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Mothers, babies and friendly bacteria

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There have been a number of reports recently that mother's breast milk may be a source of beneficial bacteria that colonise her infant's gastrointestinal tract.[1–16] The human gut is the home of a large community of bacteria which plays a part in a range of activities which contribute to our health. Occupying the colon, where their collective number of cells exceeds that of their host by a factor of 10, the 'gut microbiota' enjoy an intimate and mutually beneficial relationship with the multicellular organism they inhabit. With metabolic activities as diverse and complex as those of the liver, they can be considered an organ in their own right.[17] These 'friendly' bacteria are crucial for the maintenance of health at all stages of life, contributing to immune function and defence against infections, protecting against some cancers and digestive diseases, synthesising micronutrients and concluding the digestion of food components which escape assimilation in the small intestine.[18]

The microbiota of the gastrointestinal tract are acquired at birth and their rate, type and pattern of colonisation may have immediate and long-term positive effects on health. While it has been assumed that they are acquired chiefly during vaginal delivery from mother's lower bowel, new reports suggest that they may also have their origin inside her breast. Mother's milk supplies the newborn with a large number of non-nutritional bioactive substances which assist in adaptation of the fetus to extrauterine life,[19] and account for the superiority of human milk over artificially synthesised alternatives. While it is plausible that certain 'probiotic' bacteria (defined as endogeneous bacteria that confer health benefits to their host[20]) are among them, certain criteria must be fulfilled before they can be added to the long list of maternally derived, milkborne substances that benefit the newborn.

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MICROBIAL COLONISATION OF THE GASTROINTESTINAL TRACT

The fetal gastrointestinal tract is sterile, but is rapidly colonised once the amniotic membranes rupture, usually at birth.[21] During vaginal delivery the newborn face and mouth pass across the mother's anus and the microflora of her birth canal and rectum invade the neonatal intestinal tract, rapidly passing cranio-caudally so that the large bowel is quickly populated by bacteria.[22] Within 4 days of delivery, maternally derived faecal microorganisms can be detected in the newborn colon.[23] Aerobic species, such as *Streptococci* and *Escherichia coli* flourish initially, followed by anaerobes. Exclusively breastfed infants have a predominance of the lactic acid-producing bacteria *Lactobacillus* and *Bifidobacterium* in their faeces by day 7 after birth. These two genera account for more than 90% of the bacterial consortia in the colon of breastfed term infants.[24]

Bifidobacteria and *Lactobacilli* confer health benefits in several ways. They produce antimicrobial compounds such as *acidophillus* and other bacteriocidins,[25] they compete with enteropathogens by selectively consuming available nutrients,[26] they reduce intestinal permeability by tightening epithelial cells junctions,[27] and they produce short chain fatty acids, such as acetate and butyrate by the fermentation of unabsorbed carbohydrate, which strengthen the intestinal barrier.[28] Both bacterial genera excite local and systemic immune responses, including the production of secretory immunoglobulin A (SIgA), the modulation of phagocytosis, and the stimulation of the anti-inflammatory cytokine cascade.[29 30]

BACTERIA IN MOTHER'S MILK

Human milk is a vehicle for the transport of nutrients and other essential substances from mother to young. It is a complex emulsion of lipids, carbohydrates, proteins, vitamins, minerals, white cells and non-nutritional substances which supplies not only the nutrients and energy required for infant growth and development, but also factors which assist in microbiological protection, the maturation and regulation of defence mechanisms including the immune system, and accelerate postnatal maturation of the digestive system.[31] To make the journey from mammary gland to infant gastrointestinal tract these milkborne substances must exist in forms that preserve their integrity, stability and activity.[32] The infant digestive system must, in its turn, be equipped with mechanisms and pathways to recognise, process, utilise, absorb or reject milkborne substances, and with defences against potentially harmful, invasive or antigenic substances, including bacteria.[33] Breast and gut act in concert as a single organ, comparable to the endometrium-placenta interface, to ensure the traffic of nutrients as well as non-nutritional substances, from mother to baby.

