

Historical Aspects of Human Milk Oligosaccharides^{1–4}

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ABSTRACT

This review focuses on important observations regarding infant health around 1900 when breastfeeding was not considered a matter of importance. The discovery of lactobacilli and bifidobacteria and their relevance for health and disease was an important milestone leading to a decrease in infant mortality in the first year of life. At the same time, pediatricians realized that the fecal composition of breast-fed and bottle-fed infants differed. Observations indicated that this difference is linked to milk composition, particularly due to the milk carbohydrate fraction. Circa 1930, a human milk carbohydrate fraction called gynolactose was identified. This was the starting point of research on human milk oligosaccharides (HMO). In the following years, the first HMO were identified and their functions investigated. Studies after 1950 focused on the identification of various HMO as the bifidus factor in human milk. In the following 30 years, a tremendous amount of research was done with regard to the characterization of individual HMO and HMO patterns in milk. In this short introduction to the history of HMO research, which ends circa 1980, some outstanding scientists in pediatrics and chemistry and their pioneering contributions to research in the field of HMO are presented. *Adv. Nutr.* 3: 430S–439S, 2012.

Introduction

Interest in milk carbohydrates started around the beginning of the past century, influenced by observations that the survival rate of breast-fed infants compared with bottle-fed infants was much higher and that this might be attributed to

the unique composition of human milk. Two parallel routes of research on carbohydrates and later on human milk oligosaccharides (HMO) could be observed, one was based on physicochemical, the other on nutritional observations (1). **Table 1** shows the time periods and important investigations regarding HMO that are covered in this review.

Situation around 1900, infant mortality, and feeding

Around the turn of the century, the mortality rate ranging from 20% to 30% in the first year of an infant's life was alarmingly high. Breastfeeding was not considered a matter of importance. It was found, for example, that the mortality risk would increase dramatically if the infants could not be breast-fed. In bottle-fed infants compared with breast-fed infants, the mortality rate was 7 times higher (2).

The importance of carbohydrates in milk is intrinsically tied to the growing awareness toward the end of the 19th century that nutrition plays an essential role in the health of infants. At that time, infants were fed pure carbohydrates, chewed food, and mixtures of milk and cereals with or without added meat, or they were given native animal's milk (2). One of the first infant formulas consisting of modified cow's milk was developed by Justus Liebig (1803–1870) for his daughter's children and termed “soup for infants” (“Kindersuppe”) in 1864 (3). It was based on the use of milk and cereals. This soup

¹ Published in a supplement to *Advances in Nutrition*. Presented at the conference “The First International Conference on Glycobiology of Human Milk Oligosaccharides” held in Copenhagen, Denmark, May 16–17, 2011. The conference was supported by Glycom A/S, Lyngby, Denmark. The supplement coordinators for this supplement were Clemens Kunz, University of Giessen, Germany, and Sharon M. Donovan, University of Illinois, Urbana, IL. Supplement Coordinator disclosures: Sharon Donovan has received human milk oligosaccharides from Glycom through their HMO biology donations program. Clemens Kunz has received human milk oligosaccharides from Glycom through their HMO biology donation program. The supplement is the responsibility of the Guest Editor to whom the Editor of *Advances in Nutrition* has delegated supervision of both technical conformity to the published regulations of *Advances in Nutrition* and general oversight of the scientific merit of each article. The Guest Editor for this supplement was Mark A. McGuire, University of Idaho. Guest Editor disclosure: Mark A. McGuire consults with Abbott Nutrition, a division of Abbott Laboratories, and provides professional opinions on matters related to human lactation and infant nutrition. Further, he collaborates with Dr. Lars Bode, University of California, San Diego, in research related to human milk oligosaccharides. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, Editor, or Editorial Board of *Advances in Nutrition*.

² Supported by the German Research Foundation (DFG, KU 781/2-2; KU 781/7-2; KU 781/8-2; RU 529/4-3).

