Aust. J. Biol. Sci., 1980, **33**, 521–30

Intestinal Lactase (β-Galactosidase) and other Glycosidase Activities in Suckling and Adult Tammar Wallabies (*Macropus eugenii*)

Pattylou J. Walcott[A, B] *and Michael Messer*[A, C]

[A] Department of Biochemistry, University of Sydney, N.S.W. 2006.
[B] Present address: Plant Physiology Unit, Division of Food Research, CSIRO, School of Biological Sciences, Macquarie University, N. Ryde, N.S.W. 2113
[C] To whom correspondence should be addressed.

Abstract

The activities of various glycosidases in homogenates of the small intestinal mucosa of two adult and 18 suckling tammar wallabies (*M. eugenii*) aged from 6 to 50 weeks were investigated.

Lactase (β-D-galactosidase), β-*N*-acetylglucosaminidase, α-L-fucosidase and neuraminidase activities were high during the first 34 weeks *post partum* and then declined to very low levels. Maltase, isomaltase, sucrase and trehalase activities were very low or absent during the first 34 weeks, and then increased.

The lactase activity was unusual in being greater in the distal than the middle or proximal thirds of the intestine, and in its low pH optimum (pH 4·6), inhibition by *p*-chloromercuribenzene sulfonate but not by Tris, and lack of cellobiase activity. These properties are those of a lysosomal acid β-galactosidase rather than of a brush border neutral lactase. The maltase activity had the characteristics of a lysosomal acid α-glucosidase early in lactation and of a brush border neutral maltase in adult animals.

The significance of these findings is discussed in relation to changes in dietary carbohydrates during weaning and to the mode of digestion of milk carbohydrates by the pouch young.

Introduction

Studies on various mammalian species have demonstrated marked changes in the activities of small intestinal glycosidases during the period from birth to weaning (Henning and Kretchmer 1973). In rats, for example, the activities of lactase (β-D-galactosidase) and of the acid (lysosomal) glycosidases are high during suckling and decline at weaning, whereas those of maltase and other neutral α-D-glucosidases are low at birth and then increase (Rubino *et al.* 1961; Koldovsky 1972).

These studies were all done with eutherians (placental mammals) and there are no comparable data for marsupials, even though the milk carbohydrates of marsupials are qualitatively very different from those of eutherians; instead of lactose, they consist mainly of higher oligosaccharides which are exceptionally rich in galactose (Gross and Bolliger 1958; Jenness *et al.* 1964; Messer and Mossop 1977). Kerry (1969) investigated intestinal disaccharide activities in eight species of marsupials, but only two of the animals studied were pouch young.

In this paper we report on the activities of various intestinal glycosidases of 18 suckling tammar wallabies (*Macropus eugenii*) aged between 5 and 50 weeks, and of two adult animals.

Materials and Methods

Intestines from tammar wallabies were supplied by the Division of Wildlife Research, CSIRO, Canberra. Animals were killed by a blow on the head; adult tammar wallabies were first anaesthetized by injection of sodium methohexital into the lateral tail vein. The small intestine was

removed immediately after death, washed thoroughly with ice-cold 0·15 M NaCl solution to remove its contents, and then stored at −20°C for up to 3 months. Control experiments showed that there was no significant loss of glycosidase activities from rat small intestine when stored at −20°C for 3 months.

Each intestine was divided along its length into three equal sections (proximal, middle and distal). The mucosa of each section was squeezed out by pressing along the outside with a glass rod, weighed, and homogenized in 4 vol. (v/w) of ice-cold water using a glass homogenizer with a mechanically driven Teflon pestle. Homogenates containing toluene (10 μl/ml) were stored at −20°C for up to 2 weeks, prior to enzyme assay. Control experiments showed that there was no significant loss of glycosidase activities during this time.

Enzyme Assays

Lactase (β-D-galactosidase, EC 3.2.1.23), cellobiase (β-D-glucosidase, EC 3.2.1.21), maltase (α-D-glucosidase, EC 3.2.1.20), isomaltase (oligo-1,6-glucosidase, EC 3.2.1.10), sucrase (sucrose α-D-glucohydrolase, EC 3.2.1.48) and trehalase (α,α-trehalase, EC 3.2.1.28) activities were determined using the appropriate disaccharide substrates, as described by Dahlqvist (1964). Sodium acetate buffer, 0·1 M, was used for the pH range 3·5–5·5, and sodium maleate for pH 5·5–6·5.

