Developmental Changes of Hematioside of Rat Small Intestine

POSTNATAL HYDROXYLATION OF FATTY ACIDS AND SIALIC ACID*

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The hematioside of rat intestine is analyzed from 1 day to 60 days of age. During the first 3 weeks of life, Gm3 (N-acetyleneuraminylgalactosylglucosylceramide) contains only nonhydroxylated fatty acids and accounts for 80-90% of the ganglioside sialic acid. Its concentration is maximum at 6 days (315 µg of NeuAc/g of intestine) and falls abruptly over the next 2 weeks. It reaches 45 µg of NeuAc/g of intestine at 60 days. Between 28 and 60 days, Gm3 accounts for 72% of the total intestinal gangliosides. From 21 days on, structural modifications of Gm3 are observed. N-Acetyleneuraminic acid is replaced progressively by N-glycolyneuraminic acid and nonhydroxylated fatty acids are replaced by a-hydroxylated fatty acids. Both changes are interpreted as the result of hydroxylations of GMs components which are triggered at the time of weaning. These hydroxylations take place chiefly in epithelial cells and to a much lesser extent in nonepithelial residue, as shown by the separate analysis of both compartments of rat intestine at 38 and 60 days. In epithelial cells, the highest percentage of a-hydroxylated fatty acids and of N-glycolyneuraminic acid is found at 60 days. In addition, 4-d-hydroxysphinganine is the major base of the Gm3 of intestinal cells from birth to adulthood.

Gangliosides are recognized as markers of differentiation because their quantity and their accessibility at the cellular surface are specific of a cell type as well as of the state of differentiation of this cell (1, 2). In a previous study, we have observed that, in the intestinal epithelium of adult rat, villus cells (mature cells) contain and synthesize more GMs hematioside than crypt cells (proliferative cells) (4, 5). However, nothing indicates that the differentiation taking place for the normal regeneration of intestinal epithelium in adult rat is similar to the differentiation taking place during intestinal ontogenesis. Rat is not mature at birth and many organs and functions are completed only after birth (6). In particular, intestine, which is adapted to milk digestion during the suckling period, undergoes a rapid "redifferentiation" at the time of weaning in order to acquire its morphologic, metabolic, and kinetic properties of adult age (7). In a previous communication, we have reported on the major changes affecting the neutral glycolipid composition after birth (8). The present study is devoted to the ganglioside composition of rat intestine during postnatal development. As it appears that changes affect mainly Gm3 hematioside, we have focused our investigation on the quantitative and the qualitative evolutions of this ganglioside.

EXPERIMENTAL PROCEDURES

Animals—Wistar rats were obtained from the "Institut Merieux" (L’Arbresle, France). Day 1 was the day following birth. The entire small intestine was excised from several rats, and intestines were pooled before lipid extraction. At 1 day and at 3 days, intestines were taken from one litter (12 to 17 rats). From 6 days on, all the rats were male. At 6, 13, 21, 24, and 28 days, intestines were pooled from 10 rats. At 38 and 60 days, they were pooled from 4 rats. In addition, at 38 and 60 days, epithelial cells were isolated from the jejunum-ileum of 6 rats by the method of Weiser (9) with 10 successive steps (4). Cells isolated during the four first steps were villus cells. Cells collected during the three last steps were crypt cells. The intestinal tube from which epithelium had been removed was kept and studied as nonepithelial residue.

Lipid Extraction—Intestines were cut into small pieces and they were homogenized in methanol (7 ml/g, wet tissue) with an Ultra Turrax homogenizer for 30 s at medium speed. Chloriform was added (7 ml/g, wet tissue) and the mixture was homogenized again for 10 s. Lipids were extracted overnight under gentle stirring. The suspension was centrifuged. The supernatant was pipetted out and the pellet was homogenized in chloroform/methanol (1:1) (5 ml/g, wet tissue). After 2 h at room temperature, the suspension was centrifuged and the pellet was extracted again with chloroform/methanol (1:2) (5 ml/g, wet tissue). Combined supernatants were evaporated to dryness with a rotary evaporator. They were kept in chloroform/methanol (2:1).

