Host–Intestinal Microflora Interactions: Role of the Mucus and Gynolactose

Marie Bénédicte ROMOND,¹ Nour-eddine CHADLI,¹ Tomotari MITSUOKA²
and Charles ROMOND¹

¹INSERM 146, 369 rue Jules Guesde, Villeneuve d'Ascq, France and

²Laboratory for Intestinal Flora, FRP, RIKEN, Wako, Saitama 351–01

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Abstract Mucus and gynolactose might control host–*Bifidobacterium* interactions. Their respective roles were investigated in this study using gnotobiotic mice. By comparing glycoprotein SDS-PAGE profiles, mucus from various murine lines (NC, CF₁, BALB/c and C₃H) were differentiated. We attempted then to determine the intestinal substrates utilized by various bifidobacteria. First, NC and CF₁ germfree mice received an inoculum of a murine species, *B. animalis*. Bacteria utilized the glycosyl fraction of several CF₁ glyco-compounds. No such extensive degradation was observed in NC mice. In contrast, both NC and CF₁ mice responded to colonization by modifying hexosamine composition of their high molecular weight mucins. Three human species (*B. bifidum*, *B. breve* and *B. longum*) were then assayed for their *in vivo* capacity to degrade murine mucus from C₃H mice. *B. bifidum* utilized extensively glycoproteins from the mucus, whereas *B. longum* and *B. breve* were unable to degrade them. However, none of the human strains led to intestinal mucins modification. Origin of the strains seemed to be a factor controlling the host response. Finally, gynolactose effect was investigated in germfree, *B. breve*-associated, and infant flora-associated C₃H mice. Few modifications to mucus composition were noticed in the first two cases. In infant flora-associated mice, new intestinal glycoproteins and proteins were detected but bacterial counts were not changed. Host response to gynolactose might depend on the implantation of some unknown intestinal bacteria. It is likely that the proliferation of bifidobacteria shown in breast-fed infants does not correspond to a direct gynolactose promoting effect. But it is probably related to mucus modification induced by gynolactose.

Key words: gynolactose; mucus; *Bifidobacterium*; intestinal microflora; gnotobiotic mice

Dialyzable sugars isolated from human milk were historically divided into two groups, allolactose and gynolactose (22). The first one corresponds to well-known

lactose and is secreted in high concentration (around 70 g/liter) during the first months of lactation. The second one (about 10 g/liter) is constituted of various oligosaccharides whose molecular weights do not exceed 10 kDa (10, 18, 28). More than 50 different structures are now described (7, 18). Common constitutive sugars are D-glucose (D-Glc), D-galactose (D-Gal) and L-fucose (L-Fuc). But no mannose (Man) or N-acetylgalactosamine (NAcGal) are found. A few oligosaccharides such as 2'-fucosidolactose, 3-fucosidolactose, lactodifucotetraose, 2'-fucosido-3'-galactosidolactose, 3'-lactaminyl lactose and 6'-lactaminyl lactose, do not contain N-acetylglucosamine (NAcGlc). But the majority, called nitrogen-containing oligosaccharides, do contain NAcGlc in their structure. They are usually derived from lacto-N-tetraose or lacto-N-neotetraose (Table 1). Sialic acids (NANA) content subdivided nitrogen-containing oligosaccharides into neutral and acidic oligosaccharides (17).