β-*N*-Acetyl-D-glucosaminidase (EC 3.2.1.30) was determined as described by Koldovsky and Herbst (1971), except that the final concentration of substrate (*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) was 2·5 mM, the pH was 5·0 (unless otherwise stated) and the reaction was stopped after 5–15 min by the addition of 1 M Tris-HCl, pH 8·3 (Asp 1971).

α-L-Fucosidase (EC 3.2.1.51) was determined with *p*-nitrophenyl-α-L-fucoside as substrate (Levvy and McAllan 1961). The assay mixture contained sodium citrate 0·5 M (0·2 ml), pH 5·5, substrate solution, 2 mM (0·4 ml) and diluted intestinal homogenate (0·2 ml). The reaction was stopped after 5–20 min by the addition of 0·4 ml 1 M Tris-HCl, pH 8·3.

Neuraminidase (EC 3.2.1.18) was assayed with *N*-acetyl-neuraminyl-D-lactose as substrate as described by Dickson and Messer (1978), except that dimethylsulfoxide was used instead of butanol in the thiobarbituric acid determination of sialic acid (Skoza and Mohos 1976). Because of the instability of the enzyme (Dickson and Messer 1978), all neuraminidase assays were done immediately after preparation of the homogenate.

Homogenate protein was estimated by the method of Lowry *et al.* (1951); bovine serum albumin was used as the standard.

One unit of enzyme activity is defined as that which hydrolyses 1 μmole of substrate per minute at 37°C.

Incubation of Intestinal Mucosa with Milk Carbohydrate

A homogenate (20% w/v) of the mucosa from the distal section of the small intestine of a 23-week-old tammar wallaby pouch young was centrifuged at 1000 *g* for 5 min to remove large cell debris. The pH of the supernatant was either left unchanged at pH 6·5, or adjusted to 4·5 with 1 M acetate buffer, pH 4·0. Of the supernatant, 0·15 ml was mixed with 1 mg (in 0·05 ml water) of the carbohydrate fraction of tammar wallaby milk obtained 24 weeks *post partum* (Messer and Green 1979). Toluene, 5 μl, was added and the mixture incubated at 37°C. Samples, 1 μl, were taken at various intervals for up to 48 h, and applied to thin-layer chromatography (t.l.c.) plates. T.l.c. was done as described by Hansen (1975).

Results

Lactase

Fig. 1 shows the specific lactase activities of homogenates of the mucosa of the proximal, middle and distal thirds of the small intestine of 18 suckling and two adult tammar wallabies. All activities were measured at pH 4·6, the optimum pH throughout lactation (Fig. 1, inset). Up to 36 weeks *post partum* there was considerable variability in the activities between individual animals, even those of the same age, but in each animal the lactase activity of the distal third of the intestine was greater than that of the middle, and markedly greater than that of the proximal third. The activity in the distal and middle sections reached a peak between 26 and 30 weeks and then

rapidly declined; by 42 weeks of age the activities in all three sections were reduced to the very low levels (<2 units/g protein) found in the adult animals.

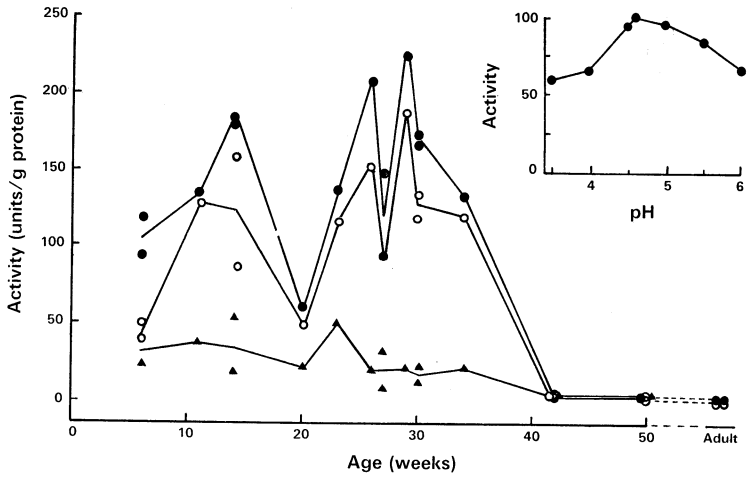


Fig. 1. Lactase activity. Specific activities of the mucosa of the small intestine of adult and suckling tammar wallabies. Each point represents the mean value of duplicate determinations of the activity of one-third (distal, middle or proximal) of the intestine of a single animal. ● Distal third. ○ Middle third. △ Proximal third. *Inset:* Activity (% of maximum) as a function of pH.