³ Author disclosure: C. Kunz, no conflicts of interest.

⁴ Dedicated to Prof. Dr. Heinz Egge on the occasion of his 81st birthday.

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Table 1. Milestones in milk carbohydrate research¹

around 1900

- observations: different fecal composition of breast-fed and bottle-fed infants
- first indications that difference is linked to milk composition
- first description of microorganisms and their importance for health

since 1930

- characterization of the first individual HMO

1950 to 1980s

- further identification of HMO and functional studies
 - biochemical interest due to similar epitopes on blood and tumor cells
 - intensive work on growth factors for microorganisms and anti-adhesive and anti-inflammatory properties
-

¹ HMO, human milk oligosaccharides.

for infants induced many others to produce infant formula to improve the overall health of infants. It was Justus von Liebig who provided a scientific basis for nutritional investigations by his technical and analytical innovations. But at the beginning, the alternative products to human milk were not very successful. This is not surprising because only later, due to scientific innovations, it was possible, for example, to perform the first balance studies (Max von Pettenkofer and C. von Voit, 1881), to determine the energy content of food (Max Rubner, 1889), or the protein content in food, after Johann Kjeldahl (1883) had developed a method for the analysis of nitrogen.

At the same time, pediatrics developed into an independent medical field, and in Europe (e.g., Vienna, Graz, Zurich, Berlin, Munich), the first professorships and chairs were established held by internationally esteemed persons (2,4). Among them, Adalbert Czerny, the author of the textbook *Des Kindes Ernährung (The Child's Nutrition)*, which was for a long time one of the leading books in this area. The tremendous impact of this book in pediatrics at that time was summarized in 1933 in a Festschrift by Henry F. Helmholtz in the *Journal of Pediatrics* as follows: “this work is a bible. ... At a time when pediatrics in America was still in its swaddling clothes, German masters took us into their clinics as students and by their inspiration and training played a part in the development of pediatrics in America of which they may feel proud” (5).

Discovery of the importance of microorganisms for health and disease

A major breakthrough for infant survival was the discovery of microorganisms and their importance to health and, at the same time, the observation that milk carbohydrates

play an important role in the growth of these microorganisms. The pioneering work in this area was done by Theodor Escherich, one of the most respected pediatricians in Europe (Table 2) (4). By 1886, Escherich (6) had published a monograph on the relationship between intestinal bacteria and physiology of digestion in the infant. This research established him as the leading bacteriologist in pediatrics.

Another pioneer in pediatrics at that time was Ernst Moro, who was born in 1874 in Slovenia (Table 3). Moro studied in Graz, and his pediatric career started in the laboratory of Escherich, who held the first Chair of Pediatrics in Graz (4). In 1900, Moro presented the first bacteriological characterization of *Lactobacillus acidophilus* (7). He attracted worldwide attention in various fields, e.g., for developing a simple percutaneous skin test for tuberculosis, which was used until the 1960s, for recommending a carrot soup as treatment for children with diarrhea, and for describing a milestone in the infant's neurological development (the “embracing reflex”) (4). Being a student of Escherich explains Moros' strong interest in bacteria and their importance for infant health.

The intimate link between basic and applied research circa 1900 is demonstrated in Figure 1, which shows Escherich at his last lecture, with Moro on the left, giving an injection to an animal, while Meinhard von Pfaundler, another renowned pediatrician, is examining a child (4).

Being fascinated by Escherich's success in the field of bacteriology and others, it was mainly Moro who stimulated research to find the growth factors of microorganisms in milk. In particular, Paul György, a student of Moro, was inspired by these ideas, as is discussed later. From then on, those who conducted research on milk carbohydrates can be divided

Table 2. Theodor Escherich: a pioneer in pediatrics and microbiology

- 1857 – 1911
- Graz (Austria), Würzburg, Munich (Germany)
- Pediatrician
- most important bacteriologist
- Monography 1886



„Die Darmbakterien des Säuglings und ihre Beziehung zur Physiologie der Verdauung“ („Infants' gut bacteria and their relation to the physiology of digestion“)

Photograph reproduced with permission from the *Österreichische Nationalbibliothek ÖNB* (Austrian National Library).

into those with a chemical background or those with a physiological/nutritional background.

