Quantitative Determination of Complex Carbohydrates in Bovine Milk and in Milk-Based Infant Formulas

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ABSTRACT
Quantitative determination of all structural families of complex carbohydrate micronutrients was performed on bovine milk samples, milk-based infant formulas, and whey-based manufacturing raw materials. Differences found between formulas depended mainly on their whey:casein ratios. A solvent separation procedure was required for quantitative estimation of the gangliosides and neutral glycolipids within the fat fraction. All infant formulas except one contained slightly more gangliosides than bovine milk. Complex carbohydrates were consistently higher in the nonfat fraction. By gel permeation chromatography, an oligosaccharide subfraction was separated from a glycopeptide one. Oligosaccharide content of infant formulas increased as a function of the whey:casein ratio, and glycopeptides were found only in formulas made with whey components. Neuraminic acids from infant formulas were associated primarily with the glycoprotein fraction, except in hydrolysate-based preparations in which "precipitable" glycoproteins were converted into "soluble" glycopeptides by trypsin treatment. Because whey-based raw materials are very rich in all bovine milk glycoconjugates and oligosaccharides, their increased use will result in high contents of these micronutrients in modern formulas.

(Key words: complex carbohydrates, glycoconjugates, infant formulas)

Abbreviation key: DWP = demineralized whey powder, IF = infant formulas, MFGM = milk fat globule membrane, NeuNAc = neuraminic acid, WPC = whey protein concentrate.

INTRODUCTION
Among mammalian milks, human milk is unique with regard to the number and structural diversity of its complex oligosaccharides (20), the concentration of which decreases as lactation progresses (33). Human milk is also very rich in glycoconjugates (glycoproteins and glycolipids) that carry complex carbohydrate moieties. Biological significance of these carbohydrate micronutrients is not understood. Human milk oligosaccharides have been suspected of being growth-promoting factors for Lactobacillus bifidus, the predominant intestinal flora of breast-fed infants (31); however, a precise relationship has not been established (3). The complex carbohydrates might protect the breast-fed baby against infectious diseases. Indeed, in vitro assays have shown that similar molecules competitively inhibit microbial adhesion and enterotoxin binding by acting as receptor analogues (2, 7, 8, 14, 23, 27). The high content of neuraminic acid (NeuNAc) in human milk oligosaccharide and glycoprotein fractions has been observed to decline exponentially over the first 2 mo of lactation (5). Highly sialylated carbohydrates in human milk might contribute to the increased concentration of NeuNAc present in cerebral and cerebellar glycoconjugates of the breast-fed baby (5).

Compared with human milk, bovine milk contains very little free saccharides other than lactose, and neuraminylactose is the only complex oligosaccharide present in both these milks (22). However, bovine milk contains numerous complex carbohydrates that are attached to protein or lipid backbones as glycoconjugates (20). Infant formulas (IF)
milk with various bovine milk fractions will contain these carbohydrate micronutrients. Quantitative analysis of these molecular species in formulas has been limited (4, 5, 23) although questions have been asked concerning the specific benefits for the bottle-fed infant of having complex carbohydrates of bovine origin in their food (6, 23). Determination of NeuNAc in IF established that most sialylated species were in the glycoprotein fraction (4) and that content of NeuNAc in the oligosaccharide fraction was dependent on the whey:casein ratio of the formula (5). The NeuNAc-containing glycolipids (gangliosides) were determined also in a whole milk-based IF (23). However, no comprehensive study has been conducted to determine quantitatively all the molecular families of carbohydrate micronutrients present in the various kinds of formulas manufactured.

The aim of the present work was to analyze the main milk constitutive fractions for their oligosaccharide and glycoconjugate components upon their isolation from three different bovine milk samples (freshly collected, pasteurized, and UHT-treated), eight milk-based IF (including hypoallergenic hydrolysate-based formulas), and two whey-based raw materials. Human milk samples were also included for some quantitative comparisons.

