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Intestinal Health

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ABSTRACT: *The intestinal microflora number in the trillions and are comprised of 100 to 400 different bacterial species. Maintaining the delicate balance of intestinal microflora is critical, because microbial balance is the key factor that determines whether substances in the intestine are converted into compounds that are beneficial or detrimental to the host. If an imbalance occurs in favor of intestinal bacteria that express virulent properties, many manifestations of ill health may result. Unfortunately, the growing arsenal of antibiotics used to fight disease is one of the main culprits in upsetting this delicate balance because antibiotics do not distinguish between healthful and pathogenic microbes. Shifting the intestinal bacterial population toward healthy microflora is essential in maintaining good health. Probiotics have been used for many years to aid in restoring and maintaining the intestinal balance in favor of healthful bacteria. Probiotics are organisms or supportive substances that improve intestinal microbial balance, and include Lactobacillus acidophilus, bifidobacteria, fiber, oligosaccharides, and bioactive proteins such as immunoglobulin A and lactoferrin. Probiotics are an important source of supportive nutrition for human intestinal health.*

In 1928 Sir Alexander Fleming, a bacteriologist at the University of London, made an unexpected discovery when he noticed that some mold growing in a culture dish in his laboratory had destroyed common bacteria that surrounded it. From this green mold, called *Penicillium notatum*, the antibiotic penicillin was first isolated. Fleming's serendipitous observation opened a new era for medicine and by the mid 1940s methods for extracting, purifying, and producing large quantities of penicillin made its clinical use a reality.

From then on an ever growing arsenal of antibiotics comprised a formidable armamentarium against such infections and diseases as tuberculosis, typhus, staphylococcal infections, streptococcal infections, bacterial pneumonia, gonorrhea, syphilis, and septicemia, to mention just a few. The proper use of antibiotics has provided a true and an unequivocal benefit to humankind, but not without some dangers and limitations.

ANTIBIOTIC RESISTANT BACTERIA

With selective amnesia the medical world had forgotten that bacteria could develop antibiotic resistance. In 1946, just 5 years after penicillin came into wide use, doctors discovered a strain of *Staphylococcus* that was invulnerable to the drug. This victory for the infectious enemy was not regarded as a threat because new antibiotics were discovered or invented that defeated the bacteria once again. Yet with inexhaustible tenacity, the microbes continued to mutate into forms capable of overcoming the latest generation of antibiotics, creating an ebb and flow that continues to this day.

Over the last 30 years, strains of resistant bacteria have turned up for every bacterial disease.[1] The seriousness of this problem is reflected in the fact that in 1992, 13,300 hospital patients died of infections that resisted every drug doctors tried and, in 1993, some 70,000 Americans died as a result of hospital acquired antibiotic resistant infections.[1,2] "It is probably the No. 1 public health issue," says Dr. Bill Jarvis of the Centers for Disease Control and Prevention in Atlanta.[1]

Reports are now common of new outbreaks of antibiotic resistant infections such as the "flesh-eating bacteria" streptococcus A, death-dealing *E. coli* in improperly cooked fast food, cholera, tuberculosis, pertussis, and many others.[3] "We are running out of drugs," says Dr. Alexander Tomasz of Rockefeller University. "The pharmaceutical industry has almost stopped trying to make new antibiotics. The rules of the game used to be if you saw resistance, you used more antibiotics. But now there are bacteria out there that are armed to the teeth."[1]

Antibiotic usage has stimulated evolutionary changes unparalleled in recorded biologic history.[2] To elaborate, overuse of antibiotics causes bacteria to mutate and develop resistance. The resistant bacteria transfers genetic material to nonresistant bacteria, causing them to become resistant. But gratuitous over-prescription of antibiotics by the medical profession is only one part of the problem. Routine consumption of antibiotics hidden in meat, poultry, and dairy products is also a significant contributor. Every year more than 35 million pounds of antibiotics are produced in the United States and their consumption is divided between livestock, poultry, and humans.[4]

