides—including lactose—and monosaccharides.

The results of t.l.c. thus confirmed the data from gel filtration.

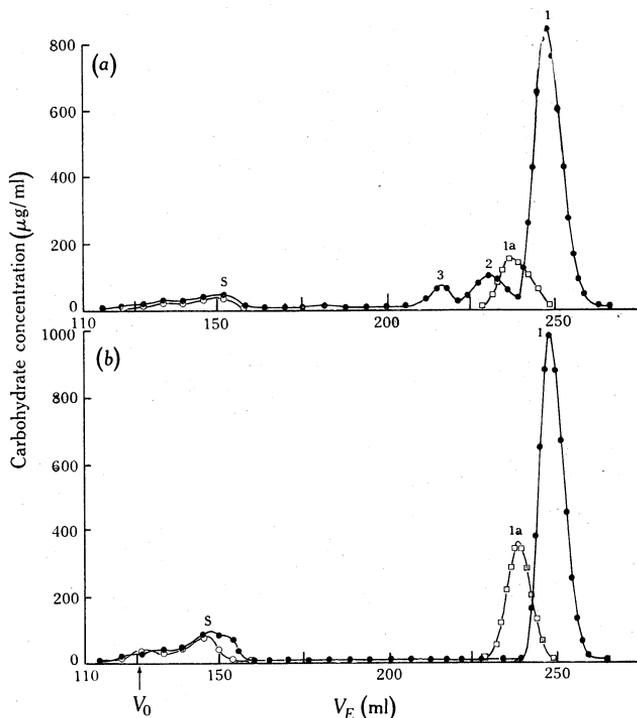


Fig. 4. Gel filtration of the carbohydrate of milk collected at 36 weeks (a) and 40 weeks (b). In both cases the carbohydrate was from milk pooled from six animals. Symbols as in Fig. 3, except that in (b) the *N*-acetylhexosamine is expressed as *N*-acetylglucosamine equivalents.

Milk Carbohydrates of Animals Suckling Simultaneously Two Young of Different Ages

Two of our animals gave birth while the young at foot were still suckling from outside the pouch. Milk was obtained from both lactating mammary glands of each animal and analysed for total hexose. The values for the milk from the glands with small teats, supplying the pouch young aged 18 days and 5 weeks, were 7.3 and 8.7 g/100 ml, respectively; those for the milk from the large teats supplying the young at foot, both aged 46 weeks, were each 1.5 g/100 ml.

When the carbohydrates were extracted from each of the four milk samples and then examined by t.l.c. (Fig. 5b) those from the milk supplying the pouch young were found to consist almost wholly of oligosaccharides, including lactose, whereas those from the milk supplying the young at foot consisted mainly of monosaccharides (galactose, glucose, *N*-acetylglucosamine and *N*-acetylgalactosamine), with only small amounts of lactose and other oligosaccharides. The chromatographic patterns of the milk carbohydrates from the small teats were thus similar to those observed

with milk from the first 26 weeks of lactation, while the patterns from the large teats were identical to those observed with milk obtained after 36 weeks *post partum* (cf. Figs 5a and 5b).

The amount of milk obtained from the small teats was insufficient for gel filtration.

