Human Gut Microecology

THE PARTICIPANTS of the UCLA Interdepartmental Clinical Case Conference are to be complimented on the timely and accurate information presented elsewhere in these pages concerning the clinical aspects of gastrointestinal bacteriology.[1] During the past decade great interest has been aroused in this field. Pioneering work on the autochronous flora was performed by René Dubois and colleagues.[2] In addition, great advances in anaerobic technology were first stimulated by Hungate and, most recently, by the tedious taxonomic studies performed at the Virginia Polytechnic Institute Anaerobe Laboratory under the direction of Drs. Moore and Holdeman.[3] Increasing interest in the importance of the flora to gastrointestinal physiology was demonstrated in several recent symposia.[4,5]

The greatest limitation in gathering information on the gut flora has been the difficulty in developing simple bacteriologic methods for obtaining reproducible results, and the rapidity of changes in bacteriologic techniques. A clear example of this is my publication, in 1968, of what I thought to be a standardization of methods for identification and culture of the intestinal microflora in man. It grew obsolete within four years. Another example of the difficulty in correlating clinical material is demonstrated in this most recent review from UCLA. Studies analyzed on subphrenic abcesses were performed without detailed anaerobic methods, whereas the studies analyzed on aspiration pneumonia were done with a gas pack anaerobic technique. However, analysis of stools to evaluate diet and cancer epidemiologic factors was done with the most recent strict anaerobic techniques as defined by Finegold to be accomplished by either a glove box or roll tube. At present, either some modification of the techniques defined by Finegold or those defined by Moore must be used to obtain useful information. When these methods are used, studies become so laborious it requires a massive laboratory effort to perform any useful human research evaluations. Consequently, the entire research field becomes restricted because of the tedious technique. This point cannot be too strongly emphasized. Information will be limited until there is some technologic breakthrough which will yield methods that can be employed at less cost and be available to a wider range of investigators. However, the interest that has been aroused in this field and the rapid change in the techniques during the past three years do presage that the breakthrough is near. Better understanding of the clinical role of the gut microflora will evolve as studies turn up information about the factors that control normal and abnormal bacteria of the gut.

At birth the gut is relatively sterile except for occasional organisms that can be cultured from meconium. The colonization of the gut then varies, depending upon the type of diet the infant is fed. The feeding of human milk results in colonizing the gut differently than cows' milk or synthetic formulas. However, there have been no recent studies employing the above mentioned anaerobic techniques. Following colonization of the human gut, the so-called "adult type" microflora gradually develops as a regular diet is eaten. Most recent studies indicate that diet affects the quantitative and qualitative aspects of the microflora. But these studies are very limited. They have not been reproduced as yet, and, as is pointed out in the UCLA Conference, tedious identifications reveal a great number of unclassified organisms. The technologic limitation to identifying all of them leaves the statistical significance of the studies totally open to question. One cannot compare unidentifiable organisms. Using less fastidious anaerobic methods, Zubrzycki and Spaulding[6] did demonstrate a definite stability of the normal fecal flora in individual subjects. However, at the time their work was done, their techniques were very limited. As was indicated above, until techniques are simplified for identification of anaerobes, the exact influence of diet on the microflora will not be defined. Epidemiologic studies on high and low risk colon cancer and colon diverticular disease patients indicate that the diet has some relationship to the flora. Once again, the statistical significance of these flora variations is still open to question, due to the inability to completely qualitate bacteria.

Factors that appear to control normal bacterial populations as well as pathogenic bacterial growth within the lumen of the gut are of prime clinical

significance. Factors involved in controlling the microflora of the mouth are poorly understood. The mouth flora exhibit tremendous variation, depending on the host and the state of oral health. Also, little is known about conditions within the esophagus. Although bacteria have been noted within the esophageal mucosa during acute and chronic inflammatory disease, there have been no detailed quantitative or qualitative studies on the autochronous flora of the esophagus. When one reaches the stomach, evidence has clearly evolved to demonstrate that bacterial growth is related to the acid content and H^+ productivity of the gastric mucosa. At a pH of less than 2, the stomach is virtually free of bacterial growth. As the pH rises above 3, a rich flora can be recovered.[7] Duodenal and proximal jejunal bacterial studies also show an excellent correlation with gastric acid productivity. This indicates that the gastric acid barrier is effective in keeping down bacterial growth in the proximal small bowel. The reasons for control of the normal bacterial populations and of pathogenic bacteria become more complex as the bacterial flora develops in complexity in the ileum, and becomes very rich in the colon. Normal peristalsis and morphologic continuity of bowel are key factors which have been demonstrated to be important in controlling bacteria within the small bowel lumen. Stasis, obstruction, resection or a blind loop can cause bacterial proliferation higher in the small bowel than usual.

Biochemical factors reported in the past to have a role in controlling bacterial growth are mucus, lysozyme, colicin and fatty acids. Most recently, simple bile acids have been demonstrated *in vitro* to control intestinal bacterial growth.[8] Fiber in the diet has been postulated to possibly bind bacteria and control bacterial populations. Immunologic relationships between the host intestinal wall and the lumenal bacteria are of great interest. Persons deficient in immunoglobulin production have variations in their intestinal flora when compared with normal persons.[9] Most recent studies point out the mechanical activity existing between antigens and antibodies within the lumen. Of particular note is the work of Jones and Rutter. They have shown that the enteropathogenic effect of the K88 antigen of Escherichia coli can be decreased by neutralizing its adhesive properties.[10] Study of this type of physical effect on antigens should produce important results in understanding the control of both healthy bacterial populations and disease-producing organisms. Although the role of the majority of antibodies produced in the wall of the gut and their possible role in the lumen is not known at present, information in this field should prove of great clinical value in future years.

Finally of keen interest is the clinical significance of infections caused by multiple organisms, as is pointed out by Bartlett and Gorbach in the UCLA Specialty Conference here under discussion. There is no question that bacteria living together produce metabolic effects which either enhance or inhibit the growth of their colleagues. These particular phenomena have best been studied in the rumen of the cow, and most recently *in vitro* by Wolin and colleagues.[11] They add several organisms to a model *in vitro* ecosystem which includes continuous addition of growth nutrient factors, continuous study of metabolic by-products and repeated bacterial counts. Results of such studies reveal that there are definite interrelationships between multiple organisms. By varying the amount of nutrients, one can vary the type of bacterial growth. By varying the type of bacteria present, one can vary bacterial by-products such as fatty acids and possibly toxins. At present we have no model ecosystem of the human small or large intestine. Development of such a model could yield valuable information to help in the assessment of multiple infections in man, and the subsequent antibiotic or chemotherapeutic treatment.

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