SPECIAL ARTICLE

Frontiers in Inflammatory Bowel Disease

The Proceedings of a Conference Sponsored by the McReynolds Foundation

Part I.

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This is a two-part article. Part II will appear next month.

Outline

Part I

INTRODUCTION

On Mar. 25, 26, and 27, 1974, an invited Conference on Frontiers in Inflammatory Bowel Disease (IBD) was held in Houston, Texas, under the auspices of the McReynolds Foundation. The meeting was suggested by Mr. Bland McReynolds and its intent was to provide a forum for ideas on directions for future investigative efforts in this enigmatic field. Those attending were Drs. Roy Shorter (Mayo Clinic) (chairman), John Bartlett (UCLA), Alan Hofmann (Mayo Clinic), Henry Janowitz (Mount Sinai, New York), Fred Kern, Jr. (University of Colorado), Joseph Kirsner (University of Chicago), Sumner Kraft (University of Chicago), Al Mendeloff (Johns Hopkins), Sidney Phillips (Mayo Clinic), Paul Sherlock (Memorial Sloan-Kettering), John Singleton (University of

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Colorado), John Stobo (Mayo Clinic), Keith Taylor (Stanford), Walt Thayer, Jr., (Brown University), David Watson (University of California, Davis), and David Shephard (Mayo Clinic) (editor). Each participant had been requested to speak on a particular topic and also to moderate a period of discussion, except for Fred Kern, Jr., whose role was to present an overall critique. Discussion, however, was not limited to the intervals between formal presentations; indeed, the freedom that the participants felt in contributing to a free-ranging and spontaneous discussion was a decidedly constructive aspect of the Conference.

The purpose of this communication is to present an edited version of the proceedings.

EPIDEMIOLOGIC FRONTIERS

The first speaker, Mendeloff, presented some considerations for future epidemiologic studies and commenced by defining some of the dimensions of the problem. First, he raised the question as to whether there are many cases of IBD that are never seen by physicians. With respect to chronic ulcerative colitis (CUC), data from Danish sources suggest that 75% of cases are definitely diagnosed within the first 3 years of symptoms and that the other 25% are correctly diagnosed over the lengthy period of 4 to 30 years. With Crohn's disease, the problem is different: definite evidence of this disorder has been noted in patients in their 70's and 80's who have had no symptoms due to it and, occasionally, as established by autopsy in patients dying of other causes. Second, assuming that one could make the diagnosis in the earliest stages—as, for example, by colonoscopy or some biochemical test—Mendeloff asked what kind of agreement one might expect from physicians on such standards for diagnosis. He emphasized that, unless there are uniform standards for diagnosis, it is impossible to determine incidence, prevalence, or trends for the entire disease spectrum. This point was stressed later by Kirsner, not only in regard to epidemiologic studies in IBD but also in many other investigational areas.

Mendeloff went on to suggest that in-depth investigations of the incidence and prevalence of IBD be carried out in population groups that vary greatly in dietary habits, social norms, associated diseases, or ways of living. He recommended that studies be made of the disease in Mormons, Seventh-Day Adventists, Christian Scientists, Amerindians, and Jews in different societies, because these might be expected to yield some etiologic clues. However, studies of this kind would be extraordinarily expensive and would have to make use of routine diagnostic measures applied equally to every person in each group. Mendeloff also suggested that serologic tests for amebiasis (HIA) be included routinely, but for Crohn's disease of the small bowel he raised the query as to what can be used besides roentgenograms, which are ethically undesirable among younger patients. This question remains unanswered but an answer must be found if these epidemiologic studies are to be contributory.

Mendeloff stressed the merit of family studies but raised the point that the main problems in the past have been those of poorly selected controls, lack of common denominators, and absence of rates. What is needed is a complete investigation of all first-degree relatives. Mendeloff pointed out that his studies of 45 families in the Baltimore area have suffered from these defects; he has studied no really large families—that is, families with many uninvolved siblings or relatives—in which he has been able to complete even a simplified study of 42 genetic markers in one blood sample from each member of the family. Furthermore, he has been unable to follow up those previously unaffected family members who may now have IBD. These limitations have been largely financial. Thus, there seems to be no alternative to a national, collaborative, prospective family study. One would need 300 index cases of each type of IBD, with full investigations of all first-degree relatives at risk, over a 10-year period,

with monitoring every 6 months. What would one evaluate? Aside from specific endoscopic or radiologic diagnostic methodology, one would need to consider various hypotheses, including those relating to immunologic factors (eg, T and B lymphocytes and immunoglobulins), hormonal factors, serum bile acids, occupational factors, exposure to radiation from x-rays or other sources, precise dietary intake, and family habits.

Such a study should relate hospital discharges to a known population at risk. This study could be combined with a study of secondary diagnoses in patients with IBD; these would include ankylosing spondylitis, aphthous ulceration, various skin diseases, and other currently recognized extraintestinal complications. A wider epidemiologic net might lead to the recognition of currently unrecognized complications of IBD.

MICROBIOLOGIC FRONTIERS

The attention of the Conference then turned to microbiologic aspects. Regarding future microbial studies in IBD, with particular reference to searching further for evidence to support a microbial etiology, Bartlett presented ideas that have been developed by him and his colleague, Gorbach. In his introduction, he stated that IBD involves mucosal surfaces that harbor luxuriant microbial populations. Histologic studies of these conditions show changes analogous to infections caused by established pathogens; namely, Crohn's disease simulating tuberculosis, and ulcerative colitis resembling bacillary dysentery. Thus, a microbial etiology is certainly attractive. Despite this logic, a specific pathogen has escaped detection after more than 50 years of investigation.

With respect to Crohn's disease, the granulomatous tissue reaction and abnormalities of delayed hypersensitivity suggest that a mycobacterium or a related agent might be involved. Although attempts to culture such an organism have been unsuccessful, the possible etiologic role of an organism is not necessarily excluded. The situation may be comparable to that of leprosy or syphilis, in which the established pathogen simply cannot be recovered in artificial media.

The most suggestive evidence for a microbial etiology in Crohn's disease has resulted from studies by Mitchell and Rees (1). These authors, whose work was stimulated by their previous demonstration of a transmissible agent in sarcoidosis, postulated a mechanism common to both conditions on the basis of similar histologic findings, immune status, and Kveim test reactions. The model they used was the mouse footpad, which had previously proved successful for passage of *Mycobacterium leprae*. Presumably, the low temperature in the footpad favors growth of the leprosy bacillus. Injection of nasal washings from patients with leprosy produces slowly evolving lesions in the footpad, with evidence of granulomas after 6 months to 2 years. The reaction is enhanced if mice are rendered immunologically deficient by thymectomy and irradiation.

Using this protocol, Mitchell and Rees injected footpads of mice with cell-free extracts of diseased ileum and draining mesenteric lymph nodes obtained from a patient with Crohn's disease. Both normal mice and immunologically compromised mice were tested. Granulomas composed principally of epithelioid cells with occasional giant cells of Langhans type were noted in 5 of 8 biopsies obtained after 26 to 46 days. At 169 to 500 days, 8 of 48 footpads showed these same changes. Positive results of the Kveim testing were also observed frequently, and small-bowel granulomas occurred in 2 of 10 mice; both the Kveim reaction and the distant granulomas occurred in mice with positive footpad tests. Cave and associates (2) produced small-bowel granulomas, by inoculating Crohn's disease directly into rabbit ileum. No abnormality was noted using ileal homogenates from normal controls.

Despite the suggestive nature of these studies, several inconsistencies remain unanswered. For

example, footpad inoculation with lymph-node homogenates obtained from persons without bowel disease also produced a granulomatous reaction, even though there was only a single positive result in 153 trials (1). Thus, though less common in controls, the reaction could not be considered necessarily specific for the "agent" of Crohn's disease. Moreover, the controls used by Mitchell and Rees consisted exclusively of lymph-node homogenates rather than of normal bowel specimens. It is possible that the footpad reaction to these two tissue sources is quite different. Another curiosity is that, in contrast to the transmission of the leprosy bacillus, more granulomas were noted in normal mice than in those that were immunologically deficient. This finding suggests a hypersensitivity of foreign-body reaction, rather than infection. The consistent failure of Mitchell and Rees to observe microorganisms microscopically also is disturbing. Finally, these footpad studies could not be reproduced by Bolton and associates (3), who worked with diseased bowel obtained from 7 patients with regional ileitis. In this study, 711 inoculation sites failed to reveal a single sarcoidlike granuloma. However, several foreign-body reactions were observed with homogenates from both patients with Crohn's disease and controls, and these were ascribed to injection of heterologous tissue. Bolton and associates noted that the previous work by Mitchell and Rees was based on material obtained from only a single patient. They concluded that, if there is a transmissible agent in Crohn's disease, it is not universally present.

Although the studies of Mitchell and Rees provide no definite answers, they do serve to emphasize that unorthodox culture techniques possibly will be required to isolate what may be a highly fastidious agent in Crohn's disease.

More extensive studies in pursuit of a microbial pathogen have been performed in connection with ulcerative colitis. Bargen (4) was one of the first to suggest a bacterial etiology when he isolated a "diplostreptococcus" from the feces of patients with CUC. Intravenous challenge with this "diplostreptococcus" in rabbits caused colonic ulceration and bloody mucoid diarrhea, and Bargen concluded that the pathogen of CUC had been identified. However, other investigators observed that similar bacteria also produced these lesions in experimental animals. The theory became invalidated with the recognition of the variability of the biochemical characteristics of diplostreptococcus; the term actually represented multiple species of Gram-positive cocci found in the normal colonic flora.

Shigella also had its day in court (5–7). The similar pathologic features of CUC and bacillary dysentery suggested a common etiologic mechanism. However, most patients with CUC do not harbor *Shigella*, and therapy directed at this organism in patients with CUC is ineffectual. Moreover, the recent decline in incidence of shigellosis in the United States and Europe has not been accompanied by a reduction in CUC.

In 1941, Dragstedt and colleagues (8) proposed another potential culprit: *Bacterium necrophorum*. This organism has taken a circuitous route through bacterial taxonomy, being subsequently reclassified as *Bacteroides funduliformis*, *Sphaerophorus necrophorus*, and, most recently, *Fusobacterium necrophorum*. Present taxonomic criteria are based on a number of biochemical tests and analysis of metabolic products by gas–liquid chromatography (9). These techniques were not available to earlier workers and it is uncertain in retrospect that they were working with a single bacterial species. More importantly, *Fusobacterium necrophorum* is now recognized as a normal component of the flora in both the intestinal tract and the oropharynx. Although subcutaneous inoculation with this organism produces abscesses, it fails to reproduce the bowel lesions of CUC in experimental animals.

After dismissing any current significance for *Entamoeba histolytica* in IBD, Bartlett stressed that *Escherichia coli* has also attracted attention, particularly in light of recent observations

that some strains are capable of penetrating the large-bowel mucosa, thus producing bacillary dysentery (10). Cooke (11) has shown that certain *E. coli* strains in stools of patients with colitis produce necrotoxins, hemolysins, or enterotoxins. Such organisms were less frequently encountered in healthy controls. Increased titers of *E. coli* antibody have been noted in patients with CUC (12, 13), though this may be a nonspecific effect related to the more frequent occurrence of portal bacteremia in inflammatory bowel diseases in general (13). Because the *E. coli* strains are susceptible to a number of antibiotics, we would expect a good clinical response to these drugs; but, unfortunately, antibiotics do not generally alter the course of CUC (14). Moreover, enterotoxic *E. coli* organisms produce an acute disease without the tendency to chronicity and relapse that is so characteristic of CUC.

All of these searches for a specific agent in CUC can be succinctly summarized in the conclusion that none have survived critical analysis. In particular, Koch's postulates have not been satisfied. Perhaps even more compelling is the evidence that many of the suggested agents cause acute self-limited diseases in man. In contrast, a characteristic hallmark of CUC is its self-perpetuating nature with periodic relapses.

Bartlett then proceeded to elaborate on another approach to the role of bacteria in IBD: the suggestion that an imbalance of intestinal microflora or intestinal "dysbiosis" contributes to the pathologic events. The microbial ecology of the bowel is generally undisturbed in mild cases of idiopathic CUC, but the number of coliforms does increase when symptoms are severe (15–17). However, similar alterations have also been noted in shigellosis, cholera, amebic dysentery, nonspecific diarrhea, traveler's diarrhea, diarrhea induced by isotonic purges, and hypolactasia in subjects fed lactose (18). Actually, three characteristic floral changes are common to all these conditions: (1) an increase in certain coliforms that are relatively uncommon in the normal flora, (2) a decrease in the concentrations of obligate anaerobes to the extent that anaerobic counts are actually smaller than aerobic counts, and (3) possible contamination of the upper small bowel with fecal flora. It would appear that these changes result from the nonspecific effects of diarrhea itself and that they cannot be implicated in the pathogenesis of any of these conditions.

Similarly, abnormalities in the small-bowel flora have been noted in approximately one-third of patients with regional enteritis, but the changes are largely determined by the anatomic features of the disease (19–22). The upper intestine harbors increased total counts, with a predominance of coliforms and anaerobes in patients with stasis or loss of the ileocecal valve. The small-bowel flora in patients with Crohn's disease who have normal intestinal motility and an intact valve is similar to that of normal persons. This illustrates the care that must be taken in interpreting floral studies of IBD. The changes appear to be secondary to the disease process rather than causally related.

