The Causes of Intestinal Dysbiosis: A Review

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Abstract

Alterations in the bowel flora and its activities are now believed to be contributing factors to many chronic and degenerative diseases. Irritable bowel syndrome, inflammatory bowel disease, rheumatoid arthritis, and ankylosing spondylitis have all been linked to alterations in the intestinal microflora. The intestinal dysbiosis hypothesis suggests a number of factors associated with modern Western living have a detrimental impact on the microflora of the gastrointestinal tract. Factors such as antibiotics, psychological and physical stress, and certain dietary components have been found to contribute to intestinal dysbiosis. If these causes can be eliminated or at least attenuated then treatments aimed at manipulating the microflora may be more successful.

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Introduction

The gastrointestinal tract (GIT) is one of the largest interfaces between the outside world and the human internal environment. From mouth to anus, it forms a nine-meter long tube, constituting the body's second largest surface area and estimated to cover approximately 250-400 m^2. Over a normal lifetime, approximately 60 tons of food will pass through the GIT.[1] Food is obviously extremely important for well-being, but its passage through the GIT can also constitute a threat to health. While the GIT functions to digest and absorb nutrients, food also provides exposure to dietary antigens, viable microorganisms, and bacterial products. The intestinal mucosa plays a dual role in both excluding these macromolecules and microbes from the systemic circulation and absorbing crucial nutrients.[2]

As mentioned above, the mucosa is exposed to bacterial products – endotoxins,[3] hydrogen sulphide,[4] phenols, ammonia, and indoles[5] – that can have detrimental effects on both mucosal and host health.[5] The presence of many of these toxic metabolites is directly dependent on the type of fermentation that occurs in the bowel. In turn, this fermentation is dependent on the type of bacteria present in the bowel, as well as the substrates available for fermentation. Diets high in protein[6] and sulfate (derived primarily from food additives)[4] have been shown to contribute greatly to the production of these potentially toxic products. The production and absorption of toxic metabolites is referred to as bowel toxemia.[7]

The bowel toxemia theory has historical roots extending as far back as Hippocrates. In 400 B.C. he stated that, "...death sits in the bowels..." and "...bad digestion is the root of all evil...."[8] More modern proponents of the bowel toxemia theory have included naturopath Louis Kuhne in the late nineteenth century,[9] as well as naturopath Henry Lindlahr[10] and Nobel prize laureate Elie

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Metchnikoff in the early twentieth century.[11] Louis Kuhne proposed that excess food intake, or the intake of the wrong types of food, resulted in the production of intestinal toxins. Fermentation of these toxins resulted in increased growth of bacteria within the bowel and, subsequently, disease. He believed a predominantly vegetarian and mostly raw diet would prevent build-up of intestinal toxins and, hence, would prevent and even cure disease.[9]

Only a few years later, Metchnikoff popularized the idea that fermented milk products could beneficially alter the microflora of the GIT. He believed many diseases, and even aging itself, were caused by putrefaction of protein in the bowel by intestinal bacteria. Lactic acid-producing bacteria were thought to inhibit the growth of putrefactive bacteria in the intestines. Thus, yogurt consumption was recommended to correct this "autointoxication" and improve composition of the microflora.[11,12]

The bowel toxemia theories eventually evolved into the intestinal dysbiosis hypothesis. The term "dysbiosis" was originally coined by Metchnikoff to describe altered pathogenic bacteria in the gut.[13] Dysbiosis has been defined by others as "...qualitative and quantitative changes in the intestinal flora, their metabolic activity and their local distribution."[14] Thus dysbiosis is a state in which the microbiota produces harmful effects via: (1) qualitative and quantitative changes in the intestinal flora itself; (2) changes in their metabolic activities; and (3) changes in their local distribution. The dysbiosis hypothesis states that the modern diet and lifestyle, as well as the use of antibiotics, have led to the disruption of the normal intestinal microflora. These factors result in alterations in bacterial metabolism, as well as the overgrowth of potentially pathogenic microorganisms. It is believed the growth of these bacteria in the intestines results in the release of potentially toxic products that play a role in many chronic and degenerative diseases.[13]

There is a growing body of evidence that substantiates and clarifies the dysbiosis theory. Altered bowel flora is now believed to play a role in myriad disease conditions, including GIT disorders like irritable bowel syndrome (IBS)[15] and inflammatory bowel disease (IBD),[16,17] as well as more systemic conditions such as rheumatoid arthritis (RA)[18] and ankylosing spondylitis.[19] Thus, knowledge of the factors that can cause detrimental changes to the microflora is becoming increasingly important to the clinician.