Not only do humans consume hidden antibiotics, they also may consume hidden antibiotic resistant bacteria in improperly cooked meat. Farm animals receive 30 times more antibiotics than humans to protect the animals from infection and to make the animals grow faster. Resistant bacteria develop in these animals, just as they do in humans, and can end up in the meat

we consume. Also, 80 different antibiotics used to prevent udder infections in dairy cows are allowed in certain concentrations in milk. A 1992 study by the Congressional General Accounting Office discovered traces of 64 antibiotics at levels "that raise health concerns"; meaning that they could produce resistant bacteria in milk drinkers.[2] In a recent study at Rutgers University, antibiotics at levels deemed safe by the FDA increased the rate at which resistant bacteria emerged from 600% to 2,700%.[2]

CONSEQUENCES OF HEALTHFUL BACTERIA DESTRUCTION

Preventing the destruction of a patient's indigenous healthful intestinal microflora is a much more difficult challenge than managing the allergic reactions or the damage to organs and tissues that is associated with antibiotic use. This difficulty lies in the fact that antibiotics do not distinguish between healthful and pathogenic microbes. "Virtually every antibiotic administered by mouth causes alterations in the intestinal microflora..." and "Pathogenic microorganisms may proliferate within the colon to fill the ecologic vacuum created by the administration of broad-spectrum antibiotics," state Drs. Garly L. Simon and Sherwood L. Gorbach.[5] Antibiotic induced, wholesale destruction of the healthful intestinal microflora has undoubtedly played a significant role in the development of the current antibiotic resistant bacterial crisis. It seems apparent and reasonable that the first step should be to reestablish, or assure, microbial balance in the intestinal tract of everyone as an immediate prophylactic measure.

The human intestinal microflora number as high as 100 trillion viable bacteria comprised of 100 to 400 different bacterial species. These bacterial species outnumber the cells of the body by a factor between 10-100:1.[5-7] Nearly one-third of the fecal dry weight consists of bacteria.[5] The out-workings of two general classes of organisms, symbiots and antagonists, living together and growing on food components that are either ingested or secreted into the intestinal tract by the host, establish an environment that maintains a delicate balance among this enormous bacterial population.

The intestinal microflora are far from static. It is a highly active society of organisms, possessing a diverse complex of enzymes that perform extremely varied functions. Microbial balance is the key factor that determines whether substances in the intestine are converted into compounds that are beneficial or detrimental to the host. Figure 1 illustrates the importance of intestinal microbial balance by depicting some primary intestinal bacteria, some of the compounds they produce, and their effect on the host.[6] If an imbalance occurred in favor of intestinal bacteria that expressed virulent properties, many manifestations of ill health and accelerated aging would be experienced. Moving the intestinal bacterial population in the opposite direction, one where the resident microflora manifest healthful properties, would be much more likely to produce and help maintain a state of good health.

Figure 1. The Importance of Intestinal Microbial Balance

Some Functions of the Intestinal Microflora	*Effects on the Host*
Healthful Properties	
1. Produces vitamins, SCFAs, and protein that are partly absorbed and utilized by the host.	Helps maintain good health.
2. Supplements the digestive and absorptive process.	Helps maintain good health.
3. Helps protect the host from overgrowth and infection by exogenous organisms such as pathogenic bacteria and yeasts.	Helps maintain good health.
4. Supports the immune system.	Helps maintain good health.
Virulent Properties	
5. Produces certain putrefactive substances (ammonia, hydrogen sulfide, amines, phenols, indoles, etc.) and secondary bile acids. 6. Produces other toxins.	These substances may cause diarrhea, constipation, and growth inhibition. They may also injure the intestine directly and be partially absorbed, potentially contributing throughout the host's life to aging and geriatric diseases such as arteriosclerosis, hypertension, liver disorders, autoimmune diseases, and immunosuppression.
7. Produces carcinogens.	May produce cancer.
8. Stimulates pathogenicity	May contribute to the establishment of a pathologic condition, i.e., spontaneous infections such as diarrhea, gastroenteritis, superinfection (cerebromeningitis, endocarditis, septicemia, urinary tract infection, brain abscess, liver abscess, pulmonary abscess).

Some Primary Bacteria Involved & Their Properties

The numbers that follow the name of the bacteria identify its properties as defined in the first column.