One factor that suggests that bacteria do not play an important role in IBD is the observation that antimicrobial agents fail to alter the course of events in the absence of septic complications (14). An exception is salicylazosulfapyridine (SAS) (Azulfidine), which, after over 30 years of use in CUC, has survived the test of time and has an apparently well-established clinical efficacy (23–26). The chemical structure of SAS is a sulfonamide moiety linked by an azo bond to 5-aminosalicylic acid. Although the precise mechanism of action of this agent remains obscure, two possible mechanisms should be considered. The first mechanism is related to the initial belief that the sulfonamide suppressed the microbial flora and the azo bond promoted affinity to the intestinal wall (27). These claims may no longer be valid. Studies of the intestinal flora of normal persons and of patients with CUC receiving SAS show minimal changes in total counts of major isolates (17, 28). However, this work cannot be considered conclusive because subtle floral

changes would not have been detected by the culture techniques used. The second mechanism is suggested by recent pharmacokinetic studies. It appears that the intestinal microflora reduces the azo bond, thereby releasing 5-aminosalicylic acid and sulfapyridine (29). The question, therefore, is which metabolite is responsible for the effect: is it the antibacterial activity of the sulfonamide or the antiinflammatory activity of the salicylate? Most authorities now favor the latter mechanism.

In considering future studies for specific pathogens in IBD, Bartlett emphasized that, despite previous failures, the possibility of microbial factors in the etiology of IBD cannot be discounted. In previous floral studies, only a small fraction of bacteria evident on Gram staining could be recovered in culture; the other morphologic forms were presumed to be nonviable. Recent use of sophisticated anaerobic culture methods has made it possible to isolate essentially all of the bacteria seen under the microscope (5, 30). Many of these organisms are extremely oxygen-sensitive (EOS) anaerobes, an appellation derived from their inability to survive exposure to air for even 10 minutes. Examples include *Eubacterium aerofaciens, Bifidobacterium adolescentes*, certain strains of *B. fragilis*, some peptostreptococci, and spirochetes (30). In concert with the improved culture techniques, there have been major advances in bacterial taxonomy. For example, the organisms that were once merely lumped together as "Bacteroides" have now been divided into separate genera, species, and even subspecies according to well-defined criteria. Recent studies suggest that 300 to 400 different bacterial types are present in a single fecal specimen (31). There are important differences among these organisms in terms of metabolic activity and pathogenic potential. For example, it is now well established that many of the bacteria found as part of the normal colonic flora are conspicuously absent from septic processes that complicate bowel disruption (32). It is curious that, despite the complexity of the inoculum associated with a ruptured appendix or perforated colon, an average of only five microbial species can be recovered from the ensuing peritonitis exudate or intraabdominal abscess (33). There even appear to be variations in pathogenic potential within a bacterial species. *B. fragilis*, the dominant colonic bacterium, has recently been accorded five subspecies; the subspecies that prevail in the indigenous flora are not the same as those found in clinical infections (34).

These observations indicate that subtle differences in the flora may easily escape detection and that the bacteriologic methods used in earlier studies must now be considered antiquated in light of modern taxonomic and technologic approaches.

An additional point of interest with regard to the intestinal flora concerns mucosal-associated bacteria. The lumen of the gut harbors a dynamic ecosystem of stable bacterial populations with specific anatomic localization. There are characteristic variations in cross-sectional arrangement and in longitudinal distribution. Certain microorganisms are associated directly with the mucosal epithelium. These have been most extensively studied in animals other than man (35–39). Specially stained histologic sections of rodent colon show large anaerobic fusiforms forming thick layers that occupy the entire mucin layer (37). These organisms are packed densely in concentrations of 10^{10}–10^{11}/g. Their ends are fixed in the mucous layer but there is no evidence of epithelial penetration. At the bases of the intestinal crypts, there is a homogeneous population of thin, motile spiral organisms that vary morphologically according to the level of bowel sampled. Both the fusiforms and spiral forms have been observed at similar locations in the large bowel of dogs as well (39). Few of these organisms are in the lumen of the gut or in the feces.

Mucosal-associated bacteria in the normal human intestine and their possible role in diseases have not been studied; such investigations are needed. These organisms are of interest be-

cause of their intimate relationship with the epithelial surface.

Nonbacterial components of the intestinal flora must also be considered. Viruses have been recovered from the intestine in up to 35% of normal persons (40). These are generally enteroviruses, though reoviruses, adenoviruses, and rhinoviruses occasionally may also be present. The significance of these isolates from normal persons and the role of viruses in many intestinal disorders have not been elucidated. Experimental attempts to produce CUC with filtrates of feces and rectal mucosa obtained from affected patients have been unsuccessful (41). On the other hand, high titers of cytomegalovirus (CMV) antibody have been noted in some patients with CUC. This effect is apparently unrelated to the duration of illness, therapy with steroids, or previous blood transfusions. CMV has also been isolated from the colonic mucosa in three of six patients with CUC compared with none of six controls (42). These findings must be interpreted with reserve, because the denuded bowel surface may simply represent an excellent culture medium for this virus. Furthermore, the pathologic changes and clinical presentation of IBD are distinctly unusual in terms of a viral infection. Similar ambiguities relate to the roles of protozoa, parasites, or mycoplasma.

In summary, the previous work in search of the "pathogen" in IBD has failed clearly to implicate a single microbe, but it has also been inadequate to exclude the infinitesimal possibilities. At the same time, a precautionary statement should be made concerning future studies of the bowel flora in these conditions. Qualitative and quantitative determination of all bacterial components according to the previously cited guidelines may require up to one year for complete analysis of a single specimen. This herculean effort is difficult to justify without more direct evidence that such an approach is likely to be rewarding.

A more logical way of studying the role of microorganisms is to approach the disease from a common underlying denominator: *penetration of the intestinal epithelium.* Under normal conditions, the mucosal surface acts as a barrier to invasion. Because the integrity of the epithelial lining could be disrupted by a number of factors, such epithelial penetration would merely serve as the initial step in a chain reaction of pathologic events.

Bacteria themselves may be responsible for the initial lesion. *Salmonella, Shigella, Entamoeba histolytica,* penetrating *E. coli, Yersinia enterocolytica,* and *Balantidium coli* are all capable of invading the intestinal epithelium.

Alternatively, invasion may result from formation of bacterial products. The lower intestinal tract is the site of intensive microbial activity that serves to control growth, maintain a low degree of oxygen reduction, and preserve a constant pH. The result is a stable resident population with resistance to implantation by certain pathogens (43, 44). However, some metabolic products of bacterial activity may be potentially injurious to the intestinal mucosa. For example, anaerobic microorganisms convert carbohydrates into short-chain organic acids. Metabolites such as acetic, butyric, and propionic acids are known irritants and may potentiate pathologic conditions. An analogous situation is the association of a common skin bacterium, *Propionibacterium acnes,* and the disease, acne vulgaris. This organism colonizes the sebaceous glands and produces large quantities of propionic acid. Comedones result from the inflammatory response to propionic acid rather than from any inherent invasive properties of the responsible bacterium. Similar short-chain fatty acids are present in the colon although their role in diseases at this location is unknown.

Endogenous constituents of microorganisms may also damage the intestinal mucosa. The wall of all Gram-negative bacteria, including Bacteroides species as well as coliforms, contains lipopolysaccharide (endotoxin). The molecular weight of this material is extremely high; it is estimated to exceed 2 million. En-

dotoxin may penetrate the intestinal mucosa in hypovolemia or septic shock. Bacteremia and endotoxemia are also common in ulcerative bowel disease (45, 46). Local effects of endotoxin may include the Shwartzman phenomenon, resulting in ischemic necrosis and ulcerations.

There is evidence that patients with CUC have an inordinately high immunologic reactivity to another component of bacterial cell-wall lipopolysaccharide: somatic antigen. When a heterogenetic antigen from *E. coli* 0:14 was used, sera from patients with CUC were found to have high levels of circulating antibody to this "common antigen" (12, 13). Increased antigen-induced reactivity has been demonstrated in lymphocytes from patients with CUC (47). Furthermore, lymphocytes from such patients have been shown to be toxic for colonic cells *in vitro* (48–50). Thus, several immunologic mechanisms lead to the production of colonic cell damage when triggered by the appropriate antigen.

Another area of considerable interest is bile acid metabolism by intestinal bacteria. Deconjugated bile acids may alter intestinal epithelium, an effect noxious enough to implicate them in the pathogenesis of bowel cancer (51).

Disruption of the integrity of the bowel can also be induced by drugs. Antibiotics such as tetracycline, chloramphenicol, clindamycin, and aminoglycoside antibiotics may cause pseudomembranous colitis (52–54). Other drugs such as indomethacin and oral potassium preparations may also cause intestinal ulceration. Denudation and ulceration of the mucosal surface are accompanied by invasion by intraluminal bacteria. It is conceivable that less pronounced forms of this condition produce only partial or superficial ulceration. This would allow penetration of bacteria or their products into the lamina propria without the obvious clinical features of pseudomembranous colitis.

Recently, there has been interest in carrageenans as a potential cause of bowel disruption (55–59). These gel- or precipitate-forming sulfated polysaccharides of high molecular weight extracted from "red" seaweed are widely used as food additives. Four different animal species fed carrageenans developed colitis that was clinically and pathologically similar to idiopathic CUC in humans. The extent of disease is dose dependent, and degraded carrageenans have proved more ulcerogenic than the parent compounds. Clinically, the disease in animals becomes manifest by weight loss, anemia, and bloody, mucoid diarrhea. Pathologic studies of the colon indicate that the lesions range from shallow mucosal erosions to deep ulcerations with cellular infiltrates, granulation tissue in the lamina propria, and multiple crypt abscesses. Other sulfated products with high molecular weights may produce similar colonic lesions in experimental animals.

Several possible mechanisms have been suggested to account for a violation of the colonic epithelial barrier. These include bacterial invasion, formation of bacterial products, immunologic activity, and use of various drugs or chemicals. As already emphasized, this may simply represent the initial insult that permits invasion of the normal colonic flora. Several mechanisms that are independent of the process causing penetration may then cause self-sustained disease. Having gained entry, the colonic flora could then be responsible for chronic disease through the action of irritating metabolic products, antigen-antibody complexes, a Shwartzman reaction, or lymphocyte-mediated cytotoxicity.

SIGNIFICANCE OF THE FECAL STREAM

Pursuant to Bartlett's presentation, Phillips presented his ideas on the possible pathophysiologic significance of the fecal stream to IBD. He suggested that the thought that a component of feces plays a role in the pathogenesis of IBD is a seductive one but, like most seductions, is provoked more by the excitement of the unknown than by a rational contemplation of established facts. Nevertheless, he perceived several points of potential interest to investigators of IBD.

As an ecosystem, feces is surpassed in complexity only perhaps by a cesspool. Known constituents of feces to be considered include water and inorganic materials, components of the diet with certain known (and unknown) additives, endogenous secretions including exuded proteins and desquamated cells, and a rich bacterial flora. As Bartlett emphasized, the fecal flora is not an inert colony of bacteria. It comprises a finely balanced array of species, capable of extensive enzymic and potentially toxic effects. Perhaps of foremost importance are the chemical reactions between bacteria and the residues of diet and secretory activity. Because of the complexities of these chemical events, their specifics are largely unknown.

Major areas in which the fecal stream could be relevant to IBD include the following: (1) etiology as an initiating, perpetuating, or aggravating factor, (2) symptoms related to the development of pain, diarrhea, and fistulas, and (3) therapy specifically relating to bypass or excisional surgery.

In terms of etiology, thousands of chemical substances are added to food consumed by people in Western countries. Although particulate matter such as silica and talc can produce granulomas in experimental preparations of intestine, we have no strong clues as to any pathogenic role of foreign material. What normal constituents of intestinal content might cause mucosal damage? There is good evidence that dihydroxy bile acids alter the mucosal structure of the jejunum and ileum in small animals (60, 61). When the hamster jejunum is perfused with dihydroxy bile acids, cytologic changes are produced at the tips of villi; when these are most pronounced, the mucosal damage progresses to a shedding of epithelial cells (60, 61). Simultaneously, mucosal function changes. Fluid absorption decreases and net fluid secretion is stimulated. However, there is little correlation between morphology and functional change. The possibility that unconjugated dihydroxy bile acids might alter ileal morphologic features in man is not unreasonable. In the blind-loop syndrome, concentrations of unconjugated bile acids in the bowel lumen are increased (62) and mucosal histology is altered (63, 64). Furthermore, even in healthy subjects dihydroxy bile acids can be recovered from the ileum (65).

Other constituents of normal feces that are known to produce functional (66) and morphologic (67) changes in ileal mucosa are certain long-chain fatty acids. It has been shown that dietary fatty acids (oleic acid) and their bacterial by-products (hydroxystearic acid) modify ileal absorptive function (66); and ricinoleic acid, the active principle of castor oil, is structurally quite similar to these fatty acids and can be shown to produce morphologic changes in intestinal mucosa (67). The argument here is less persuasive. Large amounts of fat will be found in stools only after bowel disease and steatorrhea have developed. However, bacterial interaction with the small proportion of dietary fat that is normally unabsorbed could lead to the formation of unusual hydroxylated fatty acids (68, 69) in certain individuals. Might a particular hydroxylated fatty acid be especially prejudicial to mucosal integrity?

If a constituent of feces could damage the mucosa, how might this event relate to IBD? There are several possibilities. The proposed agent might be a primary factor, actually initiating a mucosal lesion. It could potentiate or aggravate a histopathologic process begun by another stimulus. Some of these possibilities could be tested experimentally. However, the transmural nature of inflammation in Crohn's disease argues against a primary stimulus that acts only at the level of the mucosa.

In terms of the hypothesis of Shorter and associates (70), *should we be looking for mechanisms by which mucosal permeability is altered?* Is there a normal mucosal "barrier" to certain antigenic molecules, effectively excluding them from the gut wall and from intimate contact with immunologically competent tissue? Might a constituent of the fecal stream, under certain circumstances, alter mucosal per-

meability, allowing bacterial or other antigens free access to the gut wall?

