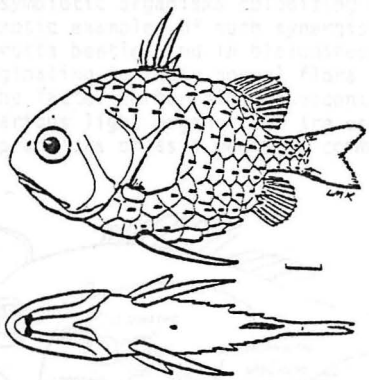
THE NORMAL FLORA

ASSET OR LIABILITY ?



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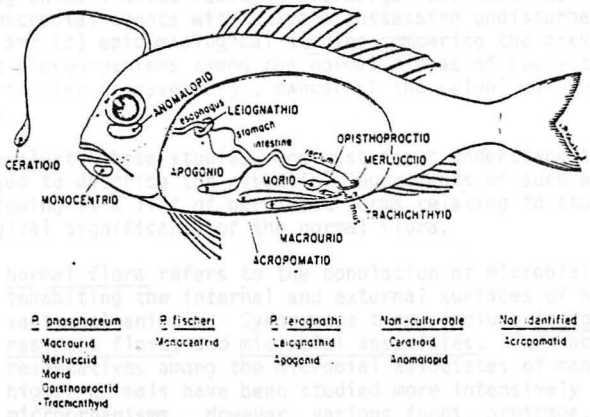
Is the normal flora an asset or a liability? Scientists of no less stature than Pasteur (1) and Metchnikoff (2) have contemplated this question since the existence of these microbial populations was first recognized. Pasteur predicted that elimination of microbial associates (i.e., production of germfree animals) would be incompatible with life. Thanks to the pioneer work of Reyniers and others at the Lobund Laboratory, University of Notre Dame, it is now clear that the germfree state can be maintained for successive generations in various experimental animals (3), thus laying to rest Pasteur's hypothesis that the normal flora is essential to life.

Metchnikoff's contention that indigenous microbes are antagonists of the host has been more difficult to refute. To be sure, there are animals within nature that profit handsomely from the activities of their indigenous microbial burden. Consider cattle and other ruminants, whose ability to use cellulose as a food source derives from the fermentation activity of symbiotic organisms colonizing their gastrointestinal tracts (4). More exotic examples of such synergistic relationships can be found in the ambrosia beetles and in bioluminescent fish. The former cultivate fungi originating in their normal flora as a chief source of food (5), whereas the latter harbor bioluminescent bacteria of the genus *Photobacterium* in various light organs that are used to attract prey, assist in escaping predators or as a means of communication (6)(Figure 1).



The "ichthylicht". A diagrammatic fish is used to indicate the approximate locations, sizes, and openings of the light organs of the several different families of luminous fishes that culture symbiotic luminous bacteria as a source of light for the organ. The taxa of bacteria known to be specifically associated with each fish group are listed at the bottom of the figure.

Figure 1

These examples would seem to vitiate Metchnikoff's contention that the normal flora is detrimental to higher animals, except that the relationships between ruminants, ambrosia beetles, and bioluminescent fish and their microbial symbiotes are highly specialized relationships that are by no means typical of those existing between the majority of higher animals (including man) and their indigenous microorganisms.

Fortunately, considerable experimental data do exist that bear directly upon the role played by the normal flora in health and disease in man and related mammals. In the present discussion, I will review these data, focusing on evidence relating to the issue of whether indigenous microorganisms help or hinder man in his efforts to cope with a hostile environment. Because the composition of the human normal flora has yet to be characterized completely, I have not attempted to delineate the individual species of microorganisms represented at various anatomical sites. I have, nevertheless, appended a table from Rosebury's Microorganisms Indigenous to Man (7) which can be used as a general guide to the composition of the normal flora of man. (See Appendix 1).

DEFINITIONS

Studies of the teleological significance of the normal flora have involved three basic types of experiments: (a) those comparing germfree or gnotobiotic animals with conventional counterparts; (b) those comparing animals whose resident microorganisms have been suppressed by antimicrobial agents with animals possessing undisturbed normal floras; and (c) epidemiological studies comparing the prevalence of specific microorganisms among the normal floras of subjects affected by a particular disease (e.g., cancer of the colon) with unaffected controls.

To evaluate these studies, one must first understand the terminology used to describe the essential ingredients of such experiments. The following is a list of pertinent terms relating to studies of the teleological significance of the normal flora:

a. Normal flora refers to the population of microbial associates inhabiting the internal and external surfaces of healthy conventional animals. Synonymous terms include indigenous flora, resident flora, and microbial associates. The bacterial representatives among the microbial associates of man and other higher animals have been studied more intensively than other microorganisms. However, various fungi, protozoa, and other microbes are also important constituents of the normal flora. Although many viruses may be cultured from otherwise healthy children, a "normal" viral flora is generally not felt to exist in man (8).

b. Conventional animal is one colonized by a full burden of resident microorganisms normally associated with its particular species.

c. Germ-free animal is one that is free from all demonstrable associated forms of life including bacteria, fungi, protozoa, viruses, and other microorganisms. In practice, the germ-free state is relative, since its definition depends upon the stringency of the tests used to establish microbial sterility (9). Physiologically, germ-free animals may differ markedly from their conventional counterparts. This is particularly ture of the gastrointestinal tract of such animals, which in certain species has a thinner lamina propria, a larger cecum, a more regular epithelium, a thinner mucosa, shallower crypts of Lieberkuh, and shorter-lived erythrocytes than in the conventional state (10). Consequently, germ-free animals are not necessarily anatomical or physiological equivalents of conventional animals *sans* their microbial associates. If a germ-free animal becomes colonized by a single microbial species, it is referred to as monoassociated; and if it becomes colonized by the full complement of microorganisms characteristic of its species, it is referred to as conventionalized.

d. Gnotobiotic is a word of Greek derivation meaning known flora. Gnotobiotic animals are either germ-free or ex-germ-free animals in which the composition of any associated microbial flora, if present, is fully defined according to the most current methodology (11).

EXOGENOUS FORCES INFLUENCING THE NORMAL FLORA

Various exogenous forces may have a pronounced effect on the delicate balance between the host and its normal flora. Diet is one such force. Restriction of carbohydrate ingestion, for example, has been shown to cause a marked reduction in the numbers of lactobacilli and *S. mutans* in the mouth (12). Synthesis of extracellular glucan from dietary glucose is critical to the latter organism's ability to adhere to tooth surfaces (13), and is a matter of no small consequence to man, owing to the pivotal role played by *S. mutans* in human cariogenesis (12). The effects of dietary components on the composition of the normal flora of other anatomical regions is largely unknown. Starvation has been shown to cause an imbalance in the gastrointestinal microbial ecosystem of rodents (14), which is felt to be at least partially responsible for the increased susceptibility of starved animals to *L. monocytogenes* infections (15). However, neither the precise dietary deficiency nor the specific indigenous microorganisms responsible for this affect have been identified.

The importance of diet as a source of microbial colonists has become increasingly apparent as clinicians have heightened their efforts to circumvent infections in immunosuppressed patients. *Ps. aeruginosa*, an important pathogen among such patients, has been shown to contaminate a high percentage of fresh vegetables finding their way to hospital kitchens (16). It has been suggested that more careful attention to eliminating such dietary sources of *Ps. aeruginosa* might be one way of reducing the high incidence of infections caused by this bacterium in immunosuppressed patients (17).

Disease and the general debilitation that accompanies diseases of unusual severity or duration are another potential source of perturbation of the indigenous flora. Johanson, et al (18,19) have shown, for instance, that the prevalence of Gram-negative bacilli among the oropharyngeal flora is low in physiologically normal subjects, even when exposed to a hospital environment (Table 1). However, the prevalence of oropharyngeal Gram-negative bacilli rises markedly when illness supervenes, presumably because sick epithelial cells are more easily adhered to by Gram-negative bacilli than are epithelial cells of healthy persons.

Table 1. Results of Single Oropharyngeal Culture Surveys

Study Group	No. of Subjects	Cultures Containing Gram-Negative Bacilli %
Normal subjects:		
Nonhospital associated	82	2
Hospital associated	47	2
Patients:		
Psychiatry service	20	0
Moderately ill	81	16
Moribund	23	57

Even in the absence of severe debilitation, certain chronic disorders induce detectable alterations in the composition of microbial populations colonizing man. Patients suffering from chronic alcoholism and diabetes mellitus have been shown to have an abnormally high prevalence of Gram-negative bacilli among the microorganisms colonizing their oropharynges (Table 2)(20). The mechanism responsible for this association is not known, but does not appear to be a deficiency of oropharyngeal Gram-positive bacteria capable of inhibiting Gram-negative bacilli (Figure 2) (21).

TABLE 2

PREVALENCE OF OROPHARYNGEAL GRAM-NEGATIVE BACILLI AMONG SUBJECTS

	Total	No. Colonized	% Colonized
Control subjects	55	13	24
Alcoholics	25	12	48
Diabetics	33	13	39

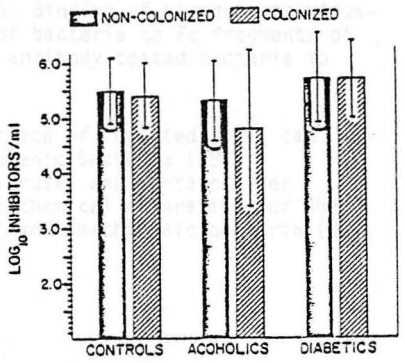


Fig. 2 Mean ± SD concentrations of total inhibitors in saline gargles from noncolonized and colonized subjects in each study population.

Perhaps the most intriguing of all the exogenous forces causing major disturbances in the composition of the normal flora are the influenza viruses and other respiratory viruses. Bacterial superinfections are frequent and occasionally devastating complications of these viral respiratory infections. Although the mechanisms responsible for the bacterial superinfections are multifactorial (22), recent evidence suggests that a critical factor in the relationships between the viral and bacterial respiratory pathogens relates to the ability of the viruses to promote colonization of the oropharynx by pathogenic bacteria (23-26) and to do so by facilitating adherence of these bacteria to pharyngeal epithelial cells (27). There are at least four mechanisms suggested by available experimental data that could account for the enhanced adherence of pathogenic bacteria to virus-infected mucosal surfaces (Figure 3).

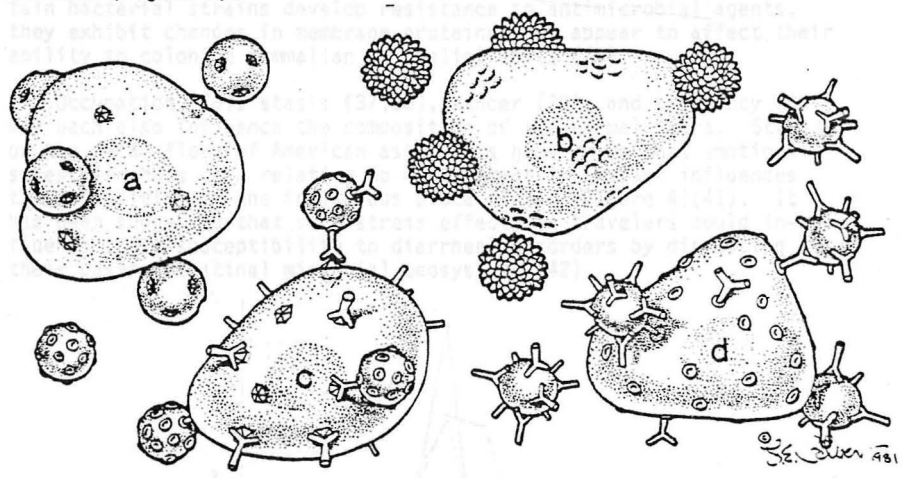


Figure 3. Possible mechanisms responsible for the enhanced adherence of pathogenic bacteria to virus-infected mucosal cells: (a) Binding of bacteria to viral antigens themselves; (b) Binding of bacteria to virus-induced membranous defects; (c) Binding of bacteria to Fc fragments of fixed antiviral antibody; (d) Binding of antibody-coated bacteria to virus-induced Fc receptors.

a. Viral antigens present on the surface of infected cells can themselves act as binding sites for pathogenic bacteria (28).

b. Infection of cells by influenza viruses and certain other respiratory viruses appear to cause physiochemical alterations of the cell membrane that may favor adherence of some pathogenic bacteria (29).

c. Adherence of bacteria such as *S. aureus* to virus-infected cells might result *in vivo* from the union of Fc fragments of fixed antivirus antibodies with bacterial Fc receptors (30).

d. Adherence of antibody-coated bacteria to virus-infected cells might also result *in vivo* from the union of Fc fragments of the antibacterial antibodies with virus-induced Fc receptors present on the surface of cells infected by certain herpes viruses (31).

