

## Changes in Milk Composition during Lactation in the Tammar Wallaby (*Macropus eugenii*)

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### Abstract

Milk samples were obtained at regular intervals throughout lactation from tammar wallabies (*M. eugenii*). Total solids represented only 12% (w/w) of the milk at the commencement of lactation and gradually increased to about 40% at 36 weeks. Milk proteins represented 4% (w/w) of whole milk during the first 18 weeks of lactation, followed by a rapid increase to around 13% (w/w) at 36 weeks. Sodium and potassium concentrations were high in early samples of milk but declined to minimal values at 30 weeks. The milk was isosmotic to the plasma at all stages.

### Introduction

There is extensive literature on lactation in eutherian mammals. This encompasses studies on the composition of milk (reviewed by Jenness and Sloan 1970), the bioenergetics of milk production and growth, and the physiology and biochemistry of lactogenesis, particularly in domesticated species (Kon and Cowie 1961; Falconer 1971; Larson and Smith 1974, 1978; Peaker 1977).

In contrast, the literature on lactation in metatherian and prototherian mammals is sparse. Studies on the composition of milk of the brush-tailed possum, *Trichosurus vulpecula* (Gross and Bolliger 1959), the red kangaroo, *Macropus rufus* (= *Megaleia rufa*) (Lemon and Barker 1967; Sharman 1970; Griffiths *et al.* 1972) and the monotremes, *Tachyglossus aculeatus* and *Ornithorhynchus anatinus* (Griffiths *et al.* 1973; Messer and Kerry 1973) indicate that major quantitative and qualitative changes in milk composition may take place during lactation. However, apart from this information little is known of the changes in the milk composition of marsupials or of the mechanisms controlling lactogenesis. Nothing at all is known of the energetics of growth in the young.

This paper presents detailed information on compositional changes in the milk with respect to total solids, protein, sodium and potassium throughout the lactational cycle of the tammar wallaby (*Macropus eugenii*). It represents part of a research program that extends to the energetics of growth in pouch young and the regulation of lactation in marsupials.

### Materials and Methods

Female tammar wallabies with pouch young were maintained in large enclosures of about 250 m<sup>2</sup>. The animals fed on grass, mainly *Phalaris tuberosa*, growing in the enclosures but lucerne hay and oats were provided as food supplements. Water was provided at all times. A more detailed description of the enclosures has been given by Calaby and Poole (1971).

During March and April, all pouch young, which were of undetermined ages, were permanently removed from their mothers. This initiated blastocyst development and about 26 days later new young were born. This synchronization of births facilitated the study of milk composition from birth to conclusion of weaning.

In 1976, 11 animals with pouch young aged 10 weeks were milked weekly until the young were 17 weeks old. During 1977, 12 tammar with 4-week-old pouch young were milked; however, in the following 8 weeks half of the young had died due to experimental manipulation. The six remaining females with young were milked fortnightly until the cessation of lactation. In 1978, a group of six animals was milked when the pouch young were 1, 2 and 3 weeks old.

#### *Milk Collection*

The pouch young were removed from the teat by gentle but firm pressure on the sides of the mouth, and placed in labelled containers which were maintained in a humid incubator at 35°C. As the pouch young became larger and grew hair, they were placed in small calico bags and wrapped in towelling for warmth.

The pouch young were kept from their mothers for about 6 h to allow milk to accumulate in the mammary glands. After this period the mothers were anaesthetized by intravenous injection of sodium methohexitone (Brietal sodium; Eli Lilly and Co., Sydney; 12 mg/kg body wt) into the lateral tail vein. The teats were cleaned with distilled water and tissue paper, after which an intramuscular injection of oxytocin (Syntocinon; Sandoz, Australia) was given at a dosage of 0.7 i.u./kg body wt. Milk started to appear within a minute but the flow was greatly assisted by the slight pressure of fingers along the teat. The milk was collected in 3-ml plastic vials after which the pouch young were assisted to take the teat into the mouth. In the earliest stages of development it was necessary to force the end of the teat into the mouth with a tapered matchstick. After recovery from the anaesthetic the animals were returned to their pens.

Milk samples were also collected from wild populations of *M. eugenii* on Kangaroo Island, S.A., during April and August 1977. The ages of pouch young at these times were 11 and 27 weeks.

#### *Milk Analyses*

##### *Total solids*

Approximately 50 or 100  $\mu$ l of milk was weighed in preweighed plastic vials. Each sample was then freeze-dried and reweighed to register total solids. All weighings were made to the nearest 0.1 mg on a Mettler balance (model K5T).

##### *Sodium and potassium*

10  $\mu$ l of milk was 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 concentrations are expressed as m-moles per litre of milk.