The Importance of Normal GIT Microflora

The microflora of the gastrointestinal tract represents an ecosystem of the highest complexity.[14] The microflora is believed to be composed of over 50 genera of bacteria[20] accounting for over 500 different species.[21] The adult human GIT is estimated to contain 10^{14} viable microorganisms, which is 10 times the number of eukaryotic cells found within the human body.[22] Some researchers have called this microbial population the "microbe" organ – an organ similar in size to the liver (1-1.5 kg in weight).[23] Indeed, this microbe organ is now recognized as rivaling the liver in the number of biochemical transformations and reactions in which it participates.[24]

The microflora plays many critical roles in the body; thus, there are many areas of host health that can be compromised when the microflora is drastically altered. The GIT microflora is involved in stimulation of the immune system, synthesis of vitamins (B group and K), enhancement of GIT motility and function, digestion and nutrient absorption, inhibition of pathogens (colonization resistance), metabolism of plant compounds/drugs, and production of short-chain fatty acids (SCFAs) and polyamines.[14,25,26]

Factors that Can Alter the GIT Microflora

Many factors can harm the beneficial members of the GIT flora, including antibiotic use, psychological and physical stress, radiation, altered GIT peristalsis, and dietary changes. This review will focus exclusively on the interactions of antibiotics, stress, and diet with the gut flora.

Table 1a. *The Effects of Some Selected Antibiotics on GIT Microflora*

Agent	Impact on			Overgrowth of resistant strains	Days to normalization of flora (post-administration)	Other
	Entero-bacteria	Entero-cocci	Anaerobic bacteria			
Ampicillin	↓↓	↓↓	↓↓	+	not stated	∇in Lactobacilli and Bifidus; ↑ in Candida; ∇ production of SCFAs[29,36,48]
Ampicillin/ Sulbactam	↓	↓	↓	+	14	∇ in Lactobacilli and Bifidus[29,48]
Amoxicillin	↓	↓	↓	+	not stated	↑ in Candida[29]
Amoxicillin/ clavulanic acid	–	–	–	+	not stated[29]	
Azlocillin	↓	↓	↓	+	not stated	∇ in Lactobacilli[29]
Aztreonam	↓↓	↑	–	+	14[29]	
Bacampillicin	–	–	↓	–	not stated	No significant change in Lactobacilli, Bifidus or yeasts[29,48]
Cefaclor	–	–	–	+	7	∇ in Bifidus; ↑ in *C. difficile*[29]
Cefaloridine	–	–	–	–	not stated[29]	
Cefazolin	–	–	–	+	not stated[29]	
Cefbuperazone	↓↓	↓	↓↓	–	28	∇ in Lactobacilli and Bifidus[29]
Cefixime	↓↓	↓	↓↓	+	14	∇ in Bifidus; ↑ in *C. difficile*[29]
Cefmenoxime	↓	–	–	+	not stated	∇ in Lactobacilli and Bifidus;↑ in Candida and Clostridia[29]

An overview of some of the research investigating the effects of selected antibiotics on the GIT microflora. ↓↓ = strong suppression (> 4 log 10 CFU/g feces); ↓ = mild to moderate suppression (2-4 log 10 CFU/g feces); ↑= increase in number of organisms during therapy; - = no significant change; ∇= decrease; + = positive result; Bifidus= *Bifidobacterium* spp.

Table 1b. *The Effects of Some Selected Antibiotics on GIT Microflora*

Agent	Impact on			Overgrowth of resistant strains	Days to normalization of flora (post-administration)	Other
	Entero-bacteria	Entero-cocci	Anaerobic bacteria			
Cefoperazone	↓↓	↓↓	↓	+	not stated	∇ in Lactobacilli and Bifidus; ↑ in *C. difficile* and Candida; 70% of drug excreted in bile[29,48,49]
Cefotaxime	↓	↓	–	–	not stated[29]	
Cefotetan	↓	↑	↓	+	not stated	∇ in Lactobacilli; ↑ in *C. difficile*[29]
Cefotiam	↓	–	–	+	not stated	↑ in Candida and Pseudomonas; ∇ in Lactobacilli[29]
Cefoxitin	↓	↑	↓	+	not stated	∇ in Lactobacilli and Bifidus; ↑ in *C. difficile* and Candida[29,48]
Ceftazadime	↓	–	–	–	not stated	∇ in Lactobacilli[29]
Ceftizoxime	↓	–	–	+	not stated	No effect on Lactobacilli; ↑ in *Citrobacter* spp. and *Proteus* spp.[29]
Ceftriaxone	↓↓	↓↓	↓	+	28	∇ in Bifidus; ↑ in Candida; 30% of drug excreted in bile[29,49]
Cephradine	–	–	–	–	not stated	No ↑ in yeast[29]
Cephrocile	↑	↓	–	+	4	↑ in *C. difficile*[29]

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Table 1c. *The Effects of Some Selected Antibiotics on GIT Microflora*