It was of interest to determine the effects of *p*-chloromercuribenzene sulfonate (*p*-CMS) and Tris, and to compare these with the effects seen in rats (see Discussion).

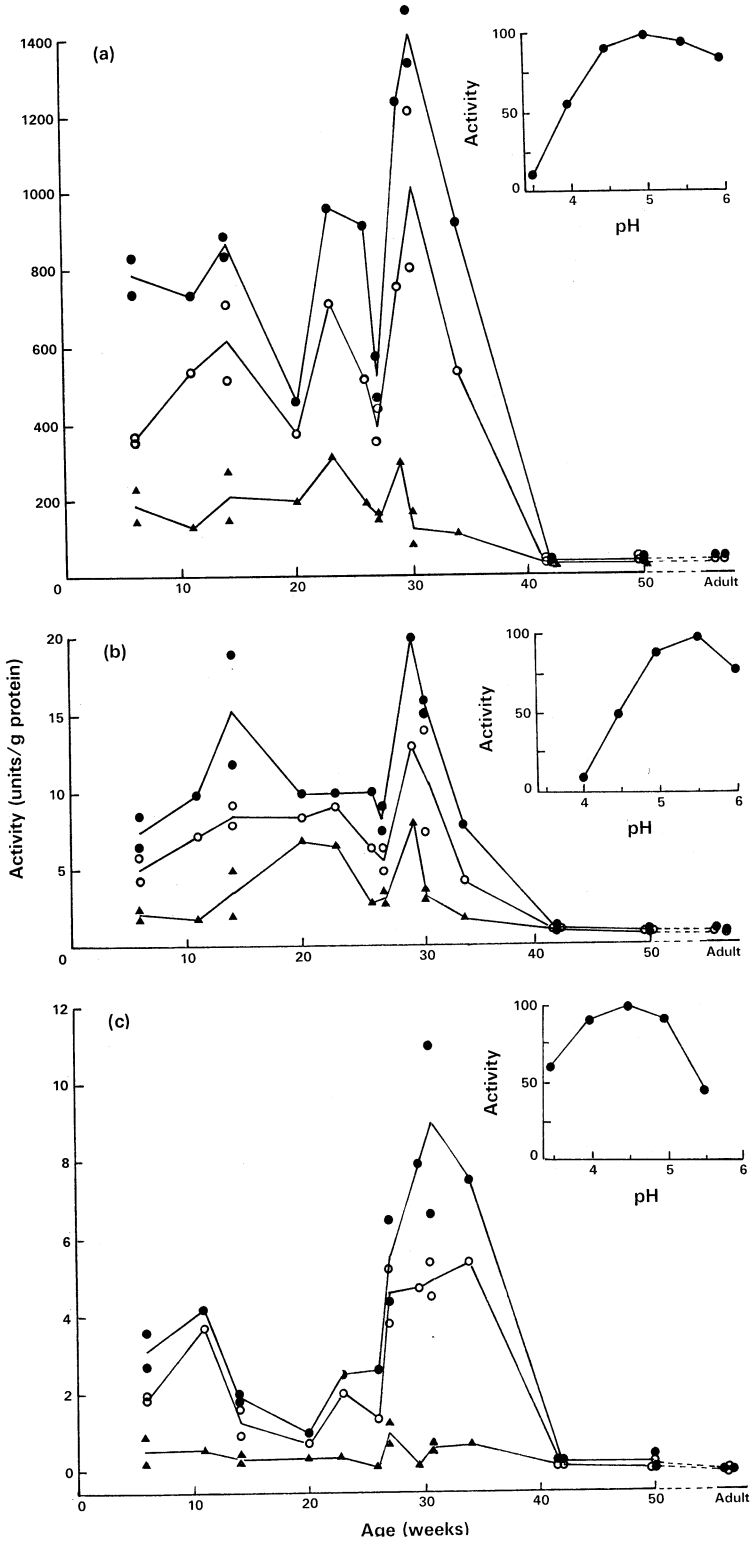
Table 1. Effects of p-CMS[A] and Tris on intestinal lactase activity

All experiments were done at pH 6·0, except for those testing the effect of *p*-CMS on tammar wallaby lactase activity, which were done at pH 4·6. Although the tammar wallaby lactase had only about 65% of maximal activity at pH 6·0 (Fig. 1, inset), the effect of Tris was tested at this pH, since the inhibiting effect of Tris on intestinal disaccharidases is known to be low at low pH values (Larner and Gillespie 1956)

Species	Age	Section of intestine	Inhibition (%) *p*-CMS (0·1 mM)	Tris (0·5 M)
Rat	8 days	Whole	13	74
	Adult	Whole	13	75
Tammar wallaby	11 weeks	Proximal	95	10
	11 weeks	Distal	99	8·9
	30 weeks	Proximal	92	15
	30 weeks	Distal	100	11

[A] *p*-Chloromercuribenzene sulfonate.