MATERIALS AND METHODS

Milk Samples, Formulas, and Dairy Raw Materials

Two pools of human milk aliquots were made from samples collected from a unique mother (blood group O, secretor H) during d 4 to 14 postpartum and the 4th mo of her lactation. Fresh bovine milk was obtained immediately after collection; pasteurized and UHT-treated milk samples were purchased in a supermarket. The IF of varied composition were from Nestlé (Vevey, Switzerland) (Table 1). Demineralized whey powder (DWP) and whey protein concentrate (WPC) were obtained from a Nestlé factory. Pure samples of lactosylceramide and ganglioside GP1 were purchased from BioCarb (Lund, Sweden). The neuraminylactose reference compound was obtained from Sigma Chemical Company (St. Louis, MO), and the caseinoglycomacropeptide was prepared as previously described (25).
Analytical Methods

Total neutral sugars (9) and total NeuNAc (17) were estimated quantitatively by colorimetry. Neutral and amino sugar compositions were analyzed by capillary gas-liquid chromatography (26). High performance thin layer chromatography of neutral glycolipids was performed by using CHCl₃-MeOH-H₂O (65:25:4, vol/vol/vol). Spots were visualized by dipping the plates (12). High performance thin layer chromatography of gangliosides was performed by using CHCl₃-MeOH-aqueous .25% CaCl₂ (65:35:8, vol/vol/vol) in two consecutive runs. Spots were visualized by spraying the plates with a solution of resorcinol reagent (28).

Figure 1. Scheme of solvent extraction of milk fat, ganglioside partition, and purification of neutral glycolipids.
Solvent Extraction

The procedure developed by Tettamanti et al. (30) was used on dialyzed milk and reconstituted powder samples. Starting volumes of human milk samples from early and late lactation were 22 and 220 ml, and bovine milk sample volumes were 500 ml. For formula reconstitution, 64 g of dry powder were dissolved in 500 ml of water; for reconstitution of whey-based raw materials, samples equivalent to 17.5 g of protein were dissolved in 500 ml of water.

All preparations were dialyzed (molecular weight cutoff: 6000 to 8000 Da) against water and freeze-dried prior to fat extraction. Fractionation steps were performed as described (30). Lyophilized material was homogenized (20%, wt/vol) into 10 mM potassium phosphate buffer (pH 6.8). Tetrahydrofuran (eight volumes) was added to the suspension prior to centrifugation at 1500 x g for 10 min at 15°C. Pellet was re-extracted three times with a mixture of phosphate buffer and tetrahydrofuran (1:4, vol/vol), and the supernatants were combined. The residual pellet (containing the non-fat fraction) was suspended repeatedly in water and concentrated under reduced pressure to remove traces of tetrahydrofuran prior to lyophilization. To the pooled supernatants, .3 volumes of ethyl ether were added. The resulting organic and aqueous layers were separated before the former was washed with .1 volume of water. Combined aqueous layers (containing the ganglioside fraction) were concentrated and dialyzed (cutoff = 1000) prior to lyophilization. The organic layer (containing the fat frac-
Physical Milk Defatting and Analysis of Subfractions

To obtain milk fractions suitable for further subfractionation, the milk and reconstituted powder samples were centrifuged at 37°C for 40 min at 2100 x g prior to cooling in an ice bath for 2 h. The pellet [material from milk fat globule membranes (MFGM)] was combined with the upper layer (cream), and the liquid layer (skim milk) was collected separately. The cream fraction, when subjected to the solvent extraction procedure, yielded a residue (MFGM proteins and glycoproteins) and a fat fraction from which the gangliosides were partitioned (Figure 2).

To the skim milk layer, an equal volume of TCA solution (24%, wt/vol) was added dropwise before the resulting mixture was centrifuged for 15 min at 4200 x g. Prior to lyophilization, the supernatant was dialyzed against water until it reached pH 5. An aliquot of this last material was applied onto a Sephadex G-50 column and eluted with 100 mM acetic acid. Column fractions were analyzed for total neutral sugars and NeuNAc, and the NeuNAc-positive fractions were collected (Figure 2). Glycopeptide fractions recovered from IF that contained maltodextrins were digested with amyloglucosidase to remove glucose-oligomer contaminants. Digestion was performed at 60°C in acetate buffer (100 mM, pH 5) with...
RESULTS

Complex Carbohydrates from the Lipid, Ganglioside, and Total Nonfat Milk Fractions

Separation procedure (Figure 1) yielded purified fractions of gangliosides and neutral glycolipids plus a nonfat fraction that contained a mixture of complex oligosaccharides, glycopeptides, and glycoproteins. Carbohydrate micronutrients present in these three fractions were estimated quantitatively by colorimetry. Specifically, total neutral sugars were determined in the neutral glycolipid fraction, NeuNAc in the ganglioside fraction, and both measurements were used to quantify the complex carbohydrates within the nonfat fraction. Across fractions, contents of NeuNAc and neutral sugars within complex carbohydrates were higher in human than in bovine milk samples (Table 2). Levels were higher also during the early relative to late human lactation period. Bovine milk samples exhibited very small differences compared with those observed among IF (Table 2).