In physiologic terms, the mucosa of the ileum and colon is a "tighter" or more restrictive membrane than that of the proximal gut. This concept has been expressed in terms of the size of "functional pores" in the distal bowel, which are smaller than those of the jejunum (71, 72). The morphologic equivalent of functional pores is unknown; currently, it is thought that the intercellular "tight junction" may not be tight at all and that this region of the mucosa might be the site of "pores." Could constituents of the fecal stream alter these permeability characteristics, just as they cause structural and other functional alterations? This phenomenon could be tested experimentally (73). It has already been shown that hydroxy fatty acids increase the permeability of colonic mucosa (74).

Although large molecules, such as intact proteins, cannot cross the gut mucosa in very large quantities, small amounts can take part in such transport. Isselbacher's group (75, 76) has shown that horseradish peroxidase (HRP) and bovine serum albumin transgress the mucosa of neonatal and adult rats in small but measurable quantities. If these findings apply to man, and if the proposal of Shorter and associates (70) has validity, any process that facilitates the movement of intact, foreign proteins across the mucosa would be of great interest.

The possible role of bacterial toxins as mediators of mucosal damage requires attention but, although the direct effects of invasive bacteria and noninvasive bacterial enterotoxins have obvious relevance to IBD, Phillips did not pursue this because of Bartlett's earlier considerations. However, Phillips pointed out that, though the production of potential mucosal toxins by the action of bacterial enzymes on the many organic materials that occur in feces raises many possibilities, at this stage there is no factual basis for such speculation.

Clinically, the observations that most strongly suggest at least a potentiating role for feces in IBD are those showing a remission of Crohn's colitis following ileal diversion (77). In some instances, fever and colonic symptoms returned when ileal contents were instilled into the defunctioned colon. No clear conclusions emerge but further observations, at least on patients whose bowel has already been diverted, seem warranted. It is unfortunate that until now, clinical observations on patients undergoing bypass (with or without exclusion) and excisional surgery for regional enteritis have been uncontrolled and no conclusions are possible.

In regard to the symptoms of established and treated disease, Hofmann and Poley (78) have characterized the pathophysiologic features of two firms of diarrhea that result from ileal dysfunction and that relate clearly to the nature of fecal contents. Therapeutic programs, aimed at altering fecal contents, have also been proposed. Other therapeutic implications of the nature of gut contents are largely unexplored. Apart from differing surgical approaches, elemental diets and "intestinal rest" with parenteral nutrition have been used, but the uncontrolled nature of these observations permits no conclusions.

Although it is a tempting concept, Phillips concluded that currently there are few existing data to support a role for the fecal stream in IBD. However, recent observations on the effects of certain endogenous constituents of feces give clues as to a different and potentially fruitful approach in this investigational area.

TESTING OF ILEAL FUNCTION

Hofmann complemented Phillips' contribution by briefly describing the rational testing of ileal function and how this might be applied to future investigations in IBD. He began his presentation with some restatements that the distal ileum is a muscular conduit wherein the sterile chyme entering from the more proximal bowel begins its transformation into the fecal material of the cecum. Its mucosa absorbs electrolytes and water, bile acids, and vitamin B_{12} and passively absorbs lipids. The ileum acts as an ion

exchanger, exchanging bicarbonate for chloride ions. The ileum secretes immunoglobulins and thus influences its own resident flora. The ileocecal valve prevents reflux of cecal contents, which should contribute to its relatively sterile state.

Rational testing of the distal ileum should assess its conduit functions, its specific and nonspecific absorptive functions, its ion-exchange functions, its immunoglobulin secretory functions, and the location and type of its flora. It should also assess macroscopic and microscopic structure as well as the structural enzymes concerned with its specific function.

With respect to conduit function, partly digested food passes rapidly through the ileum into the colon when a meal is digested. The time required for bile acids to pass from the gallbladder to the site of active absorption may be estimated by ileal perfusion techniques (79) or may be inferred by the time required for serum bile acids to reach a peak after a meal (80). The two techniques give similar results: about 90–110 minutes after a meal, a load of bile acids reaches a site where the load is rapidly absorbed. If there is obstruction proximal to the bile acid absorptive site, it should be reflected in a delayed postprandial rise of serum bile acids. Obviously, the best way to assess the conduit or transit function of the ileum would be careful roentgenographic investigation.

Considering next some specific absorptive functions, Hofmann went on to consider first the bile acids. Based on *in vitro* and *in vivo* studies, the terminal ileum has an active transport system for bile acid anions (ATSBAA) (81). The cells possessing the ATSBAA have not been identified either morphologically or histochemically. Indeed, no marker enzymes have been measured or even proposed. The ATSBAA has been present in every mammal tested, but relatively little phylogenetic exploration has been conducted. The ATSBAA transports bile acids with a single negative charge (82); bile acid sulfates, with two negative charges, are also actively transported by the ileum (83), but it is not known whether the same transport system is involved. Furthermore, alkyl sulfates also are probably absorbed from the ileum; again, it is not known whether this absorption is mediated by the ATSBAA.

The location of the ATSBAA also has not been defined. In animals, it operates in the distal one-quarter of the small intestine. In man, it is considered to lie close to the ileocecal valve, because patients with ileal resections as short as 50 cm may have symptomatic bile acid malabsorption (84). The capacity of the ATSBAA is quite large. It probably can absorb 2–3 g of bile acids per hour (85). As a consequence, the bile acid pool is conserved with extraordinary efficiency—about 95% per cycle (86) or 70% per day (87).

Various methods have been proposed for assessing bile acid absorption. The simplest approach is to label the bile acid pool with a bile acid tag in the steroid moiety (85, 88, 89). One measures fecal excretion of radioactivity during some subsequent time interval, usually 24 hours. If one does this in patients with terminal ileal resections of 50 cm or more, one generally finds that 50–100% of the label will be excreted in 24 hours. In healthy subjects, less than 10% is recovered. This striking difference suggests that a sensitive, specific test is available. However, in fact, the clear separation is deceptive. A major factor responsible for the difference between the two groups is transit time in the colon. In patients with bile acid malabsorption, the bile acids passing into the colon appear to induce water secretion, causing rapid colonic emptying (90), so that all bile acids passing into the colon are excreted during the subsequent 24 hours. In healthy subjects, about 30% of the label will enter the colon in 24 hours, but colonic emptying is so slow that little label is excreted during the 24 hours following administration.

The obvious way to correct for the colonic emptying rate is to give a nonabsorbable marker when the labeled bile acid is given parenterally (85, 91). In patients with bile acid malabsorption, one recovers both bile acid ra-

dioactivity and marker; in patients with diarrhea but no ileal disease, one recovers nearly all of the marker in 24 hours, but usually less than 40% of the administered bile acids, whereas in healthy subjects, one recovers neither marker nor bile acid. Thus, a relatively simple technique exists for measuring the degree of absorption of the bile acid pool by giving a nonabsorbable marker such as polyethylene glycol (PEG) by mouth and a labeled bile acid such as ^{14}C-cholyltaurine intravenously. One collects two 12-hour stool specimens and measures PEG and radioactivity (92).

Bile acids indicate their own malabsorption by inducing rapid colonic emptying. In Hofmann's experience, no patient with bile acid malabsorption has not had some degree of diarrhea, that is, a fecal weight exceeding 200 g/day in a 48-hour collection (85).

Hofmann stressed that the one defect in this approach of labeling the pool is that one measures the fraction of the endogenous pool absorbed, and the bile acid pool could be diminished in cases of severe bile acid malabsorption. He considered that the obvious answer to this problem would be to give a "swamping" oral load of nonradioactive bile acids, but this approach has not been seriously explored except in two instances (93, 94). What one would like to do is to titrate the oral load according to the size of the endogenous bile acid pool, but this is not practical.

Instead of using a bile acid label in the steroid moiety, one could administer cholylglycine in which the carboxyl moiety of the glycine is labeled. In cases of bile acid malabsorption, the cholylglycine will pass into the colon. Here the glycine moiety is usually liberated by cholyl amidase, an enzyme possessed by most colonic species. The glycine will then be oxidized to CO_2, which is absorbed across the colonic wall and is excreted in the breath. Breath CO_2 can be trapped quantitatively in a mixture of an organic base and ethanol by simply blowing into a liquid scintillation vial containing this solution (89). An indicator is added to signal complete titration of the base with the CO_2. Radioactivity is then determined by liquid-scintillation spectrometry to give $^{14}CO_2$ specific activity, from which the percent dose expired per unit time is readily calculated. Thus, increased $^{14}CO_2$ means increased exposure of bile acids to deconjugating bacteria, which in turn means either bacterial overgrowth in the small intestine or bile acid malabsorption.

If the glycine moiety is not degraded to $^{14}CO_2$—which can happen in patients with rapid colonic emptying—the ^{14}C would then appear in stool. In our experience (85), as well as in that of the Aalborg group (95), patients with bile acid malabsorption were uniformly found to have increased fecal ^{14}C. In patients with increased $^{14}CO_2$ in breath but no increased fecal ^{14}C, there is overgrowth of bacteria.

Obviously, it should be possible to measure bile acid absorption by perfusion techniques carried out in the distal ileum (86), but experimentally this is difficult.

A sensitive radioimmunoassay of bile acids has recently been developed (96). This radioimmunoassay is specific for conjugates of cholic acid (CCA). Using this test, one observes a striking postprandial increase in CCA in healthy subjects. In patients with bile acid malabsorption secondary to ileal resection, there is a slight increase after the first meal, a barely detectable increase after the second, and no detectable increase after the third (80). Thus, a normal rise of serum CCA after the evening meal appears to exclude bile acid malabsorption. Application of this test to patients with IBD would be of interest.

The second specific active transport system of the ileum is that for vitamin B_{12}. The location of this has not been defined well in relation to that for bile acid absorption. However, because patients in whom a short length of terminal ileum has been resected invariably have bile acid malabsorption but do not necessarily have vitamin B_{12} malabsorption, it appears that the site of vitamin B_{12} absorption is either located more proximally in the ileum or extends over a

Table 1. Comparison of Tests of Hepatic and of Ileal Function

Property measured	Function	
	Liver	Ileum
Uptake and excretion	BSP removal Bilirubin concentration Bile acid concentration	Postprandial serum bile acid concentration Bile acid absorption Fecal bile acid excretion Vitamin B_{12} absorption
Synthesis	Albumin Prothrombin	?
Biotransformation	Bilirubin conjugation Benzoic acid conjugation Galactose removal	?
Injury	Transaminase value Peptidase value	?

greater area (85). Whether the same enterocytes participate in vitamin B_{12} absorption and in bile acid absorption is unknown.

The Schilling test is based on the cumulative urinary excretion of radioactivity after ^{57}Co-labeled vitamin B_{12} and intrinsic factor have been given orally and then a flushing dose of vitamin B_{12} has been given parenterally to saturate the tissue-binding sites (97). The Schilling test is not a straightforward test of ileal absorptive function because bacteria can bind or degrade intrinsic factor, decreasing the absorption of vitamin B_{12} despite normal ileal absorption. Thus, a positive Schilling test indicates either bacterial proliferation or ileal mucosal disease, or both.

Hofmann then proceeded to speculate on the ideal test of ileal function. Obviously, a test measuring solely ileal absorptive function would have to feature a substance totally resistant to bacterial degradation. The simplest substance to use would appear to be a bile acid sulfonate, but its synthesis has never been carried out, despite the ample precedent for this in the literature dealing with detergents (98).

In concluding his remarks on absorption, Hofmann stated his belief that other absorptive functions of the ileum are much less accessible to testing. Obviously water and electrolyte conservation can be measured by perfusion techniques, as can ion-exchange capacity or passive permeability.

Hofmann then turned to considerations of the ileal flora and stressed that the population of ileal flora increases sharply as the terminal ileum is approached (99). The present impression is that biologic variability is so great that quantitative culture techniques would not be rewarding if applied to the distal small intestine. One could measure the time required for meal–bacteria interaction by two techniques. In the first, which has been carried out in a few patients, hydrogen in the breath can be measured by gas chromatography after ingestion of a meal containing a poorly absorbed carbohydrate such as lactose or raffinose (100). In the second, which has not been explored, breath $^{14}CO_2$ could be measured after a meal containing a nonabsorbable sugar tagged with ^{14}C (101). Unfortunately, interpretation of the results of any such test would probably be difficult because of variability in gastric emptying.

As a final point, Hofmann considered the relative merits of ileal versus liver tests. Table 1 compares hepatic and ileal functional tests; there is a paucity of tests for ileal function based on similar principles. Clearly, liver tests measure excretory capacity, biotransformations,

biosynthetic capacity, or specific or nonspecific enzymes released after cellular injury. Scanning techniques are based on uptake by hepatocytes or the contiguous Kupffer cells belonging to the reticuloendothelial system. In the ileum, tests of absorption of bile acids are analogous to tests of hepatic excretory function such as BSP; both measure net cellular uptake. The only test giving the dynamics of uptake appears to be the measurement of postprandial bile acids. Other tests such as fecal excretion or radioactivity are cumulative. At present, we assign no biotransformation or synthetic capacities to the ileum. No specific enzymes released in ileal injury have been identified and no scanning techniques have been proposed. A bile acid tagged with a γ-emitting isotope should be a useful scanning agent for the ileum, but no work as yet has been done along this line.

Hofmann concluded his remarks by saying that tests of ileal function are perhaps irrelevant to the diagnosis and to the management and monitoring of a patient with IBD. It may be that morphologic changes precede and are more readily detected than alterations in function or biochemical signs of injury. But Hofmann did not think so. Clearly, our knowledge of the enzymatic basis for the ileal function is almost nonexistent. With inflammatory liver disease, the ultimate criterion for disease presence or activity has been the morphologic appearance obtained by biopsy, which would often indicate smoldering disease, despite normal results of conventional blood tests for function (eg, BSP) or cell injury (eg, SGOT) (102). But now, recent data show that bile acid values are superior to biopsy findings in predicting which patients will have a relapse when therapy is discontinued (103). Thus, the insensitivity of current tests for ileal disease when these are compared to roentgenograms is most simply interpreted as indicating that we are not using the right tests.