In fact, extensive documentation on gynolactose biochemical composition and oligosaccharides structure contrasts with the lack of information on their biological properties. Considering the concentration of each gynolactose oligosaccharide isolated, it seems unlikely that they could be a source of nutrient for the infant. Some observations indicated, on the other hand, that they might participate in the protection of infant health against pathogens. Diarrhea occurred rarely in breast-fed infants compared to bottle-fed ones (9). Since infant formulas or cow's milk did not contain gynolactose (23), one might assume that human milk oligosaccharides could play a role in the prevention of the infectious process. For instance, fucose-containing oligosaccharides inhibited *Vibrio cholerae* adhesion to red cells (12, 13). In contrast, no inhibition of *Escherichia coli* adhesion to human colonocytes was shown (21). However, breast-fed infants harbored lower *E. coli* counts than bottle-fed ones (4, 20). As a concomitant higher bifidobacterial count is usually observed in the breast-fed population, repression of *E. coli* by bifidobacteria was suggested (4, 20). Such a hypothesis was supported by works using gnotobiotic models and showing a slight repression of *E. coli* by bifidobacteria (8). And the relative overproliferation of bifidobacteria observed in breast-fed infants was thought to be related to presence of growth-promoting oligosaccharides in human milk. Most of the neutral N-acetylglucosamine-containing oligosaccharides actually promote *in vitro* the growth of *B. bifidum* (11). Thus, it was tempting to explain the intestinal bifidobacterial over-growth by an *in situ* utilization of growth-promoting oligosaccharides. But, two arguments oppose this view. First, in the gut of breast-fed infants, who were either premature (27) or suffering from a vitamin K deficiency (1), colonization (when it occurred) was poor and slow, suggesting that some host-secreted substrates playing a role in implantation and maintenance of bifidobacteria were missing. Second, *B. bifidum* is the single bifidobacterial species requiring human milk oligosaccharides but other bifidobacterial species are commonly recovered from breast-fed infant stools (20). Thus, relationships between gynolactose host and microflora appeared to be more complex than expected.

For ethical and technical reasons, such complex interactions cannot be studied in human populations. Therefore we used gnotobiotic mice to investigate the

Table 1. A few structures of nitrogen-containing oligosaccharides isolated from gynolactose (10, 17, 18)

Name	Structure
Lacto- <i>N</i> -tetraose	Gal β 1-3NAcGlc β 1-3Gal β 1-4Glc
Lacto- <i>N</i> -neotetraose	Gal β 1-4NAcGlc β 1-3Gal β 1-4Glc
Lacto- <i>N</i> -hexaose	Gal β 1-3NAcGlc β 1-3Gal β 1-4Glc β 1 6 Gal β 1-4NAcGlc
Lacto- <i>N</i> -neohexaose	Gal β 1-4NAcGlc β 1-3Gal β 1-4Glc β 1 6 Gal β 1-4NAcGlc
Lacto- <i>N</i> -fucopentaose II	Gal β 1-3NAcGlc β 1-3Gal β 1-4Glc α 1 4 Fuc
Lacto- <i>N</i> -fucopentaose III	Gal β 1-4NAcGlc β 1-3Gal β 1-4Glc α 1 4 Fuc
Pentaose A	Gal β 1-3NAcGlc β 1-3Gal β 1-4Glc α 2 3 NANA
Pentaose C	Gal β 1-4NAcGlc β 1-3Gal β 1-4Glc α 2 6 NANA

respective role of each actor. We first attempted to characterize in mucus the substrates for various bifidobacteria. Two strains of mice inoculated with a murine bifidobacterial species were used in a first model to determine the adaptive capacity of bifidobacteria to different mucus. In a second step, a single murine strain was inoculated with three human *Bifidobacterium*. Finally, we analyzed host response to gynolactose administration, according to the microbial status of the mice (germ-free, *B. breve*-associated and infant flora-associated mice).

MATERIALS AND METHODS

Animals. Male CF₁ and NC mice (2 months old) were raised under germfree conditions at the Institute of Physical and Chemical Research (Riken, Tokyo, Japan). Germfree male C₃H mice were provided by the Centre d'Élevage d'Animaux de Laboratoire (CEAL, Orléans, France).

CMF pellets (Oriental Yeast Co. Ltd, Tokyo, Japan) sterilized by γ -radiation (5 Mrad) were given to CF₁ and NC mice. C₃H mice received RO₃ pellets (UAR-Epinay/Orge, France) sterilized by γ -radiation (25 Mrad). Water autoclaved for 2 hr at 121°C was given *ad libitum* to all mice.