Heat treatment of bovine milk did not cause a loss of either ganglioside-bound or nonfat fraction NeuNAc (Table 2). All IF except AL 110 contained more gangliosides than the bovine milk samples. Elevated amounts of gangliosides were found in both whey-based manufacturing materials, DWP and WPC. As expected (11), upon thin layer chromatography, lactosylceramide was identified as the major neutral glycolipid in all bovine milk and IF samples (results not shown).

Nonfat fractions contained 20 to 100 times more NeuNAc than the ganglioside fractions (Table 2). Similarly, 40 to 500 times more neutral sugars were present in nonfat complex carbohydrates than in neutral glycolipids (Table 2). The NeuNAc content of nonfat material recovered from IF increased with the whey: casein ratio. For example, formulas made with casein (AL 110) or with whole milk (LACTOGEN) contained about half the amount of nonfat NeuNAc found in bovine milk samples, whereas formulas like NAN and NAN/LP, in which DWP was added to increase the whey: casein ratio, contained the same amount of nonfat NeuNAc as bovine milk. Hypoallergenic formulations prepared with only WPC and DWP (NAN H.A. and NATIVA H.A.) contained still higher levels (Table 2).

Oligosaccharides, Glycopeptides, and Glycoproteins

Precipitation of proteins from skim milk samples yielded supernatants that contained sialylated molecular species. After dialysis, aliquots of these supernatants were subjected to gel permeation chromatography (Figure 2). Typical elution patterns are shown in Figures 1 to 3. The TCA supernatant from fresh bovine
skim milk contained only one fraction positive for NeuNAc. Based upon its elution close to the lactose peak (Figure 3A), this sialylated species was identified as neuraminylactose. Similar elution profiles were obtained from pasteurized and UHT-treated bovine milk samples and from LACTOGEN IF. The supernatant obtained from AL 110 did not contain sialylated complex carbohydrates. By contrast, the TCA supernatant originating from NA 64 IF contained two distinct fractions positive for NeuNAc (Figure 3B). The first fraction was retained moderately by the G-50 Sephadex, and the second corresponded to the neuraminylactose just described. The former fraction exhibited all the characteristics of the caseinoglycomacropeptide, based upon its lack of precipitation by TCA, apparent molecular weight, and general occurrence in formulas made with a whey component.

To ascertain the origin of this "formula-caseinoglycomacropeptide", elution profiles obtained from DWP and WPC were examined (Figure 4). The TCA supernatant of DWP exhibited the same two NeuNAc-containing fractions as the supernatant from NA 64 formula (compare Figures 3B and 4A). When derived from WPC, the TCA nonprecipitable material contained very high amounts of caseinoglycomacropeptide (Figure 4B); however, neuraminylactose no longer occurred in this supernatant. Finally, when performed on the hypoallergenic formula BEBA H.A., this analytical procedure was complicated by the presence of maltodextrins (Figure 5A). Fortunately, the glycopeptide fraction eluted after high molecular weight maltodextrins but before their low molecular weight analogues. This glycopeptide subfraction was purified after isolation by digesting residual maltodextrins with glucoamylase. Subsequent liquid chromatography removed the glucose released by this enzymatic hydrolysis (Figure 5B).

All bovine milk samples contained between 50 and 70 mg/L borne by NeuNAc the oligosaccharide neuraminylactose (Table 3). This level was significantly lower in the whole milk-based LA 20 formula but maintained in whey-containing formulas (NA 60 and NAH 2). As already noted, DWP was rich in neuraminylactose, whereas this oligosaccharide was absent from WPC (Table 3). Amount of