Finally, Hofmann defined priorities for approaches to the future testing of ileal function. First, let us define all the functions of the ileum. Does it have a synthetic role? Does it bring about biotransformation of endogenous or exogenous substances? Let us find enzymes that explain its unique transport role. Then let us develop an ileal "battery" that can be used to assess the integrity of ileal function and the release of specific signs of ileocyte injury. We must study the ileum from the viewpoint of the basic scientist rather than from that of the clinician.

The critical question is whether the unique physiologic function of the ileum—as well as its unique ecologic location—is related to the pathogenesis of IBD. Perhaps it is not. But for the moment, we must seek to relate functions to disease and look for clinical situations or animal models that may suddenly and unexpectedly provide the missing piece in the puzzle.

IMMUNOLOGIC FRONTIERS

Up to this point in the Conference, some speakers had touched on the exciting field of immunologic studies in IBD and the possible role of immune mechanisms in the etiopathogenesis. This area now was discussed by four contributors: Kraft, Watson, Stobo (with Tomasi), and Shorter.

Kraft and Watson reviewed the current status of humoral and of cell-mediated immunity, respectively. Since this Conference was held, many of the salient features they presented have been published elsewhere (104–106), and so the full contents of their reviews will not be reproduced herein. Kraft concluded that many immunologic data are consistent with the importance of immune phenomena in the etiopathogenesis or the chronicity of ulcerative colitis and Crohn's colitis. However, he stressed that anticolon antibodies may represent a secondary immunologic response to nonimmunologic bowel injury and might have a protective or healing role. A clear understanding is needed of the possible *in vivo* relationships between bacterial and colonic antigens because of the lack of documentation of specific antigen–antibody interaction in the diseased

bowel. A greater understanding is also required of the possible participation of secretory immunoglobulins, but this must await more precise identification of antigens at and near mucosal surfaces. In addition, since a hard and fast line cannot be drawn between humoral and cell-mediated immunologic studies, some problems of interpreting data on cell-mediated immunity in patients with IBD may be related to still unavailable humoral or secretory immunologic data.

Watson, in his review of cell-mediated responses in patients with IBD, discussed particularly the cytotoxicity of colitis lymphocytes for colonic epithelial cells, which has been investigated by Perlmann and Broberger (48), Watson and his colleagues (49), and, very extensively, by Shorter and his group (50, 107–112). Watson also considered the working hypothesis for the etiopathogenesis of IBD developed by Shorter and his colleagues (70) as a basis for future investigations. Such studies could be related also to suggestions made by Bartlett during the Conference and to ideas expressed by other contributors. For these reasons, Watson expounded on the hypothesis by stating that it rests on two assumptions: (1) CUC and Crohn's disease represent parts of the spectrum of a single pathogenetic process, and (2) IBD results from the establishment of a state of hypersensitivity to bacterial antigens normally present in the gastrointestinal tract of the affected individual.

The first assumption is based on the following observations: (1) the often merging histopathologic characteristics of the two disorders, (2) their similar distributions of age, sex, and race, (3) the extraintestinal manifestations shared by each, (4) the familial incidence for both that is interdependent (113), and (5) their common immunologic features (105, 106).

The second assumption can be elaborated by restating it in the form of several questions. First, what is the nature of the antigen or antigens participating in this postulated immune reaction? In view of the intimate relationship between the distal small intestine and colon and a more or less fecal type of flora, it is difficult to exclude the fecal flora from some role in the causation of two disorders largely limited to these areas. It seems likely that bacterial antigens normally gain access to the lymphoid tissue of the bowel wall and draining lymph nodes early in life, perhaps before the normal mucosal block to their uptake is established. Indeed, the normal development of the gut-associated lymphoid tissue depends on the establishment of a bacterial flora because, in the germ-free state, lymphocytes and plasma cells are sparsely evident in the lamina propria and because Peyer's patches are more rudimentary in appearance (114). In infancy, the uptake of bacterial antigens could be augmented by early exposure to cow's milk before the normal mucosal block develops. It is perhaps significant that there is reportedly a higher incidence of early weaning to cow's milk in patients with chronic IBD than in controls (115). In a similar vein, Staley and coworkers (116) have shown that *E. coli* organisms introduced via the stomach into neonatal pigs are subsequently demonstrable in the intestinal wall, and there is no theoretical reason why this should not also occur in human infants. The result would be the establishment in the gut-associated lymphoid tissues of a state of immunity to bacterial antigens.

The "near-universal" existence of immunity to a coliform antigen is suggested by the finding that lymphocytes from most normal individuals produce macrophage inhibitory factor on exposure to a phenol water extract of *E. coli* 0119:B14 (117). It seems likely, therefore, that most people are immunologically primed subsequently to react to enterobacterial antigens, given the proper challenge. However, in this hypothesis, only in genetically predisposed individuals would such a reaction be potentially harmful to the host and thus result in the clinical manifestation of IBD.

How might this rechallenge occur? After a period varying from a few weeks to many years, the normal mucosal block to the uptake of bac-

terial antigens could be temporarily lost or impaired as a result of gastroenteritis, bacillary dysentery, amebic colitis, local ischemia, or even metabolic alterations induced by psychologic stress perhaps mediated via the autonomic nervous system. Although a speculative role for psychosomatic factors may be highly controversial, IBD may follow such infectious processes as bacillary dysentery, and a secondary peak onset of ulcerative colitis in patients over 60 years of age suggests the possible involvement of vascular factors.

An important question is why all individuals thus primed and rechallenged do not develop IBD. Individual variations in immune responsiveness to a given antigen are well known but ill understood; the clinical picture of allergy does not develop in all persons exposed to different allergens. One factor in this variability is undoubtedly genetic. Kirsner and Palmer (14) first proposed the concept of individual susceptibility to the development of IBD on the basis of some sort of heritable predisposition. This concept is supported by observations concerning the ethnic and familial distribution of both forms of IBD. Both qualitative and quantitative variations in exposure to enterobacterial antigens also must be considered. The concentration and availability for uptake of potential antigens probably vary from individual to individual, from one part of the gut to another, and from time to time. Further modifying influences may be provided by such factors as changes in regional blood flow, variations in the type and amount of bile salts, and the adjuvant action of other luminal constituents such as bacterial endotoxin (118).

Once sensitization and rechallenge have been effected in those genetically predisposed individuals, how does tissue damage in the bowel (ie, IBD) take place? It seems likely that the interaction of enterobacterial antigens and sensitized lymphocytes causes the latter to release biologic mediators resulting in inflammatory changes. Specificity resides in the release mechanism rather than the activity of the mediators. Thus, enterobacterial antigens or related determinants in colonic epithelial cells interacting with specific lymphocyte receptors trigger the release of lymphotoxin and the various chemotactic factors. Lymphotoxin acts nonspecifically, affecting any cell within its range of activity. If release of lymphotoxin is triggered by antigen–lymphocyte interaction at the mucosal level, a relatively superficial contact type of colitis or "backwash ileitis" might be expected to occur, whereas if the same sequence of events occurs in the submucosa the resulting picture might be that of a transmural or granulomatous inflammation. Within this hypothesis, when the lymphocytes interact primarily with the membrane-associated antigens of the colonic epithelial cells, the features of CUC would predominate. In contrast, cross-reacting bacterial antigens gaining access to the submucosa would produce a more productive or even granulomatous type of inflammation. Therefore, Crohn's disease of the small bowel (or backwash ileitis) might be related to an interaction of sensitized lymphocytes with bacterial antigens at different levels, whereas CUC and Crohn's colitis might involve the reaction of sensitized lymphocytes to a greater or lesser extent with both epithelial cell and bacterial antigens at different points; thus, the variable and often merging pathologic features noted in the colon are accounted for. Clinical considerations lending support to this concept include the following: (1) the location of the lesions in more than 90% of cases of Crohn's disease and in all cases of CUC in those portions of the gut exposed to a fecal type of flora—the colon and terminal ileum; (2) the almost "universal" onset of CUC in the distal large bowel, where antigen concentrations are likely to be greatest and mucosal contact most prolonged; (3) the proximal extension of CUC in a contiguous fashion relating to the uniform presence of epithelial-cell antigens and the possibility that the skip areas of Crohn's disease might be due to regional variations in the uptake of bacterial antigens; and (4) the postsurgical recurrences of

Table 2. Cytotoxicity for Allogeneic Colonic Epithelial Cells of Populations of Mononuclear Cells From Patients With Idiopathic Colitis*

Population	Cell frequency (%) in each population: T	B	Cytotoxicity (%)
Unfractionated	63.6± 5.6	22.6± 2.5	27.6±8.9
SRBC rosette-enriched†	93.0± 1.4	6.3± 2.1	1.0±1.0
B-depleted‡	73.7±13.6	12.7± 6.6	35.2±3.2
B-enriched‡	27.8±12.8	68.2±17.6	8.8±6.9
Fc receptor-depleted§	75.0± 1.0	15.0± 5.0	8.7±1.1
Fc receptor-enriched§	26.0± 7.0	48.0±10.5	43.3±8.5

*Frequency of T cells in each population was determined by quantitating frequency of small round cells that bound three or more sheep red blood cells (SRBC) to their surface. Frequency of B cells was quantitated by noting percentage of small round cells positively stained with a fluoresceinated antiserum directed against human kappa and lambda chains. Cytotoxicity was determined in a 4-hour assay with allogeneic colonic epithelial cells as the target and trypan-blue exclusion as a measure of viability. Results are expressed as arithmetic mean ± SE.

†Fractions obtained by sedimenting rosette-forming cells over ficoll-Hypaque gradient.

‡Adherent and nonadherent fractions obtained from polystyrene Petri dish coated with anti-Ig.

§Adherent and nonadherent fractions from polystyrene Petri dish coated with aggregated IgG.

Crohn's disease, particularly at anastomotic sites, which may well reflect the establishment of a fecal type of flora at these sites, as well as factors promoting the uptake of bacterial antigens.

Within this hypothesis, how may patients with IBD have long periods of remission? In some situations, the temporary reduction of available antigen below a critical level might occur. This seems unlikely in the case of epithelial-cell antigens unless there is some type of synergism between bacterial and membrane-bound determinants. Even in Crohn's disease, for which it is postulated that bacterial antigens only are involved, this sort of situation is likely to be quite transient. It seems more likely that some type of selective immunosuppression of variable duration takes place in the form of desensitization or tolerance induced by continued exposure to just the right amount of antigen, with a greater or lesser stimulus resulting in persistence of hypersensitivity (119, 120). Alternatively, immunosuppression could result from the formation of antibodies to the same enterobacterial antigens with resulting selective suppression of lymphocyte-mediated immune reactions on the one hand or the protection of target cells from sensitized lymphocytes in a manner analogous to tumor enhancement on the other (121).

Although, within this hypothesis, the involvement of lymphocytes is assured, the question of whether they are T cells, B cells, or both is unanswered. Recently, this hypothesis has been discussed further by Walker and Isselbacher (122), and obviously the merit of any portion of it will be established only by research. In the meantime, it provides signposts to many potentially rewarding avenues of investigation in the continued effort to understand the etiology and pathogenesis of IBD.

Stobo and Shorter then presented some recent data from further studies of the mechanism of the cytotoxicity of colitis lymphocytes for colonic epithelial cells.

In an attempt to define better those mononuclear cells present in the peripheral blood of patients with idiopathic colitis that specifically lyse allogeneic colonic epithelial cells, Stobo and Shorter prepared subpopulations of mononuclear cells as follows: Mononuclear cells in the peripheral blood were initially isolated by centrifugation over a layer of ficoll–Hypaque. The washed mononuclear cells were then mixed with sheep red blood cells (SRBC) so that T cells bound the SRBC to their surfaces forming rosettes. Rosette-forming cells subsequently were isolated from cells that did not form rosettes by sedimentation over a density gradient. As demonstrated in Table 2, despite a relative

frequency of 93% T cells in the SRBC rosette-enriched fraction, there was a decrease by a factor of 27.6 in the cytotoxicity cells when compared to nonfractionated cells.,

As the effector cytotoxic cells thus did not appear to be contained within the bulk of SRBC rosette-forming or T cells, it was next determined whether the cytotoxic effector cells bore immunoglobulin (Ig) determinants on their surfaces, thus indicating that these cells could be classified as B cells. Mononuclear cells from the peripheral blood of patients with idiopathic colitis were suspended in RPMI 1640 medium and incubated at 37° C for 30 minutes in polystyrene Petri dishes, to which had been linked polyvalent antibody directed against human Ig. Cells not adhering to the Petri dish were washed off, and a rubber policeman was used gently to remove cells adhering to the Petri dish. The results are summarized in Table 2. Despite a depletion by a factor of 1.8 in immunoglobulin-bearing cells, when compared to unfractionated cells there was no decrease and indeed there was an increase in cytotoxicity of nonadherent cells. Adherent cells, on the other hand, despite a threefold increase in the relative frequency of immunoglobulin-bearing cells when compared to the control, manifested a decrease in cytotoxic potential. Thus, the cytotoxic cell in this system does not appear to belong to the defined, "classical" T- or B-cell populations.

That the cytotoxic cell, however, does bear a surface receptor for the Fc portion of Ig is demonstrated by the final series of experiments. Peripheral blood mononuclear cells from the patients were incubated in polystyrene Petri dishes coated with heat-aggregated human Ig. Adherent and nonadherent populations were removed in a manner similar to that described for the plates coated with antiimmunoglobulin. As Table 2 demonstrates, nonadherent cells were depleted of cytotoxic ability, but the adherent cells in contrast showed an increase by a factor of 1.6 in their cytotoxicity when compared to nonfractionated cells.

Thus it would appear that those cells from the peripheral blood of patients with idiopathic colitis that lyse allogeneic colonic epithelial cells may not belong to either the classical T- or B-cell populations but bear surface receptors that combine with the Fc portion of Ig. This finding should direct further approaches designed to elucidate mechanisms of colonic inflammation in patients with idiopathic colitis.