##### *Total nitrogen*

The dried residues from the total solid determinations were digested using the Kjeldahl procedure. Total nitrogen was estimated by microdiffusion technique (Conway 1962) and converted to estimated protein using a factor of 6.38.

##### *Osmotic concentration*

Osmoconcentration of milk and maternal plasma was measured with a Hewlett-Packard 3023 vapour pressure osmometer. Values are given as means  $\pm$  s.d.

## **Results**

The composition of milk of the tammar wallaby changed substantially throughout lactation. The milk formed during the first week or so of lactation was quite dilute,

only 12% (w/w) being solid material. By the time weaning was taking place, the milk was quite creamy with 40% (w/w) or more solid constituents (Fig. 1).

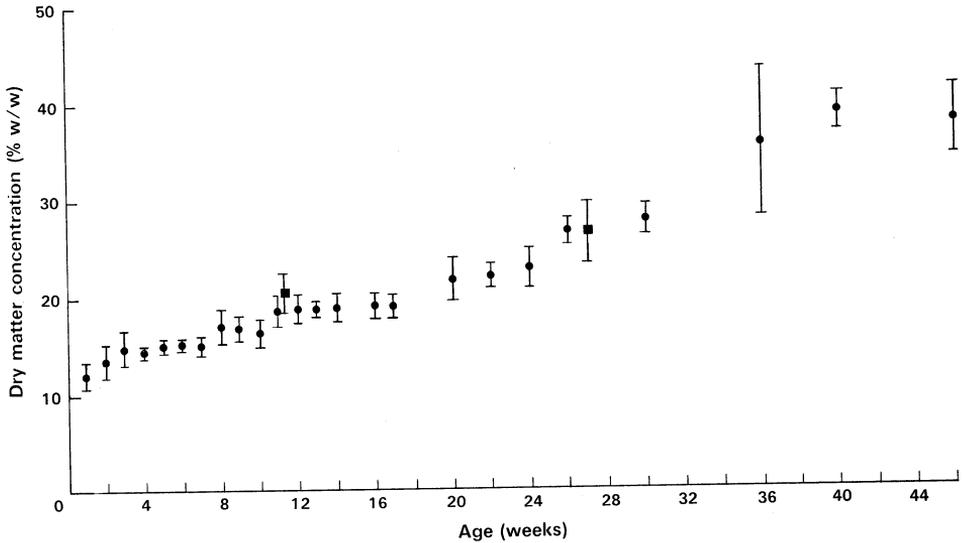


Fig. 1. Changes in total milk solids during lactation. Values are given as means  $\pm$  s.d. ■ Kangaroo I. field sample.

The electrolyte levels in the milk also showed substantial changes during lactation. Sodium concentrations in early milk were around 50 mM, but declined to approximately 17 mM by about 30 weeks (Fig. 2). The sodium concentration then increased and

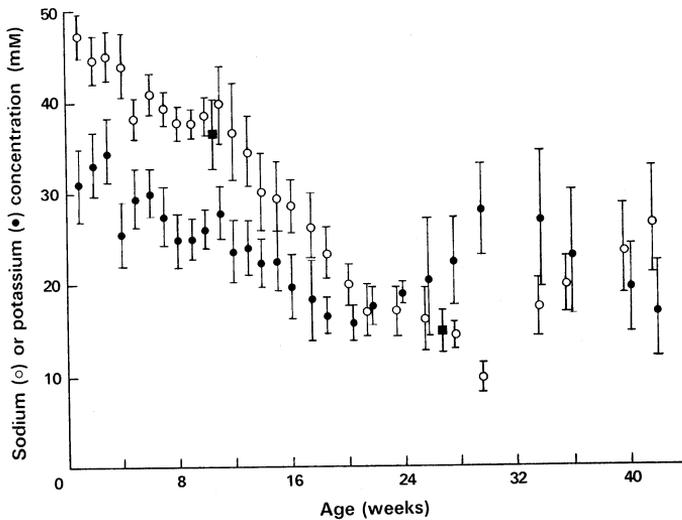


Fig. 2. Changes in sodium and potassium concentrations in milk during lactation. Values are given as means  $\pm$  s.d. ■ Kangaroo I. field sample.

finally, as the mammary gland regressed, approached plasma concentrations ( $143 \pm 2$  mM,  $n=7$ ). The concentration of potassium in the milk was about 35 mM

in the first few weeks, and it then followed a similar decline as sodium but not to such low levels. As a result, the potassium concentration curve appeared to cross that for sodium at two points (Fig. 2). In the final stages of milk secretion, the concentrations of potassium fell towards plasma levels ( $4.1 \pm 0.3$  mM,  $n=7$ ).