Table 1 shows that *p*-CMS had a much greater inhibiting effect on the intestinal lactase activity of tammar wallaby than on that of rats; Tris, on the other hand, had little effect on the lactase of tammar wallaby but significantly inhibited that of rats.


(a)
(b)
(c)
Activity (units/g protein)
Activity
pH
Adult
Age (weeks)

Cellobiase

There was no detectable cellobiase activity at either pH 4·6 or 6·0 in any part of the small intestine in any of the 18 suckling animals investigated.

β-N-Acetylglucosaminidase, α-L-Fucosidase and Neuraminidase

The activities of these enzymes showed changes with age similar to those observed with lactase; they were high from 6 weeks *post partum*, reached a peak between 26 and 30 weeks and then declined to the very low levels found in adults (Figs 2*a*, 2*b*, 2*c*). In each case the activity was highest in the distal third of the intestine. The pH optima were 5·0, 5·5 and 4·5 (insets to Figs. 2*a*, 2*b* and 2*c*), respectively.

Maltase

The specific intestinal maltase activity was low until 34 weeks of age, after which it rose rapidly towards the higher levels found in adult animals (Fig. 3*a*). During the first 20 weeks, the maltase had a pH optimum of 5·0 in all three sections of the intestine, but from 42 weeks it was optimal at pH 6·0 (Fig. 3*a*, inset). Between those ages the pH optimum was intermediate. Thus the results shown in Fig. 3*a* were obtained at various pH values, depending on the age of the animal. The maltase activity was maximal in the distal third of the intestine until 34 weeks *post partum*, after which it was maximal in the middle and proximal sections.

It was of interest to determine the effect of turanose (3-*O*-α-glucosylfructose) on the intestinal maltase activity at various ages, and to compare this with the effect in rats. In rats, the inhibition by turanose ($\simeq 33\%$) was independent of the age of the animal and the section of intestine studied (Table 2), but in tammar wallabies the effect depended on both these factors. Thus at 11 weeks of age, turanose caused marked inhibition of the maltase activity, especially in the distal section of the small intestine, whereas in adult animals the effect of turanose was similar to that in rats. At 27 weeks of age, the effect was intermediate, with greater inhibition in the distal compared with the proximal section.