T- and B-Cell-Mediated Reactions and the Secretory System

Stobo then, in collaboration with **Tomasi,** presented a general review of T- and B-cell-mediated reactions and the secretory system in the gastrointestinal tract. Because several reviews of the secretory system had been published quite recently (123, 124), Stobo and Tomasi limited their presentation to a consideration of some even more recent studies, together with their possible roles in gastrointestinal immunity.

The first topic was that of structural studies. Secretory IgA (SIgA), with a molecular weight of 390,000, contains four types of polypeptide chains: four L, four H, one J, and one secretory component (SC). With respect to SC, the amino acid sequence of both human and bovine types has been determined for a limited N-terminal sequence. These proteins show about 85% homology in the first 15 residues and the two differences represent one base change (125). This is significant because earlier there had been some question as to whether the bovine and human proteins, designated as SC, were indeed analogous. Because the bovine protein is much easier to isolate, further studies with the bovine SC, particularly in regard to the characteristics of its binding with IgA, now appear to be justified. There is no significant homology between bovine SC and either L or H chains in the first 25 N-terminal residues.

Isotopically labeled human and bovine SC, when incubated with the sera of a variety of different mammalian species, complex specifically with the IgA in that serum. It appears, there-

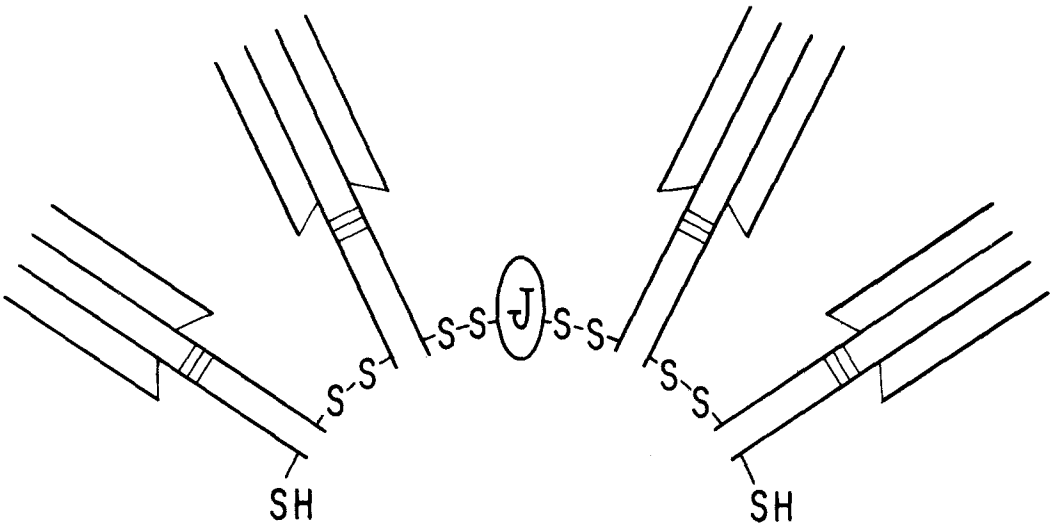

Fig 1. Schematic representation of structure of dimer and tetramer IgA. In the tetramer, J chain links two units to form a dimer and the other two units are bonded by disulfides not involving J chain.

fore, that there are highly specific attractions between SC and IgA. The complexing of SC with the human IgA_2 subclass, a major component of external secretions, appears to result in stabilization of the IgA, probably by means of a disulfide interchange. Thus, the binding of SC could stabilize the secretory IgA molecule in two ways: first, by "zippering" disulfide linkages between the L and H chains, as initially suggested by Jerry and associates (126), and, second, by conferring stability to enzymatic proteolysis, as previously reported by Tomasi and Czerwinski (127). Stability to proteolysis could, at least theoretically, facilitate the transport of IgA across the mucosal epithelial cell by protecting the IgA antibodies from intracellular degradation. Recent studies using HRP, which can be visualized by electron microscopy, have shown that SC is transported by a route similar to that of IgA but never reaches the intestinal lumen; it is apparently degraded in the lysosomal vacuoles within which it can be identified in the apical portion of the epithelial cells (Tourville D, Tomasi TB Jr: unpublished data).

The other unique polypeptide chain that is found not only in SIgA but also in serum IgM and polymeric serum IgA is the J (ie, joining) chain. There appears to be a single J chain per mole of 10*s* IgA dimer and one per mole of 19*s* IgM. It is attached to the penultimate cysteine residue and appears to join together the subunits of polymeric immunoglobulins. Studies of the N-terminal sequence as well as cysteine-containing peptides derived from the J chain indicate that it is not closely related to either L or H chains, though further and more extensive sequence work is necessary to reveal more subtle but significant homologies.

At first, it was thought that the J chain had sufficient cysteine residues to bond each of the H chains of the IgM pentamer. However, recalculation of the number of cysteine residues on the basis of newer measurements of the molecular weight of the J chain has indicated the presence of only 6–8 half-cysteines per mole of 15,000. This suggests that there are not sufficient cysteines to form symmetric bridges to each of the H chains in the IgM molecule. Studies by Halpern and Koshland (128) and by Tomasi (129) have indicated that the model shown in Figure 1 is correct. The J chain is synthesized in the plasma cell and is probably added to the IgA or IgM subunit close to its exit from

the cell. The J chain bonds two subunits together to form a dimer in both the IgM and IgA systems. The dimer apparently has a conformation that results in noncovalent interactions between other subunits and the formation of noncovalently linked polymers that subsequently undergo disulfide bonding. In favor of such a thesis is the observation that it is possible, by selective reduction of the IgM molecule with low concentrations of mercaptoethylamine, to produce a noncovalently linked pentamer (129). This dissociates in solvents such as urea into monomers and dimers, the dimer containing disulfide-bonded J chains. A similar phenomenon probably occurs with higher polymers (greater than 10*s*) in the IgA system.

Another intriguing, though incompletely understood, observation is the finding that IgA proteins have a tendency to complex with α_1-antitrypsin (α_1AT). Tomasi and Hauptman (130) have found that nearly all IgA myeloma proteins contain variable amounts of covalently bonded α_1AT. Laurell has observed that normal serum also contains a high-molecular-weight form of α_1AT that is bound to IgA (Laurell: Personal communication). However, secretory IgA does not contain α_1AT, though many secretions such as those of the gastrointestinal tract contain free α_1AT. The exact quantitative relationship between the concentration of α_1AT in serum and that in secretions has not been established, but Stobo indicated that appropriate experiments had been initiated. Stobo also stressed Tomasi's interesting observation that if one incubates a myeloma protein containing bound α_1AT (isolated from myeloma sera by affinity chromatography using an α_1AT antiserum) with intestinal fluid, the α_1AT is quickly removed from the IgM and now migrates electrophoretically in its free or unbound form. This reaction occurs within 15 minutes at 4° C and is, therefore, probably not enzymatic. These findings suggest the possibility that serum α_1AT is transported into the lumen of the bowel attached to IgA and is then released. One mechanism of release that is currently under investigation is a disulfide interchange reaction with secretory component. It has not yet been determined whether α_1AT, when attached to IgA, functions as an enzymatic inhibitor. If it does, it could play a role, in addition to SC, in protecting IgA during transport. If the postulated relationships between IgA and α_1AT prove valid, it would be important to determine carefully the α_1AT content of secretions of patients who were deficient in IgA and, conversely, the IgA content of secretions in individuals who lack α_1AT.

Stobo's second major topic concerned cellular studies. He noted that a major interest had developed recently in the distribution of various types of lymphocytes (T and B cells) at mucous membrane sites. The studies of Waldman and Henney (131) have shown that local cell-mediated immunity, probably mediated by T cells, can be elicited in the respiratory tract by local application of antigens. However, such local cell-mediated immunity has not been well demonstrated in the gastrointestinal tract.

Stobo first considered the cells in the lamina propria. Numerous studies have indicated that deposition of antigen directly onto mucous membranes leads to the local formation of antibodies, particularly of the IgA class. Experiments in several animal species and in man have shown that oral administration of antigen causes the production of circulating antibody and that systemic lymphoid tissues acquire the capacity to produce antibodies against ingested antigens. The class of serum antibodies produced after oral immunization has varied in different reports, from predominantly IgA (as detected in mice that have ingested ferritin) to a "systemic" type of response, in which IgM is formed initially and then IgG, as illustrated by oral administration of live, attenuated poliovirus in man. Since splenic plaque-forming cells can be identified following oral immunization with most antigens (132, 133), it seems likely that either free antigen or cells originating in the gastrointestinal tract and already committed

to antibody production (or both antigen and cells) reach the systemic system. It is not possible yet to make a single generalization regarding oral immunization with respect to the relative contributions of antibodies synthesized in cells found in three sites: cells located in the gastrointestinal tract, cells committed in the gastrointestinal tract but migrating to peripheral lymphoid tissues, or cells stimulated by absorbed antigen in peripheral sites such as the spleen. Evidence for the occurrence of all three events has been reported in different species with various antigens. It does appear likely, however, that the production in the serum of the IgA type of antibody elicited by a particular antigen depends more on the route of the administration of the antigen than on its chemical structure.

It has proved quite difficult to obtain lamina propria cells for studies involving both antibody formation and cellular immune reactions. Primarily this is because the presence of mucus results in the clumping of cells and also nonspecific hemolysis. Nevertheless, Robertson and Cooper (133) have reported studies concerning the lamina propria cells of rats immunized with SRBC both by the oral and the systemic routes. Lamina propria cells are able to respond to both intravenous and intrajejunal administration of this antigen, as evidenced by the formation of antibody-producing cells in the Jerne plaque technique. The response in the mesenteric nodes was particularly prominent in the orally immunized animals and was greatest in the spleen after intravenous administration of SRBC. Robertson and Cooper concluded that a definite local antibody response followed oral immunization but that this may have been augmented by cell migration from other sites, such as the spleen and mesenteric lymph nodes, into the lamina propria. This conclusion was based largely on the effect of splenectomy in greatly reducing the plaque-forming response in the lamina propria after intestinal immunization. At least in this system, there appears to be significant absorption of the intact antigen and, subsequently, migration of sensitized precursor cells from the spleen to the gastrointestinal tract.

Stobo then gave his attention to cells in Peyer's patches. Because it is much easier to obtain cells from Peyer's patches than from the lamina propria, there is much more information about the characteristics of the former. Moreover, interest in these cells was stimulated by the suggestion of Cooper and colleagues (134) that they may be the mammalian equivalent of the bursa of Fabricius of the chicken. This was based on various lines of evidence, but most directly on the observation that rabbits from which the gut-associated lymphoid tissue (GALT) had been excised were unable to form a primary antibody response. However, this was only manifest in animals in which irradiation had been followed by reconstitution with fetal liver; in other words, animals from which GALT had been removed could not be reconstituted in the same manner as sham animals. Stobo noted that more recent evidence in favor of Cooper and associates' hypothesis is the finding that Peyer's-patch cells either are not capable of producing a graft-*versus*-host reaction (135–137) or mediate the allogeneic effect (137), both of which are presumably T-cell functions that can be demonstrated easily in peripheral lymphoid tissues.

Against the idea that Peyer's patches are analogous to the bursa of Fabricius are the results of studies in mice using anti-θ antisera demonstrating that approximately 70% of the lymphocytes bear this T-cell surface marker. Also, the work of Joel and associates (138) shows that cells migrate directly from the thymus to Peyer's patches early in the neonatal period. Moreover, although graft-*versus*-host and allogeneic reactions cannot be demonstrated in studies of Peyer's-patch cells, there is evidence of other T-cell functions, such as the ability to generate carrier-specific helper cells. This suggests the possibility that only certain subpopulations of T cells may be represented in Peyer's patches. One complicating experimen-

tal factor in these studies has been the frequent presence of contaminating lamina propria cells that, for example, could be responsible for the carrier-specific cells.

Peyer's patches also contain B cells (about 30% in the adult animal, as demonstrated by surface immunoglobulin markers). However, after either oral or parenteral immunization, Peyer's patches do not contain detectable amounts of antigen (139) and do not possess plaque-forming cells (140, 141). Although *in vivo* immunization by any route does not lead to immunization of Peyer's patches, Stobo reported that stimulation by sheep cells *in vitro* using the Miscelle–Dutton culture technique resulted in a good primary response with Peyer's-patch cells (140); however, Kagnoff and Campbell (142) have reported that these cells were significantly inferior to spleen cells. Also, if Peyer's patches are removed from antigen-primed animals, a good secondary response can be elicited, which indicates that memory cells are present in the patches (140). Thus, the apparent inability of Peyer's patches to form antibodies when immunized *in vivo* may be explained on the basis of the absence of an antigen-trapping mechanism (139), and in this respect the patches differ from the lamina propria (143). It may be pertinent in this regard to note that Peyer's patches have no afferent lymphatics.

The study by Kagnoff and Campbell (142) has shown that Peyer's patches may lack an appropriate adherent-cell population (macrophages) and that antibody formation *in vitro* can be much increased by the addition of a macrophage-rich population or of β-mercaptoethanol. Moreover, there is evidence that patch cells will mediate an allograft reaction if adherent cells are added (142). The ability of patch cells to form antibodies *in vitro* (140) may be due to contamination of the patches with phagocytic cells derived from the lamina propria. Another possibility that would explain the difficulties in obtaining both T- and B-mediated reactions is the presence of a high density of suppressor cells. However, no information is presently available on the suppressor-cell population of Peyer's patches.