Ganglioside Purification—Gangliosides were purified from 20 mg of lipids. Lipids were dissolved in chloroform/methanol/water (60:30:4.5) and desalted by chromatography on a column of Sephadex G-25 (1 g) (10). Purified lipids were dried down and dissolved in chloroform/methanol/water (30:60:8). Acidic lipids were separated from neutral lipids by chromatography on a column of DEAE-Sephadex (A-25, acetate form, 0.5 g) according to the method of Ueno et al. (11). After saponification of the alkali-labile phospholipids, they were sonicated in 2 ml of water and the solution was desalted by chromatography on a Sephadex G-50 column (K15–30, Pharmacia, Uppsala, Sweden).

Chemical Assay—The sialic acid content of lipid mixtures was determined by the method of Svennerholm (12) modified by Miettinen and Takki-Lukkainen (13).

Thin Layer Chromatography—Thin layer chromatography was performed on precoated Silica Gel 60 high performance thin layer chromatography plates (0.25 mm thick) (E. Merck, Darmstadt, West Germany). The following solvents were used in this study: (A) chloroform/methanol/water (55:45:10, containing 0.02% CaCl2 · 2H2O), (B) chloroform/methanol/water (60:35:8), and (C) 1-propanol/water/concentrated ammonium hydroxide (70:28:1.5).

Analytical studies were done by high performance thin layer chromatography. Ganglioside mixtures containing 0.2–1.5 µg of sialic acid...
were lyophilized. Purified sialic acids (1-2 pg), dissolved in water, were analyzed by high performance thin layer chromatography in the vacuum generated by a water pump. Sugars were kept dry on anol/concentrated HCl/water. Fatty acid methyl esters were separated into hydroxy- and nonhydroxymethyl esters by gas-liquid chromatography on a glass column (2 mm X 2 m) packed with Supelcoport coated with 10% EGSS-X (Applied Science Laboratories, Inc., State College, PA), according to a published procedure (19). Not the Gm, was also submitted to periodate oxidation and analyzed under the same conditions as above.

An alternate method was used to analyze undegraded long chain bases. Free bases were N-acetylated and O-trimethylsilylated with the conditions given by Carter and Gaver (20). They were analyzed by gas-liquid chromatography on a OV-1 column. The oven temperature was raised to 220 °C at the time of injection and it was programmed at 3 °C/min immediately after injection. Injector and detector temperatures were set, respectively, at 250 and 300 °C.

Gas-liquid chromatographic analyses were performed on a Hewlett-Packard 5710 A gas chromatograph equipped with a double flame ionization detector. The detector signal was recorded on a Hewlett-Packard 3980 A integrator.

RESULTS

In this study, the ganglioside content of rat intestine was analyzed during the postnatal development which spans the suckling period (from birth to 21 days), the weaning period (from 21 to 28 days), and the period of puberty around 35 days.

Dramatic changes affect both the ganglioside concentration and the ganglioside pattern. Quantitative changes begin first. The ganglioside concentration, which is 4 to 5 times higher than in adult intestine during the first 2 weeks of life, decreases sharply after 5 days and it reaches the adult level at the end of the fourth week (Fig. 1). The ganglioside profile does not change at first and then it appears of increasing complexity from 21 days on (Fig. 2). At all ages, Gm, hematide is by far the major ganglioside of rat intestine. It accounts for 85% of the ganglioside content at 1 day, 90% at 6 days, 82% at 21 days, and 72% at 28 days. Thus, the fall of the ganglioside concentration is due essentially to the fall of the Gm, concentration and to a minor extent to a decreasing concentration of other gangliosides (Fig. 1). Minor gangliosides are scarcely detectable during the neonatal period. At 6 days, Gm, alone accounts for about 50% of the minor gangliosides, it is the only one which is identifiable. During the weaning period, the contribution of minor gangliosides to sialic acid content increases and they all become clearly individualized on chromatograms at 28 days. These changes explain partly the increasing complexity of the ganglioside composition which is observed on thin layer chromatograms (Fig. 2).

The most conspicuous change of the intestinal ganglioside pattern concerns Gm,5. During the first weeks of life, Gm,5 is resolved into two spots upon high performance thin layer chromatography. Spots of similar mobility are present throughout the period under study, but two other spots of higher polarity appear after 21 days and their concentration increases with age. These spots of higher polarity have the same mobility as those of the Gm, isolated from epithelial cells at 38 or at 60 days, whereas the Gm, spots of nonepithelial residue migrate at a position similar to the position of neonatal
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Postnatal Hydroxylation of the Ceramide of Intestinal G\textsubscript{M3}—Upon high performance thin layer chromatography, the lactosylceramide of G\textsubscript{M3} hematoside displays a novel pattern of evolution between 13 and 60 days (Fig. 4). This evolution does not originate in the carbohydrate portion of the molecule which is made of glucose and galactose in equimolar amount. Consequently, the increasing proportion of molecular species with a lower mobility must reflect the transformation of the ceramide constituents; fatty acids and sphingoid bases.