Chemical observations as a background for HMO research

It was observed very early that there was a not yet specified fraction detectable in human milk that could not be found in bovine milk (1). In 1888, Eschbach was the first to find that animal milk and human milk do not contain that type of lactose that is typically found in bovine milk (cited in Reference 1). He observed that in both types of milk, a mixture of various forms of lactose with different properties could be found and concluded that bovine milk contained

the most homogeneous type of lactose, whereas human milk contained the most heterogeneous type.

However, Deniges observed that both types of lactose in human and bovine milk were identical, but that mother's milk contained a carbohydrate fraction still to be identified (cited in Reference 1). Only 40 years later Michel Polonowski and Albert Lespagnol in Lille, France, established a method to specify the so far unknown carbohydrate fraction. They called this fraction, which was insoluble in methanol, gynolactose (8,9). The authors themselves, however, pointed out that this gynolactose was not homogeneous, but consisted of various components. They also found that nitrogen was an essential component of the hexosamines and that this gynolactose showed a very strong bile reaction. As we know

Table 3. Ernst Moro: a pioneer in pediatrics

- 1874 – 1951
- Graz (Austria), Heidelberg (Germany)
- student of Theodor Escherich
- pioneer investigator in pediatrics in various areas
- among the founders of pediatric science in Germany
- around 1900: Moro and Tissier (Pasteur Institute in Paris) independently found a unique predominance of bifidobacteria in the stool of breast-fed infants



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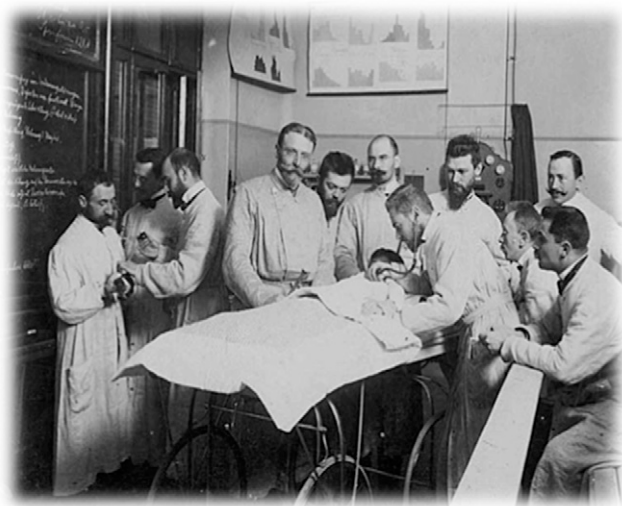


Figure 1 Escherich circa 1900 at his last lecture, with Moro on the left giving an injection to an animal while Meinhard von Pfaundler, another renowned pediatrician, is examining a child. Reproduced from Reference 4 with permission.

today, this reaction is due to the presence of *N*-acetylneuraminic acid, not yet known then. Later on, Polonowski and Lespagnol together with Montreuil, by applying 2-dimensional paper chromatography for the first time, identified the first 2 fucosyllactoses (2'-fucosyllactose and 3-fucosyllactose) in the fraction that had been unknown so far (10).

Jean Montreuil was a charismatic personality of sciences and one of the pioneers in the field of carbohydrates and glycoconjugates (Table 4). In addition to other important discoveries, he proposed that all *N*-glycans have the same core to which are attached various branches that he named antennae to suggest their mobility and potential recognition roles (11). After having built molecular models, he proposed interconvertible conformations and coined first the terms Y

and T conformations, later called bird or umbrella conformations. These original studies on the structures of glycoconjugates stimulated many researchers to study their biological roles in health and disease. As Bratosin and Mignon emphasized, enthusiasm is the key word to characterize Jean Montreuil (11).