In summarizing the current information on Peyer's patches, Stobo stated that it appears that they are not strictly analogous to the bursa of Fabricius in that they contain large numbers of T cells that have migrated from the thymus. Moreover, the Peyer's patches, though present at birth, are poorly formed and appear to develop both T- and B-cell populations similar to those of peripheral lymphoid tissues. Although Peyer's patches contain antigen-sensitive cells, there are certain differences between the patches and typical peripheral lymphoid tissue, such as their inability to mediate a graft-*versus*-host or allogeneic reaction despite the presence of T cells and their relative failure to manifest a B-cell response when immunized *in vivo* and probably also *in vitro*. In large part, these differences may result from either the lack of antigen-trapping mechanisms and the absence of other cell types (macrophages) or the factors required for the induction of T- and B-cell functions (or both of these), though the presence of a high density of suppressor cells has not been excluded.

Stobo next discussed cell migration patterns. He pointed out that another potentially important area in the immune response to orally ingested antigens concerns the recirculation pattern of lymphocytes. Gowans and Knight (144) have noted that small lymphocytes obtained from the thoracic duct lymph, when injected intravenously into rats, localized in many peripheral lymphoid tissues including the gut, predominantly in Peyer's patches. In contrast, the large lymphocytes in these preparations localized selectively in the gastrointestinal tract at various sites, including both Peyer's patches and lamina propria. By labeling lymphocytes with thymidine, Griscelli and coworkers (145) found that mesenteric lymph nodes and thoracic-duct lymph node cells showed marked homing to the lymphoid tissue adjacent to the intestine, whereas cells from peripheral lymph

nodes accumulated preferentially in peripheral lymphoid tissues. They also found that lymph node cells obtained from donors immunized with a specific antigen showed a greater tendency to accumulate in a lymph node containing that antigen than in the contralateral node. These latter experiments indicated that cells that had been sensitized previously with antigen tended to home to areas containing that antigen. This suggested that the homing pattern seen in the gut may be a consequence of the prior sensitization of cells derived from the thoracic duct or mesenteric lymph nodes by antigens commonly found in the gut. Work by Moore and Hall (146), who used labeled lymphocytes derived from the lymph of the thoracic duct in rats, has provided a clear indication that the localization occurs in subcutaneous implants of sterile fetal gut. Because it is presumed that the fetal gut was antigen free, these studies seem to have excluded antigen as being totally responsible for the homing patterns of lymphocytes. However, it is likely that, in the intact animal, both antigen-determined localization and specific homing properties of the lymphoid cells are important.

The nature of the factor or factors (other than antigen) determining homing is unknown, but homing could be attributable to specific surface receptors that interact with some element in the stroma of the gastrointestinal tract. Alternatively, there could be random seeding of cells followed by selective proliferation of certain cells in the gastrointestinal tract due to the local environment, as suggested by Craig and Cebra (147). These authors also showed that Peyer's-patch cells could be precursors of lamina propria IgA cells. Thus, rabbit Peyer's-patch cells, on injection, populated both the gastrointestinal tract and the spleen with lymphoid cells that differentiated into IgA plasma cells. Similar cell inocula derived from peripheral lymphoid tissues of the same animals were not able to seed the gut and gave rise in the spleen and peripheral lymphoid tissues to IgM and IgG cells. Thus, the Peyer's-patch cells seem to be precommitted to the production of IgA and also to possess the capacity to home specifically to the gut-associated lymphoid tissues. Whether specific homing or random seeding followed by selective proliferation occurs, it follows from this discussion that certain lymphoid cells and not others have the ability to colonize the gastrointestinal tract.

Stobo's discussion then took up the matter of T cells and IgA. He noted that several lines of evidence suggest a distinct relationship between IgA and T cells. Arnason and associates (148) found that in thymectomized rats there was a distinct deficiency in an immunoglobulin, which they termed IgX, and that both IgM and IgG remained essentially unchanged, compared to nonthymectomized controls of the same age. Thymectomized rats also showed a selective defect in their ability to form antibodies against bovine serum albumin in the IgX class. Since the mobility of IgX was similar to that of human IgA and was not found in the sera of normal rats less than 6 weeks of age, it was thought that the defect in thymectomized rats was in the IgA class rather than in the IgG or IgM. Clough and associates (149) showed that in neonatally thymectomized rabbits the antihaptene antibody response to arsanilic acid–bovine serum albumin (BSA) was strikingly diminished in the IgA class but not in IgG or IgM. It was proposed that, at least for certain antigens, the serum IgA antibody response is more dependent on T cells than the antibody responses in other immunoglobulin classes.

Clinical observations also suggest a relationship between T-cell defects and a selective deficiency of IgA. For example, patients with hereditary telangiectasia have a T-cell defect, perhaps on the basis of a thymic abnormality although this has not been proven, and the majority of these patients have a selective deficiency of IgA. Schiff and coworkers (150), in a study of IgA-deficient patients, found that the number of T cells, as measured by the SRBC rosette technique, was significantly depressed in patients with ataxia–telangiectasia and isolated

IgA deficiency. Others have reported reduced and variable responses to phytohemagglutinin (PHA) in patients with IgA deficiency (151). An intriguing question concerns the possibility that in the gut there exist specific T cells that collaborate in the IgA response; that is, there are specific helper cells for each immunoglobulin class and patients with IgA deficiency have an abnormality in their T cells rather than in their B cells. This possibility is also suggested by the finding that the majority of patients with IgA deficiency have normal numbers of circulating B cells with surface IgA (152) and that these cells are also able to synthesize IgA if stimulated by pokeweed mitogens (153).

Stobo's third and final topic was the mechanism of action of IgA antibodies. He emphasized the substantial evidence that had accumulated to indicate that secretory antibodies can neutralize viruses at their portal of entry (154). This concept results mainly from studies with respiratory tract viruses and it has been shown that resistance to infection is correlated better with titers of antibodies in secretions than with those in serum. Techniques such as aerosol immunization, which elicit efficient production of mucosal antibodies, are more effective in protection against infection than systemic immunization, which usually results in high serum titer, but only low levels of antibodies in secretions. The studies of Ogra (155) concerning the poliomyelitis virus suggest that the same principles apply to viral infections and immunizations via the oral route. Although secretory antibody appears to be of key importance in resistance to infection, once a virus colonizes a mucous membrane the complex mechanisms involved in recovery are set into motion; these mechanisms probably involve the cooperative participation of many factors, including secretory antibodies, serum antibodies, cellular immunity, and certain nonimmune substances such as interferon.

Although it is clear that secretory antibodies of local origin are effective in viral systems, the role of local immunity in bacterial infections is less clear. One of the major problems is that SIgA antibodies do not fix complement, at least by the classical pathway, and it is generally believed that the lysis of a bacteria by antibody requires activation of complement. However, aggregated human IgA myelomas are able to fix complement via the alternate pathway or C3 shunt (156). This pathway of fixation bypasses the early components (C1, C4, C2) in the complement sequence and initiates fixation at C3. However, whether specific antibodies of the IgA class will activate the shunt has not been determined. Also, the question of whether there are sufficient concentrations of the shunt components (such as properdin and C3 proactivator) in external secretions, so that this mechanism can operate at the mucosal level, has not been answered. It is known that C1 is synthesized in the intestinal epithelial cell and that macrophages from a number of sources are able to synthesize most of the other components of the classical pathway, but little is known about the sites of synthesis of properdin, C3 proactivator, and other components of the alternate pathway. Further work is needed in this important area.

A potentially important finding relating to the antibacterial activity of immunoglobulins in external secretions is the report by Williams and Gibbons (157) that antibodies of the IgG and SIgA class are capable of inhibiting the adherence of bacteria to epithelial cells. For example, SIgA antibodies directed against *Streptococcus salivarius* inhibit this bacterium from attaching to the surface of isolated human buccal epithelial cells. Fubara and Freter (158) have demonstrated a similar inhibition, by locally produced antibodies, of the adherence of *Vibrio cholerae* to the small-intestinal epithelium in rabbits. According to this theory, for most bacteria to colonize a mucosal organ effectively, the organisms first must adhere to the mucosal epithelial cell. If the initial attachment is prevented (by action of antibody), the organisms would be removed by the "cleansing function" of secretions before sufficient num-

bers were generated to have a detrimental effect. Although the antiadherence hypothesis of locally produced antibodies on antibacterial immunity is attractive, further work is needed before it can be generally accepted.

Another potentially important area concerns the role of the secretory system in preventing the mucosal absorption of nonviable materials that are inhaled or ingested. Suspicions for such a role for the secretory system came from studies of patients with a selective deficiency of IgA. These patients have high titers of antibodies against milk proteins in their serum (159), and particularly prominent are antibodies (of the IgG class) reactive with bovine milk IgM (160). Such antibodies are very rarely found in adults. More concrete experimental evidence has been provided recently by the elegant studies of Walker and associates (161) in rats; these workers showed that the extent of absorption of proteins such as BSA and HRP can be influenced by preimmunization with these antigens. Adult rats were immunized either orally or systemically with one antigen, and the degree of absorption of both proteins was determined using the inverted gut-sac technique and radiolabeled proteins. Animals immunized with BSA absorbed significantly less BSA than controls, but there was no effect of immunization on the absorption of HRP. On the other hand, immunization with HRP diminished the absorption of HRP but not of BSA. Thus, the effects of immunization on absorption were antigen specific. These results suggest the possibility that the clinical associations noted between IgA deficiency and autoimmune disease (162) could result from the continued absorption of antigens via the gastrointestinal tract, and possibly other routes, leading to immune complexlike diseases. In this regard, antibodies to bovine γ-globulin as well as circulating antigen (presumably in immune complexes) have been described in certain patients with disseminated lupus erythematosus (163). Another possibility is that patients with defective secretory systems absorb larger amounts of exogenous antigens that cross-react with certain self-antigens. This could lead to autoimmune diseases and explain the clinical association noted between these diseases and IgA deficiency. Such suggestions are intriguing, but at the present time they are highly speculative.

Finally, Stobo noted that IgE, like IgA, may be a secretory immunoglobulin. Evidence derived from plasma cell counts in various organs using the fluorescent antibody technique suggests that IgE is synthesized predominantly in secretory sites such as the respiratory and gastrointestinal tracts. Although IgE has been implicated primarily in immediate-type allergic reactions, normally it probably serves some physiologic functions. For example, it has been suggested that in the gastrointestinal tract IgE antibodies directed against intestinal parasites may be responsible for the liberation of histamine and other mediators from mast cells in the lamina propria, which leads to the self-expulsion of the parasite. Although IgE has been suggested as having a role in the resistance against viral and bacterial infections on the basis of cases of repeated infections associated with IgE deficiency, there have been several reports of patients who selectively lack IgE and apparently do not suffer from recurrent infections. No conclusions can be drawn regarding the role of IgE in host defense in the normal individual.

REFERENCES: PART I

1. Mitchell DN, Rees RJW: Agent transmissible from Crohn's disease tissue. Lancet 2:168–171, 1970
2. Cave DR, Mitchell DN, Kane SP, Brooke BN: Further animal evidence of a transmissible agent in Crohn's disease. Lancet 2:1120–1122, 1973
3. Bolton PM, Owen E, Heatley RV, Williams WJ: Negative findings in laboratory animals for a transmissible agent in Crohn's disease. Lancet 2:1122–1124, 1973
4. Bargen JA: Experimental studies on the etiology of chronic ulcerative colitis: Preliminary report. JAMA 83:332–336, 1924

5. Hurst AF: Ulcerative colitis. Guys Hosp Rep 71:26–41, 1921
6. Mackie TT: Ulcerative colitis due to chronic infection with Flexner-y bacillus. JAMA 98:1706–1710, 1932
7. Felsen J, Wolarsky W: Acute and chronic bacillary dysentery and chronic ulcerative colitis. JAMA 153:1069–1072, 1953
8. Dragstedt LR, Dack GM, Kirsner JB: Chronic ulcerative colitis: a summary of evidence implicating *Bacterium necrophorum* as an etiologic agent. Ann Surg 114:653–661, 1941
9. Virginia Polytechnic Institute, Anaerobic Laboratory: Outline of Clinical Methods in Anaerobic Bacteriology. Blacksburg, Virginia, Virginia Polytechnic Institute, 1972
10. DuPont HL, Formal SB, Hornick RB, Snyder MJ, Libonati JP, Sheahan DG, LaBrec EH, Kalas JP: Pathogenesis of *Escherichia coli* diarrhea. N Engl J Med 285:1–9, 1971
11. Cooke EM: Properties of strains of *Escherichia coli* isolated from the faeces of patients with ulcerative colitis, patients with acute diarrhoea and normal persons. J Pathol Bacteriol 95:101–113, 1968
12. Thayer WR Jr, Brown M, Sangree MH, Katz J, Hersh T: *Escherichia coli* 0:14 and colon hemagglutinating antibodies in inflammatory bowel disease. Gastroenterology 57:311–318, 1969
13. Lagercrantz R, Perlmann P, Hammarström S: Immunological studies in ulcerative colitis. V. Family studies. Gastroenterology 60:381–389, 1971
14. Kirsner JB, Palmer WL: Ulcerative colitis: Considerations of its etiology and treatment. JAMA 155:341–346, 1954
15. Seneca H, Henderson E: Normal intestinal bacteria in ulcerative colitis. Gastroenterology 15:34–39, 1950
16. Cooke EM: A quantitative comparison of the faecal flora of patients with ulcerative colitis and that of normal persons. J Pathol Bacteriol 94:439–444, 1967
17. Gorbach SL, Nahas L, Plaut AG, Weinstein L, Patterson JF, Levitan R: Studies of intestinal microflora. V. Fecal microbial ecology in ulcerative colitis and regional enteritis: relationship to severity of disease and chemotherapy. Gastroenterology 54:575–587, 1968
18. Gorbach SL: Intestinal microflora. Gastroenterology 60:1110–1129, 1971
19. Donaldson RM, McConnell C, Deffner N: Bacteriological studies in clinical and experimental blind loop syndromes. Gastroenterology 52:1082, 1967 (Abstract)
20. Krone CL, Theodor E, Sleisenger MH, Jeffries GH: Studies on the pathogenesis of malabsorption: lipid hydrolysis and micelle formation in the intestinal lumen. Medicine 47:89–106, 1968
21. Gorbach SL, Tabaqchali S: Bacteria, bile, and the small bowel. Gut 10:963–972, 1969
22. Vince A, Dyer NH, O'Grady FW, Dawson AM: Bacteriological studies in Crohn's disease. J Med Microbiol 5:219–229, 1972
23. Moertel CG, Bargen JA: A critical analysis of the use of salicylazosulfapyridine in chronic ulcerative colitis. Ann Intern Med 51:879–889, 1959
24. Lennard-Jones JE, Longmore AL, Newell AC, Wilson CWE, Jones FA: An assessment of prednisone, salazopyrin, and topical hydrocortisone hemisuccinate used as outpatient treatment for ulcerative colitis. Gut 1:217–222, 1960
25. Baron JH, Connell AM, Lennard-Jones JE, Jones FA: Sulphasalazine and salicylazosulphadimidine in ulcerative colitis. Lancet 1:1094–1096, 1962
26. Truelove SC, Watkinson G, Draper G: Comparison of corticosteroid and sulphasalazine therapy in ulcerative colitis. Br Med J 2:1708–1711, 1962
27. Svartz N: Ett nytt sulfonamidpreparat: förelöpande meddelande. [A new sulphonamide preparation.] Nord Med 9:554, 1941
28. Cooke EM: Faecal flora of patients with ulcerative colitis during treatment with salicylazosulphapyridine. Gut 10:565–568, 1969
29. Peppercorn MA, Goldman P: The role of intestinal bacteria in the metabolism of salicylazosulfapyridine. J Pharmacol Exp Ther 181:555–562, 1972
30. Attebery HR, Sutter VL, Finegold SM: Normal human intestinal flora. Anaerobic Bacteria, Role in Disease. A Balows, RM DeHaan, VR Dowell Jr, LB Guze (eds). Springfield, Illinois, Charles C Thomas, 1974, pp 81–97

