Biochem. J. (1978) **170**, 407–413
Printed in Great Britain

Intestinal Neuraminidase Activity of Suckling Rats and Other Mammals

RELATIONSHIP TO THE SIALIC ACID CONTENT OF MILK

By J. JEFFREY DICKSON and MICHAEL MESSER
Department of Biochemistry, University of Sydney, Sydney, N.S.W. 2006, Australia

(*Received* 12 *July* 1977)

1. The neuraminidase activity of homogenates of the mucosa of the middle and distal thirds of the small intestine of rats increased about 5-fold between birth and 4 to 8 days of age, and then gradually declined to the much lower adult activity by 24 days. No comparable changes occurred in the proximal third. 2. In 8-day-old rats, the neuraminidase activity of the middle and distal thirds of the small intestine was about 10 times greater than that of the proximal third, 20 times greater than that of the colon and at least 100 times greater than that of the liver, brain, gastric mucosa or pancreas. 3. In all other species investigated (mice, rabbits, cats and guinea pigs), the neuraminidase activity of the middle and distal thirds of the small intestine was greater in suckling animals than in adults. 4. The sialic acid content of rat milk increased about 2-fold between birth and 8 days *post partum* and then declined. 5. There was a highly significant positive correlation between the intestinal neuraminidase activity of suckling animals of various species and ages and the sialic acid content of milk obtained from the corresponding species and stage of lactation. 6. It is suggested that the intestinal neuraminidase of suckling mammals functions primarily to remove sialic acid from various components of milk, thus providing sialic acid for the synthesis of sialoglycoproteins and gangliosides by the young.

Sialic acid is found in milk as a constituent of casein (Woodward, 1976), whey proteins (Bezkorovainy *et al.*, 1970), glycoproteins and gangliosides of the fat globule membrane (Harrison *et al.*, 1975; Keenan, 1974) and a variety of oligosaccharides (Kobata, 1972). In rat milk a quantitatively important source of sialic acid is *N*-acetylneuraminyl-D-lactose (Kuhn, 1972).

The presence of sialic acid in milk suggests that the gastrointestinal tract of suckling mammals might contain significant neuraminidase (EC 3.2.1.18) activity, but there are few published studies on intestinal neuraminidase, and none on the intestinal neuraminidase activity during the postnatal period.

The present paper reports the neuraminidase activity of the small intestine of suckling rats and other infant mammals, and shows that this activity is related to the sialic acid content of milk.

Materials and Methods

Tissue homogenates

Rats, mice and rabbits were stunned and then killed by decapitation. Guinea pigs, cats and the possum (*Trichosurus vulpecula*) were lightly anaesthetized in diethyl ether and then killed by exsanguination. None of the animals had been starved before killing. To prepare homogenates of intestinal mucosa, the small intestine was washed thoroughly with ice-cold 0.15M-NaCl solution to remove its contents and then divided into thirds. The mucosa of each third of the intestine was squeezed out by drawing a glass rod firmly along the length of the intestine, weighed and then homogenized in ice-cold water for 2min (about 12 strokes) in a glass homogenizer with a mechanically driven Teflon pestle. For infant mice and newborn rats, the whole of the intestinal wall of each third was homogenized because the fragility of the intestine made it difficult to obtain only mucosa. With infant rats (Wistar), mice (BALB-C), cats and the possum, a 10% (w/v) homogenate was made of the tissue from the proximal third of the small intestine, whereas a 2% homogenate was made of the middle and distal thirds. With adult animals and with infant guinea pigs and rabbits (New Zealand White), a 10% homogenate was made in each case. To prepare homogenates of liver, pancreas and brain, the tissue was finely minced with scissors before homogenization. To obtain gastric mucosa the stomach was opened and the milk clot removed, the tissue was washed and the mucosa scraped off with a glass slide. Colonic mucosa was obtained as described for small-intestinal mucosa. Homogenate concentrations (w/v) were as follows: brain and liver, 20%, pancreas and gastric mucosa, 10%; colonic mucosa, 5%.