Predominantly Healthful Properties
Bifidobacteria: 1,2,3,4
Lactobacillus: 3
Eubacterium: 3

Combination of Healthful & Virulent
Bacteroidaceae: 1,2,3,4,5,7,8
Peptococcaceae: 3,8
Escherichia coli: 4,5,6,7,8
Streptococcus: 3.8

Predominantly Virulent Properties
Veillonella: 8
Clostridium perfringens: 7,8
Proteus: 7,8

EMPLOYING PROBIOTICS TO ACHIEVE BALANCE

People have used probiotics for many years in the form of fermented dairy products such as yogurt, kefir, and fermented milks. The term *probiotic* was first defined as "organisms and substances which contribute to intestinal microbial balance."[8] For the purpose of this discussion we will slightly revise that definition to read "viable organisms and/or supportive substances, which can be taken orally, that beneficially affect the host by improving its intestinal microbial balance."

The empirical evidence that, for many years, linked the use of fermented dairy products with the promotion of intestinal health is today well supported by modern science. The ability of the probiotic *Lactobacillus acidophilus* to help prevent pathogenic bacteria from proliferating and healthy bacteria from becoming toxic is well documented.[10-15] When the proper strain is chosen, it may help to maintain the proper population equilibrium, or balance, between the different forms of microorganisms, curtailing their potential overgrowth and pathogenicity.[9,13,16-19]

Bifidobacteria is another probiotic naturally occurring in the human intestine. One study observed a beneficial effect of bifidobacteria against a specific strain of enteropathogenic *E. coli* when given to infants who were either breast-fed or who were consuming fresh, raw, bacteriologically-safe human milk.[20] Another study was conducted to compare the effects of feeding infants a modified cows' milk preparation containing bifidobacteria and bifido growth-promoting substances or a buttermilk preparation. The results showed that enteric infections were 8 times more frequent in the infants given buttermilk than in those given the modified cows' milk preparation containing bifidobacteria and bifido growth-promoting substances.[21] With the focus of this article being the impact of antibiotics on intestinal health, a study by Mayer is especially encouraging: Following penicillin therapy, microscopic examination of the stool of an infant demonstrated clear evidence of *Candida albicans* overgrowth. After oral administration of a bifidus milk preparation for 7 days, a significant increase in the population of bifidobacteria and a decrease in the growth of *Candida albicans* was observed in the feces.[22,23]

What is it that enables *L. acidophilus* and bifidobacteria to help maintain the proper population equilibrium, or balance, between the different forms of microorganisms in the intestine? They produce organic acids that reduce intestinal pH and thereby inhibit the growth of acid-sensitive, undesirable bacteria. Lactobacilli produce lactic acid, hydrogen peroxide, and possibly acetic and benzoic acids.[24] Acids produced by bifidobacteria include short-chain fatty acids (SCFAs) such as acetic, propionic, and butyric acids, as well as lactic and formic acids.[5,25] The most plentiful SCFA produced by bifidobacteria is acetic acid, which exerts a wide range of antimicrobial activity against yeasts and molds as well as bacteria.[25,26] It is interesting to note that its action cannot be explained by pH reduction alone.

An undisassociated form of acetic acid penetrates the microbial cell and exerts inhibitory action.[26] Additionally, SCFAs simultaneously support normal gastrointestinal function by increasing colonic blood flow, stimulating pancreatic enzyme secretion, promoting sodium and water absorption, and potentiating intestinal mucosal growth.[27] In humans, colonic absorption of SCFAs may normally supply 5% to 10% of daily energy requirements, depending on the quantity of fiber and resistant starch in the diet.[27] The antimicrobial activity of lactic acid, propionic acid, and acetic acid varies, depending on pH. At optimal pH values they solubilize in the bacterial cell membrane, block transport of necessary growth substances, acidify the cell interior, and exert other inhibitory influences on bacterial cell growth.[26,28]

In addition to lactic and other acids, lactobacilli have the capacity to secrete numerous metabolites, or endotoxins, that kill pathogenic bacteria.[17,29-33] A variety of antibacterial/anti-yeast substances have been isolated such as lactocidin, lactobicillin, lactobreven, acidolin, etc.[16,17,29,34] Because these substances are difficult to isolate and stabilize, their value can best be obtained through the administration of those strains known to secrete these agents as a part of their life cycle.[34]