31. Moore WEC, Holdeman LV: Identification of anaerobic bacteria. Am J Clin Nutr 25:1306–1313, 1972
32. Moore WEC, Cato EP, Holdeman LV: Anaerobic bacteria of the gastrointestinal flora and their occurrence in clinical infections. J Infect Dis 119:641–649, 1969
33. Gorbach SL, Thadepalli H, Norsen J: Anaerobic microorganisms in intraabdominal infections. Anaerobic Bacteria, Role in Disease. A Balows, RM DeHaan, VR Dowell Jr, LB Guze (eds). Springfield, Illinois, Charles C Thomas, 1974, pp 399–407
34. Dowell VR Jr, Hawkins TM: Laboratory Methods in Anaerobic Bacteriology: CDC Laboratory Manual. (DHEW Publication No. 74-8272.) Atlanta, Georgia, Center for Disease Control, Jan 1974, p 28
35. Savage DC, Dubos R, Schaedler RW: The gastrointestinal epithelium and its autochthonous bacterial flora. J Exp Med 127:67–76, 1968
36. Savage DC: Associations of indigenous microorganisms with gastrointestinal mucosal epithelia. Am J Clin Nutr 23:1495–1501, 1970
37. Savage DC: Associations and physiological interactions of indigenous microorganisms and gastrointestinal epithelia. Am J Clin Nutr 25:1372–1379, 1972
38. Takeuchi A, Zeller JA: Ultrastructural identification of spirochetes and flagellated microbes at the brush border of the large intestinal epithelium of the rhesus monkey. Infect Immun 6:1008–1018, 1972
39. Leach WD, Lee A, Stubbs RP: Localization of bacteria in the gastrointestinal tract: A possible explanation of intestinal spirochaetosis. Infect Immun 7:961–972, 1973
40. Kalser MH, Cohen R, Arteaga I, Yawn E, Mayoral L, Hoffert WR, Frazier D: Normal viral and bacterial flora of the human small and large intestine. N Engl J Med 274:500–505; 558–563, 1966
41. Victor RG, Kirsner JB, Palmer WL: Failure to induce ulcerative colitis experimentally with filtrates of feces and rectal mucosa: A preliminary report. Gastroenterology 14:398–400, 1950
42. Farmer GW, Vincent MM, Fuccillo DA, Horta-Barbosa L, Ritman S, Sever JL, Gitnick GL: Viral investigations in ulcerative colitis and regional enteritis. Gastroenterology 65:8–18, 1973
43. Meynell GG: Antibacterial mechanisms of the mouse gut. II. The role of Eh and volatile fatty acids in the normal gut. Br J Exp Pathol 44:209–219, 1963
44. Hentges DJ: Inhibition of *Shigella flexneri* by the normal intestinal flora. II. Mechanisms of inhibition by coliform organisms. J Bacteriol 97:513–517, 1969
45. Brooke BN, Slaney G: Portal bacteraemia in ulcerative colitis. Lancet 1:1206–1207, 1958
46. Gilbert AP, Ravelo GZ: Serum proteins and endotoxins in chronic ulcerative colitis. Dis Colon Rectum 11:124–126, 1968
47. Bull DM, Ignaczak TF: Enterobacterial common antigen-induced lymphocyte reactivity in inflammatory bowel disease. Gastroenterology 64:43–50, 1973
48. Perlmann P, Broberger O: In vitro studies of ulcerative colitis. II. Cytotoxic action of white blood cells from patients on human fetal colon cells. J Exp Med 117:717–733, 1963
49. Watson DW, Quigley A, Bolt RJ: Effect of lymphocytes from patients with ulcerative colitis on human adult colon epithelial cells. Gastroenterology 51:985–993, 1966
50. Shorter RG, Spencer RJ, Huizenga KA, Hallenbeck GA: Inhibition of in vitro cytotoxicity of lymphocytes from patients with ulcerative colitis and granulomatous colitis for allogeneic colonic epithelial cells using horse anti-human thymus serum. Gastroenterology 54:227–231, 1968
51. Drasar BS, Hill MJ: Intestinal bacteria and cancer. Am J Clin Nutr 25:1399–1404, 1972
52. Reiner L, Schlesinger MJ, Miller GM: Pseudomembranous colitis following aureomycin and chloramphenicol. Arch Pathol 54:39–67, 1952
53. Cohen LE, McNeill CJ, Wells RF: Clindamycin-associated colitis. JAMA 223:1379–1380, 1973
54. Scott AJ, Nicholson GI, Kerr AR: Lincomycin as a cause of pseudomembranous colitis. Lancet 2:1232–1234, 1973
55. Watt J, Marcus R: Ulcerative colitis in the guinea-pig caused by seaweed extract. J Pharm Pharmacol 21 (Suppl): 187s–188s, 1969
56. Watt J, Marcus R: Carrageenan-induced ul-

ceration of the large intestine in the guinea pig. Gut 12:164–171, 1971
57. Di Rosa M: Biological properties of carrageenan. J Pharm Pharmacol 24:89–102, 1972
58. Grasso P, Sharratt M, Carpanini FMB, Gangolli SD: Studies on carrageenan and large-bowel ulceration in mammals. Food Cosmet Toxicol 11:555–564, 1973
59. Benitz KF, Golberg L, Coulston F: Intestinal effects of carrageenans in the rhesus monkey (*Macaca mulatta*). Food Cosmet Toxicol 11:565–575, 1973
60. Teem MV, Phillips SF: Perfusion of the hamster jejunum with conjugated and unconjugated bile acids. Inhibition of water absorption and effects on morphology. Gastroenterology 62:261–267, 1972
61. Low-Beer TS, Schneider RE, Dobbins WO: Morphological changes of the small-intestinal mucosa of guinea pig and hamster following incubation *in vitro* and perfusion *in vivo* with unconjugated bile salts. Gut 11:486–492, 1970
62. Tabaqchali S, Hatzioannou J, Booth CC: Bile-salt deconjugation and steatorrhoea in patients with the stagnant-loop syndrome. Lancet 2:12–16, 1968
63. Shiner M: Effect of bile acids on the small intestinal mucosa in man and rats: a light and electron microscope study. Bile Salt Metabolism. L Schiff, JB Carey Jr, J Dietschy (eds). Springfield, Illinois, Charles C Thomas, 1969, pp 41–55
64. Ament ME, Shimoda SS, Saunders DR, Rubin CE: Pathogenesis of steatorrhea in three cases of small intestinal stasis syndrome. Gastroenterology 63:728–747, 1972
65. Northfield TC, McColl I: Postprandial concentrations of free and conjugated bile acids down the length of the normal human small intestine. Gut 14:513–518, 1973
66. Ammon HV, Phillips SF: Inhibition of ileal water absorption by intraluminal fatty acids: Influence of chain length, hydroxylation, and conjugation of fatty acids. J Clin Invest 53:205–210, 1974
67. Reynell PC, Spray GH: Chemical gastroenteritis in the rat. Gastroenterology 34:867–873, 1958
68. James AT, Webb JPW, Kellock TD: The occurrence of unusual fatty acids in faecal lipids from human beings with normal and abnormal fat absorption. Biochem J 78:333–339, 1961
69. Thomas PJ: In vitro conversion of oleic acid to hydroxy stearic acid by intestinal bacteria. Clin Res 18:609, 1970 (Abstract)
70. Shorter RG, Huizenga KA, Spencer RJ: A working hypothesis for the etiology and pathogenesis of nonspecific inflammatory bowel disease (editorial). Am J Dig Dis 17:1024–1032, 1972
71. Fordtran JS, Rector FC Jr, Ewton MF, Soter N, Kinney J: Permeability characteristics of the human small intestine. J Clin Invest 44:1935–1944, 1965
72. Billich CO, Levitan R: Effects of sodium concentration and osmolality on water and electrolyte absorption from the intact human colon. J Clin Invest 48:1336–1347, 1969
73. Loehry CA, Kingham J, Baker J: Small intestinal permeability in animals and man. Gut 14:683–688, 1973
74. Bright-Asare P, Binder HJ: Stimulation of colonic secretion of water and electrolytes by hydroxy fatty acids. Gastroenterology 64:81–88, 1973
75. Warshaw AL, Walker WA, Cornell R, Isselbacher KJ: Small intestinal permeability to macromolecules: Transmission of horseradish peroxidase into mesenteric lymph and portal blood. Lab Invest 25:675–684, 1971
76. Warshaw AL, Walker WA, Isselbacher KJ: Protein uptake by the intestine: Evidence for absorption of intact macromolecules. Gastroenterology 66:987–992, 1974
77. Oberhelman HA Jr, Kohatsu S, Taylor KB, Kivel RM: Diverting ileostomy in the surgical management of Crohn's disease of the colon. Am J Surg 115:231–239, 1968
78. Hofmann AF, Poley JR: Role of bile acid malabsorption in pathogenesis of diarrhea and steatorrhea in patients with ileal resection. I. Response to cholestyramine or replacement of dietary long chain triglyceride by medium chain triglyceride. Gastroenterology 62:918–934, 1972
79. Phillips SF, Giller J: The contribution of the colon to electrolyte and water conservation in man. J Lab Clin Med 81:733–746, 1973
80. LaRusso NF, Korman MG, Hoffman NE, Hofmann AF: Dynamics of the enterohepatic

circulation of bile acids: Postprandial serum concentrations of conjugates of cholic acid in health, cholecystectomized patients, and patients with bile acid malabsorption. N Engl J Med 291:689–692, 1974
81. Dietschy JM, Salomon HS, Siperstein MD: Bile acid metabolism. I. Studies on the mechanisms of intestinal transport. J Clin Invest 45:832–846, 1966
82. Lack L, Weiner IM: Intestinal bile salt transport: Structure-activity relationships and other properties. Am J Physiol 210:1142–1152, 1966
83. Low-Beer TS, Tyor MP, Lack L: Effects of sulfation of taurolithocholic and glycolithocholic acids on their intestinal transport. Gastroenterology 56:721–726, 1969
84. Fromm H, Thomas PJ, Hofmann AF: Sensitivity and specificity in tests of distal ileal function: Prospective comparison of bile acid and vitamin B_{12} absorption in ileal resection patients. Gastroenterology 64:1077–1090, 1973
85. Krag E, Phillips SF: Active and passive bile acid absorption in man: Perfusion studies of the ileum and jejunum. J Clin Invest 53:1686–1694, 1974
86. Northfield TC, Hofmann AF: Biliary lipid output during three meals and an overnight fast. I. Relationship to bile acid pool size and cholesterol saturation of bile in gallstone and control subjects. Gut 16:1–11, 1975
87. Vlahcevic ZR, Miller JR, Farrar JT, Swell L: Kinetics and pool size of primary bile acids in man. Gastroenterology 61:85–90, 1971
88. Meihoff WE, Kern F Jr: Bile salt malabsorption in regional ileitis, ileal resection, and mannitol-induced diarrhea. J Clin Invest 47:261–267, 1968
89. Fromm H, Hofmann AF: Breath test for altered bile-acid metabolism. Lancet 2:621–625, 1971
90. Mekhjian HS, Phillips SF, Hofmann AF: Colonic secretion of water and electrolytes induced by bile acids: Perfusion studies in man. J Clin Invest 50:1569–1577, 1971
91. Woodbury JF, Kern F Jr: Fecal excretion of bile acids: a new technique for studying bile acid kinetics in patients with ileal resection. J Clin Invest 50:2531–2540, 1971
92. Balistreri WF, Partin JC, Schubert WK: Bile acid malabsorption in infantile diarrhea. Gastroenterology 66:832, 1974 (Abstract)
93. Hofmann AF, Danzinger RG, Hoffman NE, Klein PD, Berngruber OW, Szczepanik PA: Validation and comparison of deuterium/tritium and carbon-13/carbon-14 in clinical studies of bile acid metabolism. Proceedings of a Seminar on the Use of Stable Isotopes in Clinical Pharmacology, University of Chicago, November 10–11, 1971 (Conf-711115). PD Klein, LJ Roth (eds). Springfield, Virginia, National Technical Information Service, United States Department of Commerce, August 1972, pp 37–51
94. Franz B, Bode JC: Total plasma bile acid concentration in chronic hepatitis and cirrhosis: Fasting values and effect of intraduodenal bile salt administration. Klin Wochenschr 52:522–526, 1974
95. Pedersen L, Arnfred T, Hess Thaysen E: Rapid screening of increased bile acid deconjugation and bile acid malabsorption by means of the glycine-1-[^{14}C] cholylglycine assay. Scand J Gastroenterol 8:665–672, 1973
96. Simmonds WJ, Korman MG, Go VLW, Hofmann AF: Radioimmunoassay of conjugated cholyl bile acids in serum. Gastroenterology 65:705–711, 1973
97. Schilling RF: Intrinsic factor studies. II. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12}. J Lab Clin Med 42:860–866, 1953
98. Reed RM, Tartar HV: A study of salts of higher alkyl sulfonic acids. J Am Chem Soc 58:322–332, 1936
99. Gorbach SL, Plaut AG, Nahas L, Weinstein L, Spanknebel G, Levitan R: Studies of intestinal microflora. II. Microorganisms of the small intestine and their relations to oral and fecal flora. Gastroenterology 53:856–867, 1967
100. Bond JH Jr, Levitt MD: Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate absorption: study of partially gastrectomized patients. J Clin Invest 51:1219–1225, 1972
101. Salmon PR, Ajdukiewicz AB, Clamp JR, Read AE: Mannitol utilization in the stagnant loop syndrome. Gut 11:1065, 1970 (Abstract)

