INFLAMMATORY BOWEL DISEASES

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PREFACE

An international symposium on inflammatory bowel diseases was held in Jerusalem on September 7th-9th, 1981. The symposium was sponsored by the Hebrew University-Hadassah Medical School, and the Israeli Gastroenterological Society. The idea was to bring together leading workers in the field, to invite all those interested to present their new work on IBD and thus to enable exchange of information and cross-fertilization needed to improve our understanding and handling of these diseases.

The symposium was organized into four panels devoted to state of the art reviews, presentation of new findings and approaches on the following topics: New pathological concepts, etiology, pathogenesis and management of IBD. In addition, 89 abstracts were presented as posters during the symposium, all of which were published in the book of abstracts. The concluding panel outlined new directions for future research on IBD.

We owe our gratitude to Drs. J. B. Kirsner, G. L. Gitnick and C. E. Rubin, members of the Organizing Committee, without whose encouragement and help the symposium could not have taken place. The Organizing Committee owes a considerable debt of gratitude to all the contributors who presented their work in a clear and concise manner, to all those who presented posters and to all the participants who came from 27 countries. Their stimulating presentations and discussions contributed to the success of the meeting.

The keen interest which was shown in the symposium has led many to ask about a future meeting to take place in a similar framework. We hope that we shall be able to arrange a second International Symposium on Inflammatory Bowel Diseases to take place in Jerusalem in September, 1984.

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C O N T E N T S

A. New Pathological Concepts

PATHOLOGIC ASPECTS OF DIAGNOSIS, PATHOGENESIS, AND ETIOLOGY OF IDIOPATHIC INFLAMMATORY BOWEL DISEASE

John H. Yardley, M.D. and Stanley R. Hamilton, M.D.

1. INTRODUCTION

We believe that the term "Inflammatory Bowel Disease" (IBD) should be used to encompass a broad range of inflammatory disorders of the intestinal tract rather than just ulcerative colitis (UC) and Crohn's disease (CD)(1). The broader usage recognizes the total range of diagnostic possibilities in IBD, many of which are due to known and treatable causes. The more explicit term "idiopathic inflammatory bowel disease" is recommended for referring to UC, CD, and indeterminate forms.

2. DIAGNOSIS OF IBD

Basic histologic findings in IBD: The histopathologic findings in IBD are not specific for any one etiology or entity, even though certain types of pathologic change can be more characteristic of one form of IBD than another. This very fundamental fact should always be recognized when considering diagnostic criteria of IBD.

Acute and chronic inflammation are the hallmarks of "active" IBD of any etiology. Acute inflammation is relatively easy to identify since even a few polymorphonuclear leukocytes lying outside capillaries are abnormal. Chronic inflammation is more difficult to assess because plasma cells, lymphocytes and macrophages are normal constituents of the lamina propria.

In assessing active IBD, it is especially important to understand that crypt abscesses (acute inflammatory cells contained within crypt lumens) can occur in any form of active IBD, not just in UC. Likewise, inflammatory exudate can be seen overlying the mucosa in IBD due to numerous causes. When surface exudate is visible grossly, the descriptive term "pseudomembrane"

is frequently applied. Thus, although characteristically seen in some patients with antibiotic-related colitis, the pseudomembrane is not a specific feature of that disorder.