Neuraminidase assay

The standard incubation mixture consisted of 0.05ml of 0.2M-sodium acetate buffer, pH4.0,

mixed with 0.05ml of 7.5mM-*N*-acetylneuraminyl-D-lactose solution (monoammonium salt; Boehringer, Mannheim, Germany) and 0.10ml of freshly prepared tissue homogenate. Enzyme and substrate controls contained water instead of substrate solution or tissue homogenate respectively. Incubations were carried out at 37°C for 10min, except where otherwise indicated. The reaction was started by the addition of tissue homogenate and stopped by the addition of 0.2ml of periodate reagent (Aminoff, 1961); the free sialic acid was then determined as described below. The amount of sialic acid liberated by neuraminidase was calculated by subtracting the sialic acid found in the controls from that found in the complete assay mixture.

All assays were carried out on duplicate samples of homogenate. One unit of neuraminidase activity is defined as 1.0μmol of sialic acid liberated from *N*-acetylneuraminyl-D-lactose/min.

Collection of milk and determination of milk sialic acid

Rats were maintained under ether anaesthesia while being milked; other animals were not anaesthetized. The following animals were given an intraperitoneal injection of oxytocin immediately before milking, with the dose (i.u./kg body wt.) in parentheses: mouse and rat (10), guinea pig (5), cat (1) and possum (0.5). To milk rabbits, oxytocin was unnecessary. Milk was obtained by hand massage of the mammary gland and nipple, and collected in capillary micro-pipettes, calibrated 'to contain' either 10 or 50μl. The milk was diluted into an appropriate measured volume of ice-cold water, the pipette being rinsed out thoroughly. For the determination of total (free plus bound) sialic acid, 0.10ml of the diluted milk was treated with 0.10ml of 0.10M-H_2SO_4 at 85°C for 20min; these conditions gave optimal release of sialic acid. For the determination of free sialic acid, the heating step was omitted. The sialic acid was determined as described below.

Analytical

Sialic acid was determined by the thiobarbituric acid method as described by Aminoff (1961), except that the sample (0.20ml) was treated with 0.20ml of the periodate reagent for 60min at room temperature (20–25°C) rather than for 30min at 37°C; this modification, which was similar to that suggested by Ada (1963), slightly decreased the final absorbance, but had the advantage that it decreased the apparent free sialic acid of the substrate control of the neuraminidase assay by 70%. Synthetic *N*-acetylneuraminic acid (type IV; Sigma Chemical Co., St. Louis, MO, U.S.A.) was used as the standard.

When the standard conditions for the neuraminidase assay were varied by the addition of other compounds (e.g. citrate), control experiments were carried out to show that there was no interference with the determination of sialic acid.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Results

Optimum conditions for assay of infant-rat intestinal neuraminidase activity: effects of cations and other compounds

The pH optimum of the neuraminidase activity of homogenates of the small-intestinal mucosa of infant rats was 4.0 (Fig. 1). The activity in sodium acetate buffer was slightly greater than in potassium acetate or sodium maleate and considerably greater than in sodium citrate buffer (Fig. 1). The time course of the reaction over 60min is shown in Fig. 2. The amount of sialic acid released in 10min by infant-rat intestinal-mucosa homogenate was proportional to the concentration of homogenate, at least up to 2% (w/v). In experiments in which a homogenate of infant-rat intestinal mucosa had been centrifuged at 105000*g* for 60min, it was found that of the total activity recovered (82%), 77% was in the particulate fraction (resuspended in 0.15M-NaCl) and 23% was in the supernatant. Consequently, whole-tissue homogenates were used as the enzyme preparation in all experiments.