BACTERIAL STRAIN SELECTION

Much has been learned in the last few decades through intense study on many different strains of *Lactobacillus acidophilus*, bifidobacteria, and other forms of healthful microorganisms. Key to the success of probiotic nutrition is the understanding that probiotic strains vary greatly and their impact hinges upon the specificity of the strains that are used and the method of culturing, packaging, and handling of the product.[24] Many of today's healthcare professionals rely on probiotic nutritional supplements that provide selected strains of bacteria that survive in the presence of bile or stomach acid and adhere to the intestinal mucosa. The *Lactobacillus acidophilus* NCFM® strain researched at North Carolina State University is one strain that meets the strict criteria of purity and viability, being able to survive and implant in the gut. Several studies have shown that it may provide beneficial effects.[24,35-46]

Providing probiotic microorganisms according to the strict guidelines established by current scientific understanding is essential in ensuring predictable nutritional results. The viability of the selected strain can be determined through third-party laboratory analysis of the number of colony forming units (cfu) per unit weight (G) and bile resistance (oxgall bile test) for each batch produced. The laboratory assay results should be provided by the culture supplier, expressed as the ratio of bile resistant cfu/G to total cfu/G. A high ratio, above 90%, indicates high viability of the organism in the gastrointestinal tract.[24] The importance of this analysis cannot be understated, as a study conducted in 1990 suggests that there are serious problems associated with some commercial probiotic preparations.[47] Eleven products labeled *Lactobacillus acidophilus* were examined for number and type of bacteria present, and most of the products were found to actually contain only *Lactobacillus casei*. Problems with culture viability and contamination with enterococcus and clostridium were also found.

In addition to selecting viable strains, the method of packaging and storing of the product is important in maintaining viability. Temperature, moisture, light, and air can all adversely

impact viability. These variables can be controlled through the use of amber glass containers to prevent entry of oxygen, moisture, and light. Most importantly, refrigeration of the product from the time of manufacture through delivery and storage is critical in ensuring the potency of the bacterial strains.[24]

SUPPORTING PROBIOTICS WITH FIBER

In addition to viable, healthful bacteria, the revised definition of probiotics mentioned earlier included "supportive substances" that may beneficially affect the host by improving its intestinal microbial balance. Fiber is one such substance. In one study on 8 healthy adults, the effects of feeding a high cholesterol diet was compared to feeding a diet high in cholesterol with 15 grams of polydextrose (fiber) added each day. In the high cholesterol diet alone, the fecal weight decreased approximately 25% below pre-study levels and fecal pH increased by approximately 0.2%. The diet high in cholesterol with 15 grams of added polydextrose showed an increase in fecal weight by approximately 30%, while the putrefactive products (such as phenol, p-cresol, indole, and others) and the occurrence of clostridia, including *Clostridium perfringens*, were significantly reduced.[48] Mitsuoka commented on this study saying, "These results suggest that the high cholesterol diet increased putrefying bacteria and putrefactive products. Dietary fiber exerts a beneficial effect on human health by improving the balance of the intestinal flora."[6]

An interesting study with laboratory animals showed that adding fiber (cellulose powder) to a liquid diet decreased the incidence of bacterial translocation (bacteria that cross the intestinal barrier and get into systemic circulation) from 60% to 8%. It was said to help by preventing liquid diet induced alteration in mucosal structure. The authors stated, "Thus the oral administration of this fiber maintains intestinal barrier function and prevents bacterial translocation even in the absence of oral nutrients."[49]

Some complex carbohydrates known as oligo-saccharides (such as raffinose, stachyose, isomalto-oligosaccharides, galacto-oligosaccharides, and fructooligosaccharides) are fiber-like in that human digestive enzymes have little or no effect on them.[6,50] These non-nutritive carbohydrates act as a food supply for the indigenous healthful microflora, which produce organic acids, mainly volatile fatty acids.[6] They are fermented *in vitro* predominantly by *Bifidobacterium* species but also, to a limited degree, by a number of others such as *Lactobacillus* species.[6,51,52]