Germfree and ex-germfree mice were kept in isolators sterilized by a 2% peracetic acid solution.

Bacteria. *B. animalis* IR₁ was originally isolated from conventional mice at Riken Institute and identified using DNA-DNA hybridization (16). CF₁ and NC germfree mice were inoculated with brain heart infusion (BHI) broth (Difco Laboratories, Detroit, U.S.A.) cultures grown for 24 hr at 37°C.

B. bifidum AA₂₂, *B. longum* F₁ and *B. breve* BF₁ were originally isolated from infants at the Faculty of Pharmacy (Lille II, France). Identification was performed using Mitsunaka method (20) and DNA-DNA hybridization. C₃H germfree mice were inoculated with BHI cultures grown for 24 hr at 37°C.

The various strains used in this study were maintained frozen at -80°C in BHI broth.

Infant fecal microflora. Feces (around 20 mg) from a 10-day-old bottle-fed infant were collected by using a sterile "culturette" Swab (Marion Scientific, U.S.A.) in order to maintain an anaerobic atmosphere during the transport. The swab was disrupted by shaking in 10 ml of a Ringer modified solution (24). The mice were orally inoculated with 0.5 ml of the bacterial suspension.

Experimental gut colonization. Confirmation of bifidobacterial gut colonization was determined 2 or 3 days post-inoculation and at specific intervals by fecal viable counts using Ringer modified diluent (24, 25). Fresh feces were collected from each mouse and appropriate dilutions plated onto brain-heart medium supplemented with agar (10 g/liter). Plate dilutions were incubated for 3 days at 41°C in an anaerobic chamber. In ex-germfree mice inoculated with an infant flora, total aerobic and anaerobic fecal bacterial counts were determined 15 days post-inoculation and at specific intervals during gynolactose administration. Bifidobacteria and spore-forming rods were quantified at the same time (2).

Gynolactose administration. Dialyzable human milk oligosaccharides (gynolactose) were kindly given by Dr. Strecker (Biochemistry Laboratory, Lille I, France). A 1% (w/v) sterile solution was administered *ad libitum* for 7 days to germ-free and ex-germfree mice.

Analysis of mucous constituents. At specific intervals, fresh feces were collected from each of the germfree and ex-germfree mice, they were pooled and analyzed for mucous constituents. Mucous constituents were extracted by water (25). Lyophilized crude extracts were fractionated on Sephacryl S200 column (K 15; Pharmacia, Sweden) or Superose 6 FPLC column (H 16/50; Pharmacia). Elution was performed with a 0.1 M Tris-HCl buffer (pH 8.0) containing 0.02% sodium azide and 0.2 M NaCl.

Electrophoresis. Whole extract components were identified by SDS-PAGE. Proteins were detected by Coomassie Blue or by silver staining procedures. Periodic acid-Schiff (PAS) reaction was used for glycoconpounds (19).

Hexosamine determination. Analysis for hexosamines constituents was performed using the method of Morgan-Elsson after a hydrolysis of the hexosaminyl linkages with HCl (4 M) at 100°C for 4 hr (5).

RESULTS

Comparison of Mucus Composition According to the Mouse Strain

Fecal germfree crude extracts from BALB/c, C₃H, NC and CF₁ mice were analyzed for their glycoconpound composition by SDS-PAGE stained by PAS procedure. Several differences in glycosyl moieties were observed between the

Fig. 1. PAS-stained SDS-PAGE profiles of mucus from various mouse strains. Lane 1, water crude extract of FR1 food pellets; lane 2, fecal BALB/c crude extract; lane 3, fecal C₃H crude extract; lane 4, fecal NC crude extract; lane 5, fecal CF₁ crude extract; lane 6, water crude extract of CMF food pellets.

Fig. 2. Effect of *B. animalis* on NC (a) and CF₁ (b) mucus composition analyzed by PAS-stained SDS-PAGE. Lane 1, germfree fecal NC and CF₁ crude extracts; lane 2, fecal NC and CF₁ crude extracts 3 days post-inoculation; lane 3, fecal NC and CF₁ crude extracts 21 days post-inoculation. The arrow shows one glycoprotein utilized extensively by *B. animalis*. Circles correspond to partly degraded glycocompounds.