The nitrogen content of tamar milk, expressed as protein, is shown in Fig. 3. Up to around 18 weeks the protein content was about 4% (w/w) but after this time there was a pronounced increase to a peak of about 13% (w/w) of whole milk which was reached between 30 and 36 weeks. A series of oscillations in protein levels may have occurred in the final stages of lactation.

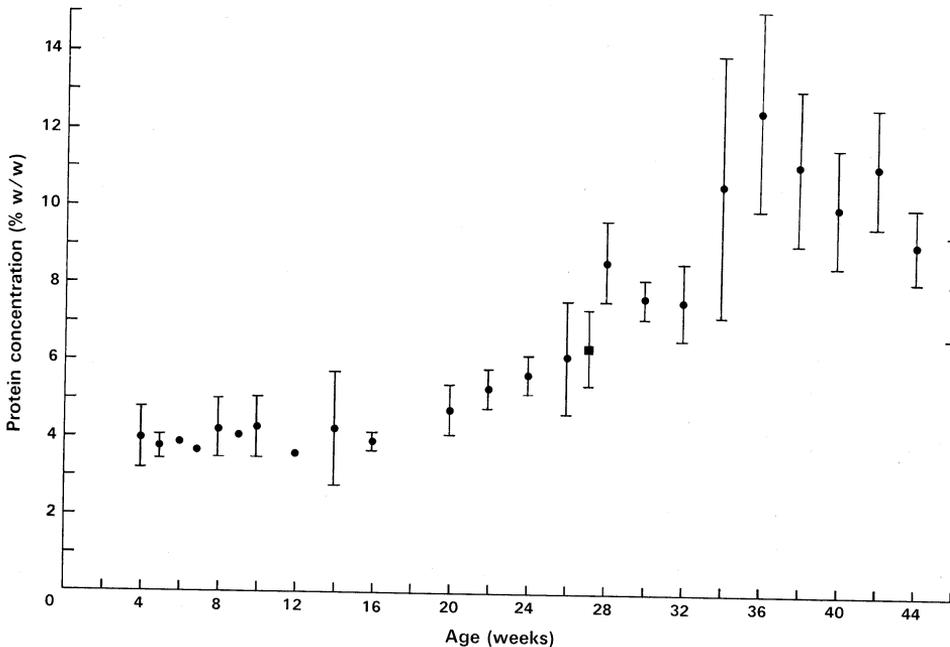


Fig. 3. Changes in the protein concentration of milk during lactation. Values are given as means  $\pm$  s.d. ■ Kangaroo I. field sample.

The osmotic concentration of the milk did not change at any stage of lactation, the mean values at 14, 20 and 38 weeks being  $278 \pm 2$ ,  $280 \pm 3$  and  $280 \pm 4$  mosmol/l, respectively. In two animals that were producing both early- and late-stage milk, the osmolarity of milk samples from each gland differed by only 4 and 8 mosmol/l respectively. The milk was isosmotic to the plasma which had a mean osmolarity of  $284 \pm 7$  mosmol/l.

The composition of milk samples from free-living tammars on Kangaroo Island was similar in all respects to samples obtained from captive animals with pouch young of the same age. The values for milk components from wild tammars are included in Figs 1, 2 and 3.

## Discussion

Since captive tammars are buffered from such factors as seasonally affected changes in diet, it may have been argued that the results for milk composition of

tammars in captivity might not be representative of those for the wild state. The two samples from free-living tammars, however, are similar to those from the captive females both in the absolute values for the parameters examined and the trends that they show between one sample and the next with respect to the ages of the pouch young. For these reasons, the results of this study are regarded as an accurate indication of compositional changes in the milk of the tammar wallaby during lactation.

The gradual increase in milk solids during lactation in the tammar wallaby is similar to the pattern reported for the brush-tailed possum by Gross and Bolliger (1959). The data of Bergman and Housley (1968) on the milk composition of the opossum (*Didelphis virginiana*) suggest a similar trend of increasing solids throughout lactation; however, these authors appear to have overlooked the consistent nature of the change.

The change in milk solids corresponds with changes in the suckling regime of the pouch young. In the early stages of development, the pouch young are continuously 'attached' to the teat and presumably spend much of the time suckling. From about 20 weeks onwards they spend less time on the teat, and after leaving the pouch suckling is reduced to only brief periods. Ben Shaul (1962) and Jenness and Sloan (1970) have proposed that the concentrations of solids in the milk of eutherians is correlated with the time spent suckling by young. It appears that macropodid marsupials fit this hypothesis quite well. Similar patterns of changing milk composition during lactation are found in other marsupials (Green, unpublished data).

In the early stages of lactation, the Na/K ratio is about 1.5, but it drops to 1.0 at 23 weeks and continues to decline to around 0.5 at 31 weeks. It then returns to about 1.0 at 40 weeks and remains slightly in excess of 1.0 for the remainder of lactation. This pattern of changes in the ratio of sodium and potassium concentrations is similar to that described for the quokka (*Setonix brachyurus*) by Bentley and Shield (1962).