It was Montreuil's ambition to describe this complex mixture in gynolactose in more detail and to specify its structures (12; see also later), although he did not realize that he would strongly compete with Richard Kuhn by doing so. Concurrent research work in Heidelberg by Kuhn and co-workers and by Montreuil and his team in Lille led to the first clear description of HMO, namely, 2'- and 3-fucosyllactose and difucosyllactose and to the identification of lacto-*N*-tetraose, lacto-*N*-fucopentaose I and II, difucosyllactose, and many others (12–16).

Since then, research in the field of HMO has continued, above all by Jean Montreuil together with L. Grimmonprez, G. Strecker, J. M. Wieruszkeski, and others; Richard Kuhn and his many co-workers; Viktor Ginsburgh; Akira Kobata; Heinz Egge; and many others (see later).

Kuhn himself had already had numerous publications together with his co-workers Bär, Brossmer, Gaue, and later Egge, identifying various oligosaccharides (for reviews, see References 1,17–19). The starting point of Kuhn's work on glycolipids in general and HMO research completely differs from that of the research of Polonowski, Lespagnol, and Montreuil.

Physiological observations as background to HMO research

To understand the physiological background, we need to go back to the turn of the 19th century. By then it was found that breast-fed infants showed a significantly greater resistance against various diseases, including infectious diarrhea, than infants fed bovine milk. This is the reason why at the

Table 4. Jean Montreuil: a pioneer in HMO research¹

- 1920 – 2010
- Lille (France)
- successor of Polonowski and Lespagnol
- pioneer in „carbohydrates/glycoconjugates
- strong competition with R. Kuhn
- first application of 2-dimensional paper chromatography to separate individual HMO



¹ HMO, human milk oligosaccharides. Photograph reproduced with permission from Reference 11.

Table 5. Paul György: a pioneer in pediatrics

- 1893 – 1976
- pediatrician
- Budapest (Hungary), Heidelberg (Germany), Cambridge (GB), Cleveland, Pennsylvania (USA)
- student of Moro
- „...superb combination of excellent investigator, sympathetic clinician and demanding teacher“ , „...a gloriously bombastic ,colorful, emphatic ward-round teacher“ (Barness and Tomarelli J Nutr 1976)
- great contributions to the discovery of B-vitamins
- pioneering work on the “bifidus factor” in human milk



Photograph reproduced with permission from Reference 22.

end of the past century, Henri Tissier of the Pasteur Institute in Paris tried to compare the intestinal microbiota of breast-fed infants with that of infants fed bovine milk (20). At the same time, Moro in Heidelberg independently found a unique predominance of bifidobacteria in the feces of breast-fed infants (4,7). In the following years, these observations led to an intensive search for microbial growth factors, mainly in human milk but also in milk from a variety of animals.

In 1926, it was Herbert Schönfeld who significantly contributed to research in the field of growth factors in human milk by proving that the bifidus factor was thermoresistant and was not contained in the nonprotein fraction of milk (21). Schönfeld drew the conclusion that this bifidus factor was a vitamin; vitamins were only just being discovered at that time.

At this point, another leading pediatrician with worldwide reputation needs to be introduced (**Table 5**).

Table 6. Major discoveries by Paul György and Richard Kuhn

- in Heidelberg (1930 ff)
 - discovery of B-vitamins
 - characterization of structures in combination with functional studies
- in Heidelberg and Philadelphia (1950ff)
 - structural and functional studies characterizing growth factors for Bifidobacteria („L . bifidus“)



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Table 7. Richard Kuhn - a pioneer in Chemistry and in HMO research¹

- 1900 to 1967
- Vienna, Munich, Zürich, Heidelberg
- Chemist, background in organic, physical and analytical chemistry combined with strong interest in biological functions
- structural/functional studies of molecules with characteristic double carbon bonds (polyenes, flavins, vitamins etc.)
- 1936 Nobel Prize in Chemistry (Vitamins, Carotinoids)
- Director at the age of 28 of the „Kaiser Wilhelm Institute for Medical Research“, Heidelberg (Germany)
- impressive series of works on glycolipids and human milk sugars together with H. Bär, R. Brossmer, A. Gauhe, F. Zilliken, H. Egge and many others



¹ HMO, human milk oligosaccharides.