<table>
<thead>
<tr>
<th>Milk sample</th>
<th>Nonfat fraction</th>
<th>Neutral glycolipids</th>
<th>Gangliosides</th>
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<tr>
<td></td>
<td>Neutral sugars</td>
<td>Total NeuNAc</td>
<td>Neutral sugars</td>
</tr>
<tr>
<td>Human (lactation d 4 to 14)</td>
<td>3419</td>
<td>933</td>
<td>6.8</td>
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<tr>
<td>Human (4th lactation month)</td>
<td>1006</td>
<td>270</td>
<td>3.6</td>
</tr>
<tr>
<td>Bovine (freshly collected)</td>
<td>220</td>
<td>166</td>
<td>5.2</td>
</tr>
<tr>
<td>Bovine (pasteurized)</td>
<td>222</td>
<td>190</td>
<td>4.7</td>
</tr>
<tr>
<td>Bovine (UHT treated)</td>
<td>232</td>
<td>192</td>
<td>5.8</td>
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<tr>
<td>Infant formulas³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL 110</td>
<td>ND</td>
<td>92</td>
<td>ND</td>
</tr>
<tr>
<td>LACTOGEN (LA 20)</td>
<td>290</td>
<td>100</td>
<td>2.5</td>
</tr>
<tr>
<td>NAN (NA 60)</td>
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<td>6.0</td>
</tr>
<tr>
<td>NAN/LP (NA 64)</td>
<td>318</td>
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<td>4.5</td>
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<td>NAN/LP (NA 3549)</td>
<td>390</td>
<td>168</td>
<td>3.9</td>
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<tr>
<td>NAN H.A. (NAH 2)</td>
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<td>ND</td>
</tr>
<tr>
<td>NATIVA H.A. (NAH 3572)</td>
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<td>250</td>
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<td>Manufacturing materials⁴</td>
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<tr>
<td>DWP</td>
<td>735</td>
<td>480</td>
<td>ND</td>
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<tr>
<td>WPC</td>
<td>665</td>
<td>685</td>
<td>10.4</td>
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</tbody>
</table>

¹Expressed in milligrams glucose equivalent or NeuNAc by liter of milk sample or reconstituted material.
²ND = Not determined.
³Reconstituted at 12.8% (dry wt/vol).
⁴Reconstituted at 3.5% (protein wt/vol), DWP = demineralized whey powder, WPC = whey protein concentrate.

TABLE 3. Quantitative determination of neuraminic acids\(^1\) borne by complex carbohydrates in subfractions obtained from milk samples, infant formulas, and manufacturing materials.

<table>
<thead>
<tr>
<th></th>
<th>Skim fraction</th>
<th>Cream</th>
<th>Gangliosides (recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Oligosaccharides</td>
<td>Glycopeptides</td>
<td>Glycoproteins(^2)</td>
</tr>
<tr>
<td><strong>Milk sample</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine (freshly collected)</td>
<td>71</td>
<td>ND(^3)</td>
<td>95</td>
</tr>
<tr>
<td>Bovine (pasteurized)</td>
<td>59</td>
<td>ND</td>
<td>131</td>
</tr>
<tr>
<td>Bovine (UHT treated)</td>
<td>53</td>
<td>ND</td>
<td>139</td>
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<td><strong>Infant formulas</strong></td>
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</tr>
<tr>
<td>AL 110</td>
<td>ND</td>
<td>ND</td>
<td>92</td>
</tr>
<tr>
<td>LACTOGEN (LA 20)</td>
<td>28</td>
<td>ND</td>
<td>72</td>
</tr>
<tr>
<td>NAN (NA 60)</td>
<td>49</td>
<td>42</td>
<td>113</td>
</tr>
<tr>
<td>NAN H.A. (NAH 2)</td>
<td>69</td>
<td>213</td>
<td>2</td>
</tr>
<tr>
<td><strong>Manufacturing materials</strong>(^5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DWP</td>
<td>88</td>
<td>96</td>
<td>296</td>
</tr>
<tr>
<td>WPC</td>
<td>9</td>
<td>244</td>
<td>432</td>
</tr>
</tbody>
</table>

\(^1\)Expressed in milligrams of neuraminic acid by liter of milk sample or reconstituted material.

\(^2\)Calculated by difference between the total nonfat neuraminic acids (from Table 2) and those occurring in the oligosaccharide and glycopeptide fractions (columns 1 and 2 from this table).

\(^3\)ND = Not detected.

\(^4\)Reconstituted at 12.8% (dry wt/vol).

\(^5\)Reconstituted at 3.5% (protein wt/vol), DWP = demineralized whey powder, WPC = whey protein concentrate.

NeuNAc measured as a probe for glycopeptides was especially high in WPC and in the NAN H.A. formula, which is based on a DWP: WPC mixture (Table 3). Monosaccharides borne by these glycopeptide molecules were NeuNAc, galactose, and N-acetylgalactosamine (Table 4), as expected for the caseinoglycomacropeptide (1). In addition, amounts of fucose, mannose, and N-acetylgalactosamine were also measured in the nonprecipitable glycopeptides from BEBA H.A. (Table 4). These last constituents originated from the whey glycoprotein carbohydrate chains normally precipitated by TCA, but, in hypoallergenic formulas, these carbohydrate units were recovered as nonprecipitable glycopeptides due to enzymatic hydrolysis of the proteins.

