BACTERIOLOGY OF THE INTESTINAL TRACT IN CERTAIN CHRONIC DISEASES

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I. Sporulating Anaerobes, Aciduric Organisms and Colon Group.

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In addition to a neurogenic mechanism the ill-defined syndrome of intestinal "intoxication", colitis or irritable colon which accompanies many chronic diseases has been explained on the basis of allergic, infectious and toxic factors. Although this suggests that bacteria may play an important part, bacteriologic studies have been of little diagnostic value. In this series we shall try to analyse the possible significance and mode of action of certain groups of bacteria. Because of the voluminous literature only those papers which have a particular bearing on the subject will be discussed.

General Considerations

There are several possible reasons why, in this group of diseases, usual bacteriologic examinations of feces may not supply diagnostic information. (a) The results are influenced by the method of collecting and handling the specimen, and the length of time it has been in the intestinal tract. (b) The rapid oxidation which takes place on contact with air is likely to harm anaerobes and micro-aerophiles. (c) Toxigenic bacteria in the bowel may not necessarily produce toxic symptoms. (d) Organisms responsible for the toxic symptoms may not be among those sought by the bacteriologist. They may be present in some other part of the body rather than in the gastrointestinal tract and affect it by an indirect mechanism. (e) The symptoms may be caused by anatomic abnormalities, adhesions, malignancy, local inflammatory processes, parasitic infestations, or by dysfunction of another organ.

Previous studies of intestinal bacteriology have followed two main objectives: (1) Classification of the flora as a whole, on the assumption that intestinal "intoxication" involves a change from "normal" to "putrefactive", "fermentative" or "mixed" types; and (2) the enumeration, isolation and biochemical differentiation of the individual bacteria. The first type of study has been discarded by most workers, and the second has not produced the information expected of it. The mere presence of an organism in the gastrointestinal tract even though it may have unusual properties has little significance. Differential tests, such as carbohydrate fermentation reactions, have given inconstant, or insignificant results, and serologic classification is still in its infancy. Demonstration of pathogenic or toxic properties should be more significant, particularly if they could be shown to affect the gastrointestinal tract.

In an effort to prove a host-parasite relationship bacteria usually have been suspected on the basis of frequency, resemblance to recognized pathogens, dermonecrotic properties, agglutinability, ability to fix com-

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plement or to grow in the patient's blood, etc. These criteria have been discarded by most workers in the diseases under discussion.

Elucidation of this problem requires a new approach or the application of new methods. We believe that we have been able to throw new light on the subject, and, therefore, will discuss this work in this series of papers.

Before describing these researches it is first necessary to show that the following bacterial groups, which have frequently been thought to be the cause of "intestinal intoxication", may have little etiologic significance. This will clear the way for the hypotheses to be presented in the next paper.

Sporulating Anaerobes

Because putrefaction is one of the outstanding characteristics of certain sporulating anaerobes it is natural that they should be suspected of playing an important role in this group of diseases where foul stools are noted frequently. Attention has been focused mainly upon *Clostridium sporogenes* although there are other important species.

The active fermentative properties of *Clostridium Welchii* have made this organism the object of intense study also.

The methods used for determining these groups are open to serious objections. For example, it is customary to heat the sample to eliminate non-sporulating bacteria, it being assumed that the number of surviving spores is proportional to the total number of anaerobes. This is far from true, as was shown by Chapman[1] for *Clostridium Welchii.* Gunnison, Althausen and Marshall[2] showed that there is no relationship between quantitative tests of *Clostridium Welchii* using the tube-dilution method and counts obtained by direct plating. They concluded that stormy fermentation of milk is not a reliable index of the presence of *Clostridium Welchii.* Even with the direct plating methods of Chapman[1] and Gunnison et al[2] it is probable that exposure of feces to oxygen-containing atmosphere, even for a few moments, will kill a large proportion of vegetative forms of these anaerobes. Thus it is difficult to make an accurate count of the number of sporulating anaerobes.

Even though it were possible to overcome these objections, the evidence suggests that they are of secondary importance.[3]

Gunnison et al[2] and Owles[3] found no relation between *Clostridium Welchii* and intestinal carbohydrate intolerance although this is one disease in which this organism, because of its strong fermentative properties, would be expected to have etiologic significance.