For example, fructooligosaccharides are widely distributed in nature and are found in honey, beer, onion, rye, asparagus, Chinese chive, banana, maple sugar, oats, and Jerusalem artichoke.[52] In one study, 8 grams of fructooligosaccharides were taken each day by 23 elderly individuals for 2 weeks. Their stools were collected and examined bacteriologically and clinically before and during the test and 8 days after the final ingestion. The numbers of bifidobacteria in the stools increased about 1000% over the level present before the fructooligosaccharides were given and the frequency of the occurrence of bifidobacteria remarkably increased from 87% to 100%. After final ingestion of the fructooligosaccharides, the numbers of bifidobacteria decreased.[52]

BIOACTIVE PROTEINS

Bioactive proteins are another class of supportive substances that qualify as probiotics because they may beneficially affect intestinal microbial balance. They are produced in the body naturally or can be obtained from various foods. To lay the groundwork for understanding their potential benefit, consider the example of the protein, immunoglobulin A (IgA). Secretory IgA is the predominant antibody, or immune protein, the body manufactures and releases in external secretions such as saliva, tears, and milk.[53,54] It is also transported through the epithelial cells lining the intestine out into the lumen. It plays a major role in the defense mechanism on the surface of the intestine by preventing the absorption of, and/or by disposing of, microbial antigens.[53,54] Mucosal IgA also neutralizes viruses and, in the case of bacterial infections, blocks the attachment of pathogens to mucosal tissues and cells.[53,54] Another important function is the binding and subsequent inhibition of absorption of soluble, dietary macro-molecular antigens.[53] Other immune proteins, such as IgM and especially IgG, may also be helpful because they are known to have remarkably similar specificities.[53,55]

It is well-known that the benefits of these immuno-globulins, or bioactive immune proteins, can be experienced when they are given orally. Consider the classic example of the nursing infant. Its immature intestine does not immediately produce appreciable amounts of mucosal IgA and IgG, leaving it initially dependent on colostrum, and then on mothers' milk as the source of this protein and the protection it offers. Phagocytic cells in human milk, which constitute 90% of the cell population, contain IgA antibodies that are released in the intestine of the infant providing what is called passive immunity.[53,54] Some recent studies have even shown the presence of antibodies to food antigens in human colostrum and colostral cells, introducing the concept that such antibodies may have potential value in preventing the entry of food antigens through the infant's intestine.[53]

Human colostrum and milk are not the only source of these orally administered, bioactive immune proteins. In a study by Brussow et al., cows were hyperimmunized with four types of human rotavirus, a pathogen that causes acute diarrhea in humans. A concentrate of bioactive immune proteins from the milk of these cows showed neutralizing activities against all four types of human rotavirus that were 100 times higher than that produced in human samples and 10 times higher than specific commercial samples. Laboratory tests showed these bioactive proteins had powerful antiviral activity, even against very high doses of infectious rotavirus.[56]

In human studies it has been shown that a concentrate of bioactive immune proteins from the milk of cows immunized with human rotavirus could provide passive immunity and prevent rotavirus gastroenteritis when added to an infant's diet.[56,57] These proteins were remarkably resistant to proteolytic digestion and may offer help in infants susceptible to this condition.[56,57]

Similar results were achieved by Mietens et al. in 60 patients, aged 10 days to 18 months, who were suffering from

diarrhea with isolation of enteropathogenic *E. coli.* They were given a concentrate of bioactive immune proteins from milk with specific activity against enterotoxigenic *E. coli.* After 10 days, the *E. coli* was eliminated in 84.2% of the patients.[58] In a double-blind controlled trial by Tacket et al., a bioactive immune protein concentrate from cows' milk with specific activity against *E. coli* was given orally to adult volunteers who were given *E. coli*. Nine of the 10 controls had diarrhea after consuming the *E. coli* but all 10 who received the protein concentrate beforehand did not. No side effects from the protein concentrate were observed in any volunteer.[59]

Lastly, an immunodeficient child developed severe vomiting and diarrhea due to cryptosporidiosis at the age of 3 years and 2 months. A concentrate of bioactive immune proteins from the milk of cows hyperimmunized with cryptosporidiosis was given to the child. His vomiting and diarrhea resolved within 5 days and the cryptosporidium was no longer seen in the stool after 8 days.[60] Cows' milk is a rich source of IgG and other immune proteins. Because the functionality of certain types of IgA and IgG are similar, IgG from milk can be considered a valuable nutritional contribution to the health of the intestinal tract.[55]