Gas-liquid chromatography of sphingoid bases as their aldehyde derivatives gives a major peak of pentadecanal (Fig. 5, left). This aldehyde is likely to come from 4D-hydroxysphinganine but it can also be generated by the periodic oxidation of C\textsubscript{17}-sphinganine. In order to assess the exact nature of the major base of intestinal G\textsubscript{M3} at birth, two other experiments were conducted. The genuine G\textsubscript{M3} molecules were submitted to periodate oxidation. We have reasoned after Crossman and Hirschberg (22) that, in this case, trihydroxy bases bearing free hydroxyl groups on C-3 and C-4 can be oxidized and yield aldehydes with 3 carbons less than the original bases while dihydroxy bases cannot be degraded. We have found that the native G\textsubscript{M3} of 1-day-old and 38-day-old rat intestine yields pentadecanal. Furthermore, upon gas-liquid chromatography of the N-trimethylsilyl derivatives of the free bases, the most prominent peak has the same retention time as standard 4D-hydroxysphinganine (Fig. 5, right). From these analyses, we conclude that 4D-hydroxysphinganine is the major base of rat intestinal G\textsubscript{M3} throughout the developmental period. Among minor bases, sphingosine, detected as hexadecanal upon periodate oxidation, can be found in significant amount only after the 4th week of life. It occurs mainly in nonepithelial tissue, analyzed at 38 and 60 days. At these ages, the G\textsubscript{M3} of nonepithelial residue contains an equal amount of 4D-hydroxysphinganine and of sphingosine.

As the sphingoid base composition does not change signifi-

![Fig. 2. Evolution of the chromatographic pattern of rat intestinal gangliosides during postnatal development. Gangliosides were purified from the entire small intestine of rats from 1 to 60 days (numbers indicate the age of rats in days), from villus epithelial cells of 38- and 60-day-old rats (St, 60b), from crypt epithelial cells, and from nonepithelial residue of 60-day-old rats (60c, 60r). Gangliosides from brains of rat fetuses taken at 17 days of gestation were used as standard (St). The ganglioside spot between G\textsubscript{M1b} and the origin is G\textsubscript{M3b}.](image)

![Fig. 3. Sialic acid of intestinal G\textsubscript{M3} during postnatal development of rat. Sialic acids were analyzed by high performance thin layer chromatography in solvent C. Numbers refer to the age of rats in days (see Fig. 2). S, N-acetylneuraminic acid standard.](image)

| Age (days) | Total intestine | Isolated villus cells | Nonepithelial residue |
|-----------|-----------------|----------------------|----------------------|
| 21        | 22              | 22                   | 15                   |
| 24        | 36              | 36                   | 15                   |
| 28        | 45              | 45                   | 15                   |
| 38        | 55              | 72                   | 15                   |
| 60        | 62              | 89                   | 20                   |

![Table I. N-Glycolyneuraminic acid appearance in intestinal hematoside during development. N-Glycolyneuraminic acid is expressed as percentage of the total response recorded by scanning of the thin layer chromatograms (Fig. 3). At 13 days and before, sialic acid is exclusively N-acetylneuraminic acid.](table)
in the fatty acid composition of G_{M3} does exist. From birth to 13 days, all the fatty acids are nonhydroxylated. At 21 days, 26% of the G_{M3} fatty acids of whole intestine is already \( \alpha \)-hydroxylated (Table II). The percentage of hydroxylation doubles at the end of the 4th week but then, it decreases and remains stable after 38 days. However, the degree of hydroxylation goes on increasing in epithelial cells. In 38-day-old rats, two-thirds of the G_{M3} fatty acids of epithelial cells are \( \alpha \)-hydroxylated and this proportion is even higher at 60 days. In nonepithelial residue, G_{M3} contains a low percentage of \( \alpha \)-hydroxylated fatty acids which increases from 5 to 9% between 38 and 60 days. The decreased percentage of \( \alpha \)-hydroxylated fatty acids in the G_{M3} of whole intestine after 28 days can be explained by a modification of the respective contribution of epithelium and of nonepithelial residue. Nonepithelial residue, which is a minor contributor before 28 days, becomes an important one after, and at 60 days, it provides 52% of intestinal G_{M3}. This finding may explain also why sphingosine, which is a long chain base of nonepithelial residue, appears only in small amounts in intestinal G_{M3} before 28 days and in higher amounts at 38 and 65 days. Thus, the developmental pattern of lactosylceramide which is illustrated by Fig. 4 reflects primarily an increased percentage of \( \alpha \)-hydroxylated fatty acids in the G_{M3} of epithelial cells and secondarily an increased contribution of nonepithelial residue to the G_{M3} of whole intestine.