Agent	Impact on			Overgrowth of resistant strains	Days to normalization of flora (post-administration)	Other
	Entero-bacteria	Entero-cocci	Anaerobic bacteria			
Ciprofloxacin	↓↓	↓↓	↓	–	7	↑ in yeast colonization; no effect on Bifidus or Clostridia[29,50]
Clindamycin	–	↑	↓↓	+	14	10% of drug excreted in bile; ∇ production of SCFAs; ∇ in Bifidus and Lactobacilli[29,36,48,49]
Doxycycline	↓	↓	–	+	not stated	No effect on SCFA production[29,36]
Enoxacin	↓↓	–	–	–	14	↑ in Candida[29]
Erythromycin	↓	↓	↓	+	not stated	No significant change in Lactobacilli or Bifidus;↑ in yeast colonization; ∇ production of SCFAs[29,36,48]
Imipenem/ cilastatin	↓↓	↓↓	↓↓	–	14	∇ in Lactobacilli and Bifidus[29]
Lomefloxacin	↓↓	–	–	–	21[29]	
Metronidazole	–	–	–	–	not stated	No significant change in Lactobacilli, yeasts, Bifidus, or SCFA production[29,36,48]
Moxalactam	↓↓	↑	↓↓	+	14	∇ in Lactobacilli and Bifidus;↑ in Candida and *C. difficile*[29,48]
Norfloxacin	↓↓	–	–	–	14[29]	
Ofloxacin	↓↓	↓	–	–		∇ in Lactobacilli and Bifidus;↑ in Candida[29]

An overview of some of the research investigating the effects of selected antibiotics on the GIT microflora. ↓↓ = strong suppression (> 4 log 10 CFU/g feces); ↓ = mild to moderate suppression (2-4 log 10 CFU/g feces); ↑= increase in number of organisms during therapy; - = no significant change; ∇= decrease; + = positive result; Bifidus= *Bifidobacterium* spp.

Table 1d. *The Effects of Some Selected Antibiotics on GIT Microflora*

Agent	Impact on			Overgrowth of resistant strains	Days to normalization of flora (post-administration)	Other
	Entero-bacteria	Entero-cocci	Anaerobic bacteria			
Pefloxacin	↓↓	–	–	–	not stated	No effect on Candida[29]
Phenoxymethyl-penicillin	–	–	–	–	14	No significant change in Bifidus; larger doses ∇ Lactobacilli[29,48,51]
Piperacillin	↓	↓	↓	–	not stated[29]	
Pivampicillin	–	–	–	+	not stated	↑ in Candida; no change in Bifidus or Lactobacilli[29,48,52]
Pivmecillinam	↓↓	↑	↓	+	not stated	∇ in Lactobacilli and Bifidus[29,48]
Talampicillin	↑	–	–	+	not stated[29]	
Temocillin	↓↓	–	–	–	not stated[29]	
Tetracycline	–	–	–	+	not stated	↑ in Candida; ∇ in Bifidus and Lactobacilli[29,48]
Ticarcillin/ Clavulanic acid	↓	↑	↓	–	not stated	∇ in Lactobacilli and Bifidus[29]
Tinidazole	–	–	–	–	not stated	No significant change in Bifidus, Lactobacilli, or SCFAs[29,48,53]

An overview of some of the research investigating the effects of selected antibiotics on the GIT microflora. ↓↓ = strong suppression (> 4 log 10 CFU/g feces); ↓ = mild to moderate suppression (2-4 log 10 CFU/g feces); ↑= increase in number of organisms during therapy; - = no significant change; ∇= decrease; + = positive result; Bifidus= *Bifidobacterium* spp.

The Impact of Antibiotics on GIT Microflora

Antibiotic use is the most common and significant cause of major alterations in normal GIT microbiota.[27] The potential for an antimicrobial agent to influence gut microflora is related to its spectrum of activity,[27] pharmacokinetics, dosage,[28] and length of administration.[29] Regarding the spectrum of activity, an antimicrobial agent active against both gram-positive and -negative organisms will have a greater impact on the intestinal flora.[27]

In terms of pharmacokinetics, the rate of intestinal absorption plays a fundamental role. Also important is whether the drug is excreted in its active form in bile or saliva. Both of these pharmacokinetic factors determine the drug's ultimate concentration in the intestinal lumen and, hence, the severity of the microfloral alteration.[27] In general, oral antimicrobials well absorbed in the small intestine will have minor impact on the colonic flora, whereas agents that are poorly absorbed can cause significant changes. Parenteral administration of antimicrobial agents is not free from these consequences, as some of these agents can be secreted in their active forms in bile, saliva, or from the intestinal mucosa, and result in considerable alterations in the colonic flora.[30]

The dosage and length of administration of an antibiotic will also determine the magnitude of impact on the intestinal flora. In general, the greater the dosage and length of administration, the larger the impact on the microflora.[29] Tables 1a-1d provide an overview of research investigating the effects of specific antibiotics on GIT microflora. In general, the trials were conducted on healthy humans and involved only a single course of antibiotics. It is possible microfloral alterations induced by a particular antibiotic might be more severe in individuals with compromised health or who have been subjected to multiple courses of antibiotics.