The TCA precipitates contained milk proteins and glycoproteins. The low relative percentage of glycoproteins allowed measurement of less than 1% total neutral sugars in these pellet fractions. Moreover, the TCA precipitation procedure caused extensive protein denaturation, which yielded pellets not readily available for colorimetric measurement of NeuNAc. Finally, these skim milk glycoproteins did not represent the total glycoprotein content of milk and IF samples, because glycoproteins associated with MFGM would be recovered with the cream layer. Consequently, NeuNAc borne by the glycoprotein subfraction was estimated by calculating the difference between the total nonfat NeuNAc (see Table 2) and the NeuNAc associated with both the oligosaccharide and the glycopeptide subfractions (Table 3). The hypoallergenic IF (NAN H.A.) contained almost no more glycoproteins, although its manufacturing materials (DWP and WPC) were very rich in these molecular species (Table 3). This finding again shows that in hydrolysate-based IF, glycoprotein cleavage yielded carbohydrate chains that occur on TCA-soluble glycopeptides.

Recovery of MFGM Components
In the Cream Layer

Generally, the MFGM components are studied after their isolation from a cream fraction prepared by physical methods (23). To allow data comparison, it was necessary to study the behavior of the material derived from MFGM during this physical milk defatting process. For
this purpose, gangliosides served as probes because they are typical of polar lipids from cell membranes. Starting with the cream layers (Figure 2), the procedure of Tettamanti et al. (30) yielded both a "cream-glycoprotein" fraction and a "cream-ganglioside" fraction. Qualitative analysis of these gangliosides was performed by thin layer chromatography. Results (not shown) confirmed that G_{P3} was the major bovine milk ganglioside (19) and established that this was true for all milk-based IF analyzed. The NeuNAc contained in the “cream-ganglioside” fractions was estimated quantitatively (Table 3) for comparison with the values obtained for gangliosides when whole milk samples were partitioned (Table 2). Ratios between these values represented the ganglioside recoveries in the cream layers (Table 3). As expected, freshly collected bovine milk yielded a cream layer made of intact milk fat globules because 95% of the total gangliosides were recovered in this layer. Notably, for bovine milk samples and for the whole milk-based LA formula, ganglioside recovery in cream increased also (Table 3).

**DISCUSSION**

To perform a comprehensive quantification of gangliosides, neutral glycolipids, and the nonfat species (glycoproteins, glycopeptides, and complex oligosaccharides), two separation procedures that involved two distinct aliquots of each sample were used. Methodology developed by Tettamanti et al. (30) for fractionating brain glycoconjugates was used to achieve the complete solvent separation of gangliosides and the purification of neutral glycolipids from milk fat (Figure 1). In addition, physical milk defatting was performed to yield skimmed and cream layers, which were subfractionated (Figure 2). The first procedure quantitatively yielded gangliosides and neutral glycolipids, and the second permitted distinction between the various species of nonfat complex carbohydrates.

With one exception, level of ganglioside-bound NeuNAc was higher in formulas than in bovine milk samples (Table 2). Gangliosides are important components of the MFGM (19, 24, 29). However, when IF ingredients are prepared from homogenized milk, these polar glycolipids associated with disrupted MFGM can migrate into various milk fractions, i.e., cream, milk SNF, or whey-based components. Upon combining these IF ingredients, resulting content of gangliosides might exceed the initial amount found in bovine milk.

It has been shown that fat layers obtained by thermal defatting had lower levels of gangliosides than those reported herein (23). Ganglioside recovery from these cream layers is not complete, especially if those layers have been heat treated (Table 3). Present results indicated that quantitative determination of gangliosides in industrially processed milk samples absolutely requires use of the solvent partition procedure (Figure 1).

Content of IF gangliosides is not always dependent on the origin of the fat component (Table 2). Although both AL 110 and LACTOGEN are made with a fat component of milk origin, the AL 110 does not contain ganglio-
sides, but LACTOGEN contains a high level of these molecular species (Table 2). If the milk fat ingredient used for manufacturing an IF is cream, these complex carbohydrates will originate partly from this component. However, if butter oil is used instead of cream, glycoconjugates will not occur in this fat ingredient because MFGM was removed with the butter milk (15). Fat fractions of NA 60 and NA 3549 formulas contained the same ganglioside level, but the former was made with milk fat and the latter with vegetable fat (Table 2). Apparently, these gangliosides did not originate from the formula fat component, but rather from the material used to increase the whey:casein ratio, namely DWP. The high NeuNAc content of the DWP ganglioside fraction strongly supported this hypothesis (Table 2). Qualitative analyses confirmed that GP3 was the major bovine milk ganglioside (13, 19, 29) and that all formulas (except AL 110) exhibited a ganglioside pattern identical to that from bovine milk (23).