Chromatographic Behavior of Intestinal G_{M3}—In order to understand the chromatographic pattern of intestinal G_{M3}, the four spots of the G_{M3} of 24-day-old rat intestine were separated by preparative thin layer chromatography and hydrolyzed with neuraminidase. The compositional analysis of the different spots is summarized in Fig. 6.

It has been found that the A spot of G_{M3} contains N-acetylenuraminic acid and nonhydroxylated fatty acids whereas the C and D spots contain N-glycolylenuraminic acid and \( \alpha \)-hydroxylated fatty acids. The intermediate B spot of G_{M3} gives rise to two spots of sialic acid and two spots of lactosylceramide. It is likely that N-glycolylenuraminic acid is associated to less polar ceramide and that N-acetylenuraminic acid is associated to more polar ceramide in such a way that both associations give G_{M3} of similar mobility. When N-acetylenuraminic acid is the only sialic acid, its removal yields a lactosylceramide which is also resolved into more spots than the original G_{M3}. This is the case for the A spot of G_{M3} at 24 days which gives two spots of lactosylceramide of similar intensity and this is the case in neonatal intestine where G_{M3} gives two spots (Fig. 2) and the related lactosylceramide gives three spots (Fig. 4). The degree of hydroxylation of the bases and of the fatty acids as well as their chain length determine the resultant polarity, and thus the mobility of the different molecular species of lactosylceramide (23, 24).

### Table II

| Fatty acids | Days after birth |
|------------|-----------------|
| Nonhydroxylated | 21 24 28 38 38v 60v 60r |
| 16-18 | 30.5 24.3 21.9 25.8 15.1 14.4 19.9 |
| 20-24 | 43.5 34.3 28.1 32.7 16.9 13.4 71.1 |
| \( \alpha \)-Hydroxylated | 20-24 | 4.7 4.9 7.7 7.1 13.1 14.6 2.4 |
| \% \( \alpha \)-hydroxylated | 20-24 | 21.3 36.5 43.2 34.4 54.9 61.8 6.6 |

FIG. 4. Lactosylceramide of intestinal G_{M3} during postnatal development of rat. Lactosylceramides were analyzed by high performance thin layer chromatography in solvent B. Spots were revealed with the α-naphthol/sulfuric acid spray. Numbers refer to the age of rats in days (see Fig. 2). S, lactosylceramide from bovine milk fat globule membrane.

FIG. 5. Chromatographic profiles of sphingoid bases of rat intestinal G_{M3}. On the left side, aldehydes obtained after periodate oxidation of the free bases were analyzed by gas-liquid chromatography on an EGSS-X column. A, position of pentadecanal; B, position of hexadecanal. Sphingoid bases of G_{M3} were taken from the entire intestine of rat at: 1, 1 day; 2, 13 days; 3, 28 days. They were taken at 60 days from: 4, epithelial cells; 5, nonepithelial residue. On the right side, free bases were analyzed as their N-acetyl O-trimethylsilyl derivatives by gas-liquid chromatography on a OV-1 column. 6, G_{M3} of 1-day intestine; 7, G_{M3} of 38-day intestine; 8, standard mixture containing: C, sphingosine; D, 4D-hydroxyphosphine.
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FIG. 6. Schematic chromatographic behavior of individual spots of intestinal GM₃ before and after hydrolysis by neuraminidase. Intestinal GM₃ of 24-day-old rats was analyzed by preparative high performance thin layer chromatography in solvent A. Individual spots A, B, C, and D were isolated and submitted to neuraminidase treatment. Lactosylceramide and sialic acid were analyzed, respectively, in solvent B and in solvent C. A scheme of their chromatographic pattern is given under the chromatographic pattern of GM₃.