There must be a considerable degree of intestinal immunity to this group otherwise gas gangrene of intestinal origin would be of common occurrence. Therefore, pathogenic effects produced by these organisms would appear to depend upon previous damage to the intestinal wall. The secondary value of *Clostridium Welchii* is also suggested by the work of Chiazarro[4] who showed that the association of this organism with a staphylococcus in certain intraocular infections produced different symptoms dependent upon the pathogenicity of the staphylococcus. When it was quite proteolytic, hemotoxic and leucotoxic it reinforced the toxicity of *Clostridium Welchii,* while a staphylococcus having none of these properties had a negligible effect on the virulence of *Clostridium Welchii.*

These facts support our contention that sporulating anaerobes probably have a secondary significance in intestinal complaints associated with chronic disease.

The Aciduric (Acidophilic) Group

Interest in this group in older children and adults centers around *Lactobacillus acidophilus*. There are two difficulties in establishing a relationship between absence of this organism and intestinal "intoxication". Kopeloff, Blackman and McGinn[5] pointed out that the accurate quantitative estimation of *Lactobacillus acidophilus* in the human intestinal tract offered well nigh insuperable obstacles due to the method of collecting and diluting the specimen, the medium selected for plating, and the close resemblance of smooth or Y type colonies of *Lactobacillus acidophilus* to other common intestinal bacteria such as enterococci, members of the colon-aerogenes group, etc.

The second difficulty, demonstrated by Gunnison et al[2] is that proteolytic organisms may occur in high frequency and in considerable numbers without regard to the presence of aciduric types. The presence of aciduric organisms in feces does not necessarily influence the stormy fermentation of milk test.[2,6] Acidophilus milk has little or no effect on the colon bacillus group[6] but it decreases the number of streptococci.[6]

On the basis of these findings it is evident that, except for a possible antagonistic effect on streptococci, aciduric bacteria do not have a constant inverse relationship to "putrefactive-- organisms, and that intestinal "intoxication" would appear to depend upon other factors than absence of this group.

Aerobacter and Friedlander Groups

Baehr, Shwartzman and Greenspan[7] reviewed the literature on the pathogenesis of Friedlander bacillus infections. They stated that the organism enters the body usually through the intestinal tract from whence it may enter the systemic or portal circulation and be excreted by the kidneys or the liver. Thus excretory infections are apt to occur if there is stasis of urine or bile. Primary infections of the lungs and the upper respiratory tract are clinical rarities in comparison with the frequency of infections of the abdominal viscera.

For simplicity in analysing results of studies to be reported later in this paper, intermediates will be grouped with Aerobacter.

The differentiation of Friedlander bacilli, often thought of as pathogens, from other closely related bacteria such as Aerobacter, which are usually considered non-pathogenic, is not as sharp as is commonly considered. The stringy characteristic of colonies is not sufficient for accurate differentiation. The mucoid type of growth of Aerobacter and Friedlander is shared also by some B. coli strains[8] and intermediates. Rough strains of Friedlander bacilli do not produce capsules.[9] Aerobacter and Friedlander are closely related serologically,[10,11,12] and Julianelle, who has made several contributions to the subject, has admitted[13] that difficulties in differentiation have been increased by recent studies.

If Friedlander is so closely related to Aerobacter that refined technics are required to differentiate them, and if Aerobacter represents a subgroup in the colon group rather than a specific division[14] as suggested by the occurrence of numerous intermediate types and the conversion of B.

coli into atypical variants,[15] then until a better differential method has been worked out, the significance of Friedlander and Aerobacter groups, except in acute infections, would seem to be similar to that of the colon group in general.

Because of difficulties in demonstrating pathogenicity or toxicity of members of the Friedlander-Aerogenes group it is difficult to determine the exact significance of any particular strain isolated from feces.

Paracolon Bacilli ("Non-Lactose" Fermenters)

Most non-lactose or slow lactose fermenting members of the colon group which are not specific pathogens are now recognized as non-pathogenic degraded colon bacilli and are often referred to as "paracolon" bacilli. Some types, such as *"B. coli mutabile"*, produce both fermenting and non-fermenting daughter colonies, even from single-cell isolations.[15,17] Hershey and Bronfenbrenner[18] produced lactose stable variants.