Table 2. Hexosamines concentration in fractionated mucous constituents from germfree (GF) and ex-germfree (EGF) NC and CF₁ mice

Fractions M.W. (kDa)	GF		EGF ^{a)}	
	NC	CF ₁	NC	CF ₁
>5,000	42	36	112 ^{b)}	169 ^{b)}
1,000–5,000	31	17	26	13
100–1,000	34	31	31	10 ^{c)}
40–300	58	33	29	8 ^{c)}
30–45	54	78	81	13 ^{c)}
30–40	43	47	36	34

^{a)} Analysis was performed 3 weeks after *B. animalis* inoculation.

^{b)} Increase of hexosamines content.

^{c)} Decrease of hexosamines content.

murine lines (Fig. 1). Some glycocompounds were common to all mouse strains such as mucin-like glycoproteins detected at the top of the gel. But others were excreted by a single mouse strain. Glycoproteins seemed to be endogenous in origin as no such bands were identified in the water extract from the food pellets.

Experimental Gut Colonization of NC and CF₁ Mice with a Murine Bifidobacterial Strain

B. animalis IR₁ colonized the gut of each murine line within 24 hr. Colonization levels were similar in both groups 3 weeks post-inoculation (9.1 ± 0.6 cfu/g

Fig. 3. Gynolactose effect on S200 chromatographic profiles from germfree mucus. 3a: control. 3b: 7-day gynolactose administration. White squares correspond to $A_{280\text{nm}}$ elution profiles and black ones to carbohydrates elution profiles determined according to Dubois method (6).

in NC mice, 9.5 ± 0.1 in CF_1 mice).

No obvious change in electrophoretic patterns of glycoproteins was noticed after bifidobacterial colonization of NC gut (Fig. 2a). On the contrary, one glycoprotein (around 40 kDa) secreted by CF_1 mice disappeared within three weeks after inoculation with *B. animalis* (Fig. 2b). Three other glycoproteins and mucin-like glycoprotein were less stained, suggesting an incomplete utilization by *B. animalis* IR₁.

NC and CF_1 germfree and ex-germfree crude extracts were then applied to a Superose 6 FPLC column. Chromatographic elution profiles were similar in their

Fig. 4. Gynolactose effect on S200 chromatographic profiles from ex-germfree mucus. 4a: control. 4b: 7-day gynolactose administration. White squares correspond to $A_{280\text{nm}}$ elution profiles and black ones to carbohydrates elution profiles determined according to Dubois method (6).

absorbance at 280 nm. Six different fractions were collected in each case and analyzed for their hexosamines content. Hexosamines concentration in NC and CF₁ germfree fractions were almost identical (Table 2).

Surprisingly, *B. animalis* colonization led to an increase of the hexosamines concentration in NC and CF₁ fraction 1. Both fractions 1 contained exclusively mucin-like glycoproteins as demonstrated by SDS-PAGE (data not shown). NC glycocompounds of lower molecular weight did not show any modification to their hexosamines content. In contrast, a significant decrease was observed for CF₁ glycocompounds ranging in molecular weight between 30 and 1,000 kDa (Table 2).

Experimental Gut Colonization of C₃H Mice with Human Bifidobacterial Strains

B. bifidum AA₂₂, *B. longum* F₁ and *B. breve* BF₁ colonized the mouse gut within 24 hr. Bifidobacterial counts were similar to *B. animalis* ones. Two C₃H glycoproteins were used extensively by *B. bifidum*, as demonstrated by SDS-PAGE (data not shown). In contrast, *B. longum* and *B. breve* did not degrade any C₃H fecal glycoproteins.

Gynolactose Effect on the Host and on the Bacterial Floras

No modification to the bacterial counts was noticed under gynolactose either in *B. breve*-associated mice or in infant flora-associated mice.