Antimicrobial agents are capable of causing the most rapid and the most radical changes in the normal flora of any exogenous factors influencing these populations. Aside from their ability to destroy microbial associates outright, antimicrobial agents may impair adherence of microorganisms to epithelial cells even when present in subinhibitory concentrations (32-34). Furthermore, as certain bacterial strains develop resistance to antimicrobial agents, they exhibit changes in membrane proteins that appear to affect their ability to colonize mammalian epithelial cells (35).

Occupation (36), stasis (37,38), cancer (39), and pregnancy (40), may each also influence the composition of the normal flora. Studies of the fecal flora of American astronauts have shown that emotional stress (in this case relating to confinement in Skylab) influences the composition of the indigenous biota of man (Figure 4)(41). It has been suggested that such stress effects in travelers could influence their susceptibility to diarrheal disorders by disrupting their gastrointestinal microbial ecosystems (42).

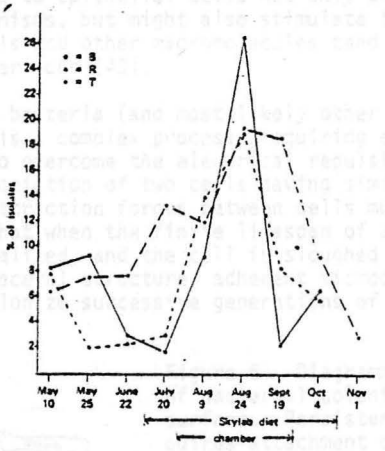


Figure 4. Quantitative stool cultures obtained from three astronauts before, during, and after confinement in the Skylab chamber. Percentage of isolates, from different fecal specimens, that were *B. fragilis* subsp. *thetaiotaomicron*.

MECHANISMS OF COLONIZATION

The mechanisms by which microorganisms colonize epithelial surfaces fall into one of two broad categories: those concerned with adherence to the animal surfaces and those that enable microorganisms to survive in the surface environment (38). Normal microbial associates and pathogenic microorganisms alike must overcome a variety of obstacles encountered on animal epithelial surfaces if they are to successfully colonize these surfaces (Table 3). The continuous unidirectional flow of material through most visceral channels --

Table 3. Obstacles Encountered by Microorganisms Attempting to Colonize the Body Surfaces of Higher Animals

1. Unidirectional flow	6. Non-specific Host Antimicrobics
2. Mucociliary Clearance Systems	7. Variations in pH or Redox Potential
3. Epithelial Cell Turnover	8. Microbial Competitors
4. Local Immune Systems	
5. Receptor Analogues	

either as a result of peristaltic activity, gravitational forces, or mucociliary clearance systems -- dictates that organisms within channels such as the gastrointestinal tract will be swept away with other intraluminal materials if not attached to underlying epithelial surfaces. Adherence to epithelial cells not only prevents the expulsion of the microorganisms, but might also stimulate their growth, since nutrient materials and other macromolecules tend to concentrate at solid-liquid interfaces (43).

Adherence of bacteria (and most likely other microorganisms) to mammalian cells is a complex process, requiring expenditure of sufficient energy to overcome the electrical repulsion barrier posed by the intimate association of two cells having similar electrical charges (13). The attraction forces between cells must be strong and yet reversible, so that when the finite lifespan of a colonized epithelial cell has been realized, and the cell is sloughed into the intraluminal stream of the visceral structure, adherent microorganisms can free themselves to colonize successive generations of new epithelial cells (Figure 5)(44)

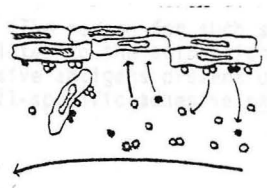


Figure 5. Diagrammatic representation of bacterial colonization on a mucosal surface. Persistent colonization requires attachment of dislodged progeny. Differences between the innate abilities of bacterial species (black and white) to attach are multiplied because of the cyclical nature of the events. More feebly adhering organisms become eliminated over time. Species unable to attach (gray) are removed by the flowing secretions.

In vitro studies of bacterial adherence to epithelial cells have shown a remarkable degree of specificity of microorganisms for particular cell types. Avian lactobacilli, for example, adhere to avian epithelial cells in such experiments, but not to the epithelial cells of other animals (45a). Similarly, rat lactobacilli adhere preferentially to rat epithelial cells (46a). Observations of this nature suggest that the ability of microorganisms to adhere to epithelial cells of a particular animal species is a critical determinant of whether they are able to colonize that species.

Perhaps even more intriguing are the observations in similar *in vitro* experiments, that bacterial adherence to epithelial cells is also cell-specific. In fact, different epithelial cell types within an anatomical area as confined as the human oropharynx demonstrate striking differences in suitability for adherence by given species (Table 4)(44). The fact that the specific microorganisms exhibiting

Table 4. Ability of bacteria to attach and their proportions found indigenously[a]

	Relative Indigenous Proportions			Experimentally Observed Adherence		
Bacteria	Tooth	Tongue Dorsum	Buccal Mucosa	Tooth	Tongue Dorsum	Buccal Mucosa
Streptococcus salivarius	low	high	mod.	low	high	mod.
Streptococcus mitis	high	mod.	high	high	mod.	high
Streptococcus sanguis	high	mod.	mod.	high	mod.	mod.
Streptococcus mutans	low to high[b]	low	low	low to high[b]	low	low
Veillonella	low	high	low	low	high	low
Lactobacilli	low	low	low	low	low	low
Neisseria	low	low	low	low	low	low

[a] Data derived from (71, 86, 134, 135, 209–211).
[b] High under the influence of dietary sucrose.

the strongest affinity for particular epithelial cell types in *in vitro* adherence assays are the same microorganisms colonizing the cell types *in vivo*, adds further credence to the concept that adherence is a critical determinant of a microorganism's ability to colonize epithelial surfaces. It is noteworthy, however, that even microorganisms with limited ability to directly colonize particular host surfaces, may nevertheless do so *in vivo* through a process of interbacterial aggregation. Whittenberger, et al (45b), for instance, have shown that glucosyltransferase produced by *S. salivarius* greatly facilitates adhesion of *Veillonella* to smooth surfaces. Presumably, a similar process of interbacterial adhesion is responsible for the ability of *Veillonella* to colonize the smooth surface of teeth *in vivo*.

The reason for such striking cell specificity almost certainly relates to the attraction of species-specific microbial adhesins (adhesive antigens present on the surface of microbes) to complementary cell-specific adhesive receptors present on the surfaces of host cells

(46b). Many such adhesins and receptors have been identified. In general, adhesins are proteinaceous antigens present as filamentous projections on the surface of bacterial cells (Figure 6). These bind to

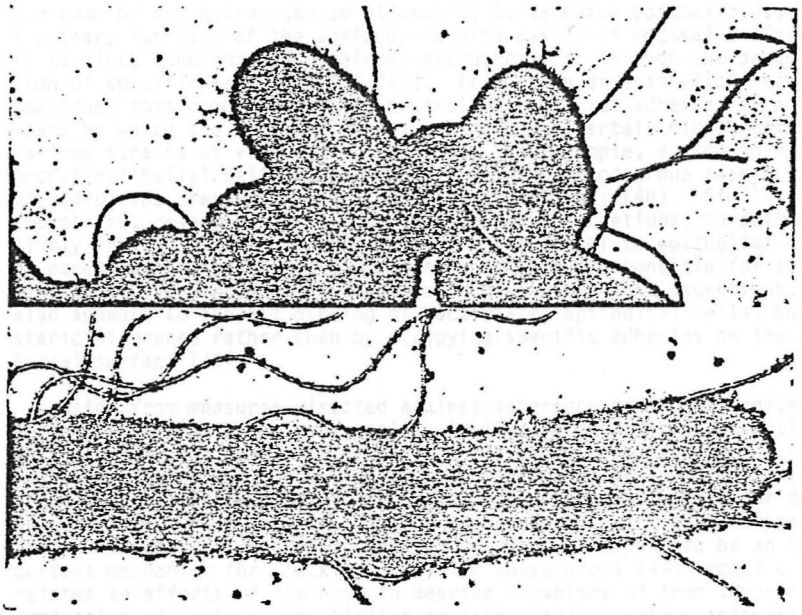


Figure 6. Formation of filaments and lack of fimbriae induced by growth of *Escherichia coli* in subminimal inhibitory concentrations of penicillin. Although the filaments (*bottom*) lacked the fimbriae present on organisms grown without penicillin (*top*), the formation of flagella did not appear to be affected by penicillin. (See earlier discussion regarding the effect of subinhibitory antibiotic concentrations on adherence).

specific receptors on the cell membrane of epithelial cells. Receptors for Gram-negative bacteria have generally been identified as carbohydrate moieties. Mannose, for example, has been shown to be the specific receptor for such species of Enterobacteriaceae. The receptor for *S. pyogenes* -- the most thoroughly studied Gram-positive organism in this regard -- appears to reside in an albumin-like protein or glycoprotein present in the membrane of oral epithelial cells.

Host mechanisms aimed at blocking adherence of microorganisms to

epithelial surfaces have evolved *pari passu* with those microbial mechanisms promoting adherence of microorganisms to epithelial surfaces. In general, inhibitory host mechanisms are more active against potential pathogens than normal microbial associates, but are obstacles to be overcome by any microorganism attempting to colonize epithelial surfaces. A primary function of the antibody-forming system of mucosal surfaces is to block adherence of unwelcome microorganisms through the production of specific secretory IgA (47). Production of epithelial receptor analogues that combine with and neutralize bacterial adhesins is another means by which the host may thwart adherence of certain microorganisms. Various strains of viridans streptococci, for example, attach to human buccal epithelial cells by selectively binding blood group reactive glycoproteins present on the surface of these cells (48). Similar glycoproteins, released by the host into salivary secretions, competitively inhibit binding of the viridans streptococci to epithelial cells by occupying the bacterial glycoprotein adhesins responsible for such binding. Lysozyme, another constituent of certain host secretions, also appears to inhibit binding of bacteria to epithelial cells, but by steric hindrance rather than by occupying specific adhesins on the bacterial surface (49).

Aside from measures directed against adherence of microorganisms to epithelial cells, other non-specific inhibitory mechanisms are active on epithelial surfaces that discourage microbial colonization. These represent the primary obstacles to colonization of stagnant anatomical areas such as the cecum of lower animals, the large intestine, or dental crevices, where adherence is not a prerequisite for surface association. In general, nutrient deprivation does not appear to be an important mechanism for checking growth in these areas (44) except as it relates to efforts of the host to deprive organisms of iron through the production of various iron binding proteins (50). Various antibacterial substances are produced by the host, which may further impair microbial growth on some surfaces. Unconjugated bile is one such substance, whose toxicity is probably at least partially responsible for the low number of microorganisms present in the small intestine (51). Certain anatomical sites, because of an unfavorable pH or oxidation-reduction potential, are suitable for growth of some, but not other, groups of microorganisms. In the mouth, for instance, the low oxygen tension within the gingival crevice makes it the primary ecological niche of obligate anaerobic microorganisms (44). If this niche is eliminated, as it is in the edentulous patient, concentrations of anaerobic microorganisms within the oropharynx drop precipitously.