The classification of this group is very unsatisfactory.[19] Blood cultures and agglutination tests give negative results.[20] Mackie[21] found that complement fixing properties were similar to those of colon bacilli. Most of them behave like Salmonella in all respects except agglutination.[17] Kriebel[18] concluded that they resembled more closely the colon-paratyphoid groups, and later[17] considered them transitional stages between non-pathogenic colon bacilli and pathogenic Salmonella.

They can be considered as Aerogenes,[22] *B. coli*,[17,22,42] or intermediates[42] in which lactose fermenting power is partially or completely lost. Parr[22] showed that Aerobacter is much more easily degraded than *B. coli*. Our own results support this contention. For example, when pathogenic streptococci were not found in feces, the ratio of the average number of Aerobacter to the average number of paracoli was 11.3 : 1 and 96.7 : 1, depending upon whether or not pathogenic staphylococci were found in the upper respiratory tract. However, when pathogenic streptococci were found in feces, the average ratios were 1 : 7.9 and 1 : 5.8 (table 1) indicating that, under the influence of pathogenic streptococci and to a slight extent pathogenic staphylococci, there is degradation of Aerobacter with a consequent reduction in its proportion to paracoli (approximately 10 and 100 to 1 in the absence, but approximately 1 to 6 in the presence of pathogenic cocci. Under similar conditions *B. coli* is only slightly transformed to paracolon types the average ratio remaining almost constant (table I).

TABLE I

Ratios of Escherichia, Aerobacter, and paracolon strains to total colon bacilli in feces. Considered in relation to the presence of pathogenic streptococci in the feces and pathogenic staphylococci in the nose and throat.

Pathogenic streptococci in feces	Pathogenic staphylococci in nose & throat	R A T I O S			
		Coli: Total.	Aerogenes: Total	Paracoli: Total	Aerogenes: Paracoli
0	0	1 : 1.4	1 : 3.9	1 : 384	96.7 : 1
0	+	1 : 1.3	1 : 4.7	1 : 53	11.3 : 1
+	0	1 : 1.6	1 : 17.5	1 : 3.0	1 : 5.8
+	+	1 : 2.7	1 : 14.1	1 : 1.8	1 : 7.9

Some of the difficulty in interpreting results of lactose fermentation tests is due to unstandardized experimental conditions[23]. Many of these bacteria will ferment lactose if given a long enough incubation period. In

Kriebel's[17] series 55 per cent finally fermented lactose. Hershey and Bronfenbrenner[18] induced variation in both directions.

Not all strains produce gas in dextrose, which is characteristic of the *B. coli,* Aerobacter and Salmonella groups. Some strains produce acid only in this sugar, making them resemble dysentery bacilli. Denison and deHoll[24] found bacteria giving typical fermentation reactions of dysentery bacilli in 18 per cent of their cases. They were not agglutinable, neither did they absorb agglutinins for dysentery bacilli.

B. Morgani, or more precisely *B. Morgani I,* is closely related to, if not identical with this group. It does not seem to be a sharply defined species. Jordan et al[23] cited authorities who thought that it was related to *B. dysenteriae,* Salmonella, and Escherichia but concluded that it was more closely related to the colon group (slow lactose fermenters). Rauss[25] showed that it had certain characteristics in common with Proteus. Kahn[26] stated that *B. Morgani I* may assume pathogenic properties in a small number of cases but that the mere recovery of this organism in a diarrheal stool is not proof of its pathogenicity.

The significance of paracolon strains is further complicated by the possibility that specific pathogens may become degraded into similar types. Gilbert and Coleman[27] produced evidence that *B. alkalescens* (Andrewes) may be a variant *B. typhosus.* Larkum[28] and Grinnell[29, 30] showed that certain variants of typhoid bacilli are deficient in pathogenic power and Poston[31] described atypical typhoid fever due to atypical strains. Warren and Iredale[32] quoted Ledingham and Penfold and Bruce White that anaerogenic paratyphoid strains are of common occurence. While these strains did not produce gas in any media they were serologically similar to other paratyphoid B strains.

These observations illustrate the difficulty of classifying or of attaching etiologic importance to lactose degraded forms in chronic intestinal disease. However, they suggest that, while they may have little significance as pathogens except when they give specific reactions (typhoid, paratyphoid and dysentery), their presence in feces may indicate degeneration of colon bacilli brought about by pathogenic streptococci, and to a certain extent by pathogenic staphylococci.