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The name of Paul György is associated by most of us with a period around the turn of the past century when the first vitamins were discovered and isolated (22). György was a pediatrician with a strong interest in basic sciences, especially in the field of nutrition in general and nutrition of children and adolescents in particular. From the beginning,

he had promoted the benefit of breast milk for children, although at that time, those recommendations were not at all very popular. György was born on April 7, 1893. Shortly after World War I, he joined Moro's group in Heidelberg. Therefore, it is not surprising that György had all his life been engaged in research on the intestinal microbiota and

Table 8. Akira Kobata: a pioneer in HMO research¹

- born 1933
- Washington (USA), Kobe, Tokyo (Japan)
- well respected for his glycoconjugate research
- living legend on HMO research
- joined V. Ginsburg's group (NIH, Washington, USA)
- intensive work on methods to isolate and characterize HMO
- elucidation of the biosynthetic pathway of ABO and Lewis antigens using milk oligosaccharides as source



¹ HMO, human milk oligosaccharides.

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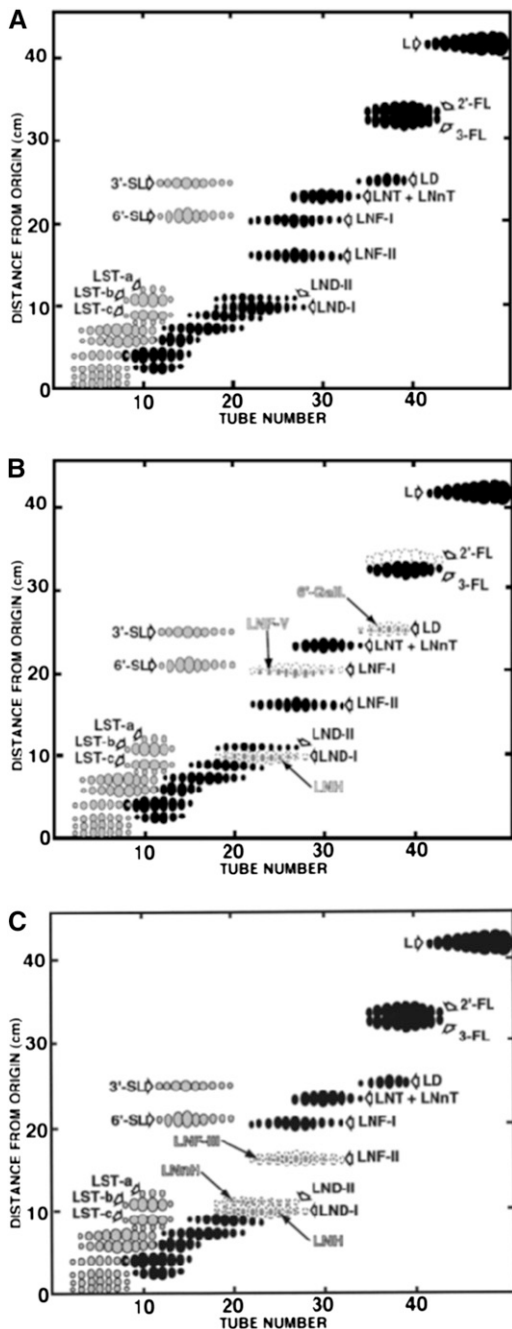