The hypothesis that that some bacterial species found in the gastrointestinal tract of infants derive from mother's breast and are transferred directly in her milk derives from two observations: that the colonic microbiota of exclusively breastfed infants differs from those exclusively formula fed (taking into account other factors such as mode of delivery, postnatal and gestational age)[14]; and that human milk contains a spectrum of 'commensal bacteria', which inhibit *Staphylococcus aureus*, a known causative agent of mastitis.[5] Following the discovery of identical *Lactobacillus* species in breast milk and neonatal stool samples of mother–infant pairs[10] the principal bacteria that have been studied are *Bifidobacterium breve, bifidum, longum* and *pseudocatenulatum*.[15] Given that these lactic-acid producing bacteria are strict anaerobes and difficult to culture, determination of their genetic identity requires molecular techniques, including random amplification of polymorphic DNA, pyrosequencing and PCR methods related to the 16S rRNA gene. Studies of the potential probiotic functions of these bacteria suggest that they possess characteristics which not only confer benefit to the infant, but also facilitate their safe passage from maternal lactating breast to infant colon.[11]

These novel studies provide evidence that the strictly anaerobic bacterial strains identified within breast milk are unlikely to be contaminants from the skin. Gestationally dependent hormonally induced changes in the mammary gland may be conducive to the development of an anaerobic environment and promote the growth of lactic-acid producing bacteria by creating a biofilm

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within the ductule to which they adhere and thrive.[10] Such a specialised environment favours *Bifidobacteria* and *Lactobacilli*, which may be destined to become the dominant bacterial genera found in the infant colon. These reports that human milk is a source of bacteria which come to reside in the infant gut raise the question of their significance to the health of the newborn.[12]

TESTING THE ASSERTION THAT MILKBORNE BACTERIA ARE BENEFICIAL TO THE BABY

To test the assertion that bacteria found in the lactating mammary gland are a source of a beneficial colonic microbiota in the suckling newborn, criteria comparable to Koch's postulates must be fulfilled (box 1, A). Peaker and Neville[34] proposed such postulates when they addressed the biological significance of trophic and other non-nutritional substances in mother's milk to the health of the newborn. They argued that if a substance in mother's milk is to be shown to play a part in neonatal development and have specific positive effects in the newborn then certain criteria must be met. While these criteria make sense for the evaluation of the potential biologic effects of milkborne macromolecules, such as SIgA[35] and epidermal growth factor,[36] they must be revised to echo Koch's postulates when used to test the potential health-promoting (as opposed to pathogenic) effects of microorganisms. Such criteria require demonstration of the true origin and viability of milkborne bacteria throughout the journey from maternal breast to infant colon, and their beneficial effects when they reach the infant colon (box 1, B).

These criteria do not exclude the possibility that components of microorganisms (living or dead), such as DNA or protein, may stimulate an immune reaction or in other ways promote a healthy response. Nor do the criteria rule out other potential routes of transfer of 'friendly' bacteria and/or their products, such as transplacental or transpulmonary.[37] If bacteria in mother's milk are to be shown to play a part in neonatal development and have specific positive effects in the newborn then precise criteria must be met against which the true significance of the novel reports that mother's milk is a source of probiotic bacteria which colonise and thrive in the newborn gastrointestinal tract can be judged.

Studies performed so far offer strong circumstantial evidence that mother's breast milk is a likely source of probiotic bacteria that come to reside in the infant's gastrointestinal tract. However, while criteria 1 and 2 appear to have been satisfied, published studies have not demonstrated positive health-promoting effects in the newborn or its digestive system of bacteria that derive from mother's breast alone (rather than from mother's gut) which are denied to those babies who do not receive them. Satisfaction of criterion 3 requires observations or experiments that are capable of distinguishing the separate origin and independent effects of milk borne and faecally derived bacteria (box 1, C).

Box 1 Criteria by which to test the pathogenic, biological or probiotic effects of bacteria and/or bioactive substances.

A: Koch's postulates: pathogenic effects of bacteria
1. The microorganism must be isolated from the diseased organism and grown in pure culture.
2. The cultured microorganism must cause disease when introduced into a healthy organism.
3. The microorganism must be re-isolated from the inoculated, diseased host and identified as identical to the original specific causative organism.