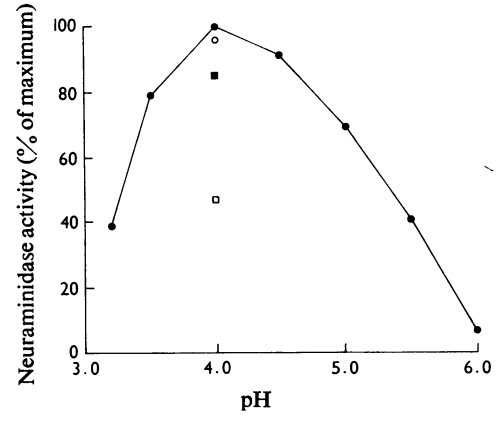

Fig. 1. *Effect of pH and various buffers on rat intestinal neuraminidase activity*

A homogenate prepared from the intestinal mucosa of a 4-day-old rat was assayed for neuraminidase activity as described in the Materials and Methods section, except that the pH of the incubation mixture and the type of buffer solution used was varied as indicated. The final concentration of buffer solution was 50mM in each case. ●, Sodium acetate; ○, potassium acetate; ■, sodium maleate; □, sodium citrate.

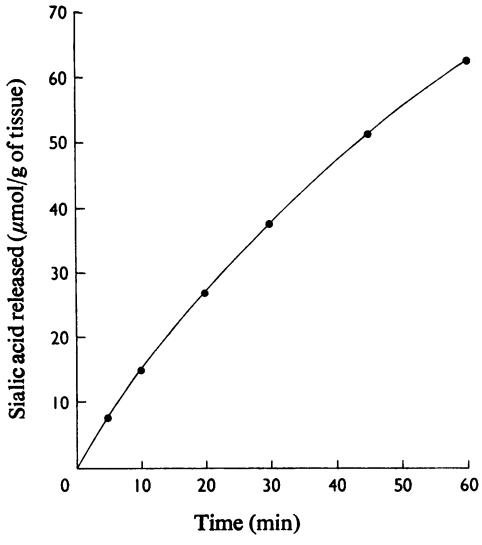

Fig. 2. *Time course of the action of rat intestinal neuraminidase on N-acetylneuraminyl-D-lactose*
A homogenate (2%, w/v), prepared from the intestinal mucosa of a 13-day-old rat, was assayed for neuraminidase activity as described in the Materials and Methods section, except that the time of incubation was varied as indicated.

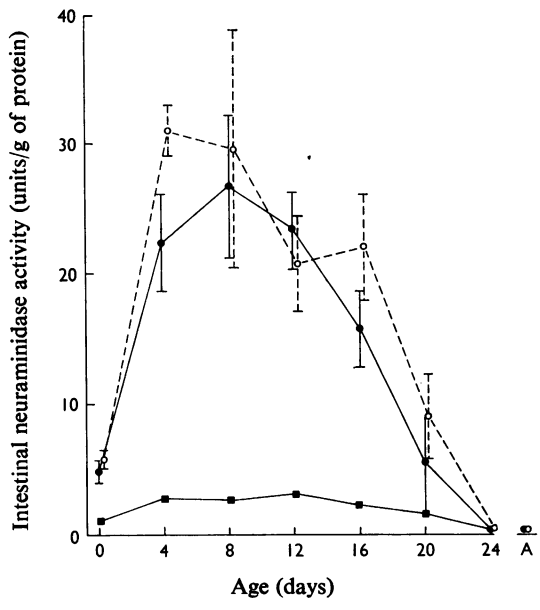

Fig. 3. *Postnatal developmental changes in the specific neuraminidase activities in different regions of the small intestine of rats*
Homogenates of the mucosa from the proximal (■), middle (●) and distal (○) thirds of the small intestine of rats of various ages were assayed for neuraminidase activity as described in the Materials and Methods section. Each point represents the mean (±S.E.M.) of determinations in three animals from different litters, except that at 12 days four animals were used, and at 24 days and in adults (A) only two animals were used. At 0 and 4 days, the tissue from a pair of littermates was combined to provide sufficient for assay, so that at these times the points represent means of three pairs of littermates.