A 7-day gynolactose administration to C₃H germfree or *B. breve*-associated mice did not modify SDS-PAGE glycoprotein profile. However, comparison of S200 chromatographic profiles during gynolactose intake showed extensive changes in protein and carbohydrates patterns after a 7-day gynolactose intake (Fig. 3a, b). No such modification was noticed 2 days following the first intake. Three days post-administration, gynolactose effect on S200 chromatographic profile was no longer observed (data not shown).

Experimental gut colonization with the human infant microflora led to the degradation of some proteins and a glycoprotein as demonstrated by SDS-PAGE (data not shown). Confirmation of protein utilization was shown on S200 chromatographic profile (Fig. 4a).

Gynolactose administration to ex-germfree mice led to an extensive modification to SDS-PAGE profiles and S200 chromatographic patterns (Fig. 4b). New glycoprotein bands (above 40 kDa) were detected on PAS-stained SDS-PAGE. Two new protein bands (around 20 kDa) were shown after a 4-day gynolactose intake. In addition, protein surface ratio of peak 1 on peak 2 was inversed compared to the control (Fig. 4a, b).

DISCUSSION

Each mouse strain excreted mucus characterized by its glycoprotein composition as shown on SDS-PAGE profiles (Fig. 1). *B. animalis* proliferation in NC and CF₁ mice was almost identical, suggesting that the strain found its substrates for growth in both murine hosts. *In vitro*, *B. animalis* utilized mainly *N*-acetylglucosamine-containing compounds (data not shown). However, no NC glycoprotein was degraded after *B. animalis* implantation (Fig. 2a) but protein concentration dramatically decreased in fractions containing glycoproteins ranging in molecular weight between 100 and 5,000 kDa (data not shown). *B. animalis* probably utilized the protein core of NC high-molecular-weight glycoproteins. It contrasted with the glycosyl structures the bifidobacteria utilized in CF₁ mucus (Fig. 2b, Table 2). Unability of *B. animalis* to utilize glycosyl components in NC mice could partly explain the poor bifidobacterial colonization observed in this murine line (14). For instance, administration of a clostridial flora led to the repression of *B. animalis* in NC monoassociated mice. No such effect was observed

in CF₁ mice (15). Clostridia proteases are well known for their capacity to digest a wide variety of proteins. *B. animalis* probably could not compete for the protein substrates it utilized in NC mice as it possesses few protease activities. On the contrary, the bifidobacteria were adapted to CF₁ glycoproteins and could compete with clostridia with efficiency. However, the ability to utilize intestinal glycosyl components seemed in some cases insufficient. Human *B. bifidum*, a species which required *N*-acetylglucosamine-containing glycoproteins, degraded to a great extent C₃H mucins and glycoproteins as demonstrated by SDS-PAGE (26). However, Boureau et al (3) showed that infection with *Clostridium cochleatum* led to the reduction of the bifidobacterial population. They confirmed that *C. cochleatum* possessed the necessary enzymatic activities to degrade mucin oligosaccharides but it did not possess mucin proteins-degrading enzymes. *C. cochleatum* was unable to colonize germfree mice, but it did when *B. bifidum* was implanted. Having a wider variety of osidases and utilizing the same glycoproteins, *C. cochleatum* might reduce *B. bifidum* population by competition for common intestinal glycosyl substrates (3). Interestingly, human *B. bifidum* strain did not lead to an increase of mucin hexosamines content (data not shown). It seems that the host did not respond to human oligosaccharides-dependent bifidobacteria as it did after inoculation of murine ones. It raises the question of the ecological meaning of such a host response. We might assume that new mucin structures secreted by the host would favor oligosaccharides-dependent bifidobacteria and allow their maintenance at high level (which was the situation for *B. animalis*).