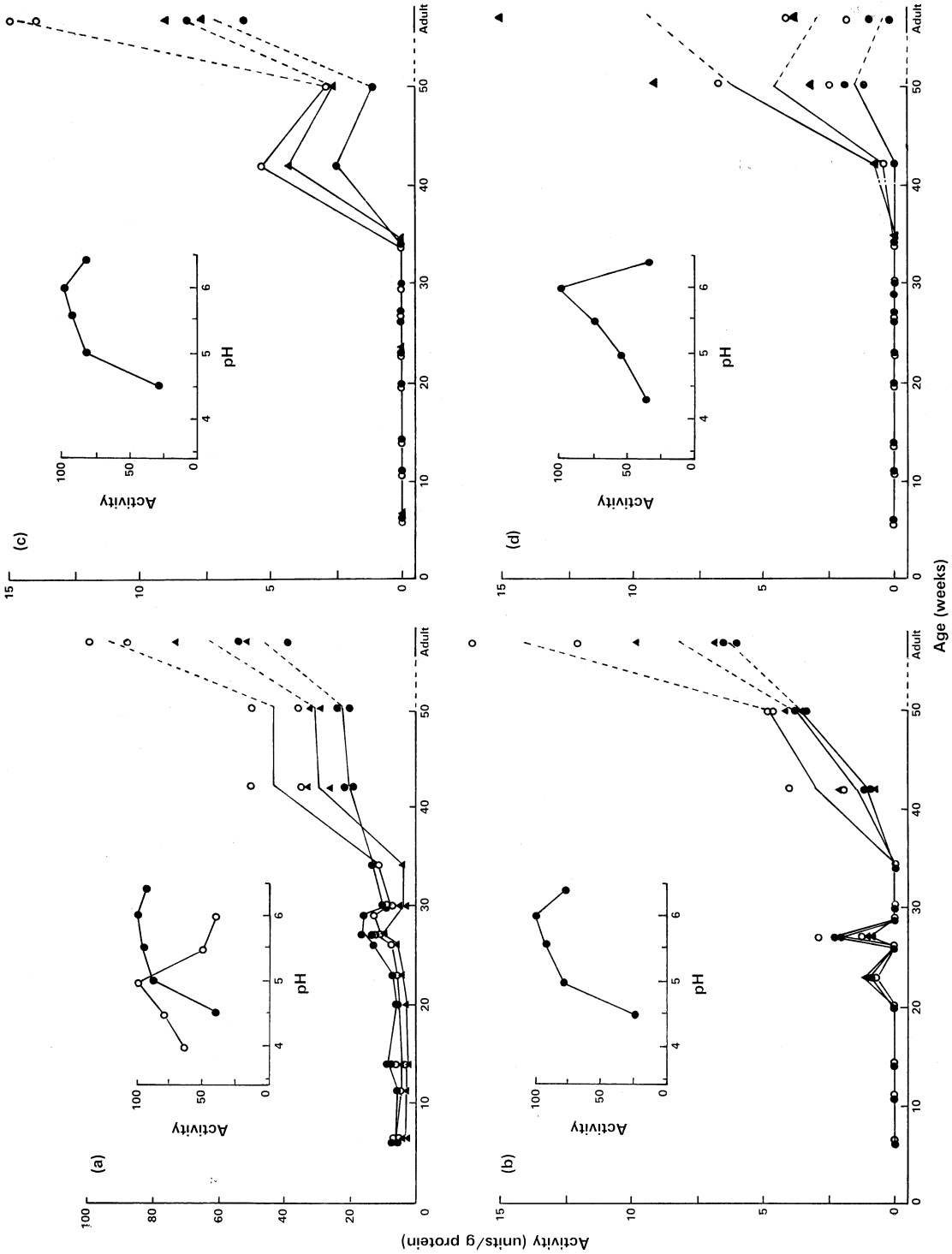
Sucrase, Isomaltase and Trehalase

Activities of these enzymes were all very low or undetectable up to 34 weeks of age, after which they rose to the higher levels found in adult animals (Figs 3*b*, 3*c* and 3*d*, respectively). All three activities were maximal at pH 6·0, but the pH-activity curve for trehalase (Fig. 3*d*, inset) differed from the curves for sucrase and isomaltase (insets to Figs 3*b* and 3*c*), which were identical with each other and with that for maltase at 42 weeks (cf. Fig. 3*a*, inset). Sucrase and isomaltase activities were both maximal in the middle section of the intestine, whereas trehalase was maximal in the proximal third.

Degradation of Milk Carbohydrate by Intestinal Mucosa

When tammar wallaby milk carbohydrate was incubated at pH 4·5 with a homogenate of intestinal mucosa from a 23-week pouch young (see Materials and Methods),

Fig. 2. (*a*) *β-N*-Acetylglucosaminidase activity. (*b*) α-L-Fucosidase activity. (*c*) Neuraminidase activity. For explanation and symbols, see Fig. 1. Insets as in Fig. 1.


(a)
(b)
(c)
(d)
Activity
pH
Adult
Age (weeks)
Activity (units/g protein)

the oligosaccharides completely disappeared within 24 h and were replaced by monosaccharides, predominantly galactose; glucose and traces of *N*-acetylglucosamine and sialic acid were also observed. When the experiment was repeated at pH 6·5, only partial hydrolysis took place.

Table 2. Effect of turanose on intestinal maltase activity

Species	Age	Section of intestine	pH	Inhibition by 60 mM turanose (%)
Rat	3 days	Proximal	6·0	31
	3 days	Distal	6·0	35
	Adult	Proximal	6·0	33
	Adult	Distal	6·0	35
Tammar wallaby	11 weeks	Proximal	5·0	78
	11 weeks	Distal	5·0	92
	27 weeks	Proximal	5·5	36
	27 weeks	Distal	5·5	57
	Adult	Proximal	6·0	28
	Adult	Distal	6·0	32

Discussion

The intestinal lactase activity of tammar wallaby, like that of other mammals, was found to be high during suckling and low in adult animals. This confirmed Kerry's (1969) finding of high intestinal lactase activity in a pouch young ringtail possum and a grey kangaroo, relative to the activities found in adult marsupials. The values observed by us during the suckling period (from 8·0 to 227 units/g protein) were similar to those found in suckling rats (Rubino *et al.* 1964) and human infants (Kerry and Townley 1965).