When the substrate solution was replaced by 0.60mM-*N*-acetylneuraminic acid in the standard assay mixture, there was a 100% recovery of the added sialic acid after 30min of incubation. This showed that there was no significant *N*-acetylneuraminate aldolase (EC 4.1.3.3) activity under the conditions of the neuraminidase assay (cf. Tsvetkova, 1965).

Addition of 10mM-Ca^{2+}, -Mg^{2+} or -Mn^{2+} as their chlorides or 5mM-EDTA or 0.1mM-*p*-chloromercuribenzoate had no effect on the intestinal neuraminidase activity. Addition of a detergent, Triton X-100, resulted in 15% inhibition of activity at 0.05% (w/v) concentration and in 25% inhibition at a concentration of 0.3% (w/v). The presence of 0.1M-Tris/HCl buffer decreased the activity by 20%.

The homogenates rapidly lost their neuraminidase activity at 37°C; after 2h of incubation before assay, only 27% of the initial activity remained. Storage of the homogenate at 4°C resulted in 32% loss of activity over 2h, whereas storage at −15°C for 1 week resulted in a 20% loss of activity; one cycle of freezing and thawing without storage resulted in a 5% loss of activity. Owing to the instability of the intestinal neuraminidase activity, only freshly prepared homogenates were used in the following experiments.

Neuraminidase activity of rat small intestine during postnatal development

The specific neuraminidase activity of the middle and distal thirds of the small intestine rose rapidly between birth and 4 days of age, reached a peak at 4–8 days and then gradually fell, reaching the far lower values of the adult animal by 24 days of age (Fig. 3). In the proximal third, the neuraminidase activity was much lower than in the middle and distal thirds, and developmental changes in activity were small.

The neuraminidase activity per unit weight of tissue showed developmental changes similar to those in specific activity, with peaks of 2.2 and 2.9 units/g of mucosa at 4 days of age in the middle and distal thirds of the small intestine respectively.

Neuraminidase activity in various tissues of the suckling rat

The middle and distal thirds of the small intestine had by far the greatest neuraminidase activity of a variety of tissues of suckling rats. At 8 days of age, the activities in colonic mucosa, brain, liver, gastric mucosa and pancreas were 1.3, 0.21, 0.08, 0.15 and 0.05 units/g of protein respectively, compared with 2.8, 27 and 30 units/g of protein (see Fig. 3) in the proximal, middle and distal thirds of the intestine respectively. The activities in the latter four tissues were so low that the standard incubation period (10min) was not sufficient to obtain measurable differences in absorbance between the tests and the controls in the sialic acid determination. Incubations were therefore carried out for periods of up to 3h; there was no decline in enzyme activity during these periods.

Intestinal neuraminidase activities of other mammals

Table 1 presents the results of experiments in which the neuraminidase activities of different regions of the small intestine of various infant and adult mammals were measured. In the mouse, rabbit, guinea pig and cat, as in the rat, intestinal neuraminidase activity was higher during the suckling period than in the adult. It was also relatively high in the infant brush-tailed possum. In all the infant eutherians, neuraminidase activity was greatest in the distal third of the small intestine, but in the infant possum, a marsupial, activity was greatest proximally. Except in the mouse, neuraminidase activity was almost uniformly distributed along the small intestine of adult animals.

Relationship of intestinal neuraminidase activity to the sialic acid content of milk

To determine whether the intestinal neuraminidase activity of suckling mammals (Fig. 3 and Table 1) was related to the milk sialic acid concentration, the amount of sialic acid in milk from rats and other species, obtained at various stages of lactation, was determined. The concentration of free sialic acid was negligible in all milk samples. In rat milk the total sialic acid concentration was 13μmol/ml at parturition, reached a peak of 27μmol/ml at 8 days *post partum* and then fell through the remainder of lactation to reach a value of 3.7μmol/ml after 24 days. Among other species, the milk sialic acid concentration was high in the mouse, lower in the possum and rabbit and still lower in the guinea pig and cat.