LACTOFERRIN

Another equally interesting bioactive protein is lactoferrin. Similar to secretory IgA, it is found in external secretions, such as saliva, tears, nasal, bronchial and gastrointestinal secretions, and polymorphonuclear leukocytes, and is very plentiful in milk.[61] Most microorganisms are dependent upon iron for growth. Lactoferrin, an iron-binding protein similar to transferrin, has been speculated to play a role in the primary defense system against invading pathogenic organisms, probably by depriving them of iron.[62] Its antibacterial activity, being just one of its properties, has been observed repeatedly.[62-66] It works in concert with, or apart from, naturally occurring secretory antibodies.[62] *In vitro* and, to a lesser degree, *in vivo* testing has demonstrated its effectiveness against a variety of different microorganisms, some of which include *Escherichia coli, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus aureus, Vibrio cholerae, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Candida albicans*, while it does not affect *Lactobacillus caseii* and actually may promote the growth of *Bifidobacterium*.[61-67]

Lactoferrin is able to retain iron at low pH levels and may pass through the acidic environment of the stomach and enter the intestine unaltered. Even if it was partially hydrolyzed, some studies have shown that treatment of lactoferrin with acid or acidic proteases (like pepsin) produces hydrolysates with strong potential bacterial activities.[64,68] A peptide isolated from a pepsin hydrolysate of bovine lactoferrin was highly effective against a broad range of gram-positive and gram-negative bacteria *in vitro*.[64,68]

LACTOPEROXIDASE

Lactoperoxidase is another enzyme occurring in the various secretions of exocrine glands like saliva, tears, bronchial, nasal, and intestinal secretions.[61,69] It is also the second most prominent enzyme in bovine milk.[69] It has no antibacterial activity itself but forms, with hydrogen peroxide and thiocyanate, a potent natural antibacterial system known as the LP-system.

The action of the LP-system is described as follows: Lactoperoxidase catalyses a reaction where hydrogen peroxide oxidizes thiocyanate to form hypothiocyanate. The hypothiocyanate has the actual antimicrobial action by oxidizing functional sulfhydryl groups in vital metabolic enzymes and proteins of the microorganisms.[61,69,70] The mechanisms of the antimicrobial action of the LP-system have been studied extensively and are found to be mainly based on the following: damage to the bacterial cytoplasmic membrane and inhibition of essential transport mechanisms, like those for glucose and amino acids; inhibition of the syntheses of proteins, RNA, DNA; and vital metabolic enzymes such as those of the glycolysis system.[61,69,70]

The antimicrobial activity of the LP-system has been studied extensively *in vitro* and, to a lesser degree, *in vivo* with a wide range of microorganisms being inhibited. They include a number of gram-positive bacteria such as *Staphylococcus aureus, Campylobacter jejuni*, which is recognized as a cause of acute enteritis, *Campylobacter coli, Listeria monocytogenes, Streptococcus* species and *Bacillus* species.[69,71-75] A partial list of gram-negative bacteria include *Escherichia coli, Salmonella* species, and *Pseudomonas* species.[69,71-75] Some lactic acid bacteria are unaffected because they contain a "reversal enzyme" called the NAD(P)-OSCN-oxidase reductase, which prevents the antimicrobial activity of the LP- system.[69]

Lactoperoxidase is a highly active enzyme and only very low concentrations, along with low concentrations of hydrogen peroxide and thiocyanate, are needed to obtain an effective system. Hydrogen peroxide is known to be produced in many species of naturally occurring lactobacilli, and thiocyanate is widely distributed in animal and human tissues, body fluids, and secretions.[69,70] From a toxicological point of view the levels of thiocyanate used in the LP-system, as well as the generated oxidation products, are reported to be harmless. The widely *in vivo* occurrence of the LP-system underlines this conclusion.

Examining these probiotic and supportive substances in light of the desire to help reestablish, or assure, microbial balance in the intestinal tract is highly interesting and encouraging. They warrant the initiation of further studies to explore the spectrum of activity, dosage, and practicality of providing probiotics as a source of supportive nutrition for human intestinal health.

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