Figure 2 A, Fingerprinting of the oligosaccharide fraction obtained from milk samples of Le^{a+b+} individuals (19). Fraction numbers as indicated by TUBE NUMBER in abscissa were obtained by Sephadex G-25 column chromatography of human milk oligosaccharide fraction. Aliquots of the fractions were spotted at the origin of a sheet of a filter paper and subjected to chromatography using ethyl acetate/pyridine/acetic acid/water (5:5:1:3) as solvent. Black dots represent oligosaccharides visualized by alkaline-AgNO₃ reagent, and hatched ones encircled by black line represent those detected by both alkaline-AgNO₃ reagent and thiobarbituric acid reagent (Kobata). Reproduced from Reference 19 with permission. B, Fingerprinting of the oligosaccharide fraction obtained from milk samples of nonsecretor individuals. The condition of fingerprinting was the same as in the legend for A. Dots shown

its relevance for the health of breast-fed infants. Due to the Nazi regime, György had to leave Germany in 1933. Via Cambridge (England), he finally came to Cleveland, Ohio, and later to Pennsylvania (22).

In the 1950s, György and Kuhn started their impressive collaboration on HMO research. György, in Philadelphia since 1936, contacted Kuhn, and thus ~30 years after the beginning of their joint research on lactoflavines in Heidelberg, the 2 research groups renewed their collaboration (Table 6). They started to investigate the importance of human milk compared with cow's milk in the infant's resistance against infections. The cooperation between the 2, being extremely successful, led to the proof that the growth factors for *Lactobacillus bifidus* in human milk consist of oligosaccharides containing *N*-acetylglucosamine and polysaccharides (1,17–19). In contrast, the nitrogen-free fraction in human milk did not show any bifidogenous effect.

Kuhn was interested in science at a very young age (Table 7) (23). He did not go to elementary school but was educated by his mother before entering secondary school. When he was 13 years old, he helped a family friend who was a professor of chemistry in Zurich to prepare the experimental classes for his chemistry students. At this time he received as a present the newest chemistry textbook, about which Richard Kuhn was very excited. Therefore, it is not that surprising that at the age of 21, he started his PhD under the supervision of Richard Willstätter (the recipient of the Nobel Prize in Chemistry in 1915) in 1921 and finished 1 year later. The title of his PhD thesis was "The Specificity of Enzymes in Carbohydrate Metabolism."

The 1950s were a second period of enormous research by Richard Kuhn, consisting of investigations on immune factors and associated research on oligosaccharides in human milk and gangliosides in the brain (23). Based on studies of bifidus factors in milk and the virus-inhibiting effects of human milk, Kuhn, together with Bär, Gaue, Brossmer, and others, focused on the identification and characterization of oligosaccharides in human milk (1,16,17,23). Since 1954, numerous oligosaccharides have been purely presented and structurally elucidated by excellent techniques. Isolated in 1954, lacto-*N*-tetraose was identified as a key substance of HMO, and during the following years, further components were identified (16).

Kuhn had always been interested in the biological significance of isolated compounds. The findings that oligosaccharides containing lactaminic acid (called sialic acid today) are cleavable by influenza virus was of great interest. 3'- and 6'-lactaminyl lactose were found to be the most easily cleavable component. It showed, for example, that influenza virus cleaves the glycosidic linkage of lactaminic acid, and in

by broken lines were missing in the pattern. Gray dots detected at the positions of missing oligosaccharides are minor oligosaccharides hidden under the major oligosaccharides (19). C, Fingerprinting of the oligosaccharide fraction obtained from milk samples of Le a-b- individuals. The condition of fingerprinting and the descriptions of various spots were the same in the legend for A and B (19).