These results are presented graphically in Fig. 4, in which the intestinal neuraminidase activities of infant rats and other mammals of various ages are plotted against the sialic acid concentration of milk collected from the mothers at the corresponding times *post partum*. Generally, in those species and at those stages of lactation where the intestinal neuraminidase activity of the young was high, the milk sialic acid concentration was also high. Conversely, when the intestinal neuraminidase activity was low, so was the milk sialic acid concentration. This positive correlation between intestinal neuraminidase activity and milk sialic acid concentration was highly significant ($r = +0.84$, degrees of freedom $= 14$, $P < 0.01$).

Discussion

The present study shows that the mucosa of the distal and middle thirds of the small intestine of suckling rats and other mammals contains very high neuraminidase activity. In the rat, this activity was much greater, by two orders of magnitude, than the activity of other tissues and of the intestine of adults.

Neuraminidase activity has been detected previously in the small intestine of adult rats (Carubelli *et al.*, 1962), but at an activity less than 1% of that found in the present investigation. In the earlier report, however, only the soluble activity was

Table 1. *Neuraminidase activities in different regions of the small intestine of various species of mammals and at various ages*
Homogenates of the mucosa from each third of the small intestine were prepared and assayed for neuraminidase activity as described in the Materials and Methods section. The results for rat are as in Fig. 3. For other species, one animal only was used at each age, except for 8-day-old guinea pigs and 11-day-old mice, where the values are the means from two animals. The activities of adult (A) animals were measured by using incubation periods of 1h as well as the standard one of 10min; these activities were linear with respect to time during this period.

		Neuraminidase activity (units/g of protein)															
Region of small intestine	Age (days) ...	Rat		Mouse			Rabbit			Possum	Guinea pig			Cat		
		8	A	8	11	A	0	15	A	120	0	8	A	4	11	A
Proximal		2.8	0.27	5.4	3.3	0.31	0.22	0.20	0.23	2.3	0.36	0.42	0.64	0.18	0.10	0.10
Middle		27	0.24	13	13	0.50	1.3	2.3	0.16	0.89	0.89	0.39	0.54	0.21	0.16	0.12
Distal		30	0.35	17	14	1.1	5.1	7.4	0.16	0.83	1.7	0.41	0.57	0.91	1.6	0.10

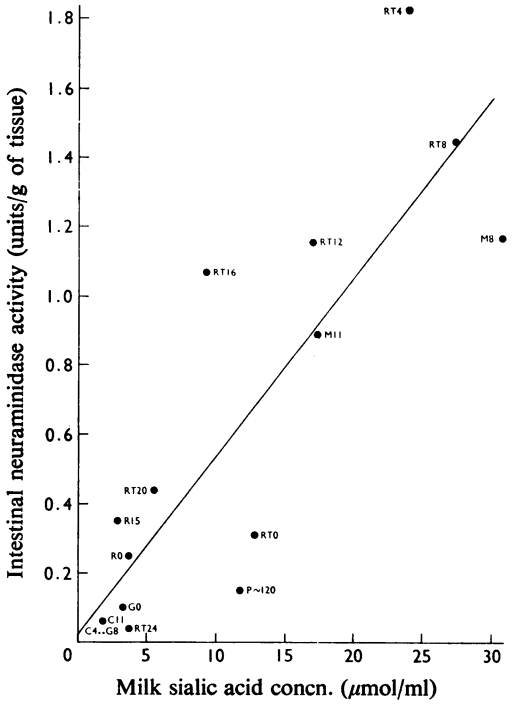

Fig. 4. *Relationship between intestinal neuraminidase activity and milk sialic acid concentration in various species*