Finally, the microorganisms themselves pose a barrier to colonization. The nature and teleological significance of this competitive barrier is considered in a later section.

CARCINOGENESIS AND THE NORMAL FLORA

Some of the most persuasive evidence favoring Metchnikoff's contention that the normal flora is a threat to the host, derives from studies of the carcinogenic potential of the normal flora. Prime examples of such studies are those dealing with cycasin. This glycoside of cycad nuts has been shown to be carcinogenic when eaten by man or animals (52). Given parenterally to rats, it has no adverse effects nor is it carcinogenic when fed in larger amounts to germ-free rats. Spatz, et al have shown that cycasin is carcinogenic only when given orally to conventional animals, because the true carcinogen, methylazoxyimethanol, is formed only after hydrolysis of cycasin by intestinal bacteria (53).

Recent concern has arisen as to the possibility that carcinogenic nitrosamines might be produced *in vivo* by microbial reduction of nitrates and secondary amines used as food preservatives (54). This concern has heightened with the demonstration that the intestinal microflora is capable of catalysing the synthesis of nitrosamines at neutral pH and that some strains of intestinal bacteria can also reduce nitrate to nitrite (55). Fortunately, in man, nitrate is most likely absorbed prior to reaching regions of the gut heavily colonized by such bacteria.

Various "sentinel microorganisms" have an apparent predilection for colonizing patients with underlying malignant neoplasms. *Streptococcus bovis* ((56), *Clostridium septicum* (57), various non-typhoidal strains of salmonella (58), and the Epstein-Barr virus (59) are examples of such microorganisms. Production of a human choriogonadotropin-like substance by certain tumor-associated bacteria -- some of which continue to produce the substance even after having been subcultured for years -- suggests that exchange of genetic material between tumor cells and tumor-associated bacteria might take place in some cases (60). However, it is not yet known whether sentinel microorganisms are simply "innocent bystanders" or are actively involved in the carcinogenic process. The animal data reviewed above would suggest the latter; and yet, studies comparing the incidence of neoplastic diseases in germ-free and conventional animals have found malignant neoplasms to be evenly distributed between the two groups (61,62).

NUTRITIONAL CONTRIBUTIONS OF THE NORMAL FLORA

If the normal flora does contribute to the well-being of the host, as Pasteur hypothesized, it most likely does so in one of two ways: by satisfying certain nutritional needs of the host or by functioning as a barrier to infection. In nature, there are numerous clear-cut examples of symbiotic relationships between the normal flora and animal hosts, whereby critical nutritional needs of the host are satisfied

by resident microorganisms. The ambrosia beetles, already mentioned, are some of the more spectacular examples of such symbiotic arrangements. The ruminants are a much more familiar example, but are no less extraordinary in their ability to capitalize on the nutritional assets of their own intestinal flora. In fact, Hungate has suggested that "... utilization of microorganisms as food recalls the numerous plankton feeders of the ocean, but whereas typical plankton feeders have numerous devices for straining the organisms out of large volumes of water, the ruminant grows the "plankton" continously in tremendous numbers in a small volume and then harvests them. In a sense, the ruminant can be termed a plankton feeder, but its adaptations go much further since it utilizes not only the bodies of microorganisms, but also some of the waste products formed during their growth " (63).

Does man derive any comparable nutritional benefits from his own normal flora? At the present time, this question must be answered by pointing out that there are no data to suggest that man's normal flora or that of any closely related animals is essential to the nutritional well-being of the host. Nevertheless, certain representatives within the resident flora, particularly nutritionally fastidious bowel inhabitants, are capable of synthesizing vitamins in excess of their own metabolic needs (Table 5). It is probable, though not yet certain, that some of these vitamins are absorbed by man (7).

Table 5. Examples of Vitamins Synthesized by the Gastrointestinal Flora of Nonruminant Animals

Vitamin	Animal	Reference
K	Man, rat	7
Folate	Man, rat, chicken, pig, dog	64-67
B_{12}	Man	66
Pyridoxine	Man	68
Biotin	Man, rat	69,70
Pantothenate	Man, rat	71,72
Riboflavin	Man, rat	73,74

However, the synthesizing activity of the intestinal flora of nonruminant species has almost invariably been shown to be supplementary or contributory rather than indispensible. Furthermore, certain vitamins may be diverted from the host by vitamin-requiring bacteria among the resident flora, or (in the case of ascorbic acid) as a result of decomposition of vitamins by such microbes (7). In man, these processes are responsible for vitamin B_{12} deficiency that occasionally complicates the various intestinal overgrowth syndromes (75).

It has been speculated that excess ammonia produced as a result of degradation of urea by the gastrointestinal flora might be available to the host for synthesis of nonessential amino acids and amination of nitrogen-free analogues of essential amino acids (76,77). Mitch recently analyzed urea metabolism in patients with chronic renal failure before and during antibiotic suppression of the intestinal flora to see if these bacteria might benefit uremic patients by providing an internal source of nitrogen or by functioning as an alternative means of clearing waste products (78). He found no evidence to suggest that nitrogen derived from bacterially degraded urea was used by uremic patients for amino acid synthesis. In fact, he found that nitrogen balance was improved when the intestinal flora of the patients was suppressed by antibiotics. These findings suggest that the intestinal flora does not contribute in a positive way to protein metabolism in man.

In rodents, bile acid metabolism (79), cholesterol synthesis (80), and modification of polyunsaturated fatty acids (9) have been shown to be under the influence of intestinal flora. Whether or not such activities are important to the hosts' nutritional needs appears to depend on many factors. Their relevance to the relationship between man and his own indigenous flora is uncertain.

THE ROLE OF THE NORMAL FLORA IN RESISTANCE TO INFECTION

Mechanisms of Pathogen Inhibition

An important principle of infectious diseases is that pathogenic microorganisms generally have to attain certain critical population densities on the host before they can successfully invade the host (81). A single pathogenic microbial cell rarely, if ever, is sufficiently virulent to cause disease in a susceptible host. Therefore, pathogenic microorganisms first must establish themselves on the skin or mucosal surfaces of the host and then proliferate to population sizes sufficient for invasion.

Much of the research into the role of the normal flora in resistance to infection has dealth with studies of its ability to limit the growth of pathogenic microorganisms on mucosal surfaces. Although such experiments have yet to prove the importance of the normal flora as a barrier to infection, they have established the existence of numerous mechanisms by its members which may inhibit growth of pathogenic microorganisms (Table 6).

Of the direct mechanisms by which indigenous microorganisms suppress potential pathogens, bacteriocin production is the one best characterized. Production of these high molecular weight proteinaceous antibiotics by resident viridans streptococci is felt to repre-

Table 6. Mechanisms by Which Indigenous Microorganisms Inhibit Potential Pathogens. (Adapted from Savage, ref. 42)

Direct Effects:

- Bacteriocin production
- Production of toxic metabolic end-products
- Induction of low oxidation-reduction potential
- Depletion of essential nutrients
- Suppression of adherence
- Inhibition of translocation
- Degradation of toxins

Indirect Effects:

- Enhancement of antibody production
- Phagocyte stimulation
- Augmentation of interferon production
- Bile acid deconjugation
- Stimulation of clearance mechanisms

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sent an important barrier to colonization of the oropharynx by pathogenic bacteria such as *S. pneumoniae* (82), *S. pyogenes* (83-85), and Gram-negative bacilli (86,87). It is also likely that bacteriocin production is the principal means by which indigenous bacteria of the bowel and lower genitourinary tract suppress potential pathogens attempting to colonize these two anatomic sites. That this mechanism is responsible for suppressing pathogenic microorganisms on integuemental surfaces is suggested by studies demonstrating a reduced incidence of secondary infections in patients with eczematous or varicose ulcerations that are colonized by antibiotic-producing bacteria (88).

In the intestine, production of toxic short-chain fatty acids by the indigenous flora might be an important means by which pathogenic microorganisms are discouraged from colonizing intestinal ecosystems (89). There are probably many other metabolic end products of the indigenous flora that are either directly toxic to potential pathogens or inhibit growth of pathogenic microorganisms indirectly by lowering local oxidation-reduction potentials (42). Although depletion of essential nutrients might occur as a result of such metabolic processes, the few *in vitro* studies examining this mechanism have not found it to be important in suppressing potential pathogens (90).

The all too frequent development of candidiasis as a consequence of suppression of the resident flora by broad-spectrum antibiotics suggests that inhibitory mechanisms of the normal flora are active against fungal, as well as bacterial, pathogens. In fact, competitive bacteria within the normal flora appear to be more important than the

immune system of experimental animals in resistance to acute candidiasis (Figure 7)(91). *Streptococcus mitis* and *Lactobacillus acidophilus*

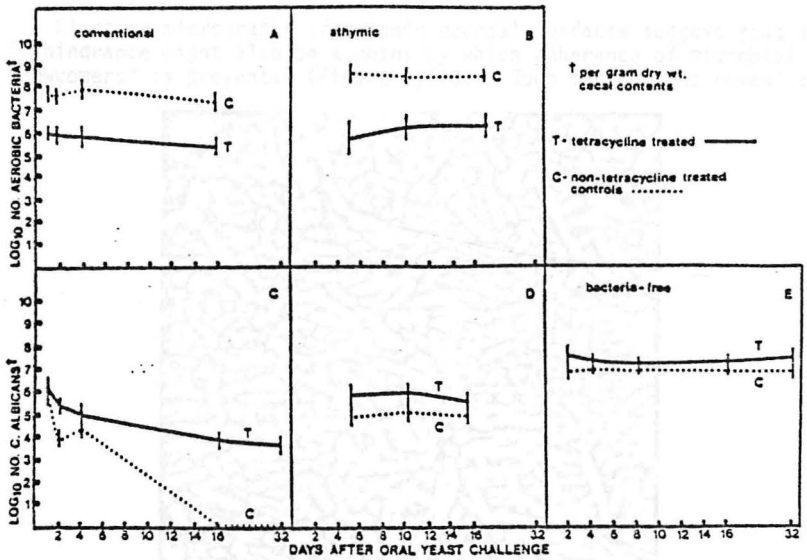

FIG. 7. *Effect of oral tetracycline treatment on the cecal, aerobic bacterial flora and C. albicans colonization in BALB/c mice, Each data point represents mean ± the standard error of at least four animals sacrificed at each data point. (A) Bacterial counts from tetracycline-treated and control conventional mice; (B) bacterial counts from athymic tetracycline-treated and control mice; (C) yeast counts from tetracycline-treated and control conventional mice; (D) yeast counts from tetracycline-treated and control athymic mice; and (E) yeast counts from tetracycline-treated and control bacteria-free mice.*

and other H_2O_2-generating resident bacteria have been found to inhibit fungi and certain viruses *in vitro* when combined with peroxidase and a halide (92). However, the role of this inhibitory system in controlling either fungal diseases such as candidiasis or viral infections remains uncertain.

Some of the most innovative studies of microbial inhibitory mechanisms have considered the effect of resident microbes on adherence of pathogens to epithelial surfaces. In germ-free mice, for example, twice as many cells of *Candida albicans* have been shown to adhere to oral epithelial cells as in conventional animals (93). Kuramitsu and Paul have recently shown that adherence of *Actinomyces viscosus* (an etiological agent in root surface caries and periodontal bone loss) to hydroxyapatite is suppressed by various bacterial species found among the normal flora of the human oropharynx (94). In one instance, adherence of *A. viscosus* appeared to be impaired because its attachment sites partially overlapped those of the indigenous microorganism

(*S. sanguis*), whereas in another case, inhibition of adherence appeared to be mediated through the effect of a bacteriocin (produced by *S. mutans*).