It is likely that *B. longum* and *B. breve*, whose growth did not depend on mucin oligosaccharides, are not influenced by such mechanisms. They did not utilize intestinal glycoproteins but easily colonized C₃H germfree mice. Furthermore, *B. breve* colonization did not improve delay of host response to gynolactose administration. After a 7-day gynolactose intake, modifications to S200 chromatographic patterns occurred for both germfree and monoassociated mice, whereas similar changes were observed sooner in ex-germfree mice associated with the infant flora. In germfree and *B. breve*-monoassociated mice, increase of peak 1 to peak 2 *A*₂₈₀ ratios was probably not related to a protein modification since Coomassie Blue-stained SDS-PAGE profile was similar to the control one. It is likely that mucins release was increasing but PAS-stained SDS-PAGE did not allow us to confirm this hypothesis. In mixed flora-associated mice, the phenomena were more obvious as new glycoproteins and proteins were detected. Only four days of gynolactose administration led to an extensive change in mucous composition. The bacterial flora seemed to act as an activator of the host response to gynolactose intake. Predominant bacterial counts did not increase, suggesting that they are probably not involved in the process.

In conclusion, various factors influenced microbial equilibriums *in vivo*: quality of mucous constituents secreted by the host and ability of the bacterial strain itself to induce a host response. In this context, it appears that gynolactose does not act directly on the microbial flora but induces a host response amplified by the presence of intestinal bacteria. Modification to microbial flora might occur in a second

step by utilizing the new substrates secreted. But further investigations are required to confirm this hypothesis.

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REFERENCES

- (1) Benno, Y., K. Sawada, and T. Mitsuoka. 1985. The intestinal microflora of infants: fecal flora of infants with vitamin K deficiency. *Microbiol. Immunol.* **29**: 243–250.
- (2) Bezirtzoglou, E., M.B. Romond, and C. Romond. 1989. Modulation of *Clostridium perfringens* intestinal colonization in infants delivered by caesarean section. *Infection* **17**: 232–236.
- (3) Boureau, H., C. Guichet, M.B. Romond, and P. Bourlioux. 1990. Anaerobic strains responsible for the colonisation resistance to *Clostridium difficile*: importance of the first strain colonising the digestive tract of axenic mice, p. 107–112. *In* S.P. Borriello (ed.), *Clinical and molecular aspects of anaerobes*, Wrighston Biomedical Publishing Ltd., London.
- (4) Bullen, C.L., P.V. Tearle, and M.G. Stewart. 1977. The effect of “humanized” milks and supplemented breast-feeding on the fecal flora of infants. *J. Med. Microbiol.* **10**: 403–413.
- (5) Chaplin, M.F. 1986. Monosaccharides, p. 1–8. *In* M.F. Chaplin and J.F. Kennedy (eds.), *Carbohydrate analysis, a practical approach*, IRL Press, Oxford.
- (6) Dubois, M., K. Gilles, J.K. Hamilton, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**: 350–356.
- (7) Egge, H., A. Dell, and H. von Nicolai. 1983. Fucose containing oligosaccharides from human milk. *Arch. Biochem. Biophys.* **224**: 235–253.
- (8) Faure, J.C., D. Schellenberg, A. Bexter, and H.P. Würzner. 1982. Barrier effect of *B. longum* on *E. coli* in the germfree rat. *Int. Vitam. Nutr. Res.* **52**: 225.
- (9) Feachem, R.G., and M.A. Koblinsky. 1984. Interventions for the control of the diarrhoeal diseases among young children: promotion of breast-feeding. *Bull. WHO* **62**: 271–291.
- (10) Grimmonprez, L. 1977. Les glucides du lait maternel. *Med. Nutr.* **13**: 343–348.
- (11) György, P., F. Robert, R.F. Norris, and C.S. Rose. 1954. Bifidus factor I, variant of *Lactobacillus bifidus* requiring a special growth factor. *Arch. Biochem. Biophys.* **48**: 193–201.
- (12) Holmgreen, J., A.M. Svennerholm, and M. Lindblad. 1981. Non immunoglobulin fraction of human milks inhibits bacterial adhesion (hemagglutination) and enterotoxin binding of *E. coli* and *V. cholerae*. *Infect. Immun.* **33**: 136–141.
- (13) Holmgreen, J., A.M. Svennerholm, and M. Lindblad. 1983. Receptor-like glycoproteins in human milk that inhibit classical and El Tor *V. cholerae* cell adherence (hemagglutination). *Infect. Immun.* **39**: 143–154.
- (14) Itoh, K., T. Mitsuoka, K. Sudo, and K. Suzuki. 1983. Comparison of fecal flora of mice based upon different strains and different housing conditions. *Z. Versuchstierkd.* **25**: 135–146.
- (15) Itoh, K., T. Oowada, and T. Mitsuoka. 1985. Characteristic faecal flora of NC mice. *Lab. Anim.* **19**: 7–15.
- (16) Johnson, J.L., C.F. Phelps, C.S. Cummins, J. London, and F. Gasser. 1980. Taxonomy of the *Lactobacillus acidophilus* group. *Int. J. Syst. Bacteriol.* **30**: 53–68.
- (17) Khun, R., and A. Gauhe. 1962. Isolierung einer nieder-molekularen, durch influenzavirus spaltbaren substanz aus milch. *Chem. Ber.* **95**: 513–521.
- (18) Kobata, A., K. Kamashita, and Y. Tachibana. 1978. Oligosaccharides from human milk. *Methods Enzymol.* **50**: 216–220.
- (19) Mantle, M., and A. Allen. 1978. A colorimetric assay for glycoproteins based on the periodic acid/Schiff stain. *Biochem. Soc. Trans.* **6**: 607–609.
- (20) Mitsuoka, T. 1984. Taxonomy and ecology of bifidobacteria. *Bifidobacteria Microflora* **3**: 11–28.