The intestinal neuraminidase activity represents an average for the mucosa of the whole length of the small intestine, calculated from the enzyme activity in each third of the intestine (data of Fig. 3 and Table 1, but expressed in units/g of tissue). The values for milk sialic acid concentration are each based on the analysis of one milk sample only, except in the rat, where they are the means of values obtained with milk from two animals. C, cat; G, guinea pig; M, mouse; P, brush-tailed possum; R, rabbit; RT, rat. The numbers after the abbreviations for the animals give the ages of the young in days. The diagonal line is the line of regression, for which the equation, calculated by linear regression analysis, was $y = 0.0515x + 0.0249$.

measured, the substrate concentration (0.15 mM) was considerably lower and the enzyme may not have been assayed near its pH optimum because the pH of the reaction mixture was not stated. Activity towards *N*-acetylneuraminyl-D-lactose similar to that found in the present study has been reported in adult human small-intestinal and colonic mucosa (Ghosh *et al.*, 1968), but in that case most of the enzyme was in the 105000*g* supernatant. Kolodny *et al.* (1971) have described an enzyme of rat small intestine that cleaves *N*-acetylneuraminic acid from Tay–Sachs ganglioside (G_{M2}-ganglioside). This enzyme, like the neuraminidase of infant rat small intestine, was found mainly in the particulate fraction and was inhibited by Triton X-100, but differed in being inhibited by Ca^{2+} and Mg^{2+} cations and *p*-chloromercuribenzenesulphonate. More importantly, *N*-acetylneuraminyl-D-lactose did not inhibit enzyme activity towards Tay–Sachs ganglioside, and therefore was probably not a substrate for this enzyme; this suggests that the enzyme described by Kolodny *et al.* (1971) is not identical with the neuraminidase investigated in the present paper.

Our results for the total sialic acid concentration of rat milk at various stages of lactation were very similar to those of Kuhn (1972) for the *N*-acetylneuraminyl-D-lactose concentration. At 4, 8, 12 and 16 days *post partum*, when the total sialic acid concentration was 24, 27, 17 and 9.2 μmol/ml respectively (Fig. 4), the *N*-acetylneuraminyl-D-lactose concentration, as determined by Kuhn (1972), was 26, 22, 23 and 4.1 μmol/ml respectively. Comparable values for other sialic acid-containing rat milk constituents are available only for casein, the sialic acid content of which is 20 μmol/g (Woodward & Messer, 1976). If the mean casein concentration in rat milk is 7.6% (w/v; Luckey *et al.*, 1954) then casein, the main protein of milk, contributes only 1.5 μmol of the total sialic acid/ml. It is evident therefore that, at least during the first 12 days *post partum*, *N*-acetylneuraminyl-D-lactose must be quantitatively the major sialic acid-containing constituent of rat milk.

The present results show that in rats at various stages of lactation and in other species there was a positive correlation between the intestinal neuraminidase activity and the sialic acid concentration of milk (Fig. 4). This correlation suggests that the main function of intestinal neuraminidase in suckling mammals is to participate in the digestion of sialic acid-containing milk components, such as *N*-acetylneuraminyl-D-lactose, thus enabling free sialic acid to be supplied to the young mammal for use in the synthesis of glycoproteins and glycolipids. In rats, the suckling period is known to be the time of most rapid synthesis of brain sialoglycoproteins and gangliosides (Roukema *et al.*, 1970). Even though circulating sialic acid may not be able to enter the brain during this period, an exogenous supply of sialic acid might nevertheless be advantageous for species such as rats and mice, whose young are born in a relatively immature state. In these species the liver may not have the full adult capacity for sialic acid synthesis during the early postnatal period, since it has been shown that the activity of UDP-*N*-acetylglucosamine 2-epimerase, which catalyses the first step unique to the biosynthesis of sialic acid (Kornfeld *et al.*, 1964), is low in the livers of newborn rats, rising to a maximum only at 14–21 days after birth (Kikuchi *et al.*, 1971). It is noteworthy in this connection that sialic acid is a significant com-

ponent of the milk carbohydrates of monotremes and marsupials (Messer & Kerry, 1973; Messer & Mossop, 1977), whose young are hatched or born respectively in a semi-embryonic state.