Electron micrographs of colonic mucosal surfaces suggest that steric hindrance might also be a means by which adherence of microbial "newcomers" is prevented (Figure 8)(38). Such micrographs reveal a

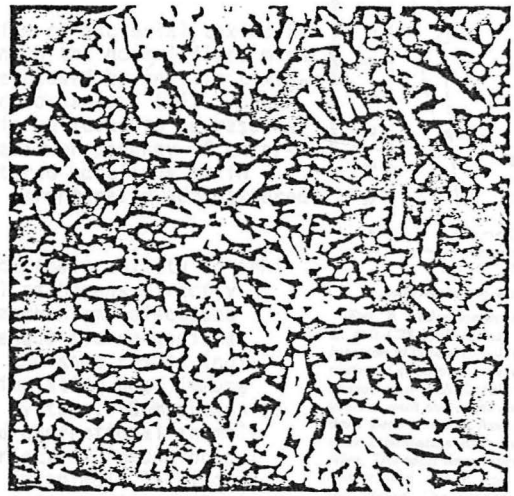

Figure 8 Microorganisms associated with stratified squamous epithelium of stomachs of adult CD-1 mice. Note end-on attachment of bacteria to epithelium (SEM, ×3800). Micrograph courtesy of D. Savage; reproduced from *Infect. Immun.* 10, 242 (1974) by copyright permission of the Rockefeller University Press, New York.

carpet of indigenous microorganisms so complete that it is difficult to conceive of a means by which potential pathogens might find access to the underlying mucosal surface, short of burrowing through this carpet. This form of competition, if important, would obviously be less evident in more sparsely populated surfaces such as the skin or small bowel mucosa.

Numerous studies in conventional mice have demonstrated reduced translocation (i.e., passage of bacteria from the gastrointestinal tract through the epithelial mucosa, the lamina propria and into mesenteric lymph nodes) of indigenous and autochthonous microorganisms in the presence of a viable normal flora (Figure 9)(95). By comparison, in germ-free mice the mechanisms limiting translocation are either absent or greatly reduced. Although the specific mechanisms involved

have not been identified. It has been speculated that the indigenous flora inhibits translocation by reducing microbial population densities within the bowel lumen, and this in turn is accomplished through stimulation of peristalsis.

Degradation of bacterial toxins is a means by which the normal flora might limit disease caused by certain toxin-producing microorganisms (96). Such degradation is believed to be responsible for the lower potency of enterally administered botulinus toxin (compared to parenterally administered toxin).

Aside from their ability to inhibit potential pathogens directly through the mechanisms discussed above, indigenous microorganisms might obtain the same result indirectly by stimulating host immune or clearance systems. Comparison of germ-free and conventional animals reveals a variety of immunological deficiencies in animals lacking indigenous microbial associates (Table 7). Conventional animals have been shown to have higher levels of natural antibodies (97) and more responsive microphages (Figure 10)(98) than their germ-free counterparts. Tippestad and Midtvedt have also reported that

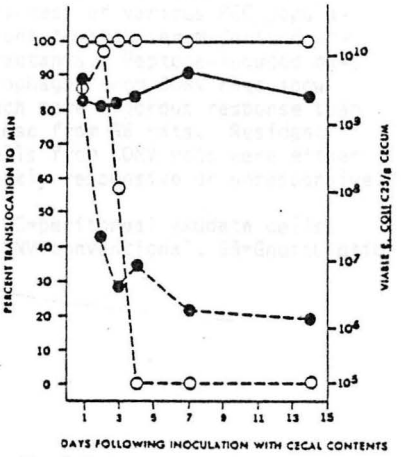

FIG. 9. *Reduction in the cecal population levels of E. coli C25 by bacterial antagonism inhibits translocation of viable E. coli C25 to the mesenteric lymph nodes of gnotobiotic mice. Germfree mice were monoassociated with E. coli C25 for 1 week before inoculation with the cecal contents from SPF mice. The data were obtained from these mice at various days after inoculation with the cecal contents from SPF mice. The solid lines represent gnotobiotic mice monoassociated with E. coli C25 throughout the entire test period. The dashed lines represent gnotobiotic mice monoassociated with E. coli C25 for 1 week followed by inoculation with the cecal contents from SPF mice. The solid circles represent the mean numbers of viable E. coli C25 per gram of cecum. The open circles represent the incidence of translocation of viable E. coli C25 to the mesenteric lymph nodes determined by the percentage of mice exhibiting viable E. coli C25 in their mesenteric lymph nodes.*

Table 7. Relative Immunological Deficiencies Observed in Germ-Free Animals

Deficiency	Ref. No.
Low levels of natural antibodies	97
Hyporesponsive macrophages	98
Hypresponsive neutrophils	99
Underdeveloped lymphoid tissues	102,103
Subnormal interferon production	104

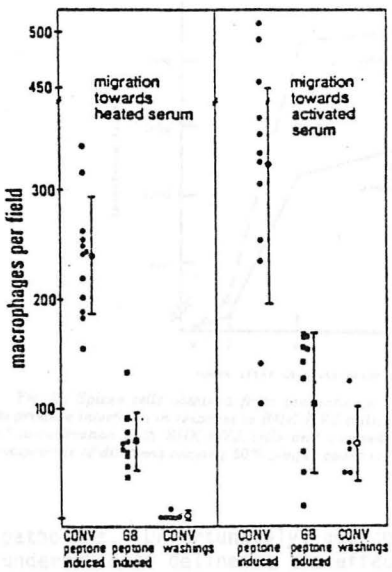

Figure 10. Chemotactic responsiveness of various PEC populations towards serum-derived attractants. Peptone-induced macrophages from CONV rats show much more vigorous response than those from GB rats. Resident cells from CONV rats were either weakly responsive or unresponsive.*

*PEC=peritoneal exudate cells; CONV=conventional; GB=Gnotobiotic

accumulation of neutrophils in casein-induced exudates is diminished in germ-free as compared to conventional rats (99). However, other investigators have failed to substantiate these observations (98). Conventional mice but not germ-free counterparts exhibit increases in serum colony stimulating factor following lethal irradiation, implying that the normal flora might be one of the determinants of serum colony stimulating factor levels (100). Germ-free animals occasionally exhibit lower total white blood cell counts than conventional controls (101) and have comparatively underdeveloped lymphoid tissue and smaller lymph nodes early in life (102,103). Populations of wandering cells within the lamina propria of the intestinal mucosa have also been reported to be diminished in germ-free animals (103). Nevertheless, the differences reported between germ-free and conventional animals have generally been minor; where differences in immune function have been identified, they have been quantitative rather than qualitative, and are rapidly eliminated with exposure of the germ-free animal to conventional microbial associates (11).

Interferon production in response to various interferon inducers has been shown to be diminished in germ-free as compared to conventional mice (Figure 11)(104). These observations illustrate a mechanism by which indigenous microorganisms might enhance resistance to viral

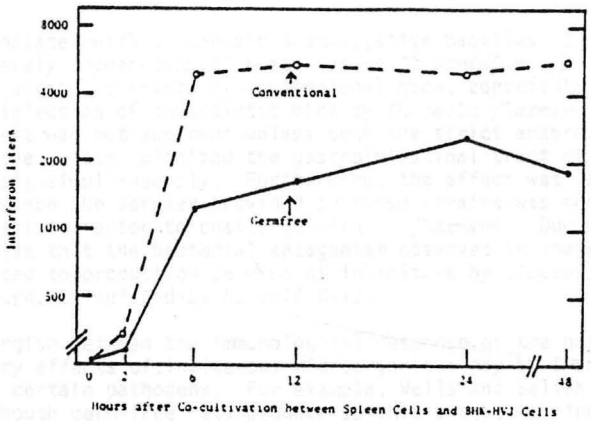

FIG. 11. *Spleen cells obtained from conventional or germfree mice were tested in vitro for their ability to produce interferon in response to BHK-HVJ cells. Culture fluid was obtained after 2, 6, 12, 24, and 48 h of co-cultivation with BHK-HVJ cells and assayed for interferon. Interferon titers are expressed as the reciprocals of dilutions causing 50% plaque count reduction.*

pathogens. Unfortunately, appropriate studies have not yet been undertaken to delineate the effect of the normal flora on resistance to viral infections (105).

Since many bacteria are destroyed *in vitro* by deconjugated bile acids, deconjugation of bile acids has been proposed to be an indirect means by which the normal gastrointestinal flora contributes to resistance to intestinal pathogens (42). Some intestinal pathogens, however, resist the toxic effects of bile acids, and other (sensitive) pathogens avoid exposure to the acids by colonizing areas of the upper small intestine where concentrations of deconjugated bile acids are low.

Finally, the normal flora may heighten host clearance mechanisms at least to the extent that it appears to stimulate intestinal peristalsis (42). In this regard, it is likely that indigenous microorganisms limit the capacity of intestinal pathogens to establish themselves by facilitating the expulsion of such pathogens from the gastrointestinal tract.

If the resident flora does have a function in defense against infection, it is unlikely that a single species is responsible for the inhibitory effect of the flora on potential pathogens. Rather, synergistic relationships between members of the normal flora appear to be critical in the process of suppression of potential pathogens. Ducluzeau and associates have verified the importance of such synergistic relationships in experiments with *Shigella flexneri* (106). They showed that

when associated with an aerobic Gram-negative bacillus (*E. coli* K-12), two extremely oxygen-sensitive strains of *Clostridium spp.* obtained from the digestive tracts of conventional mice, constitute a barrier against infection of gnotobiotic mice by *Shigella flexneri*. The barrier effect was not apparent unless both the strict anaerobe and the facultative aerobe colonized the gastrointestinal tract of experimental animals simultaneously. Furthermore, the effect was largely preventive since the barrier provided by these strains was most effective when it existed prior to challenge with *S. flexneri*. Ducluzeau, et al hypothesize that the bacterial antagonism observed in their experiments was related to production *in vivo* of inhibitors by *Clostridium spp.* that is, in turn, stimulated by *E. coli* K-12.

Synergism between the immunological response of the host and direct inhibitory effects of indigenous microorganisms may be important in inhibiting certain pathogens. For example, Wells and Balish have reported that although germ-free rats produce splenic plaque-forming cells in response to challenge with sheep erythrocytes, immune potentiation of the response by *P. acnes* is not observed unless rats are first exposed to a conventional resident flora (107). Similarly, Shedlofsky and Freter have observed enhanced resistance of germ-free mice to *Vibrio cholerae* following immunization if these animals are simultaneously colonized by microorganisms from the intestinal flora of conventional animals (108).

In vivo Data Indicative of a Positive Role

Indigenous microorganisms of the oropharynx have been scrutinized more carefully than any of man's resident microorganisms for evidence that the normal flora functions as a barrier to infection. *In vitro* observations have established that various strains of viridans streptococci inhabiting the oropharynx suppress growth of such diversified respiratory pathogens as *Streptococcus pyogenes* (83-85), *Streptococcus pneumoniae* (82), *Neisseria meningitidis* (83), *Staphylococcus aureus* (83), *Mycobacterium tuberculosis* (109), *Legionella pneumophila* (110), and Gram-negative bacilli (86-87). More importantly, numerous investigators have shown that elimination of inhibiting oropharyngeal bacteria *in vivo* is rapidly followed by the emergence of many of these same pathogens in the oropharynx.