These findings show that the analysis of GM₃ by high performance thin layer chromatography resolves partially the different molecular species occurring in rat intestine. It has been sufficient to detect that major changes of composition occur during the developmental period. However, in order to characterize these changes, it has been necessary to analyze separately the sialic acids and the lactosylceramide.

DISCUSSION

We have already discovered that, in adult rat, GM₃ hematosite containing N-glycolyneraminic acid is the prominent ganglioside of epithelial cells of small intestine, that is at least five times more concentrated in villus cells (mature cells) than in crypt cells (proliferative cells) (4, 5), and that, in both cell types, its fatty acids are highly α-hydroxylated (25). These findings have been confirmed by Breimer et al. (26, 27). Besides GM₃, which accounts for 17% of the glycosphingolipids of epithelium, the other major sphingolipids, except sphingomyelin, namely free ceramide, glucosylceramide, globotriaosylceramide (5, 26), and tetrahexosylceramide (27) are also affected by the cellular differentiation occurring in adult rat intestine. It is not known at the present time whether the numbers, but quantitatively minor, complex glycolipids that have been recently identified in the intestinal epithelium of the white rat by Breimer et al. (25) are also affected by this type of differentiation. The regeneration of the intestinal epithelium of adult rat provides a good system for studying a normal type of differentiation in vitro, thanks to the method of Weiser (9) which allows a separation of the cells according to their stage of differentiation. But, up to now, it was not known whether the differentiation of epithelial cells in adult rat intestine mimics the differentiation occurring during the development of intestine.

The major discoveries of the present study are that: (i) GM₃ hematosite is the major ganglioside of neonatal intestine; (ii) it is 7 times more concentrated than in adult intestine; (iii) its fatty acids and its sialic acid, nonhydroxylated at birth, are progressively hydroxylated, starting at the beginning of the 4th week of life.

In a previous study, we have already demonstrated that glucosylceramide is by far the major neutral glycolipid of neonatal intestine and that globotriaosylceramide, which is a major glycolipid of crypt cells of adult intestine, is detected in noticeable amount only after 21 days (8).

Therefore, under all these aspects, namely GM₃ concentration and structure and neutral glycolipid composition, the cell differentiation taking place during the postnatal growth of rat intestine is different from the crypt to villus cell differentiation taking place during the regeneration of the epithelium of adult rat intestine.

The present study was conducted on the entire intestine of 1-day-old to 28-day-old rats because the fragility of the intestinal wall prevented us from using the method of Weiser (9) in order to separate epithelial cells from nonepithelial residue. Epithelium and nonepithelium were separated in 38- and 60-day-old rats. This procedure has led us to observe that the hydroxylation of the fatty acids and sialic acid of GM₃ takes place mainly in epithelial cells and that the adult degree of hydroxylation of both components is not reached by the end of puberty which occurs around 35 days in male Wistar rat. At 60 days, nonepithelial residue contains half the intestinal GM₃ and all the gangliosides more complex than GM₃ whereas epithelium contains the other half of GM₃. Thus, the GM₃ of nonepithelial residue is a more important contributor to the intestinal GM₃ of adult rat than it is suggested by Angstrom et al. (29) but, like these authors, we find that nonepithelial GM₃ is less hydroxylated in its fatty acids and in its sialic acids than epithelial GM₃.

The α-hydroxylation of fatty acids appears to follow a similar evolution in GM₃ and in glucosylceramide which is the most abundant glycolipid of epithelial cells (5, 8, 28). However, at all ages of the developmental period, the degree of hydroxylation is lower in GM₃ than in glucosylceramide. This difference may originate in the fact that intestinal GM₃ comes from the epithelium as well as from the nonepithelial residue while glucosylceramide comes almost exclusively from the epithelium. It is also possible that the difference in the degree of hydroxylation of the fatty acids of GM₃ and of glucosylceramide expresses the delay between the completion of GM₃ and the synthesis of its distant precursor glucosylceramide.