Recent epidemiological research has shown that individuals who had taken only one course of antibiotics had significantly lower serum concentrations of enterolactone up to 16 months post-antibiotic use compared to individuals who had remained antibiotic-free during the same time period ($p<0.05$). As serum concentrations of enterolactone are dependent on colonic conversion of plant lignans to enterolactone by the intestinal microflora (via beta-glycosidation), this study suggests infrequent antibiotic use has much longer-lasting effects on the microflora and its metabolic activities than was previously believed.[31] This negative association between serum enterolactone levels and antibiotic use has clinical importance due to recent studies showing correlations between high serum enterolactone concentrations and protection from cardiovascular mortality[32] and breast cancer.[33]

If an antimicrobial agent severely impacts the microflora, negative repercussions on host health can result, and include:

• Overgrowth of already-present microorganisms, such as fungi or *Clostridium difficile*.[34] Overgrowth of these organisms is a frequent cause of antibiotic-associated diarrhea, and overgrowth of *C. difficile* can develop into a severe life-threatening infection.[35]

• Decreased production of SCFAs, which can result in electrolyte imbalances and diarrhea.[36] Short-chain fatty acids play a vital role in electrolyte and water absorption in the colon.[37] Reduced production of SCFAs post-antibiotic use may be a causative factor in antibiotic-associated diarrhea.[38] Short-chain fatty acids also contribute to host health in other ways, such as improving colonic and hepatic blood flow,[39] increasing the solubility and absorption of calcium,[40] increasing the absorptive capacity of the small intestine,[41] and maintaining colonic mucosal integrity.[42]

• Increased susceptibility to intestinal pathogens due to the decrease in colonization resistance.[43] A decrease in colonization resistance after antibiotic administration has been observed in animal models. Such experiments have shown that disruption of normal microflora decreases the number of pathogens necessary to cause an infection and lengthens the time of infection.[44]

• Decreased therapeutic effect of some medicinal herbs and phytoestrogen-rich foods.[31] The activity of many medicinal herbs depends on bacterial enzymatic metabolism in the colon. Of the many enzymes produced by intestinal flora, bacterial beta-glycosidases probably play the most significant role, as many active herbal constituents are glycosides and are inert until the active aglycone is released via enzymatic hydrolysis.[45] Herbs such as willow bark (*Salix spp.*), senna (*Cassia senna*), rhubarb (*Rheum palmatum*), devil's claw (*Harpagophytum procumbens*), soy

(*Glycine max*), and red clover (*Trifolium pratense*) would be essentially inactive without this colonic metabolism.[45,46] Based on the results of the above-described epidemiological study,[31] it can be inferred that antibiotic use interferes with microbial beta-glycosidation in the GIT for a considerable period post-antibiotic administration, which could significantly impact the efficacy of many phytotherapeutic agents prescribed post-antibiotic use.

Hence, antimicrobial agents should be used sparingly and selected carefully in order to minimize the impact on GIT microflora.[47]

The Effect of Stress on GIT Microflora

To determine whether psychological stress results in an altered gastrointestinal environment, Bailey and Coe investigated changes in indigenous GIT microflora in primates after maternal separation. GIT microflora was evaluated in 20 infant rhesus macaques ages 6-9 months who were separated from their mothers for the first time. All infant monkeys were found to have typical fecal bacterial concentrations at baseline. A brief increase in Lactobacilli shedding on the first day post-separation ($p<0.05$) was followed by a significant decrease in the concentration of Lactobacilli in the feces ($p<0.001$). An inverse relationship was also found between the fecal concentration of shed pathogens (*Shigella spp.* and *Campylobacter spp.*) and shed Lactobacilli ($p= 0.07$). The study demonstrates that psychological stress can alter the integrity of indigenous microflora for several days.[54]

Other authors have also theorized the Lactobacilli population responds to stress-induced changes in GIT physiology, such as inhibition of gastric acid release,[55] alterations in GIT motility,[56] or increased duodenal bicarbonate production.[57] These changes may result in an intestinal environment less conducive to Lactobacilli survival, adherence, and replication. Alterations in GIT milieu may lead to detachment of Lactobacilli from the intestinal epithelium and subsequent passage through the GIT, thus resulting in decreased numbers of replicating Lactobacilli. This would explain the increased shedding of Lactobacilli found on the first day of stress, followed by a dramatic decrease in numbers of Lactobacilli over the next six days.[54]

The effects of psychological stress on the intestinal environment have been studied in Soviet cosmonauts. In general, it was found that on return from space flight there was a decrease in fecal Bifidobacteria and Lactobacillus organisms (Table 2). These changes were attributed primarily to stress, although a diet low in fiber may also have contributed.[58]

Table 2. *Stress-associated Changes to GIT Microflora*

	L. acidophilus	*L. casei*	*L. plantarum*
During preparation	4.0	3.5	2.6
After short flight	1.7	0	0.5
After long flight	2.9	0	0

Changes in the Lactobacillus fecal flora in Soviet Cosmonauts (log/mL).[58]

The change in microflora observed by Lizko led to a subsequent decline in colonization resistance, which in turn resulted in increased numbers of potentially pathogenic organisms. It has been found that exposure to psychological stress results in a significant reduction in the production of mucin and a decreased presence of acidic mucopolysaccharides on the mucosal surface.[58] Since both mucin and acidic mucopolysaccharides are important for inhibiting adherence of pathogenic organisms to the gut mucosa, a decrease in either contributes significantly to successful colonization by pathogenic organisms.[59]

Lizko states that exposure to stress results in decreased production of immunoglobulin A (IgA). As IgA plays a vital role in the defense against pathogenic organisms by inhibiting bacterial adherence and promoting their elimination from the GIT, Lizko postulates that any decrease in IgA secretion would most likely increase intestinal colonization by potentially pathogenic microorganisms (PPMs).[58]

A 1997 study assessed the effects of psychosocial stress on mucosal immunity, specifically the effect of emotional stress on secretory IgA (sIgA) levels.[60] The study was conducted on children ages 8-12 years (mean age 9.4 years). Ninety children were included in the trial – half of whom had a history of recurrent colds and flu, while the other half were healthy controls. The results demonstrated that stressful life events correlated with a decreased salivary ratio of sIgA to albumin. The ratio of sIgA to albumin controls for serum leakage of sIgA and is thought to give a clearer indication of mucosal immunity than total sIgA concentration. This result provides additional evidence of the likelihood of stress effectively decreasing mucosal immunity and, thus, diminishing intestinal colonization resistance.