Sprunt has reported that antibiotic-induced suppression of the normal oropharyngeal flora of man produces an ecological vacuum that is rapidly filled by resistant Gram-negative bacilli (Figure 12)(86). If, however, inhibitory bacteria within the resident oropharyngeal flora are resistant to the antibiotic administered, colonization of the oropharynx by Gram-negative bacilli does not occur (87). These observations, in conjunction with ones showing that children who become colonized by *S. pyogenes* possess fewer bacteria capable of inhibiting *S. pyogenes* among their resident oropharyngeal flora than children who do not become colonized by this pathogen (85), provide strong epidemiological evidence that the normal

flora acts as a barrier to colonization of the oropharynx by pathogenic bacteria. Unfortunately, careful microbiological assays performed on persons colonized by Gram-negative bacilli and non-colonized controls -- neither of which were receiving antimicrobial drug therapy at the time of examination -- have failed to demonstrate quantitative differences in oropharyngeal inhibitory bacteria (21). Thus, additional data are needed to clarify the relevance of *in vitro* observations of inhibition of pathogens by resident microbes to the capacity of the host to resist colonization by these pathogens *in vivo*.

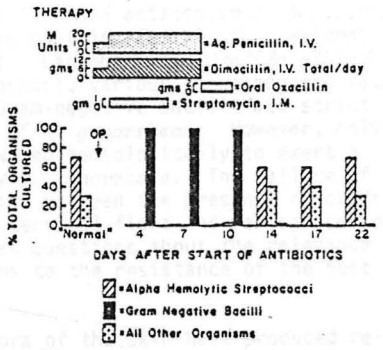

FIG. 12 Organisms cultured from posterior pharynx of Patient L. D. before, during, and after therapy with antibiotics noted.

Of the studies available, those concerned with the gastrointestinal tract have provided some of the most compelling evidence of the importance of the resident flora as a defense against infection. In numerous *in vitro* experiments, resident microorganisms of the gastrointestinal tract have been shown to inhibit growth of important pathogens such as *Vibrio cholerae*, salmonella, and shigella (89,108, 111, 112). Furthermore, suppression of these same resident bacteria (by antibiotics) reduces substantially the resistance of experimental animals (and to a lesser extent, man) to invasion by intestinal pathogens (89). In addition to suppressing growth of intestinal pathogens, the resident microflora of the bowel appears to protect the host by degrading bacterial toxins (96), a function that almost certainly accounts for the increased resistance of conventional animals to *Clostridium botulinum* as compared to germ-free controls (Table 8)(113).

Table 8. Resistance to intestinal botulinum infection when germ-free adult mice are conventionalized by exposure to normal mice for different periods.

Days[a]	No. tested	LD_{50} in intestine[b]
0	7	300–9,900[c]
3	6	Nil[d]
6	3	Nil
9	4	Nil[e]
12	3	Nil

[a] Days in room with normal mice before receiving spore challenge.

[b] Three days after intragastric dose of 10^5 spores.

[c] One with 300 LD_{50}, remaining six with 1,000 LD_{50} or more.

[d] Three homogenates tested with 1:25 to 1:625 dilutions.

[e] Two homogenates tested with 1:25 dilution.

Saigh and others have demonstrated *in vitro* antagonism of *Neisseria gonorrheae* by various microbial species contained within the resident flora of the human endocervix (90,114). Lactobacilli, *Candida albicans*, staphylococci, corynebacteria, streptococci, various nonpathogenic *Neisseria spp.*, and a variety of aerobic Gram-negative bacilli and strict anaerobes all inhibit *in vitro* growth of *N. gonorrheae*. However, only inhibitory lactobacilli have been shown epidemiologically to exert a protective effect against infection by *N. gonorrheae*. The failure of Saigh, et al to establish an association between the presence of other inhibitory species among the resident cervical flora and resistance to infection by *N. gonorrheae* again raises questions about the relevance of these kinds of *in vitro* observations to the resistance of the host *in vivo*.

Investigations of the resident flora of the skin have produced results that closely parallel those of the normal residents of other body surfaces. Patients with eczematous or varicose ulcerations that are colonized by antibiotic-producing bacteria have been reported to have fewer secondary infections of these ulcers than patients deficient in antibiotic-producing bacteria (88). Thus, the resident flora of integuemental surfaces appears to be a barrier to infection, but only in certain cases.

A number of investigators have observed diminished delayed-type hypersensitivity reactions in germ-free animals and have therefore maintained that indigenous microorganisms play a role in resistance against intracellular pathogens (115). This conclusion is supported by investigations demonstrating increased susceptibility of germ-free animals to infections caused by *Listeria monocytogenes* (116), *M. tuberculosis* (98), and *Nocardia asteroides* (117). However, the conclusion is not supported by observations that viral infections in germ-free animals generally produce diseases that are indistinguishable from those produced in conventional controls (118).

In vivo Data Indicative of a Negative Role

For every investigation demonstrating a protective effect of the resident flora against infection, at least one other has demonstrated an antagonistic effect on host resistance. The latter reports do not necessarily invalidate the former, but rather emphasize the complexity of the relationship between the host and its indigenous microbial burden.

A number of experimental observations suggest that the normal flora is a liability rather than an asset in infections in which the host immune response plays an important part in the pathogenic process. Lymphocytic choriomeningitis is a case in point. In the mouse, neural damage during lymphocytic choriomeningitis results from activation of the host animal's own immune response rather than by a direct (virus-induced) cytopathic effect (119). Germ-free mice suffer less than

half the mortality of conventional mice when infected by lymphocytic choriomeningitis virus and appear to enjoy this increased resistance to the infection because of a decreased cellular immune response (120). Recovery from hepatitis of adult mice rendered susceptible to the hepatitis virus by treatment with cortisone or X-irradiation may be accelerated in germ-free animals for similar reasons (121).

Resistance to the lethal effects of bacterial lipopolysaccharide has long been recognized to be higher in germ-free experimental animals than in controls (11,122,123). Kiyono, et al have recently shown that germ-free animals exhibit greater mitogenic and immunologic responses to lipopolysaccharide *in vitro* and are more refractory to lipopolysaccharide than are conventional animals (124). In contrast to the findings with lymphocytic choriomeningitis, these observations suggest that, under certain circumstances, indigenous microorganisms induce immunological tolerance to bacterial toxins and theoretically might concomitantly decrease resistance to infection by pathogens producing these toxins.

In rare cases indigenous microorganisms appear to assist pathogenic microorganisms in their efforts to penetrate the host or to avoid destruction during therapeutic intervention (22). *Entamoeba histolytica* is a prime example of a pathogenic microorganism that depends on the resident flora to establish itself within the host. Germ-free animals are highly resistant to this pathogen, which normally exists as a commensal in the large intestine of its host, where it feeds on resident bacteria and superficial mucosal cells. Rarely, *E. histolytica* becomes highly invasive, attacking the colonic wall, liver, and other organs of its host. Observations in experimental animals suggest that these rare episodes of enhanced pathogenicity could result from virulence factors obtained from intestinal bacteria (125).

In the case of pathogenic shigella, *in vitro* observations suggest that, whereas the normal flora antagonizes the growth of shigella, it may simultaneously augment the capacity of pathogenic shigella to adhere to intestinal epithelial cells (126). However, in view of the reported antagonism of shigella by the normal flora *in vivo* (127), the *in vitro* observations of enhanced adherence are probably less important to the host than those concerned with growth inhibition.

Transmission of drug-resistant factors (R factors) affords another means by which resident microorganisms occasionally enhance the pathogenicity of invasive microorganisms. As a result of such transfer of R factors, pathogenic microorganisms may then enjoy increased resistance to one or more antibiotics normally used to terminate the infections they produce.

Practitioners of clinical medicine need no reminder of the potential of indigenous microorganisms -- no matter how innocuous -- for

becoming true pathogens under appropriate conditions. In fact, when the normal immune functions of higher animals are compromised either by disease or experimental design, the indigenous microorganisms of these animals frequently turn on their hosts with a fury that belies any attempt to attribute a beneficial role to the normal flora. Aside from being the major source of "opportunistic" infections in the immuno-suppressed host, there is considerable evidence that resident microorganisms are also the etiologic agents responsible for graft versus host disease following bone marrow transplantation (118), and the runting syndrome associated with neonatal thymectomy (128). Perhaps even more important are the observations that suppression of the normal flora by antibiotics or maintenance of the germ-free state substantially reduces the incidence of these complications in susceptible subjects.

Finally, indigenous microorganisms, while having no apparent enhancing or inhibitory effect on certain infections may, nevertheless, aggravate these infections by acting as secondary invaders (22). A classic example of this phenomenon is the superinfection of acute influenzal pneumonia by strains of *S. pneumoniae* originating from the ranks of the indigenous microbes of the oropharynx. Secondary invasion of the blood stream by normal residents of the large intestine during acute shigellosis (129) and overwhelming strongyloidiasis (130) are additional examples of the capacity of the indigenous flora for complicating rather than alleviating infections caused by exogenous pathogens.

Attempts to Build a Better Normal Flora

In a cleverly written note to The Annals of Internal Medicine, Rees recently described the hypothetical case of a Gene Splice, Inc. technician who enjoyed remarkable freedom from illness as a result of becoming colonized by an interferon-producing bacteria with which he had been working (131). According to the tale, he then loses his heightened resistance to infection when a court-ordered tonsillectomy terminated his carrier state.

Actually, numerous investigators have sought to increase resistance in just such a way -- that is, by supplementing the normal flora with nonpathogenic bacteria that are more effect inhibitors of these pathogens. The most extensive efforts in this regard have been devoted to attempts to control epidemic staphylococcal infections. These studies have demonstrated that interference between strains of *Staphylococcus aureus* can be exploited to curtail epidemis of staphylococcal disease in nurseries and to interrupt cycles of a recurrent furunculus in older persons (132). In these studies the "nonpathogenic" 502A strain of *Staphylococcus aureus* has been the primary re-

placement agent used to prevent colonization by more virulent strains of staphylococci. Unfortunately, nonpathogenicity has been a relative, rather than an absolute characteristic of this bacterium, as illustrated by reported instances of disease caused by *S. aureus* 502A (133).

Investigators in South Africa have also attempted to eliminate carriage of multiply resistant pneumococci by inducing nasopharyngeal colonization by *Streptococcus faecalis* (134). In spite of the fact that this bacterium produced inhibition of the multiply resistant organism *in vitro*, it did not eradicate carriage of the pneumococcus. Priority of colonization has been a crucial factor in that when two competing organisms vie for a particular ecological niche, the one reaching the niche first will generally prevail (135). The failure of investigators to terminate an established carrier state in patients colonized by resistant pneumococci may have been related to these experimental observations.

In addition to these attempts to increase the effectiveness of the normal flora as a barrier to infection by introducing new inhibitory microorganisms into its ranks, some investigators have sought to manipulate the normal flora through a process of "selective decontamination" (136,137). According to this process, antimicrobial agents that suppress aerobic microrganisms while leaving the obligate anaerobic flora undisturbed are administered to neutropenic patients. This is done with the knowledge that obligate anaerobes rarely cause infections in granulocytopenic patients, and, in fact, might constitute a limited barrier to colonization by aerobic bacteria that are responsible for the majority of infections seen in these patients. Such attempts to capitalize on the "colonization resistance" of the anaerobic constituents of the normal flora, while minimizing the invasive potential of the aerobic constituents have already met with some success in clinical trials in leukemia patients receiving chemotherapy (138-140). However, the ultimate question of whether selective decontamination enhances survival in such patients has not been resolved.