Neutral glycolipids are also important components of the MFGM (11). Reported levels of 70 mg of lactosylceramide and 38 mg of glucosylceramide per 10 L of bovine milk are in agreement with current results (Table 2). The NeuNAc are monosaccharides commonly found in complex carbohydrates of animal origin, but they do not occur in all glycoconjugates or oligosaccharides. Among the milk complex carbohydrate families, neutral glycolipids alone do not carry NeuNAc residues. Considering the low concentration of milk glycolipids relative to nonfat glycoconjugates and oligosaccharides (Table 2) and the general occurrence of NeuNAc in milk complex carbohydrates, content of total nonfat NeuNAc is an appropriate probe for these milk carbohydrate micronutrients. Consequently, a clear relationship appeared between the level of nonfat NeuNAc in IF and the amount of DWP and WPC used for their manufacture (Tables 1 and 2), because both contained very high levels of nonfat NeuNAc (Table 2). Other reports (4, 5) support this observation. Neuaraminylactose is the only complex human milk oligosaccharide also found in milk of other mammalian species (22). It was identified as the major sialylated free oligosaccharide in formulas (4). Formulas manufactured by mixing whey protein and casein in a 60:40 ratio have been reported to contain fivefold more NeuNAc in their oligosaccharide fraction than that found in this fraction from cow’s milk and formulas made with a 18:82 ratio (72 vs. 14 mg/L, (5)). In this last study, neuraminylactose was not conclusively identified, because the fractionation procedure used to obtain these “oligosaccharides” did not separate them from the caseinoglycomacropeptide, a glycopeptide that, upon cleavage from β-casein by rennin during cheese making, occurs in whey and whey-based raw materials (1). To resolve this analytical problem, gel permeation liquid chromatography was used herein to separate the glycopeptide and oligosaccharide fractions (Figures 3 to 5). Quantitative estimations clearly showed that IF content of neuraminylactose increased with the whey:casein ratio, but not in the proportion reported (5). Glycopeptide fraction occurred only in formulas made with one or more whey components (Table 3). The DWP material contained similar amounts of neuraminylactose and caseinoglycomacropeptide, but WPC had a high glycopeptide content but no oligosaccharide (Table 3). The ultrafiltration or diafiltration step used to process the WPC resulted in retention of the whey glycopeptides, and whey oligosaccharides were lost with lactose.

Milk proteins can be divided in two main categories: caseins and whey proteins. Among the various casein subcomponents, only β-casein has been confirmed to be a glycoprotein (20). Regarding the carbohydrate chains from bovine mature milk β-casein, three structures have been established: NeuNAcc2 → 3Galβ1 → 3GalNAC, Galβ1 → 3( NeuNAcc2 → 6)GalNAC and NeuNAcc2 → 3Galβ1 → 3( NeuNAcc2 → 6)-GalNAC (16, 32). By contrast, numerous whey glycoproteins have been described (20). Glycoproteins are not only found in skim milk but also in the milk fat fraction, more precisely on the MFGM (18, 21). Among the monosaccharide constituents commonly occurring in milk glycoprotein carbohydrate chains, only sialic acids have been quantified in IF (4, 5). Present primarily on glycoproteins (4), NeuNAC values of 56 to 67 mg/L were found in milk-derived formulas (5), whereas a value of 146 mg/L was obtained for cows’ milk (5). Present data (Table 3) confirmed that most of the sialic acid residues from milk glycoconjugates are borne by
glycoproteins. The case of hypoallergenic formulas is an exception, because glycoproteins are converted into TCA-soluble glycoconjugates by trypsin hydrolysis. An analysis of the composition of the various glycoconjugate fractions from IF corroborated this observation (Table 4); fucose, mannose, and N-acetylgalactosamine were only found in the glycoconjugate fraction of BEBA H.A.

CONCLUSION
Most milk-based infant formulas contained complex carbohydrates in amounts similar to those found in bovine milk. In whey-based raw materials, elevated levels of all bovine milk glucosaccharides and oligosaccharides were evident. Consequently, modern formulas prepared from these raw materials will contain increased amounts of these micronutrients.

REFERENCES
Milk Glycoconjugates in Infant Formulas