102. Soloway RD, Summerskill WHJ, Baggenstoss AH, Geall MG, Gitnick GL, Elveback LR, Schoenfield LJ: Clinical, biochemical, and histological remission of severe chronic active liver disease: A controlled study of treatments and early prognosis. Gastroenterology 63:820–833, 1972
103. Korman MG, Hofmann AF, Summerskill WHJ: Assessment of activity in chronic active liver disease: Serum bile acids compared with conventional tests and histology. N Engl J Med 290:1399–1402, 1974
104. Watson DW: Ulcerative colitis, autoimmune epiphenomena, and colonic cancer. Cancer 34 Suppl:867–871, 1974
105. Kraft SC, Kirsner JB: The immunology of ulcerative colitis and Crohn's disease: Clinical and humoral aspects. Inflammatory Bowel Disease. JB Kirsner, RG Shorter (eds). Philadelphia, Lea & Febiger, 1975, pp 60–80
106. Watson DW, Shorter RG: The immunology of ulcerative colitis and Crohn's disease: Cell-mediated immune responses. Inflammatory Bowel Disease. JB Kirsner, RG Shorter (eds). Philadelphia, Lea & Febiger, 1975, pp 81–98
107. Shorter RG, Cardoza M, Spencer RJ, Huizenga KA: Further studies of in vitro cytotoxicity of lymphocytes from patients with ulcerative and granulomatous colitis for allogeneic colonic epithelial cells, including the effects of colectomy. Gastroenterology 56:304–309, 1969
108. Shorter RG, Cardoza MR, ReMine SG, Spencer RJ, Huizenga KA: Modification of in vitro cytotoxicity of lymphocytes from patients with chronic ulcerative colitis or granulomatous colitis for allogenic colonic epithelial cells. Gastroenterology 58:692–698, 1970
109. Shorter RG, Huizenga KA, ReMine SG, Spencer RJ: Effects of preliminary incubation of lymphocytes with serum on their cytotoxicity for colonic epithelial cells. Gastroenterology 58:843–850, 1970
110. Shorter RG, Huizenga KA, Spencer RJ, Aas J, Guy SK: Inflammatory bowel disease: Cytophilic antibody and the cytotoxicity of lymphocytes for colonic cells in vitro. Am J Dig Dis 16:673–679, 1971
111. Shorter RG, Huizenga KA, Spencer RJ, Guy SK: Inflammatory bowel disease: the role of lymphotoxin in the cytotoxicity of lymphocytes for colonic epithelial cells. Am J Dig Dis 17:689–696, 1972
112. Shorter RG, Huizenga KA, Spencer RJ, Weedon D: Lymphotoxin in nonspecific inflammatory bowel disease lymphocytes. Am J Dig Dis 18:79–83, 1973
113. Singer HC, Anderson JGD, Frischer H, Kirsner JB: Familial aspects of inflammatory bowel disease. Gastroenterology 61:423–430, 1971
114. Miyakawa M: The lymphatic system of germfree guinea pigs. Ann NY Acad Sci 78:221–236, 1959
115. Acheson ED, Truelove SC: Early weaning in the aetiology of ulcerative colitis: A study of feeding in infancy in cases and controls. Br Med J 2:929–933, 1961
116. Staley TE, Jones EW, Corley LD: Attachment and penetration of *Escherichia coli* into intestinal epithelium of the ileum in newborn pigs. Am J Pathol 56:371–392, 1969
117. Watson DW, Martucci R: Inhibition of peripheral leucocyte migration by E. coli 0119:B14 antigen in normal individuals and patients with chronic inflammatory bowel disease (CIBD). Gastroenterology 64:818, 1973 (Abstract)
118. Neter E: Endotoxins and the immune response. Curr Top Microbiol Immunol 47:82–124, 1969
119. Uhr JW, Pappenheimer AM Jr: Delayed hypersensitivity. III. Specific desensitization of guinea pigs sensitized to protein antigens. J Exp Med 108:891–904, 1958
120. Asherson GL: Antigen-mediated depression of delayed hypersensitivity. Br Med Bull 23:24–29, 1967
121. Snell GD, Winn HJ, Stimpfling JH, Parker SJ: Depression by antibody of the immune response to homografts and its role in immunological enhancement. J Exp Med 112:293–314, 1960
122. Walker WA, Isselbacher KJ: Uptake and transport of macromolecules by the intestine: Possible role in clinical disorders. Gastroenterology 67:531–550, 1974
123. Tomasi TB Jr, Bienenstock J: Secretory immunoglobulins. Adv Immunol 9:1–96, 1968
124. Tomasi TB Jr, Grey HM: Structure and func-

tion of immunoglobulin A. Prog Allergy 16:81–213, 1972
125. Cunningham-Rundles C, Lamm ME, Franklin EC: Studies on the primary structure of human secretory component. The Immunoglobulin A System. J Mestecky, AR Lawton (eds). New York, Plenum Publishing Corporation, 1974, pp 241–243
126. Jerry LM, Kunkel HG, Adams L: Stabilization of dissociable IgA2 proteins by secretory component. J Immunol 109:275–283, 1972
127. Tomasi TB Jr, Czerwinski DS: The secretory IgA system. Birth Defects 4:270–275, 1968
128. Halpern MS, Koshland ME: Novel subunit in secretory IgA. Nature 228:1276–1278, 1970
129. Tomasi TB Jr: Production of a noncovalently bonded pentamer of immunoglobulin M: Relationship to J chain. Proc Natl Acad Sci USA 70:3410–3414, 1973
130. Tomasi TB Jr, Hauptman SP: The binding of α-1 antitrypsin to human IgA. J Immunol 112:2274–2277, 1974
131. Waldman RH, Henney CS: Cell-mediated immunity and antibody responses in the respiratory tract after local and systemic immunization. J Exp Med 134:482–494, 1971
132. Bazin H, Levi G, Doria G: Predominant contribution of IgA antibody-forming cells to an immune response detected in extraintestinal lymphoid tissues of germ-free mice exposed to antigen by the oral route. J Immunol 105:1049–1051, 1970
133. Robertson PW, Cooper GN: Immune responses in intestinal tissues to particulate antigens: Plaque-forming and rosette-forming cell responses in rats. Aust J Exp Biol Med Sci 50:703–714, 1972
134. Cooper MD, Perey DY, Gabrielsen AE, Sutherland DER, McKneally MF, Good RA: Production of an antibody deficiency syndrome in rabbits by neonatal removal of their organized intestinal lymphoid tissues. Int Arch Allergy Appl Immunol 33:65–88, 1968
135. Perey DYE, Guttmann RD: Peyer's patch cells. Absence of graft-*versus*-host reactivity in mice and rats. Lab Invest 27:427–433, 1972
136. Heim LR, McGarry, MP, Montgomery JR, Trentin JJ, South MA: Potentials of spleen, lymph node, and Peyer's patches to reconstitute lymphoid tissue and produce graft-versus-host reaction. Transplantation 14:418–423, 1972
137. Katz DH, Perey DYE: Lymphocytes in Peyer's patches of the mouse: Analysis of the constituent cells in terms of their capacities to mediate functions of mature T and B lymphocytes. J Immunol 111:1507–1513, 1973
138. Joel DD, Hess MW, Cottier H: Magnitude and pattern of thymic lymphocyte migration in neonatal mice. J Exp Med 135:907–923, 1972
139. Bienenstock J, Dolezel J: Peyer's patches: Lack of specific antibody-containing cells after oral and parenteral immunization. J Immunol 106:938–945, 1971
140. Henry C, Faulk WP, Kuhn L, Yoffey JM, Fudenberg HH: Peyer's patches: Immunologic studies. J Exp Med 131:1200–1210, 1970
141. Coppola ED, Aboul-Enein A, Kopycinski CF, DeLuca F: Synergism between Peyer's patch cells and spleen cells in humoral antibody production. J Immunol 108:831–833, 1972
142. Kagnoff M, Campbell S: Functional characteristics of Peyer's patch lymphoid cells. I. Induction of humoral antibody and cell-mediated allograft reactions. J Exp Med 139:398–406, 1974
143. Hunter RL Jr: Antigen trapping in the lamina propria and production of IgA antibody. J Reticuloendothel Soc 11:245–252, 1972
144. Gowans JL, Knight EJ: The route of recirculation of lymphocytes in the rat. Proc R Soc Lond 159:257–282, 1964
145. Griscelli C, Vassalli P, McCluskey RT: The distribution of large dividing lymph node cells in syngeneic recipient rats after intravenous injection. J Exp Med 130:1427–1451, 1969
146. Moore AR, Hall JG: Evidence for a primary association between immunoblasts and small gut (letter to the editor). Nature 239:161–162, 1972
147. Craig SW, Cebra JJ: Peyer's patches: an enriched source of precursors for IgA-producing immunocytes in the rabbit. J Exp Med 134:188–200, 1971
148. Arnason BG, St-Cyr C de V, Relyveld EH: Role of the thymus in immune reactions in rats. IV. Immunoglobulins and antibody formation. Int Arch Allergy Appl Immunol 25:206–224, 1964
149. Clough JD, Mims LH, Strober W: Deficient

IgA antibody responses to arsanilic acid bovine serum albumin (BSA) in neonatally thymectomized rabbits. J Immunol 106:1624–1629, 1971

150. Schiff RI, Buckley RH, Gilbertsen RB, Metzgar RS: Membrane receptors and in vitro responsiveness of lymphocytes in human immunodeficiency. J Immunol 112:376–386, 1974
151. McFarlin DE, Oppenheim JJ: Impaired lymphocyte transformation in ataxia-telangiectasia in part due to a plasma inhibitory factor. J Immunol 103:1212–1222, 1969
152. Lawton AR, Royal SA, Self KS, Cooper MD: IgA determinants on B-lymphocytes in patients with deficiency of circulating IgA. J Lab Clin Med 80:26–33, 1972
153. Wu LYF, Lawton AR, Greaves MF, Cooper MD: Evaluation of human B lymphocyte differentiation using pokeweek mitogen (P.W.M.) stimulation. Proceedings of the Seventh Leucocyte Culture Conference, Laval University, Quebec, Canada. F Daguillard (ed). New York, Academic Press, 1973, pp 485–500
154. Chanock RM: Local antibody and resistance to acute viral respiratory tract disease. The Secretory Immunologic System (Proceedings of a conference on the Secretory Immunologic System, December 10–13, 1969, Vero Beach, Florida). DH Dayton Jr, PA Small Jr, RM Chanock, HE Kaufman, TB Tomasi Jr (eds). Washington DC, Government Printing Office, 1971, pp 83–92
155. Ogra PL: The secretory immunoglobulin system of the gastrointestinal tract. The Secretory Immunologic System (Proceedings of a conference on the Secretory Immunologic System, December 10–13, 1969, Vero Beach, Florida). DH Dayton Jr, PA Small Jr, RM Chanock, HE Kaufman, TB Tomasi Jr (eds). Washington DC, Government Printing Office, 1971, pp 259–279
156. Meinke GC, Spiegelberg HL: Studies of light and "J" polypeptide chains of human γM. Fed Proc 30:468, 1971 (Abstract)
157. Williams RC, Gibbons RJ: Inhibition of bacterial adherence by secretory immunoglobulin A: A mechanism of antigen disposal. Science 177:697–699, 1972
158. Fubara ES, Freter R: Source and protective function of coproantibodies in intestinal disease. Am J Clin Nutr 25:1357–1363, 1972
159. Buckley RH, Dees SC: Correlation of milk precipitins with IgA deficiency. N Engl J Med 281:465–469, 1969
160. Tomasi TB, Katz L: Human antibodies against bovine immunoglobulin M in IgA deficient sera. Clin Exp Immunol 9:3–10, 1971
161. Walker WA, Isselbacher KJ, Bloch KJ: Intestinal uptake of macromolecules: Effect of oral immunization. Science 177:608–610, 1972
162. Ammann AJ, Hong R: Selective IgA deficiency: Presentation of 30 cases and a review of the literature. Medicine 50:223–236, 1971
163. Carr RI, Wold RT, Farr RS: Antibodies to bovine gamma globulin (BGG) and the occurrence of a BGG-like substance in systemic lupus erythematosus sera. J Allergy Clin Immunol 50:18–30, 1972