Table 9. Heinz Egge: a pioneer in HMO research¹

- born in 1931
- Heidelberg, Marburg, Bonn (Germany)
- PhD with Richard Kuhn
- close collaboration with and friend of Jean Montreuil
- living legend of HMO research
- well respected for his work on glycoconjugates
- established one of the first FAB-MS instruments (1981)



¹ HMO, human milk oligosaccharides.
Photograph from the author's private collection.

the following years, lactaminyl oligosaccharides were identified as receptors for influenza virus, thus explaining the virus-inhibiting effect of human milk (23,24). In his lecture on resistance problems at the 100th Meeting of the Society of German Natural and Medical Scientists in 1958, Kuhn explained that cells not developing such lactaminyl oligosaccharide structures on the surface had to be resistant to influenza virus (16,23,24), an observation with long-lasting influence on research even until today in the fields of chemistry, biology, and medicine.

From the beginning of their collaboration on HMO research, György in Philadelphia and Kuhn in Heidelberg found out that there was a connection between the work of Tissier on bacteria and the milk fraction research of Polonowski and Lespagnol, called gynolactose (8). In the course of their investigations, György found a *Lactobacillus bifidus* mutant solely growing in the presence of milk, which he named after its place of discovery *L. bifidus* var. *pennsilvanicus* (25).

György also realized that the bifidus factor activity is somehow related to the intestinal defense system. In 1953, he published one of his key papers "A Hitherto Unrecognized Biochemical Difference Between Human Milk and Cow's Milk" (26). He, together with Kuhn, Rose, and Zilliken, found the greatest activity in human colostrum, followed by rat colostrum, human milk, rat milk, and cow colostrum. The milk of ruminants (i.e., cows, ewes, goats) showed only weak activity, if any. A large number of organic compounds including known microbial growth factors, yeast extract, vitamins not present in the original *Lactobacillus* growth medium, carbohydrates, and several vegetable extracts were all ineffective in replacing the bifidus factor. In this close collaboration between Philadelphia and Heidelberg, many HMO such as lacto-*N*-tetraose, lacto-*N*-fucopentaose I, lacto-*N*-fucopentaose II, and lacto-*N*-difucohexaose have been characterized by classic methods (1,17,19).

On the basis of their observations that *L. bifidus* grew only in the presence of *N*-acetylglucosamine, they postulated that those bacteria were not able to synthesize acetylglucosamine themselves (23). They concluded at that time that if an infant is fed *N*-acetylglucosamine containing oligosaccharides, growth of *L. bifidus* will increase, thus inhibiting the invasion of pathogenic bacteria in 2 ways. One was the production of milk and acetic acid by which the activity of *L. bifidus* leads to a decrease in the intestinal pH, inhibiting the growth of various pathogenic bacteria. The other way is to support the growth of *L. bifidus*, which will lead to a lack of nutrients for pathogenic microorganisms. These underlying mechanisms had been discovered in the mid-1950s, but are still research topics, as the current strong interest in investigating probiotics and prebiotics and their importance in health and disease shows.

Elucidation of the ABO and secretor pathway of HMO

This topic is intimately linked to Akira Kobata, another pioneer HMO investigator (Table 8).

Beginning at about 1960, the blood group ABO and secretor system were investigated by Watkins (27) and others. Due to their large amounts in human milk, HMO had been used at that time as an optimal source for the elucidation of the blood group specificity. In 1967, Grollmann and Ginsburg (28) proved that 2'-fucosyllactose is not detectable in milk samples of nonsecretor women. These women express ABO blood group epitopes on their erythrocytes corresponding to their genetic ABO background, but do not express them in glycoproteins secreted by epithelial cells of mucus-producing gland.

Kobata, first in Ginsburg's laboratory in Washington and later with his co-workers in Japan, developed a new method to determine the oligosaccharide pattern using only a small amount of milk (reviewed in Reference 19). This method