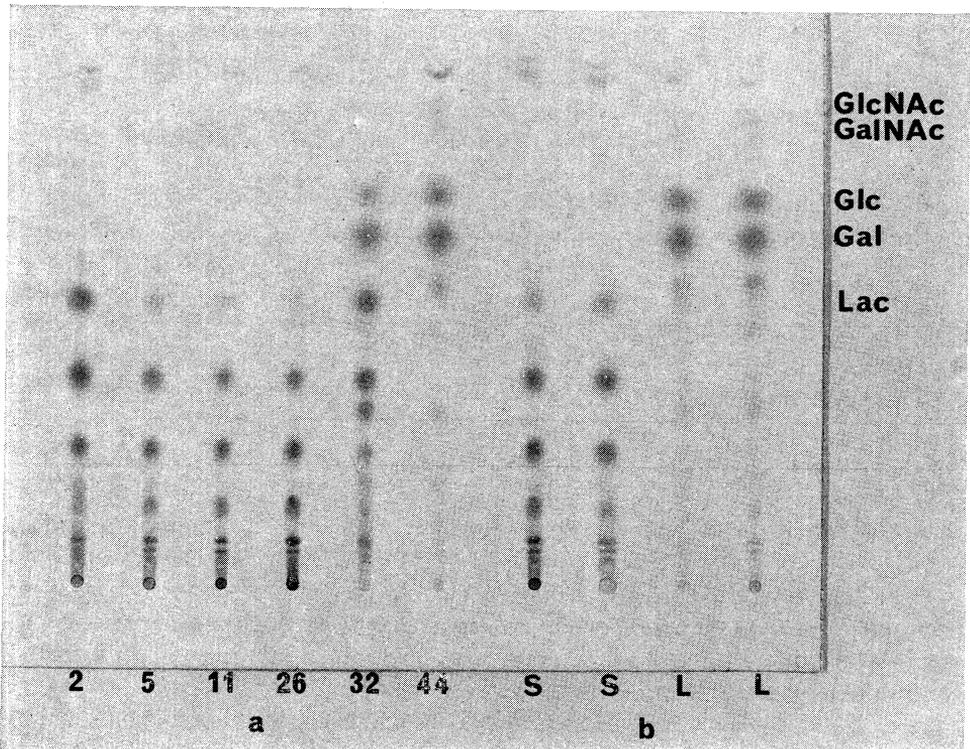


Fig. 5. Thin-layer chromatography of milk carbohydrates. (a) Carbohydrate of milk collected at various stages of lactation; the figures refer to weeks *post partum*. In each case the carbohydrate was from milk pooled from six animals except for that collected at 32 weeks, which was from a single animal (cf. Fig. 3c). (b) Carbohydrate of milk from two animals, each suckling simultaneously two young of different ages. S, milk from small teat supplying pouch young. L, milk from large teat supplying young at foot (see text for ages of young). Lac, lactose; Glc, glucose; Gal, galactose; GlcNAc, N-acetylglucosamine; GalNAc, N-acetylgalactosamine.

Discussion

Quantitative Changes

The data of Table 1 and Fig. 2 demonstrate marked quantitative changes in milk carbohydrates during the course of lactation in the tammar wallaby. The results are in contrast to those of Gross and Bolliger (1959), who found that the 'lactose' content of milk of the brush-tailed possum, *Trichosurus vulpecula*, remained fairly constant around a mean value of 3.2% during growth of the young. Lemon and Barker (1967) similarly reported that the reducing sugars of the red kangaroo showed only slight fluctuations around 2% throughout lactation. The difference in results could be due to differences between the species investigated, but this seems less likely in the case of red kangaroo, which is closely related to the tammar wallaby. A more important

point to consider is the method of analysis used. The values for carbohydrate reported by the above authors were based on measurements of the reducing power of unhydrolysed samples. Since the milk of both possum and red kangaroo contains oligosaccharides other than lactose during at least part of lactation (Gross and Bolliger 1958; Jenness *et al.* 1964), and since, on a weight basis, such oligosaccharides have a lower reducing power, the reported values must represent underestimates of the total carbohydrate. This was recognized by Lemon and Barker (1967), who noted a marked discrepancy between the estimated total solids and the combined fat, protein and reducing sugars, and suggested as an explanation that the latter comprised only a small fraction of the total carbohydrate. Consequently the method of analysis used may have precluded the possibility of observing any changes in the concentration of milk carbohydrate during lactation.

In the present work, the carbohydrate content of milk was estimated by a method in which the oligosaccharides are hydrolysed, and in which galactose and glucose give equal colour yields. We believe that this method gives more reliable estimates of the total non-amino carbohydrate content of marsupial milk than the methods used previously.

A notable feature of our results was the very high values for carbohydrate during the first 6 months of lactation. During all but the first 2 weeks of this period the milk contained over 7% of hexose, while from weeks 24 to 28 it contained over 11% (Table 1). By comparison, the highest value for milk carbohydrate previously recorded for any species is 10.2% (green monkey; Jenness and Sloan 1970); that for marsupials is 6.7% (red kangaroo; Jenness and Sloan 1970). Although criticisms can be made of previous methods of analysis (see Methods), we believe that the high values observed by us with tammar wallaby are characteristic of that species. These values are in fact underestimates of the total carbohydrate, since they do not include contributions made by aminosugars (see Table 2).

After 26 weeks *post partum*, there was a dramatic fall in the milk carbohydrate content, which took place over a relatively short time of 6–10 weeks. We observed that this period coincided with that during which the young began to leave the pouch. By the time it ended at about 36 weeks, the young had left the pouch permanently and were beginning to consume herbage. In the red kangaroo, the young leave the pouch permanently at 34 weeks *post partum* (Sharman and Calaby 1964) by which time they have developed a ruminant-type digestion through appropriate changes in their stomachs (Griffiths and Barton 1966). Assuming that a ruminant-type digestion develops by about 36 weeks in the young tammar wallaby, the remarkably low carbohydrate content of the milk after this time may be associated with the consumption of carbohydrates (cellulose and starch) in herbage.

Lemon and Barker (1967) observed a marked decrease in the percentage of fat-free solids in the milk of the red kangaroo, beginning at about 30 weeks *post partum*. Since this decrease was not accompanied by any significant reduction in the concentration of protein, it is likely to have been caused by a decrease in the percentage of carbohydrate, similar to that found in this study.