The subcellular localization of the intestinal neuraminidase was not investigated in detail, but it is known that in liver and other tissues neuraminidase activity is found in various cell fractions, including lysosomes (Mahadevan *et al.*, 1967; Tulsiani & Carubelli, 1971; Kishore *et al.*, 1975). Several of our findings suggest that the intestinal neuraminidase of suckling rats is also at least partially lysosomal. The enzyme had an acid pH optimum, was found mainly in the particulate fraction and exhibited a pattern of postnatal development and distribution along the small intestine that was very similar to that of acid β-galactosidase (Koldovský & Chytil, 1965), an enzyme that has been shown to be localized in the lysosomes of adult rat and human small-intestinal mucosa (Alpers, 1969). In all these respects the neuraminidase also resembled other intestinal acid hydrolases that are generally presumed to be lysosomal (Hsu & Tappel, 1964; Koldovský & Herbst, 1971), such as β-glucuronidase (Heringová *et al.*, 1965), arylsulphatase (Danovitch & Laster, 1969), *N*-acetylglucosaminidase (Koldovský & Herbst, 1971) and α-galactosidase (Jumawan *et al.*, 1972). A partial explanation for the high neuraminidase activity in the distal and middle thirds of the small intestine of suckling rats, as compared with adult rats, might therefore lie in a higher concentration of intestinal lysosomes. However, as noted by Danovitch & Laster (1969) and Koldovský *et al.* (1972), there are substantial variations in the ratios of activities of various acid hydrolases during development and along the length of the small intestine. The results of Koldovský *et al.* (1972; Table 2) show, for example, that the ratios of enzyme activities in the distal third of the small intestine of 14-day-old rats to those of adult rats were 41 for acid β-galactosidase, 19 for β-glucuronidase, 29 for α-galactosidase and 13 for *N*-acetylglucosaminidase. The present results (Fig. 3) indicate that with neuraminidase, for which the peak of activity occurred earlier than with the other hydrolases, this ratio was 89 for 4-day-old and 60 for 12-day-old rats. Therefore the high intestinal neuraminidase activity of infant as compared with adult rats must be at least partially specific to that enzyme.

If the intestinal neuraminidase of suckling mammals is lysosomal, then the cleavage of sialic acid from various constituents of milk would take place within the lysosomes rather than in the intestinal lumen or on the microvilli. This would imply entry of these constituents, or their partial breakdown products, into the mucosal epithelial cells via pinocytosis, in line with much morphological and cytochemical evidence pointing to large-scale pinocytosis and absorption of immunoglobulins, other proteins, lipid and particulate matter in the distal small intestine of rats and other mammals during the suckling period (Clark, 1959; Cornell & Padykula, 1969; Williams & Beck, 1969; Clarke & Hardy, 1969). *N*-Acetylneuraminyl-D-lactose might be incorporated into pinocytotic vesicles along with macromolecules; after release of the sialic acid by lysosomal neuraminidase, the lactose moiety could be hydrolysed by the acid β-galactosidase (Asp & Dahlqvist, 1968). This mechanism for the intestinal digestion of *N*-acetylneuraminyl-D-lactose would provide a possibly important function for the lysosomal β-galactosidase of the intestine of suckling rats.

This work was supported by the Australian Research Grants Committee and by a scholarship to J. J. D. from the Medical Research Committee of the University of Sydney.