In addition to lactase, three other glycosidase activities, viz. *β*-*N*-acetylglucosaminidase, α-L-fucosidase and neuraminidase, were found to be high in suckling animals compared with adults. The observed activities were of the same order as those of *β*-*N*-acetylglucosaminidase (Koldovsky and Herbst 1971), α-L-fucosidase (J. M. Rowley and M. Messer, unpublished data) and neuraminidase (Dickson and Messer 1978) of the small intestine of suckling rats. The activities of all four enzymes began to decline towards adult levels after about 30 weeks *post partum*, a time which correlates reasonably well with that at which there is a marked fall in the carbohydrate content of tammar wallaby milk beginning at about 26 weeks *post partum*, and with the period during which the animals begin to leave the pouch and to consume herbage (Messer and Green 1979).

The carbohydrate of the milk of tammar wallaby and other marsupials consists of a mixture of neutral oligo- and polysaccharides and sialyl saccharides, whose major monosaccharide constituent is galactose; glucose, *N*-acetylglucosamine and sialic acid are also present (Messer and Mossop 1977; Messer and Green 1979). Recent

Fig. 3. (*a*) Maltase activity. (*b*) Sucrase activity. (*c*) Isomaltase activity. (*d*) Trehalase activity. For explanation and symbols, see Fig. 1. Insets as in Fig. 1. Inset to Fig. 3*a*: ○ 20 weeks. ● 42 weeks.

studies have shown that the neutral oligosaccharides consist mainly of β-(1→3)-linked galactose residues, with lactose at their reducing ends (Messer *et al.* 1980; J. G. Collins, J. H. Bradbury, E. Trifonoff and M. Messer, unpublished data). The formation of galactose and other monosaccharides from the tammar wallaby milk carbohydrate, during *in vitro* incubation with intestinal homogenate from a pouch young, was therefore likely to have been due to the combined actions of lactase (a β-galactosidase), β-*N*-acetylglucosaminidase and neuraminidase. It can be reasonably assumed that these three enzymes play a similar digestive role *in vivo*. The function of α-L-fucosidase is problematical since the milk carbohydrate of tammar wallaby does not contain fucose (Messer and Green 1979), but this enzyme may be involved in the digestion of other constituents of milk, such as glycoproteins or glycolipids.

In rats, humans and other eutherians, most of the intestinal lactase activity is due to an enzyme, often called 'neutral lactase', which is localized in the microvillous (brush border) membrane of the epithelial cells, is more active in the proximal than the distal part of the small intestine, has a pH optimum of 5·5–6·0, is inhibited by Tris but not by *p*-chloromercuribenzoate (*p*-CMB) or *p*-CMS, and is active towards cellobiose as well as lactose. A small part of the total lactase activity is due to an acid β-galactosidase which is associated with lysosomes (Alpers 1969), is most active in the distal part of the intestine, has a pH optimum of 3·5–4·5, is inhibited by *p*-CMB but not by Tris, and has no cellobiase activity (Gray and Santiago 1969; Asp *et al.* 1970; Kraml *et al.* 1972). In this study we found, unexpectedly, that the intestinal lactase activity of tammar wallaby had the properties of an acid β-galactosidase rather than a neutral lactase. It was greatest in the distal part of the intestine, was optimal at pH 4·6, was inhibited by *p*-CMS but not by Tris, and there was no cellobiase activity. These observations therefore suggest that the intestinal lactase activity of tammar wallaby is associated with the lysosomes rather than microvilli. A lysosomal localization is supported by the observation that its pattern of post-natal development and distribution along the intestine was similar to the patterns shown by β-*N*-acetylglucosaminidase, α-L-fucosidase and neuraminidase, all three of which are presumed to be lysosomal (Koldovsky and Herbst 1971; Barret and Heath 1977; Dickson and Messer 1978). Furthermore, the almost complete inhibition of the lactase activity by *p*-CMS and the absence of cellobiase activity suggest that the neutral lactase of eutherians is entirely absent.