Qualitative Changes

Jenness *et al.* (1964), using paper chromatography, noted the presence of significant amounts of free galactose and glucose, in addition to lactose and other oligosaccharides, in the milk of the red kangaroo, the quokka (*Setonix brachyurus*) and

the opossum (*Didelphis virginiana*). Since the milk samples from red kangaroo and opossum obtained late in lactation were found to contain more free monosaccharides than those obtained earlier, Jenness *et al.* (1964) suggested that the stage of lactation was a major factor influencing the composition of marsupial milk. This suggestion is amply supported by the present results. Up to about 26 weeks *post partum*, the milk carbohydrate contained only oligosaccharides and no monosaccharides, whereas from about 36 weeks it contained almost only monosaccharides, and no neutral oligosaccharides. Between these times the milk exhibited an intermediate pattern of a mixture of lower oligosaccharides and free monosaccharides.

The period during which the milk carbohydrate changed from oligosaccharides to monosaccharides coincided with the time during which there was a marked fall in the concentration of total hexose. Thus, prior to 26 weeks the milk contained relatively large amounts of carbohydrate consisting of oligosaccharides, whereas after 36 weeks it contained much less, and this consisted almost wholly of monosaccharides.

Milk appears to be necessarily isosmotic with plasma, and its osmolarity is determined mainly by its content of carbohydrate and salts (Linzell and Peaker 1971). According to Jenness and Sloan (1970), the observed osmotic pressure of milk would be achieved by a lactose concentration of 11% if no other solutes were involved. The presence of large amounts of carbohydrate (up to 13%) in tammar wallaby milk presumably suits the nutritional requirements of the young during the first 6 months *post partum*, but if this carbohydrate consisted only of lactose it would clearly raise the osmotic pressure of the milk above that of plasma. The fact that it consists mainly of oligosaccharides larger than lactose obviates this problem; a hexasaccharide, for example, exerts only about one-third of the osmotic pressure of an equal weight of lactose. In line with this hypothesis, is the finding of a progressive increase in the average molecular size of the milk oligosaccharides during the first 26 weeks, concomitant with the increase in carbohydrate content (cf Figs 3a and 3b). Furthermore by 36 weeks, when the hexose content of the milk was less than 2% and the osmotic effect of the milk carbohydrate was therefore much smaller, almost all the oligosaccharides had disappeared and been replaced by monosaccharides.

The disappearance of the oligosaccharides was gradual, beginning with the higher oligosaccharides and ending with lactose. This suggests a progressive loss of activity of the various enzymes of the mammary gland which are responsible for the synthesis of these oligosaccharides. In the later stages of lactation even lactose had almost entirely disappeared, indicating a loss of lactose synthase activity. Lactose synthase consists of two proteins, galactosyl transferase and α -lactalbumin, both of which are normally found in milk as well as in the mammary gland (Ebner and Schanbacher 1974). Its activity is controlled by the amount of α -lactalbumin, and milk secreted by mammary glands that contain no α -lactalbumin, e.g. during pregnancy or in certain aquatic species such as the California sea lion (Johnson *et al.* 1971), is devoid of lactose. The virtual absence of lactose from the milk of tammar wallaby after 36 weeks may therefore be due to a cessation of α -lactalbumin synthesis by the mammary gland. Lemon and Bailey (1966) have indeed observed that a protein, which migrated electrophoretically in the region of cow α -lactalbumin, was present in milk from red kangaroo obtained at either 77 or 210 days *post partum*, but not in milk obtained at 330 days.

Although the late-lactation milk contained almost no lactose, it did contain free monosaccharides; in this respect it differed from the milk of California sea lion and

other pinnipeds, which contains virtually no carbohydrates of any kind (Kerry and Messer 1968). The major monosaccharides were galactose (presumably derived from UDP-galactose) and *N*-acetylglucosamine; their presence implies that the mammary gland, even late in lactation, contains all the enzymes required for their synthesis. Their function is not immediately apparent since, as already mentioned, the young at that stage of lactation can be expected to obtain carbohydrates from herbage and to have developed a ruminant-type digestion in which these carbohydrates are fermented to volatile fatty acids (Tyndale-Biscoe 1973).

Milk Carbohydrates of Animals Suckling Two Young of Different Ages

In red kangaroos suckling simultaneously a new-born pouch young and a young at foot, the two mammary glands secrete two kinds of milk which show marked qualitative differences in their whey proteins (Bailey and Lemon 1966) and quantitative differences in the fatty acid composition of their triacylglycerols (Griffiths *et al.* 1972). The present work shows that in the tammar wallaby, the two mammary glands secrete milk which differs, qualitatively as well as quantitatively, with respect to carbohydrates. The differences observed were in accord with the previously described data on changes in milk carbohydrates during lactation; thus the milk supplying the pouch young, obtained early in lactation, had a high hexose content and contained oligosaccharides, whereas that supplying the young at foot, obtained late in lactation, had a low hexose content and contained mainly free monosaccharides.

It is not known whether marsupials in general show the quantitative and qualitative changes in milk carbohydrates that we have observed in the tammar wallaby. A survey on the composition of marsupial milk is required to determine whether macropodids are unique among marsupials in exhibiting such profound changes in milk carbohydrates.

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