Conclusions

Those like Pasteur, who would regard the normal flora as essential to the well-being of higher animals have only to compare the life spans of germ-free animals with conventional controls. In general, they are comparable, and when differences have been demonstrated, germ-free animals have been shown to enjoy greater longevity than their conventional counterparts (11). Man, however, does not live in a germ-free environment. Rather, he lives in a world heavily populated by microorganisms of almost incomprehensible diversity. That the comparatively noninvasive microbial agents normally populating his skin and mucosal surfaces provide a degree of protection against colonization and/or invasion by pathogenic microorganisms has been convincingly demonstrated in studies

of the effects of antibiotic suppression of the normal flora on resistance to infections caused by *Candida albicans*, shigella, salmonella, and cholera. That these same microbial benefactors can destroy, as well as protect their natural host, either through their own efforts or in concert with exogenous pathogens, is just as convincingly demonstrated by the plethora of opportunistic infections occurring among patients with impaired immunological defenses. Thus, indigenous microorganisms cannot be viewed in absolute terms as to their capacity to benefit or menace the host. Each member of the normal resident flora is capable of helping or harming the host under appropriate circumstances.

Cover: Line drawing of *M. japonicus*, one of the luminous fishes. Light organs (solid black areas) located on the ventral surface of the lower jaw are actually aggregate collections of bioluminescent bacteria belonging to the genus *Photobacterium*. Light emitted by these bacterial symbionts is thought to benefit the fish by serving as a means of intraspecies communication or by illuminating the surroundings.

REFERENCES

1. Schottelius M: Die bedeutung der darmbakterien für die ernährung. II Arch Hyg 1902; 42:48-70.

2. Metchnikoff E: Sur la flore du corps humain. Manchester Lit Phil Soc 1901; 45:1-38.

3. Reyniers JA, Trexler PC, Ervin RF, Wagner M, Luckey TD, Gordon HA: A complete life-cycle in the gremfree Bantam chicken. Nature (London) 1949; 163:67-68.

4. Hungate RE: Symbiotic associations: the rumen bacteria. *In* Symbiotic Associations, ed. by P.S. Nutman and B. Mosse. The University Press, Cambridge, 1963. pp 266-297.

5. Baker JM: Ambrosia beetles and their fungi, with particular reference to *Platypus cylindrus* FAB. *In* Symbiotic Associations, ed. by P.S. Nutman and B. Mosse. The University Press, Cambridge, 1963. pp 232-264.

6. Nealson KH, Hastings JW: Bacterial luminescence: its control and ecological significance. Microbial Rev 1979; 43:496-519.

7. Rosebury T: Microorganisms Indigenous to Man. McGraw-Hill Book Co., Inc., New York, 1962. pp 310-384.

8. Reed SE, Tyrrell DAJ: Viruses associated with the healthy individual. *In* The Normal Microbial Flora of Man, ed. by F.A. Skinner and J.G. Carr. Academic Press, New York, 1974. pp 255-257.

9. Coates ME: Gnotobiotic animals in research: their uses and limitations. Lab Animal 1975; 9:275-282.

10. Whitt DD, Savage DC: Kinetics of changes induced by indigenous microbiota in the activity levels of alkaline phosphatase and disaccharides in small intestinal enterocytes in mice. Infect Immun 1980; 29:144-151.

11. Gordon HA, Pesti L: The gnotobiotic animal as a tool in the study of host microbial relationships. Bacteriol Rev 1971; 35:390-429.

12. Hoover CI, Newbrun E, Mettrow G, Graf H: Microflora and chemical composition of dental plaque from subjects with hereditary fructose intolerance. Infect Immun 1980; 28:853-859.

13. Corpe WA: Microbial components involved in adsorption of microorganisms onto surfaces. *In* Adsorption of Microorganisms to Surfaces, ed. by G. Britton and K.C. Marshall. John Wiley & Sons, New York, 1980. pp 105-144.

14. Tannock GW, Savage DC: Influences of diet and environmental stress on microbial populations in the murine gastrointestinal tract. Infect Immun 1975; 9:591-598.

15. Seeliger HPR: Listeriosis. Second ed. Hafner Publishing Co., Inc., New York, 1961.

16. Kominos SD, Copeland CE, Grosiak B, Postic B: Introduction of *Pseudomonas aeruginosa* into a hospital via vegetables. Appl Microbiol, 1972; 24:567-570.

17. Remington JS, Schimpff SC: Please don't eat the salads. N Engl J Med 1981; 304:433-434.

18. Johanson WG, Pierce AK, and Sanford JP: Changing pharyngeal bacterial flora of hospitalized patients. N Engl J Med 1969; 281:1137-1140.

19. Johanson WG, Pierce AK, Sanford JP, Thomas GD: Nosocomial respiratory infections with gram-negative bacilli. The significance of colonization of the respiratory tract. Ann Int Med 1972; 77:701-706.

20. Mackowiak PA, Martin RM, Jones SR, Smith JW: Pharyngeal colonization of aspiration-prone persons by gram-negative bacilli and other potential pathogens. Arch Int Med 1978; 138:1224-1227.

21. Mackowiak PA, Martin RM, Smith JW: The role of bacterial interference in the increased prevalence of oropharyngeal gram-negative bacilli among alcoholics and diabetics. Am Rev Resp Dis 1979; 120: 589-593.

22. Mackowiak PA: Microbial synergism in human infection (Parts 1 and 2). N Engl J Med 1978; 298:21-26; 83-87.

23. Eichenwald HF, Kotsevalov O, Fasso LA: Some Effects of viral infection on aerial dissemination of staphylococci and on susceptibility to bacterial colonization. Bact Rev 1961; 25:274-281.

24. Young LS, LaForce FM, Head JJ, et al: A simultaneous outbreak of meningococcal and influenzal infections. N Engl J Med 1972; 287: 5-9.

25. Smith CB, Golden CA, Kanner RE, Renzetti AD: Haemophilus influenzae and Haemophilus parainfluenzae in chronic obstructive pulmonary disease. Lancet 1976; 1253

26. Gwaltney JM Jr, Sande MA, Austrian R, Hendley JO: Spread of *Streptococcus pneumoniae* in families. II. Relation of transfer of *S. pneumoniae* to incidence of colds and serum antibody. J Infect Dis 1975; 132:62-68.

27. Fainstein J, Musher DM: Bacterial adherence to pharyngeal cells in smokers, nonsmokers, and chronic bronchitis. Infect Immun 1979; 26:178-182.

28. Sanford BA, Shelokov A, Ramsay MA: Bacterial adherence to virus-infected cells: a cell culture model of bacterial superinfection. J Infect Dis 1978; 137:176-181.

29. Mackowiak P, Marling-Cason M, Smith J, Luby J: Enhanced adherence of *N. meningitidis* to influenza A-infected monolayers. Abstracts of the 21st Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, 1981.

30. Austin RM, Daniels CA: The role of protein A in the attachment of staphylococci to influenza-infected cells. Lab Invest 1978; 39:128-132.

31. McTaggart SP, Burns WH, White DO, and Jackson DC: Fc receptors induced by herpes simplex virus. I. Biologic and biochemical properties. J Immun 1978; 121:726-730.

32. Svanborg-Eden C, Sanberg T, Stenqvist K, Ahlstedt S: Decrease in adhesion of *Escherichia coli* to human urinary tract epithelial cells in vitro by subinhibitory concentrations of ampicillin. Infection 1978; 6(Suppl 1):S121-S124.

33. Eisenstein BI, Ofek I, Beachey EH: Interference with the mannose binding and epithelial cell adherence of *Escherichia coli* by sublethal concentrations of streptomycin. J Clin Invest 1979; 63: 1219-1228.

34. Vosbeck K, Handschin H, Menge E-B, Zak O: Effects of subminimal inhibitory concentrations of antibiotics on adhesiveness of *Escherichia coli* in vitro. Rev Infect Dis 1979; 845-851.

35. Onderdonk A, Marshall B, Cisneros R, Levy SB: Competition between cogenic *Escherichia coli* K-12 strains in vivo. Infect Immun 1981; 32:74-79.

36. Jones DM, Davis P: Upper respiratory tract and aural flora of saturation divers. J Clin Pathol 1978; 31:721-723.

37. Aly R, Shirley C, Cunico B, Maibach HI: Effect of prolonged occlusion on the microbial flora, pH carbon diazide and transepidermal water loss on human skin. J Invest Dermatol 1978; 71:378-381.

38. Lee A: Normal flora of animal intestinal surfaces. *In* Adsorption of Microorganisms to Surfaces. Ed. by G. Britton and K.C. Marshall. John Wiley & Sons, New York, 1980. pp 145-173.

39. Mackowiak PA: Clinical uses of microorganisms and their products. Am J Med 1979; 67:293-306.

40. Thadepalli H, Chan WH, Maidman JE, Davidson EC Jr: Microflora of the cervix during normal labor and the puerperium. J Infect Dis 1978; 137:568-572.

41. Holdeman LV, Good IJ, Moore WEC: Human fecal flora: variations in bacterial composition within individuals and a possible effect of emotional stress. Apple Environ Microbiol 1976; 31:359-375.

42. Savage DC: Colonization by and survival of pathogenic bacteria on intestinal mucosal surfaces. *In* Adsorption of Microorganisms to Surfaces. Ed. by G. Britton and K.C. Marshall. John Wiley & Sons, New York, 1980. pp 175-251.

43. Marshall KC, Britton G: Microbial adhesion in perspective. *In* Adsorption of Microorganisms to Surfaces. Ed. by G. Britton and K.C. Marshall. John Wiley & Sons, New York, 1980. pp 1-5.

44. Gibbons RJ, van Houte J: Bacterial adherence in oral microbial ecology. Ann Rev Microbiol 1975; 29:19-44.

45a. Fuller R: Ecological studies on the Lactobacillus flora associated with the crop epithelium of the fowl. J Appl Bacteriol 1973; 36: 131-139.

45b. Whittenberger CL. Beaman AJ. Lee LN. McCabe RM. Donkersloot JA: Possible role of *Streptococcus salivarius* glucosyltransferase in adherence of *Veillonella* to smooth surfaces. *In* Microbiology-1977. Ed. by D. Schlessinger. American Society for Microbiology, Washington, D.C., 1979. pp 417-421.

46a. Suegara N, Morotomi M, Watanabe T, Kawai Y, Mutai M: Behavior of microflora in the rat stomach: adhesion of Lactobacilli to the keratinized epithelial cells of the rat stomach in vitro. Infect Immun 1975; 12:173-179.

46b. Beachey EH: Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J Infect Dis 1981; 143:325-345.

47. Williams RC, Gibbons RJ: Inhibition of bacterial adherence by secretory immunoglobulin A: A mechanism of antigen disposal. Science 1972; 171:697-699.

48. Williams RC, Gibbons RJ: Inhibition of streptococcal attachment to receptors on human buccal epithelial cells by antigenically similar salivary glycoproteins. Infect Immun 1975; 11:711-718.

49. Iacono VJ, Taubman MA, Smith DJ, Garant PR, Pollock JJ: *In* Immunological Aspects of Dental Caries, Ed. by W.H. Bowen, R.J. Genco, and T.C. O'Brien. Information Retrieval Service, Inc., Washington, D.C., 1976. p 75.

50. Bullen JJ, Rogers HJ, Griffiths E: Bacterial iron metabolism in infection and immunity. *In* Microbial Iron Metabolism - A Comprehensive Treatise. Ed. by J.B. Neilands. Academic Press, New York, 1974. p 518.

51. Binder HJ, Filburn B, Floch M: Bile acid inhibition of intestinal anaerobic organisms. Am J Clin Nutr 1975; 28:119-125.

52. Laqueur GL: Carcinogenic effects of cycad meal and cycasin, methylazosymethanol glycoside in rats and effects of cycasin in germfree rats. Fed Proc 1964; 23:1386-1387.

53. Spatz M, Smith DWE, McDaniel EG, Laqueur GL: Role of intestinal microorganisms in determining cycasin toxicity. Proc Soc Exp Biol Med 1967; 124:691-697.