If the intestinal β-galactosidase activity of tammar wallaby is exclusively intracellular, then the *in vivo* digestion of the milk carbohydrates could not take place on the brush border membrane and would require a mechanism for their entry into the cell prior to their degradation. A mechanism for the intestinal digestion of the sialyl lactose of rat milk, involving entry into the epithelial cells via pinocytosis prior to its digestion by lysosomal neuraminidase and acid β-galactosidase, has been proposed for suckling rats (Dickson and Messer 1978). This is based on numerous demonstrations of extensive pinocytosis and absorption of antibodies and other macromolecules in the small intestine of infant rats and other eutherians (see Henning and Kretchmer 1973). Unfortunately it is not known whether similar pinocytosis occurs in the intestine of suckling marsupials, but there is evidence that pouch young of the tammar wallaby, quokka (*Setonix brachyurus*) and possum (*Trichosurus vulpecula*) acquire antibodies from milk by absorption through the intestine (Yadav 1971).

The intestinal activities of the α-glucosidases were either low (maltase, sucrase) or absent (sucrase, isomaltase, trehalase) during the first 34 weeks *post partum*, and then rose towards the levels found in adult animals. These changes are similar to those observed in suckling rats (Rubino *et al.* 1964) and appear to correlate in time with the dietary change from milk carbohydrates to cellulose and other carbohydrates obtained from herbage. The activities were somewhat lower than those found in most eutherians, but Kerry (1969) similarly observed low α-glucosidase activities in the grey kangaroo, and attributed this to its specialized ruminant-like digestion (Tyndale-Biscoe 1973); the activities of α-glucosidases are low in ruminants such as sheep (Walker 1959) and cattle (Siddons 1968), in which dietary carbohydrates are fermented mainly to volatile fatty acids.

The presence of intestinal sucrase activity in the tammar wallaby was surprising, since sucrase was found to be absent in ruminants (Walker 1959; Siddons 1968) and the grey kangaroo (Kerry 1969). It was, however, consistent with the finding of isomaltase activity; in eutherian intestine the sucrase and isomaltase activities are due to a single protein with two active sites (Conklin *et al.* 1975). The tammar wallaby sucrase and isomaltase activities were almost identical with respect to the effect of pH and the patterns of post-natal development and distribution along the intestine, and may similarly be due to a single sucrase–isomaltase.

The maltase activity of the adult animals was similar to that of eutherians in its pH optimum, distribution along the intestine, and inhibition by turanose, and there is no reason to suppose that it is not located in the brush border membrane. The maltase activity of animals aged up to 20 weeks, however, had a relatively low pH optimum (5·0), was greater in the distal than the proximal section and was strongly inhibited by turanose (Table 2), and thus resembled an acid maltase, believed to be lysosomal, found in rat intestine (Galand and Forstner 1974). These results therefore suggest the possibility that during development of the pouch young a lysosomal maltase is replaced by a brush border maltase.

Further studies will be needed to clarify the intracellular localization and physiological roles of the intestinal glycosidases of marsupial pouch young. The apparent lack of brush border β-galactosidase activity suggests, however, that the *in vivo* mode of intestinal digestion of milk carbohydrates in suckling marsupials differs fundamentally from that for lactose in suckling eutherians.

Acknowledgments

We thank Dr B. Green, Mr J. C. Merchant and Mr K. W. Newgrain, Division of Wildlife Research, CSIRO, Canberra, for supplying the intestines from tammar wallaby. This work was supported by the Australian Research Grants Committee.

References

Alpers, D. H. (1969). Separation and isolation of rat and human intestinal β-galactosidases. *J. Biol. Chem.* **244**, 1238–46.

Asp, N.-G. (1971). Human small intestinal β-galactosidases. Separation and characterization of three forms of an acid β-galactosidase. *Biochem. J.* **121**, 299–308.

Asp, N.-G., Dahlqvist, A., and Koldovsky, O. (1970). Small intestinal β-galactosidase activity. *Gastroenterology*, **58**, 591–3.

Barrett, A. J., and Heath, M. F. (1977). Lysosomal enzymes. In 'Lysosomes'. (Ed. J. T. Dingle.) 2nd Edn. p. 86. (North-Holland Publishing Co.: Amsterdam.)

Conklin, K. A., Yamashiro, K. M., and Gray, G. M. (1975). Human intestinal sucrase–isomaltase. *J. Biol. Chem.* **250**, 5735–41.

Dahlqvist, A. (1964). Method for assay of intestinal disaccharidases. *Anal. Biochem.* **7**, 18–25.

Dickson, J. J., and Messer, M. (1978). Intestinal neuraminidase activity of suckling rats and other mammals. Relationship to the sialic acid content of milk. *Biochem. J.* **170**, 407–13.