Other studies on college students have found sIgA concentrations decrease during or shortly after examinations.[61] Salivary concentrations of sIgA are inversely associated with norepinephrine concentrations, suggesting sympathetic nervous system activation suppresses the production and/or release of sIgA.[60] Thus, frequent suppression of mucosal immunity by the sympathetic nervous system during stressful experiences could increase colonization of the intestinal mucosa by PPMs.

Holdeman et al studied factors that affect human fecal flora. They noted a 20-30 percent rise in the proportion of *Bacteroides fragilis* subsp. *thetaiotaomicron* in the feces of individuals in response to anger or fearful situations. When these situations were resolved, the concentration of these organisms in the feces decreased to normal levels.[62] This effect may be mediated via epinephrine, which has been shown to stimulate both intestinal motility and bile flow. As growth of *B. fragilis* subsp. *thetaiotaomicron* is enhanced by bile, this may partly explain the increased numbers of organisms in response to increased epinephrine release.[63]

In vitro experiments conducted by Ernst and Lyte have demonstrated that several neurochemicals have the ability to directly enhance the growth of PPMs. The influence of the catecholamines norepinephrine, epinephrine, dopamine, and dopa were assessed on two strains of Enterobacteriaceae – *Yersinia enterocolitica* and *Escherichia coli*, and one strain of Pseudomonadaceae – *Pseudomonas aeruginosa*.[64] All three bacterial species are potential pathogens, with *Y. enterocolitica*[65] and *E. coli*[66] involved in GIT infections and *P. aeruginosa* in gastrointestinal, respiratory, and urinary tract infections.[67] The concentrations of catecholamines used in the experiment were equivalent to those found in plasma. The addition of norepinephrine, epinephrine, dopamine, and dopa to the cultures of *E. coli* resulted in increased growth when compared to non-catecholamine-supplemented control cultures. However, the largest increase in growth was observed with the addition of norepinephrine. Norepinephrine caused a large increase in growth of *Y. enterocolitica*, while both dopa and dopamine produced only small, but significant, increases in growth. Epinephrine demonstrated no effect. Norepinephrine also markedly increased the growth of *P. aeruginosa*, while the other catecholamines appeared to have no effect on this organism.[64]

In vitro experiments performed by Lyte et al showed exposure of enterotoxigenic and enterohemorrhagic strains of *E. coli* to norepinephrine resulted in increased growth and the expression of virulence factors, such as the K99 pilus adhesin, which is involved in the attachment and penetration of the bacterium into the host's intestinal mucosa. Growth of the enterohemorrhagic *E. coli* was also increased, as was its production of Shiga-like toxin-I and Shiga-like toxin-II. The capability of norepinephrine to enhance both bacterial virulence-associated factors and growth was shown to be non-nutritional in nature – in other words, the bacteria did not use norepinephrine as a food; rather, the effect was via an unknown mechanism.[68]

Additional experiments by Lyte et al demonstrated that upon exposure to norepinephrine, *E. coli* produces a growth hormone known as an

"autoinducer of growth."[69] This autoinducer showed a high degree of cross-species activity with other gram-negative bacteria, resulting in increased growth of other organisms. It was later found to stimulate 10- to 10^4-fold increases in the growth of 12 of 15 gram-negative microorganisms tested.[70]

Exposure to stress has been documented to result in dramatic and sustained increases in catecholamine levels. This high concentration of catecholamines, and especially norepinephrine, may result in increased growth of PPMs in the intestines.[61] The GIT has abundant noradrenergic innervation and a high amount of norepinephrine is present throughout.[71] Studies conducted by Eisenhofer et al showed 45-50 percent of the total body production of norepinephrine occurs in the mesenteric organs.[72,73] Lyte suggests spillover of norepinephrine into the lumen of the intestinal tract undoubtedly occurs due to the concentration gradient present within the mesenteric organs.[74] Thus, there would be no requirement for an active transport system. This spillover effect has previously been demonstrated for serotonin following its release from gut enterochromaffin cells.[75] As such, the GIT represents an area in which neuroendocrine hormones like norepinephrine coexist with indigenous microflora.[74] Thus far, catecholamines have not been found to induce the growth of gram-positive bacteria.[70]

The effect of norepinephrine on gut flora was recently demonstrated in a murine model. The release of norepinephrine into the systemic circulation, caused by neurotoxin-induced noradrenergic neuron trauma, resulted in increased growth of gram-negative bacteria within the GIT. The total gram-negative population increased by 3 log units within the cecal wall and 5 log units within the cecal contents inside a 24-hour time period. The predominant species of gram-negative bacteria identified was *E. coli*.[74]

To summarize, stress can induce significant alterations in GIT microflora, including a significant decrease in beneficial bacteria such as Lactobacilli and Bifidobacteria and an increase in PPMs such as *E. coli*. These changes may be caused by the growth-enhancing effects of norepinephrine on gram-negative microorganisms or by stress-induced changes to GIT motility and secretions.