54. Walker R: The influence of gut micro-organisms on the metabolism of drugs and food additives. Proc Nutr Soc 1973; 32:73-78.

55. Hawksworth G, Hill MJ: The formation of nitrosamines by human intestinal bacterial. Biochem J 1971; 122:28P.

56. Klein RS, Reeco RA, Catalano MT, et al: Association of Streptococcus bovis with carcinoma of the colon. N Engl J Med 1977; 297:800.

57. Alpern RJ, Dowell VR Jr: Clostridium septicum infections and malignancy. JAMA 1969; 209:385.

58. Black PH, Kunz LJ, Swartz MN: Salmonellosis - review of some unusual aspects. N Engl J Med 1970; 262:811; 864; 921.

59. Chang RS, Lewis JP, Renolds RD, et al: Oropharyngeal excretion of Epstein-Barr virus by patients with lymphoproliferative disorders and by recipients of renal homografts. Ann Intern Med 1978; 83:34.

60. Backus BT, Affronti LF: Tumor-associated bacteria capable of producing a human choriogonadotropin-like substance. Infect Immun 1981; 32:1211-1215.

61. Gordon HA, Bruckner-Kardoss E, Wostmann BS: Aging in germfree mice: life tables and lesions observed at natural death. J Gerontol 1966; 21:380-387.

62. Walburg HE, Cosgrove GE: Aging in irradiated and unirradiated germfree ICR mice. Exp Gerontol 1967; 2:143-158.

63. Hungate RE: The Rumen and its Microbes. Academic Press, New York, 1966. pp 5-6; 442.

64. Denko CW, Grundy WE, Wheeler NC, Henderson CR, Berryman GH, Friedmann TE, Youmans JB: The excretion of B-complex vitamins by normal adults on a restricted intake. Arch Biochem 1946; 11:109-117.

65. Ford JE, Henry KM, Kon SK, Porter JWG, Thompson SY, Wilby FW: The effect of sulfonamides and of cooking or grinding the diet on the excretion of vitamins of the B-complex. Br J Nutrition 1953; 7:67-83.

66. Mickelsen O: Intestinal synthesis of vitamins in the nonruminant. Vitamins and Hormones 1956; 14:1-95.

67. Coates ME, Ford JE, Harrison GF: Intestinal synthesis of vitamins of the B-complex in chicks. Br J Nutr 1968; 22:493-500.

68. Linkswiler H, Reynolds MS: Urinary and fecal elimination of B_6 and 4-pyridoxic acid on three levels of intake. J Nutrition 1950; 41:523-532.

69. Neilson E, Skull GM, Peterson WH: Response of bacteria, yeast, and rats to peroxide-treated biotin. Intestinal synthesis of biotin in the rat.

70. Gardner J, Parsons HT, Peterson WH: Human utilization of biotin from various diets. Am J Med Sci 1946; 211:198-204.

71. Skeggs HR, Wright LD: Vitamin B complex studies with diets differing in the carbohydrate component. J Nutrition 1946; 32:375-386.

72. Denko CW, Grundy WE, Porter JW, Berryman GH, Friedemann TE, Youmans JB: The excretion of B-complex vitamins in the urine and feces of seven normal adults. Arch Biochem 1946; 10:33-40.

73. Najjar VA, Johns GA, Medairy GC, Fleischmann G, Holt LE Jr: The biosynthesis of riboflavin in man. J Am Med Assoc 1944; 126:357-358.

74. Obermeyer HG, Wurtz E, Emerson GA: Proc Soc Exptl Biol Med 1945; 59:300-302.

75. Greenberger NJ, Isselbacher KJ: Disorders of adsorption. *In* Harrison's Principles of Internal Medicine, Ninth edition. Ed. by K.J. Isselbacher, R.D. Adams, E. Braunwald, R.G. Petersdorf and J.D. Wilson. McGraw-Hill Book Col, New York, 1980. pp 1401.

76. Giordano C: Use of exogenous and endogenous urea for protein synthesis in normal and uremic subjects. J Lab Clin Med 1963; 62:231.

77. Richards P, Metcalfe A, Ward EE, Wrong OM, Houghton BJ: Utilization of ammonia nitrogen for protein synthesis in man, and the effect of protein restriction and uremia. Lancet 1967; 2: 845-849.

78. Mitch WE: Effects of intestinal flora on nitrogen metabolism in patients with chronic renal failure. Am J Clin Nutrition 1978; 31:1594-1600.

79. Eyssen HJ, Parmentier GE, Merteus JA: Sulfated bile acids in germ-free and conventional mice. Eur J Biochem 1976; 66:507-514.

80. Ukai M, Tomura A, Ito M: Cholesterol synthesis in germfree and conventional rats. J Nutr 1976; 106:1175-1183.

81. Noble WC: Skin as a microbial habitat. *In* Selected Topics in Clinical Bacteriology. Ed. by J. DeLouvois. Williams and Wilkins Co., Baltimore, 1976. pp 235-256.

82. Johanson WG. Blackstock R, Pierce AK, Sanford JP: The role of bacterial antagonism in pneumococcal colonization of the human pharynx. J Lab Clin Med 1970; 75:946-952.

83. Sanders E: Bacterial interference. I. Its occurrence among the respiratory tract flora and characterization of inhibition of group A streptococci by viridans streptococci. J Infect Dis 1969; 120:698-707.

84. Sanders WE Jr, Harrowe DJ: Bacterial interference: Effects of oral antibiotics on the normal throat flora and its ability to interfere with group A streptococci. Infect Immun 1976; 13:808-812.

85. Crowe CC, Sanders WE, Longley S: Bacterial interference. II. Role of the normal throat flora in prevention of colonization by group A streptococcus. J Infect Dis 1973; 128:527-532.

86. Sprunt K, Redman W: Evidence suggesting importance of role of interbacterial inhibition in maintaining balance of normal flora. Ann Intern Med 1968; 68:579-590.

87. Sprunt K, Leidy GA, Redman W: Prevention of bacterial overgrowth. J Infect Dis 1971; 123:1-10.

88. Selwyn S: Natural antibiosis among skin bacteria as a primary defence against infection. Br J Dermatol 1975; 93:487-493.

89. Blacklow NR, Dolin R, Fedson DS, et al: Acute infectious nonbacterial gastroenteritis: Etiology and pathogenesis. Ann Int Med 1972; 76:993-1008.

90. Morin A, Saheb SA, Bisaillon JG, Beaudet R, Sylvestre M: In vitro inhibition of *Neisseria gonorrhoeae* growth by strict anaerobes. Infect Immun 1980; 28:766-770.

91. Helstrom PB, Balish E: Effect of oral tetracycline, the microbial flora, and the athymic state on gastrointestinal colonization and infection of BALB/C mice with *Candida albicans*. Infect Immun 1979; 23:764-774.

92. Klebanoff SJ, Belding ME: Virucidal activity of H_2O_2-generating bacteria: requirement for peroxidase and a halide. J Infect Dis 1974; 129:345-348.

93. Liljemark WF, Gibbons RJ: Suppression of *Candida albicans* by human oral streptococci in gnotobiotic mice. Infect Immun 1973; 8:846-849.

94. Kuramitsu HK, Paul A: Role of bacterial interactions in the colonization of oral surfaces by *Actinomyces viscosus*. Infect Immun 1980; 29:83-90.

95. Berg RD, Owens WE: Inhibition of translocation of viable *Escherichia coli* from the gastrointestinal tract of mice by bacterial antagonism. Infect Immun 1979; 25:820-827.

96. Allison MJ, Maloy SE, Matson RR: Inactivation of *Clostridium botulinum* toxin by ruminal microbes from cattle and sheep. Appl Environmental Microbiol 1976; 32:685-688.

97. Scheffel JW, Kim YB: Role of environment in the development of "natural"hemagglutinins in Minnesota miniature swine. Infect Immun 1979; 26:202-210.

98. Jungi TW, McGregor DD: Impaired chemotactic responsiveness of macrophages from gnotobiotic rats. Infect Immun 1978; 19:553-561.

99. Tippestad A, Midtvedt T: Chemotaxis of polymorphonuclear leukocytes from germ-free rats and generation of chemostatic activity in germ-free rat sera. Clin Exp Immunol 1971; 8:639-646.

100. Quesenberry PJ, Bealmear P, Ryan M, Stohlman F Jr: Serum colony stimulating and inhibiting factors in irradiated germfree and conventional mice. Br J Haematol 1974; 28:531-539.

101. Gordon HA, Bruckner-Kardoss E, Staley TE, Wagner M, Wostman BS: Characteristics of the germfree rat. Acta Anat 1966; 64:367-389.

102. Miyakawa M: The lymphatic septum of germfree guinea pigs. Ann NY Acad Sci 1959; 78:221-236.

103. Abrams GD, Bishop JE: Germfree techniques in experimental pathology: a survey of morphologic changes in, and the research potential of, the germfree guinea pig. Univ Mich Bull 1961; 27:136-147.

104. Ito Y, Nisiyama Y, Shimokata K, Kimura Y, Nagata I, Kunii A: Interferon-producing capacity of germfree mice. Infect Immun 1976; 13: 332-336.

105. Sweet C, Smith H: Pathogenicity of influenza virus. Microbiol Rev 1980; 44:303-330.

106. Ducluzeau R, Ladire M, Callut C, Raibaud P, Abrams GD: Antagonistic effect of extremely oxygen-sensitive clostridia from the microflora of conventional mice and of *Escherichia coli* against *Shigella flexneri* in the digestive tract of gnotobiotic mice. Infect Immun 1977; 17:415-424.

107. Wells CL, Balish E: Immune response modulation by colonization of germfree rats with *Proprionibacterium acnes*. Infect Immun 1979; 26: 473-478.

108. Shedlofsky S, Freter R: Synergism between ecologic and immunologic control mechanisms of intestinal flora. J Infect Dis 1974; 129:296-303.

109. Darling CL, Hart GD: Antagonistic action of *Streptococcus salivarius* and *Streptococcus faecalis* to *Mycobacterium tuberculosis*. J Clin Microbiol 1976; 4:375-378.

110. Flesher AR, Kasper DL, Modern PA, Mason EO Jr: *Legionella pneumophila:* growth inhibition by human pharyngeal flora. J Infect Dis 1980; 142:313-317.

111. Tannock GW, Savage DC: Indigenous microorganisms prevent reduction in cecal size induced by *Salmonella typhimurium* in vaccinated gnotobiotic mice. Infect Immun 1976; 13:172-179.

112. Collins FM, Carter PB: Growth of salmonellae in orally infected germ free mice. Infect Immun 1978; 21:41-47.

113. Moberg LJ, Sugiyama H: Microbial ecological basis of infant botulism as studied with germfree mice. Infect Immun 1979; 25:653-657.

114. Saigh JH, Sanders CC, Sanders WE Jr: Inhibition of *Neisseria gonorrheae* by aerobic and facultatively anaerobic components of the endocervical flora: evidence for a protective effect against infection. Infect Immun 1978; 19:704-710.

115. Ueda K, Yamagaki S, Someya S: Hyporeactivity to tuberculin in germfree mice. J Reticuloendothel Soc 1975; 18:107-117.

116. Zachar Z, Savage DC: Microbial interference and colonization of the murine gastrointestinal tract by *Listeria monocytogenes*. Infect Immun 1979; 23:168-174.

117. Beaman BL, Gershwin ME, Scates SS, Ohsugi Y: Immunobiology of germfree mice infected with *Nocardia asteroides*. Infect Immun 1980; 29: 733-743.

118. Pollard M, Chang CF, Srivastava KK: The role of microflora in development of graft-versus-host disease. Transplant Proc 1976; 8: 533-536.