Pathogenicity of B. Coli

We have not attempted to classify colon bacillus strains into "species" or "varieties" because our experience confirmed the conclusion of Dudgeon, Wordley and Bawtree[16], since held by most other workers in this field, that fermentation reactions are of little value in grouping them. In this paper we use the term "colon bacillus" to signify any member of the coli-aerogenes group as a whole, and *"B. coli"* to designate any member of the Escherichia group.

Apart from the ability of some strains to invade organs such as the kidney and bladder, *B. coli* is thought by some to possess toxic properties which act on the body generally without the organism penetrating farther than the superficial membranes. Plantenga[33] described experiments on guinea pigs which tended to prove that the colitoxin was a compound one, composed in part of an aggressin, a product of the growth of the colon bacillus. He found that, when acting alone, the fractions were non-toxic. Vincent[34] claimed that *B. coli* produced two toxins: (1) A neurotropic

exotoxin, destroyed at 72° C; and (2) an enterotropic endotoxin, destroyed at 96-98° C. Gioja[35] was unable to demonstrate exo- and endo-toxins but found that heat killed broth cultures had a variable (usually low) degree of toxicity for rabbits. We have been unable to produce toxic effects in mice with filtrates of *B. coli* cultures but killed whole cultures of these strains were pathogenic for rabbits and mice when injected intraperitoneally in large doses or intracerebrally into mice in smaller doses. There was only a slight difference between the toxic power of different strains, which agrees with Gioja's[35] findings, and is in harmony with our results of intradermal tests on human beings.

Numerous animal inoculation experiments have been made in an attempt to differentiate *B. coli* strains on the basis of "virulence". The irregular results obtained when only one animal was used for each test eliminate many of the results reported in the literature. The unsatisfactory state of affairs was reflected in the proposal of the coordinating committee of standard methods of the American Public Health Association[36] which suggested the appointment of a committee to investigate this matter because of increasing reports on variable reactions of laboratory animals to experimental infection.

Some workers have stressed the hemolytic property of certain *B. coli* strains but, while they appear to be slightly more pathogenic than non-hemolytic strains, there is only meager evidence connecting them with intestinal "intoxication".

Using the Falk capillary cell to measure the electrophoretic migration velocity (P.D.) of the colon bacillus group[37], Lieb and Chapman[38] showed a relationship between the P.D. of colon bacilli and the complement fixing power of the blood serum of the person from whom the strains had been isolated. Joffe, Hitchcock and Mudd[39] showed that smooth strains of certain intestinal pathogens carry a very low surface charge while rough variants have a high charge. The strain which we have used for "implantations" has an extremely high charge (as measured by its P.D.) and has never produced toxic effects when injected per rectum in doses of 1 to 10 trillions. These results suggest that there may be some relationship between electrophoretic migration velocity and pathogenicity. However, experiments on thousands of strains of colon bacilli in this laboratory have failed to show sufficient specificity of the test to be of practical value.

Attempts to establish pathogenicity for the particular patient have centered around intradermal tests. Because of the significance frequently attached to these tests and the extent of misunderstandings about them, the subject will be discussed in a separate monograph. For the present the reader is referred to the excellent summaries of Steinberg and Wiltsie[40] and Short, Dienes and Bauer[41]. The former showed that normal persons give a positive reaction to intradermal injections of *B. coli* toxic filtrate and washed organisms and concluded that under the conditions of their experiments the skin reaction for the determination of the presence of colon bacillus infection was of uncertain value. The latter authors pointed out the necessity of using several subjects as controls and explained variations in the skin reactions by differing irritability of the patients' skins, natural toxicity of the bacterial species, or possibly a sensitization to certain bacterial groups.

It is concluded that there is not enough evidence of differences in pathogenicity of *B. coli* strains to warrant considering them as factors in

chronic diseases.

Thus, it has been shown that sporulating anaerobes, degraded colon bacilli, Aerobacter, Friedlander bacilli, *B. coli*, and aciduric types do not have an easily demonstrable direct or inverse relationship to intestinal complaints accompanying chronic diseases.

Conclusions

An analysis of existing knowledge regarding the possible relationship between the presence of sporulating anaerobes, the colon group, Klebsiella, and aciduric organisms and symptoms attributed to the intestinal tract in certain chronic diseases indicates that the evidence is insufficient to establish an etiologic relationship.

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