Diet and Intestinal Microflora

The composition of the diet has been shown to have a significant impact on the content and metabolic activities of the human fecal flora.[20] Some diets promote the growth of beneficial microorganisms, while others promote microfloral activity that can be harmful to the host.

Sulfates

Sulfur compounds, including sulfate and sulfite, have been shown to increase the growth of PPMs or increase production of potentially harmful bacterial products in the GIT. In the colon is a specialized class of gram-negative anaerobes known as sulfate-reducing bacteria (SRB). SRB include species belonging to the genera Desulfotomaculum, Desulfovibrio, Desulfobulbus, Desulfobacter, and Desulfomonas.[76] The principal genus, however, is Desulfovibrio, which accounts for 64-81 percent of all human colonic SRB.

Sulfate-reducing bacteria utilize a process termed "dissimilatory sulfate reduction" to reduce sulfite and sulfate to sulfide.[4] The consequence of this process is the production of potentially toxic hydrogen sulfide, which can contribute to abdominal gas-distension.[76] Hydrogen sulfide can also damage colonic mucosa by inhibiting the oxidation of butyric acid, the primary fuel for enterocytes. Butyrate oxidation is essential for absorption of ions, mucus synthesis, and lipid synthesis for colonocyte membranes.[77] This inhibition of butyrate oxidation is characteristic of the defect observed in ulcerative colitis and leads to intracellular energy deficiency, as well as disruption of essential activities.[4] Sulfide has also been shown to cause a substantial increase in mucosal permeability, presumably due to the breakdown of the polymeric gel structure of mucin through the cleavage of disulfide bonds.[4]

Sulfate-reducing bacteria are not present in all individuals and there appears to be considerable variation in SRB concentrations depending on geographical location, a variation hypothesized to be connected to dietary differences. Sulfate-reducing bacteria directly compete with methanogenic bacteria (MB) for vital substrates,

such as hydrogen and acetate. In fact, methanogenesis and sulfate reduction appear to be mutually exclusive in the colon. In the presence of sufficient amounts of sulfate, SRB have been shown to outcompete MB for both hydrogen and acetate; whereas, under conditions of sulfate limitation the reverse occurs.[78] The amount of dietary sulfate that reaches the colon appears to be the primary factor in determining the growth of SRB. On the other hand, endogenous sources of sulfate (e.g., sulfated glycoproteins, chondroitin sulfate) appear to have little impact on SRB levels.[79]

Sources of dietary sulfate include preservatives, dried fruits (if treated with sulfur dioxide), dehydrated vegetables, shellfish (fresh or frozen),[80] packaged fruit juices, baked goods,[81] white bread, and the majority of alcoholic beverages.[6] It also appears probable that ingestion of foods rich in sulfur-containing amino acids encourages both the growth of SRB and the production of sulfide in the large bowel.[4] Major amounts of sulfur-containing amino acids are found in cow's milk, cheese, eggs, meat, and cruciferous vegetables. Consumption of large amounts of these foods may significantly increase sulfide production in the colon.[77] Research conducted in the 1960s found elimination of milk, cheese, and eggs from the diet of ulcerative colitis sufferers resulted in substantial therapeutic benefit, suggesting that reducing the intake of sulfur-containing amino acids decreases colonic production of sulfide.[82]

High Protein Diet

Consumption of a high-protein diet can also increase the production of potentially harmful bacterial metabolites. It has been estimated that in individuals consuming a typical Western diet (containing ~ 100 g protein/day) as much as 12 g of dietary protein per day can escape digestion in the upper GIT and reach the colon.[83,84] This is in addition to host-derived proteins, such as pancreatic and intestinal enzymes, mucins, glycoproteins, and sloughed epithelial cells.[5] Undigested protein is fermented by the colonic microflora with the resultant end-products of SCFAs, branched-chain fatty acids (e.g., isovalerate, isobutyrate, and 2-methylbutyrate), and potentially harmful metabolites – ammonia, amines, phenols, sulfide, and indoles.[5,77,85]

Ammonia has been shown to alter the morphology and intermediate metabolism, increase DNA synthesis, and reduce the lifespan of mucosal cells.[6] It is also considered to be more toxic to healthy mucosal cells than transformed cells and, thus, may potentially select for neoplastic growth.[5] Ammonia production and accumulation is also involved in the pathogenesis of portal-systemic encephalopathy.[86] Indoles, phenols, and amines have been implicated in schizophrenia[87] and migraines.[88] Indoles and phenols are also thought to act as co-carcinogens[5] and may play a role in the etiology of bladder and bowel cancer.[83]