References

Ada, G. L. (1963) *Biochim. Biophys. Acta* **73**, 276–284
Alpers, D. H. (1969) *J. Biol. Chem.* **244**, 1238–1246
Aminoff, D. (1961) *Biochem. J.* **81**, 384–392
Asp, N. G. & Dahlqvist, A. (1968) *Biochem. J.* **106**, 841–845
Bezkorovainy, A., Grohlich, D. & Pinter, J. K. (1970) *J. Agric. Food Chem.* **18**, 624–628
Carubelli, R., Trucco, R. E. & Caputto, R. (1962) *Biochim. Biophys. Acta* **60**, 196–197
Clark, S. L. (1959) *J. Biochem. Biophys. Cytol.* **5**, 41–48
Clarke, R. M. & Hardy, R. N. (1969) *J. Physiol. (London)* **204**, 113–125
Cornell, C. A. & Padykula, H. A. (1969) *Am. J. Anat.* **125**, 291–316
Danovitch, S. H. & Laster, L. (1969) *Biochem. J.* **114**, 343–349
Ghosh, N. K., Kotowitz, L. & Fishman, W. H. (1968) *Biochim. Biophys. Acta* **167**, 201–204
Harrison, R., Higginbotham, J. D. & Newman, R. (1975) *Biochim. Biophys. Acta* **389**, 449–463
Heringová, A., Jirsová, V. & Koldovský, O. (1965) *Can. J. Biochem.* **43**, 173–178
Hsu, L. & Tappel. A. L. (1964) *J. Cell Biol.* **23**, 233–240
Jumawan, J., Koldovský, O. & Palmieri, M. (1972) *Biol. Neonate* **20**, 380–384
Keenan, T. W. (1974) *J. Dairy Sci.* **57**, 187–192
Kikuchi, K., Kikuchi, H. & Tsuiki, S. (1971) *Biochim. Biophys. Acta* **252**, 357–368
Kishore, G. S., Tulsiani, D. R. P., Bhavanandan, V. P. & Carubelli, R. (1975) *J. Biol. Chem.* **250**, 2655–2659
Kobata, A. (1972) *Methods Enzymol.* **28**, 262–271
Koldovský, O. & Chytil, F. (1965) *Biochem. J.* **94**, 266–270
Koldovský, O. & Herbst, J. (1971) *Biol. Neonate* **17**, 1–9
Koldovský, O., Palmieri, M. & Jumawan, J. (1972) *Comp. Biochem. Physiol. B* **43**, 1–8
Kolodny, E. H., Kanfer, J., Quirk, J. M. & Brady, R. O. (1971) *J. Biol. Chem.* **246**, 1426–1431
Kornfeld, S., Kornfeld, R., Neufeld, E. F. & O'Brien, P. J. (1964) *Proc. Natl. Acad. Sci. U.S.A.* **52**, 371–379
Kuhn, N. J. (1972) *Biochem. J.* **130**, 177–180

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Luckey, T. D., Mende, T. J. & Pleasants, J. (1954) *J. Nutr.* **54**, 345–359
Mahadevan, S., Nduaguba, J. C. & Tappel, A. L. (1967) *J. Biol. Chem.* **242**, 4409–4413
Messer, M. & Kerry, K. R. (1973) *Science* **180**, 201–203
Messer, M. & Mossop, G. S. (1977) *Aust. J. Biol. Sci.* **30**, 379–388
Roukema, P. A., van den Eijnden, D. H., Heijlman, J. & van der Berg, G. (1970) *FEBS Lett.* **9**, 267–270
Tsvetkova, I. V. (1965) *Biokhimiya* **30**, 349–355
Tulsiani, D. R. P. & Carubelli, R. (1971) *Biochim. Biophys. Acta* **227**, 139–153
Williams, R. M. & Beck, F. (1969) *J. Anat.* **105**, 487–501
Woodward, D. R. (1976) *Dairy Sci. Abstr.* **38**, 137–150
Woodward, D. R. & Messer, M. (1976) *Comp. Biochem. Physiol. B* **55**, 141–143