consisted of a combination of gel filtration and paper chromatography, which offered the possibility to show 14 oligosaccharides at the same time in the form of fingerprinting using ~10 mL of milk (Fig. 2A). They could prove that 3 different oligosaccharide patterns were detectable using this method. Eighty percent of all milk samples showed 14 spots corresponding to the 14 oligosaccharides shown in Figure 2A. In contrast, 15% of the milk samples showed a pattern shown in Figure 2B. The lack of 4 oligosaccharides is characteristic of this pattern. A further interesting observation was that all mothers whose milk showed this pattern were nonsecretors, which means that they neither expressed ABO blood group determinants nor Lewis determinants in their secreted glycoproteins. The structures of the 4 lacking oligosaccharides clearly showed that they were all lacking fucose linked to galactose in the α 1,2 position. This means that the secretor organs of these nonsecretor persons did not have a fucosyltransferase, which is responsible for the generation of the disaccharide. The remaining 5% of the milk samples showed the pattern in Figure 2C, in which 3 oligosaccharides are lacking. The examination of those women's blood group types revealed that they were all Lewis negative, which means that they had neither Lewis A nor Lewis B antigens in their secreted glycoproteins or on their erythrocytes (19).

As shown in Table 8, Kobata and co-workers have a tremendous list of classic publications on the development of methods to separate and characterize HMO (18,19,29,30).

Development of new methods for the analysis of complex HMO

Gain in knowledge of HMO has only been possible by establishing advanced methods for the identification and characterization of HMO within the past 60 years of research. One of the leading pioneers in this field is Heinz Egge (Table 9).

Egge was involved in glycoconjugate research throughout his entire career, starting as a graduate student with Richard Kuhn at the Max Planck Institute for Medical Research in Heidelberg (31). In the late 1950s, Kuhn's laboratory elucidated the first structure of a ganglioside, a major glycolipid component of neuronal plasma membranes. Classic methods of the time required large amounts of material; hence, Egge started his structural analysis of gangliosides from 350 kg of bovine brain! Later, following Fritz Zilliken to Bonn and then being appointed Director of the Institute of Physiological Chemistry, he was able to follow his interests in glycoconjugate research, focusing on spectroscopic methods for structural elucidation. His collaboration with others (e.g., H. von Nicolai, P. Hanfland, J. and U. Dabrowski) resulted in a number of publications, which became classics for glycosphingolipid analysis (17,32,33). He also was one of the first to introduce fast atom bombardment MS for HMO and to establish it as a general method for mapping and sequencing of native and derivatized glycoconjugates.

In his publication on fucose-containing HMO in 1983, Egge and co-workers used 10 L of pooled human milk and applied classic chromatographic separation methods together with high-performance thin-layer liquid chromatography,

HPLC, MS, and $^1\text{H-NMR}$ spectroscopy to analyze reduced neutral and peracetylated HMO (34). Compared with conventional methods of determination of carbohydrate constituents, this method was highly reliable, sensitive, and specific. It allowed an exact determination of the molecular composition and also the analysis of mixtures not resolved by chromatographic methods. Because the determinations could be performed with microgram quantities of substance, it was the method of choice. In view of the enormous variability of higher carbohydrate structures, of which only the tip of the iceberg was identified, Egge recommended in 1973 that it would certainly be necessary to analyze the milk of single donors to reduce the number of possible isomers. In the following years, some of Egge's ideas about HMO have been addressed and are still investigated by his previous co-workers (35–42).

Final remarks

This short review on the development of HMO research ends in the 1980s. However, there is still a continuing strong fascination with these components, which are, together with other components, the reason for the special character of human milk. It is impressive how clearly 50 years ago many of the HMO could be characterized by classic analytical methods, and functions like the influence on microbial composition or the effects on the immune system were investigated. Still today, these main areas of research are given top priority (e.g., see contributions in these proceedings). I would like to stress that apart from the mentioned individuals who influenced HMO research substantially, there are numerous others not mentioned who have also made important contributions, leading to our current knowledge of HMO.

This brief review ends with a quote from Jean Montreuil who wrote in 1993: "History demonstrates the importance of the knowledge of the primary structure to approach the metabolism, molecular biology and mechanisms of the biological activity of HMO. Only the collaboration of scientists belonging to different domains allows to solve the problems" (1).

Acknowledgments

The sole author had responsibility for all parts of the manuscript.

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