The production of these potentially toxic compounds has been found to be directly related to dietary protein intake,[6] a reduction of which can decrease production of harmful by-products.[89] The production of these potentially harmful by-products can also be attenuated by the consumption of diets high in fiber[89] and/or indigestible starch (both of which reduce intestinal pH).[83]

Diets High In Animal Protein

In comparison to diets high in overall protein, diets especially high in animal protein have specific effects on intestinal microflora. While not appearing to dramatically alter the bacterial composition of the flora compared to control diets, ingestion of large amounts of animal protein does increase the activity of certain bacterial enzymes,[90] such as beta-glucuronidase, azoreductase, nitroreductase, and 7-alpha-hydroxysteroid dehydroxylase, in animals[91,92] and humans.[93] This can have important ramifications to the host, as any increase in activity of these enzymes will result in increased release of potentially toxic metabolites in the bowel. For instance, bacterial azoreductase can reduce the azo bond found in many synthetic food-coloring agents, releasing substituted phenyl and napthyl amines, some of which are known to be potent carcinogens.[90] Another example is the action of the bacterial beta-glucuronidases. Many xenobiotics are processed in the liver by a series of reactions that result in glucuronic acid conjugation. These glucuronides are then passed, via the biliary system, to the intestines. When these compounds reach the colon

Table 3. *The Effects of Various Diets on GIT Microflora*

Microorganisms	American/ Mixed Western Diet	American/ Seventh Day Adventist Vegetarian Diet	English/ Mixed Western Diet	Japanese/ Japanese Diet	Japanese/ Mixed Western Diet	Ugandan/ Vegetarian Diet	Indian/ Vegetarian Diet
Total anaerobes	10.2^{a}	–	10.1^{a}	9.9^{a} 11.4^{b}	11.5^{b}	9.3^{a}	9.7^{a}
Total aerobes	7.5^{a}	–	8.0^{a}	9.4^{a} 9.8^{b}	9.6^{b}	8.2^{a}	8.2^{a}
Bacteroides spp.	9.8^{a}	11.7^{b}	9.8^{a} $9.7^{a\Psi}$	9.4^{a} 10.1^{b}	11.1^{b}	8.2^{a} $8.2^{a\Psi}$	9.2^{a}
Enterococci	5.5^{a}	6.5^{b}	5.8^{a} $5.7^{a\Psi}$	8.1^{a} 8.4^{b}	8.4^{b}	7.0^{a} $7.0^{a\Psi}$	7.3^{a}
Bifidobacteria	10.0^{a}	8.1^{b}	9.8^{a} $9.9^{a\Psi}$	9.7^{a} 8.2^{b}	9.5^{b}	9.3^{a} $9.3^{a\Psi}$	9.6^{a}
Lactobacilli	7.3^{a}	10.0^{b}	6.5^{a} $6.0^{a\Psi}$	7.4^{a} 5.7^{b}	4.0^{b}	7.2^{a} $7.2^{a\Psi}$	7.6^{a}
Clostridia	4.4^{a}	8.6^{b}	5.0^{a} 4.4^{a}	5.1^{a} 9.7^{b}	9.5^{b}	4.6^{a} 4.0^{a}	5.0^{a}
Yeasts	–	–	$1.3^{a\Psi}$	–	–	$3.1^{a\Psi}$	–

Effect of 'Western' vs. vegetarian or high carbohydrate diets on the human fecal flora (- = no data; Ψ = a significant difference between groups; ^{a} = log10 mean count/g wet weight of feces; ^{b} = log10 mean count/g dry weight of feces.)
From: Salminen S, Isolauri E, Onnela T. Gut flora in normal and altered states. *Chemother* 1995;41 (suppl 1):5-15.

they can be hydrolyzed by beta-glucuronidase produced by the microflora, resulting in the release of the original xenobiotic, which then re-enters enterohepatic circulation and is recirculated several times before eventually being eliminated through the feces. If the original xenobiotic is mutagenic, carcinogenic, or otherwise toxic, this process can be detrimental to the host.[94] The nutrient calcium D-glucarate exerts its potentially beneficial effects by inhibiting beta-glucuronidase.

High Simple Sugar/Refined Carbohydrate Diet

Kruis et al observed that diets high in simple sugars slow bowel transit time and increase fermentative bacterial activity and fecal concentrations of total and secondary bile acids in the colon.[95] A consequence of slower bowel transit time may be an increased exposure to potentially toxic bowel contents.[96] The mechanism by which high-sugar diets increase bowel transit time is not yet known.[95]

The increase in colonic fermentative activity noted in the Kruis study may not be directly associated with changes in microflora composition, but rather be caused by direct exposure of the colon to simple sugars. Refined sugars are metabolized quickly in the ascending colon; whereas, high-fiber foods, containing substantial amounts of insoluble fiber, are metabolized more slowly, releasing fermentation end-products (e.g., hydrogen gas and SCFAs) more gradually.[97]

It is possible, however, that high sugar intake does cause alterations in the microflora. It has been observed that high sugar intakes increase bile output. Some species of intestinal bacteria utilize bile acids as food and, hence, any increase in their production will result in a competitive advantage for this group of bacteria.[63] The changes observed in bacterial fermentation in this study may or may not be related to changes in the species composition of the microflora. Since this was not adequately assessed in this study, the significance of these results requires further investigation.