- (21) Neeser, J.R., A. Chambaz, M. Golliard, H. Link-Amster, V. Fryder, and E. Kolodziejczyk. 1989. Adhesion of colonization factor antigen II-positive enterotoxigenic *E. coli* strains to human enterocytelike differentiated HT-29 cells: a basis for host-pathogen interactions in the gut. *Infect. Immun.* **57**: 3727-3734.
- (22) Polonski, M., and A. Lespagnol. 1930. Un nouveau constituant glucidique du lait de femme, le gynolactose. *C.R. Soc. Biol.* **104**: 553-555.
- (23) Romond, C., H. Beerens, C. Neut, and J. Montreuil. 1980. Contribution à l'étude de la maturation des laits: influence *in vitro* du lait maternel, du lait de vache et des laits maternisés sur la croissance de *Bifidobacterium*. *Ann. Microbiol. (Inst. Pasteur)* **131**: 309-314.
- (24) Romond, C., C. Neut, and H. Beerens. 1981. Etude comparative de deux moyens de transport destinés à maintenir en survie les anaérobies. *Rev. Inst. Pasteur Lyon*, **14**: 133-139.
- (25) Romond, M.B., C. Romond, H. Beerens, and P. Bourlioux. 1989. The "*in vivo*" effect of dietary buffering capacity on the bifidogenic activity of human milk oligosaccharides. *M.E.H.D.* **2**: 29-36.
- (26) Romond, M.B., M. Hamze, P. Bourlioux, and C. Romond. 1990. Etude des interactions hôte-*Bifidobacterium* chez la souris axénique: caractérisation partielle des facteurs bifidogènes du contenu intestinal. *Can. J. Microbiol.* **36**: 286-291.
- (27) Stark, P.L., and A. Lee. 1982. The bacterial colonization of the large bowel of protein low birth weight neonates. *J. Hyg.* **15**: 59-67.
- (28) Viverge, D., L. Grimmonprez, G. Cassanas, L. Bardet, H. Bonnet, and M. Solere. 1985. Variations of lactose and oligosaccharides in milk from women of blood types secretor A or H, secretor Lewis and secretor H/non secretor Lewis during the course of lactation. *Ann. Nutr. Metab.* **29**: 13-17.