119. Thomsen AR, Volkert M, Marker O: The timing of the immune response in relation to virus growth determines the outcome of the LCM infection. Acta Path Microbiol Scand 1979; 87C:47-54.

120. Szeri I, Anderlik P, Banos Z, Rádnai B: Decreased cellular immune response of germfree mice. Acta Microbiol Acad Sci Hung 1976; 23: 231-234.

121. Pollard M: The use of germfree animals in virus research. Progr Med Virol 1965; 7:362-376.

122. Jensen SB, Mergenhogen SE, Fitzgerald RJ, Jordan HV: Susceptibility of conventional and germfree mice to lethal effects of endotoxin. Proc Soc Exp Biol Med 1963; 113:710-714.

123. Thompson GE: Control of intestinal flora in animals and humans: implications for toxicology and health. J Environ Path Toxicol 1977; 113-123.

124. Kiyono H, McGhee JR, Michalek SM: Lipopolysaccharide regulation of the immune response: comparison of responses to LPS in germfree, *Escherichia coli*-monoassociated and conventional mice. J Immunol 1980; 124:36-41.

125. Wittner M, Rosenbaum RM: Role of bacteria in modifying virulence of *Entamoeba histolytica*. Studies of ameba from axenic cultures. Am J Trop Med Hyg 1970; 19:755-761.

126. Prizont R, Reed WP: Possible role of colonic content in the mucosal association of pathogenic shigella. Infect Immun 1980; 29:1197-1199.

127. Maier BR, Onderdonk AB, Baskett RC, Hentges DJ: Shigella, indigenous flora interactions in mice. Am J Clin Nutr 1972; 25:1433-1440.

128. Wilson R, Sjodin K, Bealmear M: The absence of wasting in thymectomized germfree (axenic) mice. Proc Soc Exp Biol Med 1964; 117:237-239.

129. Haltalin KC, Nelson JD: Coliform septicemia complicating shigellosis in children. JAMA 1965; 192:97-99.

130. Scowden EB, Schaffner W, Stone WJ: Overwhelming strongyloidiasis. An unappreciated opportunistic infection. Medicine 1978; 57:527-544.

131. Rees MK: The case of the man who felt too well. Ann Int Med 1981; 94:133.

132. Aly R, Mailbach HI, Shinefield HR, Mandel A, Strauss WG: Bacterial interference among strains of *Staphylococcus aureus* in man. J Infect Dis 1974; 129:720-724.

133. Drutz DJ, van Way MH, Schaffner W, et al: Bacterial interference in the therapy of recurrent staphylococcal infections. N Engl J Med 1966; 275:1161.

134. CDC: Follow-up on multiple-antibiotic-resistant pneumococci - South Africa. Center for Disease Control MMWR 1978; 27:1-7.

135. Wickman K: Studies of bacterial interference in experimentally produced burns in guinea pigs. Acta Path Microbiol Scand 1970; 78B:15-28.

136. van der Waaij D, Berhuis-deVries JM, Lekkerkerk-van der Wees JEC: Colonization resistance of the digestive tract and the spread of bacteria to the lymphatic organs in mice. J Hyg (Camb) 1972; 70: 335-342.

137. de Vries-Hospers HG, Sleijfer DT, Mulder NH, van der Waaij D, Nieweg HO, van Saene HKF: Bacteriological aspects of selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients. Antimicrob Agents Chemother 1981; 19:813-820.

138. Gurwith MJ, Brunton JL, Lank BA, Harding GKM, Ronald AR: A prospective controlled investigation of prophylactic trimethoprim/sulfamethoxazole in hospitalized granulocytopenic patients. Am J Med 1979; 66:248-256.

139. Sleijfer DT, Mulder NH, de Vries-Hospers HG, Fidler V, Nieweg HO, van der Waaij D, van Saene KF: Infection prevention in granulocytopenic patients by selective decontamination of the digestive tract. Eur J Cancer 1980; 16:859-869.

140. Wade JC, Schimpff SC, Hargadon MT, Fortner CL, Young VM, Wiernik PH: A comparison of trimethprim-sulfamethoxazole plus nystatin with gentamicin plus nystatin in prevention of infections in acute leukemia. N Engl J Med 1981; 304:1057-1062.

APPENDIX A

MICROORGANISMS COMMONLY FOUND ON HEALTHY HUMAN BODY SURFACES*

Species or group	Skin		Conjunctiva	Upper respiratory tract			Mouth			Lower intestine			Genitourinary tract		
	General	External auditory canal		Nasal passages	Nasopharynx	Oropharynx-tonsil	Predentulous	Saliva-tooth surfaces	Gingival crevice	Upper levels	Feces: Infant	Feces: Adult	External genitalia	Anterior urethra	Vagina
A. Gram-positive cocci:															
Coagulase-negative staphylococci	**88–100** 2–6/cm²	**27–100**	**37–94**	**90**	++	± †	+	**75–100** 1–4/ml	-		**31–89** ‡	+ 2–4/Gm	+	++	**34–78**
Coagulase-positive staphylococci	**5–24** §	**12–20**	**0–10**	**22–55** **9–100**ᵃ	++	**36–42**		+ (16–35) ¶		+	**10–92**ᵃ	++ §		±	**5–15**
Anaerobic micrococci	±					+		+		+					+
Str. mitis and undifferentiated α and γ streptococci	±0	**0.2–23**	**0.9–1**	±	**24–99**	**100**	++	**100** 0–8/ml	**100** 6/mg	+	**14–32**	+	+	±	**10–21** **47–80**ᵇ
Str. hominis (*salivarius*)					+	++	**100**	**100** 7/ml	++	+	**0–6**	+			
Enterococci or group D streptococci						+ᶜ	+	**4–22**ᵈ		+	**87** 6–9/Gm	**100** 3–8/Gm	+	**2–10**ᵉ	+
Str. pyogenes (usually group A unless noted)	**0–4**		**0.3–2.5**	**0.1–6**	**0–9**	**5–56**ᶠ		**12–68**/ 3–6/ml/			**0.7–13**ᵍ	**16**ʰ			**4.4–10**ⁱ
Anaerobic streptococci						+	+	++	++ 6/mg			+	+		**12–59**
D. pneumoniae		+	**0–6**	**0–17**/	**0–60**	**5–71**	+	**26**							±
B. Gram-negative cocci:															
N. catarrhalis and other spp.			**2.3**	**12**	**10–97**	**98**		**95–100** 5–7/ml	+					+	+
N. meningitidis					+										
V. alcalescens					+	+	+	**100** 6–8/ml	+						+
C. Gram-positive bacilli:															
Lactobacilli							+	**95**ʰ 0–6/ml		+		**50**ʰ –7/Gm			**49–73**
Aerobic corynebacteria	**53** 3/cm²	**56**	**3–53**	++	+	+		**59**			**10–21**	**6**	+	+	**44–74**
C. acnes	**45–100** 6/cm²			+			±								
Mycobacteria	+			±	±	±						+	+	±	
Cl. perfringens, other spp.								±		++	**13–19**	**25–35**			
Cl. tetani												1–35			
Actinomyces bifidus							+	+			**18–60**ᵍ 90ᵐ				**26–72**
A. israelii						+		+	+		7–11/Gmᵐ				
Leptotrichia buccalis								++ 0–3/ml	++						±
L. dentium								+							

±0, rare: ±, irregular or uncertain (may be only pathologic): +, common; ++, prominent.

* Boldface values (e.g., **31–59**) = range of incidence in per cent, rounded, in different surveys. Values given with units (e.g., 3–6/ml) = range of concentrations expressed as log₁₀: 0 = >5 × 10⁰ <5 × 10¹.

† + in newborn; more common in school children than in adults.
‡ Predominant first day; decreasing during first month.
§ Associated with nasal carriage.
¶ Per cent of strains isolated.
ᵃ In infants and children; highest in hospital nursery infants.
ᵇ Children.
ᶜ In newborn.
ᵈ More common below age 20.
ᵉ More common in school children; see text.
/ Associated with presence in throat.
ᵍ "Hemolytic"; Lancefield group not given.
ʰ Groups B, C, F, and G; no A.
ⁱ Children; not group A.
ʲ More common in children.
ᵏ Especially in dental caries.
ˡ Bottle-fed infants.
ᵐ Breast-fed infants.

APPENDIX A (cont'd)

MICROORGANISMS COMMONLY FOUND ON HEALTHY HUMAN BODY SURFACES

Species or group	Skin			Conjunctiva	Upper respiratory tract			Mouth			Lower intestine			Genitourinary tract		
												Feces				
	General	Feet	External auditory canal		Nasal passages	Nasopharynx	Oropharynx–tonsil	Predentulous	Saliva–tooth surfaces	Gingival crevice	Upper levels	Infant	Adult	External genitalia	Anterior urethra	Vagina
D. Aerobic Gram-negative bacilli:																
Undifferentiated "coliforms"			4–8	2.1		21–23	+	+	65 0–3/ml			86–100	100 7–9/Gm	+	+	17–36[a] 3–12
Escherichia coli						±			4.2		++	67–99	100		+	+
"Intermediates"			0.1–0.4				±		31			28–52	+			6[a]
Klebsiella aerogenes			+	0.1		±	+		52		++	19–48	32–48		+	
Proteus mirabilis, other spp.			0.2–1	0.4			+	+				48	5–53 –6/Gm		+	+[a]
Pseudomonas aeruginosa			0–1.3				±					+	2–11		+	
Alcaligenes faecalis			1.1–1.8	±								0–2.1	+		±	
Vibrio alcaligenes									±				±			
Moraxella lacunata				±	+	±								±	±	
Mima polymorpha				±		±							±			+
M. vaginicola				±		±							±		5	+
Haemophilus influenzae				0.4–25	12	43–90	3–97		25–100							+[a]
H. parainfluenzae						5	20		25							
Hemolytic hemophili						+	77		+							
H. aegyptius				+												
H. vaginalis															±	+
E. Anaerobic Gram-negative bacilli, vibrios, spirilla, and spirochetes; PPLO, etc.:																
Bacteroides fragilis, other spp.						+	+		+	+	+		100? 7–10/Gm	+		±
B. nigrescens									+	+			+	+		
										6/mg						
Fusobacterium fusiforme							+	+	14–68	+			+	+		
									3–5/ml	4/mg						
P. gingivalis										+			+			±
Spirillum sputigenum						+		±	+	+			+?	+		
Vibrio sputorum						+	+	±	+	+						+
Treponema dentium and *Borrelia refringens*							+			60–85 6/mg	+	18[a]	28	+		
PPLO, etc.							+		+				+	+	±	+
F. Fungi:																
Candida albicans	±	±	+				6–28		6–49 0–5/ml		+	+	14–31 0–4/Gm	+	±	28–46[a]
Other candidas	1–15	+	+				3–10		1–4				1–12			4–6
Torulopsis glabrata							±		+				+	+	±	+
Pityrosporum ovale	100[c]															
P. orbiculare	++															
Dermatophytes		2–41														
G. Protozoa:																
Entamoeba gingivalis							+			0–72.6						
Ent. coli													8.0–32.1[c]			
Endolimax nana													9.3–16.0			
Dientamoeba fragilis													0.2–5.9			
Iodamoeba bütschlii													1.4–6.0			
Trichomonas tenax							+			4.0–33.3						
T. hominis													0.3–4.1			
T. vaginalis															+	9.9–24.9
Giardia lamblia													2.9–14.7 17.6[a]			
Chilomastix mesnili													0.4–6.1			
Enteromonas hominis													0.1–3.2			
Retortomonas intestinalis													0.1–1.3			

[a] Children.
[b] After the second week.
[c] *C. stellatoidea*.
[d] Especially scalp and nasal folds; also other skin areas.
[e] Values in this column are for North America and western Europe; see Chap. 3.