B: Peaker and Neville's postulates: bioactive effects of milk macromolecules
1. An effect in the offspring must be obtained in response to exposure to the substance in milk.
2. This effect must be abolished by removal of that substance from milk and the effect must be restored when it is returned.
3. The substance must be present and active in milk.
4. The substance must retain its biological activity in the offspring to the site where it is postulated to act.

C: Beattie and Weaver's postulates: probiotic effects of milkborne bacteria
1. Bacteria in the human lactating mammary gland must be identifiable in situ, demonstrated to be live and to belong to a genetically stable population identical to that found in the infant gastrointestinal tract.
2. These bacteria must be shown to be present in human milk, and survive the journey from maternal breast to infant gastrointestinal tract.
3. Such bacteria must be shown to thrive, reproduce and have a biological or health-promoting (probiotic) effect in the newborn which is not present if they are absent.

BIOLOGY OF LACTATION

The evolutionary biology of lactation and the co-evolution of mammals and microbes offer some clues as to the potential biological importance of the milkborne transfer of probiotic bacteria. Mammals have co-existed with microorganisms for many millions of years and their gastrointestinal tracts provide a relatively stable and secure microenvironment for bacteria which have become adapted to this unique habitat.[38] The human stomach is colonised by *Helicobacter pylori* (the stomachs of many mammals harbour species of *Helicobacter*), which lives in relative harmony with its host in most circumstances.[39] The abomasum (forestomach) of ruminants, for example, is a microbial fermentation chamber that converts plant cellulose into short chain fatty acids to the benefit of bacteria and host. Co-evolution of host and bacteria has come about through the selection of the genotypes of each, leading to mutual adaptation to life together.

Lactation is thought to have originated in small reptilian ancestors of primitive mammals (therapsids) as a modification of the secretions of skin glands that secreted antibacterial substances to protect the newborn from infection.[40 41] Mammalian milk, which is rich in antibacterial, antiviral and other protective substances, may well have had a greater importance initially as a source of defence than of nutrition. During the diversification and radiation of mammals in the Cenozoic era, lactation has been adapted and preserved as the principal mode of feeding of the young.[42] Indeed milk feeding is a defining characteristic of all mammals.

Mother and young share 50% genetic identity and therefore many genes in common which favour the health of both.[43] Mother supplies her fetus in utero with immunoglobulins (particularly IgG)

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and other substances that confer protection against microbial and other antigens via the placenta, and after birth, through the entero-mammary immune system she targets specific SIgA directly to the microorganisms to which she and her baby are exposed.[44] Her breast milk is also the source of a huge portfolio of non-specific immuno-defensive, modulatory, anti-inflammatory and other protective factors.[45] The transfer to her infant of a population of 'friendly bacteria' adds to this health-promoting endowment. Perpetuation of the harmonious co-existence of mother and baby demands not just the transfer of genes, nutrients, protective and trophic substances, but also microbiota that are genetically identical, safe and of proven advantage.[46]

Human milk induces a predominance of *Bifidobacteria* and *Lactobacilli* in the infant gut microbiota. Conversely the artificially fed baby, deprived of mother's milk, has fewer *Bifidobacteria* and *Lactobacilli* within its faecal microbiota, in spite of exposure to her lower gastrointestinal tract at delivery. To safeguard transfer and colonic colonisation of the infant, mother's lactating breast may be a special reservoir of these microorganisms, which have become uniquely adapted to live and travel in milk. Nature favours 'redundancy' (dual vital organs, long intestinal tract with reserve capacity, for instance) because it offers 'fail-safe' systems and multiple mechanisms to safeguard essential functions.[47] The abundance of defence factors in milk strengthens the battery of non-lactational immune and non-immune protective mechanisms that have evolved to guard against infection in infancy.[48] Whether this milkborne source of *Bifidobacteria* and *Lactobacilli* is a 'back-up' to ensure the transfer of a beneficial microbiota from mother to young, or a 'relic' of a biological system that has become redundant through the availability of a more ready and abundant maternal faecal supply, is a question posed by these reports.

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