Galand, G., and Forstner, G. G. (1974). Soluble neutral and acid maltases in the suckling rat intestine. *Biochem. J.* **144**, 281–92.

Gray, G. M., and Santiago, N. A. (1969). Intestinal β-galactosidases. Separation and characterization of three enzymes in normal human intestine. *J. Clin. Inv.* **48**, 716–28.

Gross, R., and Bolliger, A. (1958). The occurrence of carbohydrates other than lactose in the milk of a marsupial (*Trichosurus vulpecula*). *Aust. J. Sci.* **20**, 184–5.

Hansen, S. A. (1975). Thin-layer chromatographic method for identification of oligosaccharides in starch hydrolysates. *J. Chromatogr.* **105**, 388–90.

Henning, S. J., and Kretchmer, N. (1973). Development of intestinal function in mammals. *Enzyme* **15**, 3–23.

Jenness, R., Regehr, E. A., and Sloan, R. E. (1964). Comparative biochemical studies of milks. II. Dialyzable carbohydrates. *Comp. Biochem. Physiol.* **13**, 339–52.

Kerry, K. R. (1969). Intestinal disaccharide activity in a monotreme and eight species of marsupials (with an added note on the disaccharidases of five species of sea birds). *Comp. Biochem. Physiol.* **29**, 1015–22.

Kerry, K. R., and Townley, R. R. W. (1965). Genetic aspects of intestinal sucrase–isomaltase deficiency. *Aust. Paediat. J.* **1**, 223–35.

Koldovsky, O. (1972). Hormonal and dietary factors in the development of digestion and absorption. In 'Nutrition and Development'. (Ed. M. Winick.) (John Wiley: New York.)

Koldovsky, O., and Herbst, J. (1971). *N*-Acetyl-β-glucosaminidase in the small intestine and its changes during postnatal development of the rat. *Biol. Neonate* **17**, 1–9.

Kraml, J., Kolinska, J., Ellederova, D., and Hirsova, D. (1972). β-Glucosidase (phlorizin hydrolase) activity of the lactase fraction isolated from the small intestinal mucosa of infant rats, and the relationship between β-glucosidases and β-galactosidases. *Biochim. Biophys. Acta* **258**, 520–30.

Larner, J., and Gillespie, R. E. (1956). Gastrointestinal digestion of starch. II. Properties of the intestinal carbohydrases. *J. Biol. Chem.* **223**, 709–25.

Levvy, G. A., and McAllan, A. (1961). Mammalian fucosidases. 2. α-L-Fucosidase. *Biochem. J.* **80**, 435–9.

Lowry, O., Rosebrough, N. J., Farr, A. L., and Randall, J. (1951). Protein measurement with the phenol reagent. *J. Biol. Chem.* **193**, 265–75.

Messer, M., and Green, B. (1979). Milk carbohydrates of marsupials. II. Quantitative and qualitative changes in milk carbohydrates during lactation in the tammar wallaby (*Macropus eugenii*). *Aust. J. Biol. Sci.* **32**, 519–31.

Messer, M., and Mossop, G. S. (1977). Milk carbohydrates of marsupials. I. Partial separation and characterization of neutral milk oligosaccharides of the eastern grey kangaroo. *Aust. J. Biol. Sci.* **30**, 379–88.

Messer, M., Trifonoff, E., Stern, W., Collins, J. G., and Bradbury, J. H. (1980). Structure of a marsupial milk trisaccharide. *Carbohydr. Res.* **83**, 327–34.

Rubino, A., Zimballati, F., and Auricchio, S. (1964). Intestinal disaccharidase activities in adult and suckling rats. *Biochim. Biophys. Acta* **92**, 305–11.

Siddons, R. C. (1968). Carbohydrase activities in the bovine digestive tract. *Biochem. J.* **108**, 839–44.

Skoza, L., and Mohos, S. (1976). Stable thiobarbituric acid chromophore with dimethyl sulphoxide. Application to sialic acid assay in analytical de-*O*-acetylation. *Biochem. J.* **159**, 457–62.

Tyndale-Biscoe, H. (1973). 'Life of Marsupials.' (E. Arnold: London.)

Walker, D. M. (1959). The development of the digestive system of the young animal. III. Carbohydrase enzyme development in the young lamb. *J. Agric. Sci.* **53**, 374–80.

Yadav, M. (1971). The transmission of antibodies across the gut of pouch-young marsupials. *Immunology* **21**, 839–51.

Manuscript received 31 March 1980, accepted 7 July 1980