The reasons for the changes in electrolyte concentrations in milk are not clear. They may be related, however, to structural and functional changes occurring in the kidneys of pouch young. It is possible that the kidneys of young less than 20 weeks are not able to regulate urinary losses of sodium and potassium to a marked extent, and the resulting electrolyte losses must be replaced from the milk. The kidneys of *S. brachyurus* pouch young less than 120 days (17 weeks) old are unable to produce a concentrated urine, even when dehydrated or injected with antidiuretic hormone; however, older animals are able to form hypertonic urine in response to these treatments (Bentley and Shield 1962). Similar differences are found between the urinary concentrating abilities of tammars less than 20 weeks and those that are older (Thomas, personal communication). The changing Na/K ratios in milk may also reflect alterations in the relative growth of intra- and extracellular fluid compartments (Bentley and Shield 1962).

The sodium content of eutherian milk is generally quite low, in the region of 10–20 mM. However, it is much higher in the rabbit, around 50 mM or more (Lebas *et al.* 1971; Peaker and Taylor (1975) and Linzell *et al.* (1975) have suggested that this is due to the paracellular leakage of plasma sodium through the zonulae occludentes of the secretory epithelial cells. Peaker (1977) suggests, on limited data, that a similar paracellular leakage of sodium may occur in macropodids and that in the later stages of lactation the zonulae occludentes become tighter so that all milk secretions are

then transcellular. This suggestion could possibly be resolved by ultrastructural study of the secretory epithelia to determine whether the tight junctions are 'leaky' in early lactation in macropodids.

The protein levels (w/w) of the milk of the tammar wallaby remain at 4% of whole milk for the first 18 weeks of lactation and then rise to a maximum of around 13% at 36 weeks. The protein level slowly declines during the remainder of lactation and just before the conclusion of weaning represents around 8% of whole milk. The changing levels of protein reflect the overall changes in total milk solids, protein representing a constant 25% of milk solids throughout lactation except for a slight rise to 35% at 36 weeks.

The quokka appears to have slightly lower levels of protein in the milk than the tammar, averaging around 3% up to 21 weeks. After this there is an increase in milk protein, to around 6% in the late stages of lactation (Jordan and Morgan 1968). The protein content of the milk of the red kangaroo is similar to that of *S. brachyurus* and reaches a maximum value of approximately 8% (Lemon and Barker 1967). Specific proteins appear in the whey fractions of the milk of the red kangaroo and of the grey kangaroo (*M. g. giganteus*) after about 30 weeks (Bailey and Lemon 1966; Lemon and Poole 1969). It is not known if similar proteins appear in the late milk of tammar wallabies but it is likely that they do.

Messer and Green (1979) have shown that early milk of the tammar wallaby is rich in carbohydrate, rising to an average of 13% (w/v) of whole milk by 26–30 weeks. This milk consists mainly of oligosaccharides with only small amounts of monosaccharides. The carbohydrate levels reflect total milk solids, representing 53% (w/v) of the solid components. However, by 36 weeks the carbohydrate levels drop markedly to less than 1% (w/v) of whole milk (2% (w/v) of total solids). Concomitant with this decline is a change in the carbohydrate moiety from oligosaccharides to mono- and disaccharides.

The peaks in carbohydrate and protein levels in milk coincide with the times when the pouch young commence to leave, and finally remain outside, the pouch. During this period the young commence to consume herbage and, in the red kangaroo at least, the stomach develops to the adult condition (Griffiths and Barton 1966). Presumably, the changes in milk components during this time are associated in some way with the development of the digestive tract and its associated microflora. However, the precise relationship between milk composition and the gut physiology of the young remains unknown.

In this study, milk composition has been related to the age of pouch young. However, some young grow faster than others and complete the lactational cycle earlier. It appears that body weight of the pouch young may correlate more closely with changes in milk composition than age, and if so could account for the increasing size of the variances associated with each parameter of milk composition from about 30 weeks onwards. The time at which lactation ceases is also quite variable, ranging between 46 and 65 weeks in this study, and is also presumably due to differential growth rates of the young.

Although the composition of milk varies widely within the Eutheria as a whole (Jenness and Sloan 1970), the composition of postcolostrum milk does not change greatly within most species. Certainly nothing like the dramatic changes in qualitative composition that occur in macropodids are seen in eutherian species. It would be interesting to know if the milk composition of other marsupial groups varies in the

same way as it does in macropodids. Limited evidence (Gross and Bolliger 1959; Bergman and Housley 1968) indicates that major quantitative changes in milk components occur in other marsupials. However, a broad survey of marsupial groups is required to resolve the extent of quantitative and qualitative differences.

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