Other researchers have postulated that when intake of dietary carbohydrates is insufficient, increased fermentation of the protective layer of mucin may occur due to the limited quantity of carbon sources reaching the colon. This may compromise mucosal defense and lead to direct contact between colonic cells and bacterial products and antigens. This, in turn, may lead to inflammation and increased mucosal permeability. Such a situation may encourage the growth of potentially pathogenic bacteria and perpetuate the inflammatory response.[98,99] This theory, however, is yet to be supported by direct evidence.

General Dietary Factors

The effect of the overall diet on the composition and metabolic activities of GIT microflora has been the subject of research since the late 1960s. It was initially believed that changing the content of the diet (in terms of meat, fat, carbohydrate, and fiber content) would dramatically alter the bacterial species composition of the colonic flora. However, when the diets of various population groups consuming different diets were analyzed, the changes noted were not dramatic.[93] Only minor changes were noted among the groups, although these changes were considered to be caused by differences in diet.[100] Table 3 outlines results of several studies comparing the fecal flora of individuals consuming the typical Western diet (high in fat and meat) to that of individuals eating vegetarian and/or high complex-carbohydrate diets.

In general, it appears populations consuming the typical Western diet have more fecal anaerobic bacteria, less Enterococci, and fewer yeasts than populations consuming a vegetarian or high complex-carbohydrate diet. Although one study found a significant difference between a mixed Western diet and a vegetarian diet, overall there appear to be relatively few trends.

In spite of these findings, Gorbach argues that due to the sheer number of bacteria present in the stool (approximately 10^{11} viable bacteria/g) and the enormous variety (around 500 anaerobic species, not to mention aerobic and facultative species), the classical method of quantifying flora is, at best, a crude approximation. Thus, these methods may be unable to differentiate changes due to variations in diet.[90]

In an attempt to create a more sensitive method to detect changes in human microflora, Peltonen et al utilized gas-liquid chromatography (GLC) to analyze profiles of bacterial cellular fatty acids. This method measures bacterial cellular fatty acids present in the stool that accumulate to form a GLC fatty acid profile, with each peak in the profile representing relative amounts of a particular fatty acid in the stool. Similar bacterial compositions should yield similar fatty acid profiles, while distinctions can be quantified by the extent to which profiles differ from each other. The researchers utilized this technique to analyze the effects of a vegan, raw food diet on the intestinal microflora. The one-month diet consisted of a variety of sprouts, fermented vegetables, fruits, seaweed, nuts, and seeds. Differences in the GLC profile between the test and control groups were statistically significant ($p<0.05$), as were the differences in test group GLC profiles before and

during the diet. No significant changes in the fecal flora could be detected in either group using the traditional isolation, identification, and enumeration bacteriological. While GLC may be a more sensitive method to determine changes in fecal flora, it cannot identify particular components of the flora.[101]

Newer techniques such as fluorescence *in situ* hybridization (FISH) or polymerase chain reaction assays coupled with denaturing gel electrophoresis[102] are more sensitive to minor alterations in microflora and allow for bacterial identification that would otherwise be impossible to culture.[103] The use of these modern techniques in future diet studies will shed more light on this contentious area. Interestingly, recent research utilizing the FISH technique has indicated the majority of bacteria in the colon are not culturable and have yet to be described. This finding suggests how little is actually known about the composition of GIT microflora.[104]

In summary, research has shown that consumption of foods rich in sulfur compounds, high in protein, and/or high in meat may produce detrimental effects on the host. These changes may be mediated through alterations in composition of the microflora or through increased production of bacterial metabolites. The impact of a high refined-carbohydrate intake on the microflora has yet to be clearly elucidated. Similarly, the relationship between the overall diet and composition of the microflora awaits further clarification using modern microbiological techniques.

Conclusion

Alterations in bowel flora and its activities are now believed to be contributing factors to many chronic degenerative diseases. Ample evidence in the literature exists to confirm dysbiosis as an important clinical entity. It is therefore imperative to know what factors play a causative role in this increasingly common condition. Antibiotics, psychological and physical stress, and dietary factors contribute to intestinal dysbiosis. Armed with knowledge of the factors that contribute to dysbiosis, clinicians are better equipped to deal with the causes of this condition. Diets can be altered, the effects of stress attenuated, and antibiotics used sparingly, in order to minimize the effects of these factors on intestinal microflora. If the causes of dysbiosis can be eliminated or at least attenuated, then treatments aimed at manipulating the microflora may become more successful and longer-lasting in effect.

Future research using molecular microbiology techniques will provide definitive answers to currently unanswered questions regarding the effects of various factors on the GIT microflora. Older studies that evaluated the effects of different dietary regimes on the GIT flora should be reconducted utilizing modern microbiology techniques. These techniques will also provide accurate information regarding how specific drugs or herbs affect microbial populations in the GIT. This information will allow far greater precision in both dietary and herbal prescribing.

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