An age-dependent increase of the degree of hydroxylation of fatty acids has been found in the galactosylceramide of rat brain by Kishimoto and Radin (30). This change depends on an α-hydroxylating system which appears in brain after 9 days, reaches a maximum activity around 21 days, and then declines rapidly (31). We have demonstrated that, in intestinal epithelium also, a large part (up to 75%) of the fatty acids of the glycolipids is α-hydroxylated (25), and in the present study we show that this α-hydroxylation is a process under developmental regulation. However, intestine and brain are different on two counts. First, the α-hydroxylation of intestinal epithelium is not likely to decline after 21 days since the percentage of α-hydroxylated fatty acids increases greatly in GM₃ after this age and since intestinal epithelial cells have a mean lifetime which does not exceed 48 h (32). Consequently, in intestinal epithelial cells, an α-hydroxylating activity must be maintained during the whole life. The second difference between intestine and brain is that intestinal GM₃ contains a high percentage of α-hydroxylated fatty acids whereas brain gangliosides contain only nonhydroxylated fatty acids (33).

This study on GM₃ hematosite shows that the development of rat intestine is characterized by a hydroxylation of fatty acids and sialic acid after weaning, while bases do not change. 4D-Hydroxyphosphinganine, which is a trihydroxy base distinctive of intestinal epithelium in many species including human (25, 34, 35), is already present at birth in the GM₃ of rat intestine, as in its glucosylceramide (8). It is also present in GM₃ and in NeuAcα2→6 GlcNAcTetraose (36) as in neuronal glycolipids (37, 38) of human fetal intestine analyzed at birth in meconium. But, unlike what happens in rat intestine, human 4D-hydroxyphosphinganine is already associated to α-hydroxylated fatty acids at birth. One must keep in mind that rat is an animal with a short gestational period and that
it undergoes only after birth some of the stages of development which are completed before birth in species with a long gestational period such as man (59). Therefore, the study of the glycolipids of the developing rat intestine gives evidence that the synthesis of 4-D-hydroxyphosphatidic acid, which requires the addition of an hydroxyl group on C-4 of sphingosine (40), occurs at an earlier stage of development than the α-hydroxylation of the fatty acids of glycolipids.

N-glycolylneuraminic acid derives from N-acetylneuraminic acid by the action of a N-acetyl hydroxylase (21). It is the prominent sialic acid of the GM₃ of the intestinal epithelium of adult rat (4, 29). The present study shows that, at birth, GM₃ contains only N-acetylneuraminic acid and that the progressive hydroxylation of the molecule is concomitant with the α-hydroxylation of fatty acids, beginning at the time of weaning. N-Glycolynearaminic acid is a common sialic acid of glycolipids and glycoproteins but, up to now, it has never been suspected that its occurrence may be under developmental control. Further investigations are needed to know whether this control is specific of the development of rat intestine or whether it is a general phenomenon occurring in other tissues and other species.

As it is known that rat intestine undergoes important transformations in its structure as well as in its way of absorbing the nutrients during postnatal development, which likely that the modifications of the GM₃ content are an expression, at the molecular level, of the changes affecting the plasma membranes of intestinal cells. After birth, the only nutrient, milk, is absorbed by pinocytosis, which is replaced gradually by an absorption of the adult type, first in the proximal intestine, then in the ileum, and it is terminated at 21 days (59).

Simultaneously, the activity of lactase, a brush border enzyme, which is maximum during the days following birth, declines during the 2nd and the 3rd week of life and reaches its low adult level at 21 days (6). Both processes are likely to involve important transformations of the plasma membranes of epithelial cells. Our results show that GM₃ is at its highest level at 6 days and that it falls abruptly afterward. This evolution suggests that a correlation may exist between the GM₃ content of plasma membranes, the pinocytotic capability, and/or the lactase activity.

By 20 days begins what has been called a "redifferentiation" period because of extensive functional changes in rat intestine (7); the cell turnover is accelerated (41) and new proteins such as sucrase-isomaltase (6) and the adult form of alkaline phosphatase (42, 43) are inserted into the brush border membrane. It is likely that the increased hydroxylation of GM₃ together with the changes in the neutral glycolipid composition (8) are parts of a reorganization of the structure of the plasma membrane. In adult rat, 20% at least of the lipids of the brush border membrane are glycolipids (44). This particular composition may be responsible for the low fluidity that characterizes this membrane (45). The additional hydroxyl groups that appear during the "redifferentiation" period in the carbohydrate as well as in the ceramide part of GM₃ may contribute significantly to the exceptional cohesion of the membrane components (46, 47).

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