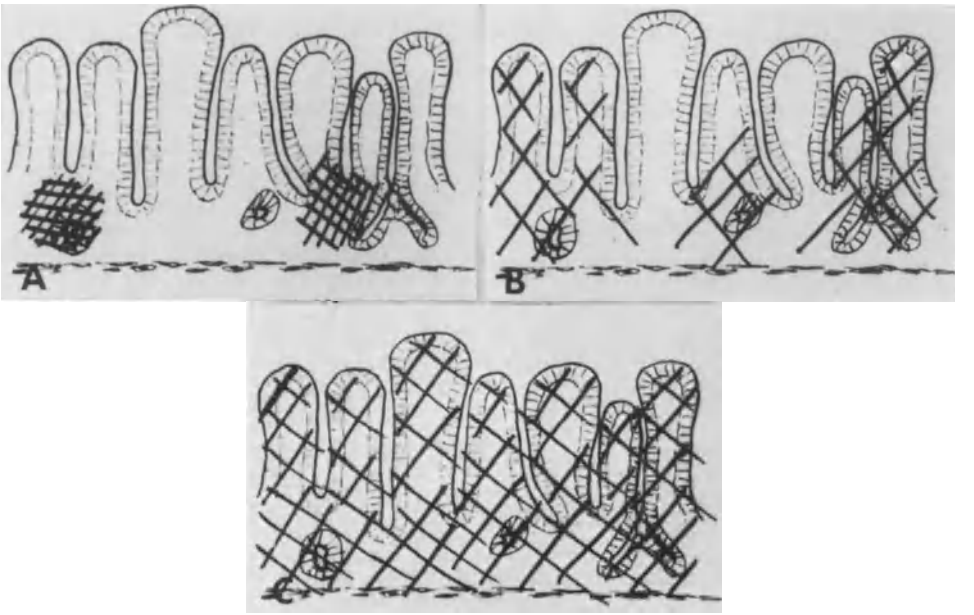

Fig 1. Schematic drawing depicting distribution patterns of inflammation (cross-hatched areas) in IBD when: (A) Focal, (B) Patchy, and (C) Diffuse.

The distribution pattern of inflammation in the lamina propria is characterizable as diffuse, focal, or patchy (Fig.1). When diffuse, acute and chronic inflammation is more or less uniform everywhere in the mucosa. At the other extreme, inflammation is focal when it occurs in localized areas in a mucosa that is not actively inflamed everywhere. The term patchy describes a distribution which lies somewhere between the extremes of diffuse and focal. Patchy inflammation shows variation in intensity from one area to the next, but at least some inflammation is seen almost everywhere. The various distribution patterns are not per se specific for any disease entity, but in

Table 1. Classification of Inflammatory Bowel Disease (IBD)

Bacterial IBD
- Dysenteric (Shigella, Salmonella)
- Yersinia enterocolitis
- Campylobacter enterocolitis
- Gonococcal proctitis
- Antibiotic-related (Clostridium difficile)
- Syphilitic proctitis
- Tuberculosis
- Whipple's disease

Chlamydial IBD
- Lymphogranuloma venereum (LGV)
- Non-LGV proctitis

Viral IBD
- Cytomegalovirus
- Herpes

Parasitic IBD
- Amebiasis
- Schistosomiasis
- Balantidiasis
- Cryptosporidiosis

Fungal IBD
- Histoplasmosis

Drug-, Chemical-, and Foodstuff- Related IBD
- Antibiotic (see Bacterial IBD)
- Heavy metal
- Cytotoxic drugs (e.g. 5-fluorouracil)
- Milk protein allergy

Irradiation-Induced IBD
- Acute irradiation colitis
- Postirradiation colitis

IBD of Intrinsic Origin
- Ischemic colitis
- Necrotizing enterocolitis of infancy
- Hirschsprung's-associated
- Obstruction-related
- Diverticulitis
- Solitary ulcer syndrome
- Graft vs host reaction (post bone marrow transplant)

Idiopathic IBD
- Ulcerative colitis and proctitis
- Crohn's disease
- Indeterminate

idiopathic IBD they can be helpful in distinquishing between CD and UC (see below).

A granuloma is a well-demarcated collection of epitheloid macrophages, often with one or more multinucleated giant cells. Granulomas are not limited to CD. They may also be seen in tuberculosis, histoplasmosis, lymphogranuloma venereum and possibly other forms of IBD. In CD they are typically hard (sarcoid-like), but this feature, too, may be noted in other entities. Scattered or ill-defined collections of macrophages are sometimes observed, and we refer to these as "granulomatous features".

Epithelial cells are highly sensitive to injurious agents, and are commonly involved in IBD. In addition to overt evidence of injury, as with necrosis in ischemia, there can be more subtle changes such as increased crypt mitosis, reduced epithelial mucin, and nuclear atypia.

Inflammatory pseudopolyps are most common in UC, but like the other features described above, they too can be noted in any form of chronic IBD, including CD and known causes of IBD such as schistosomisis where they are seen regularly.

IBD due to known causes: The diversity of infectious and other agents as well as intrinsic factors which can lead to IBD is shown in Table 1. It is also apparent from Table 1 why a diagnosis of idiopathic IBD should only be made after considering and searching for evidence of known forms of IBD. It is noteworthy, and an encouraging sign, that the list of causes of IBD continues to grow. For instance, within the past few years, we have come to recognize campylobacter, non-LGV forms of chlamydia, and cryptosporidia as causes of IBD. Also, Clostridium difficile is now known to be the principal cause of antibiotic colitis.

Idiopathic IBD: Since a large proportion of all biopsies showing IBD come from patients considered to have either UC or CD, chosing between these two entities is often the major diagnostic objective. The important gross (endoscopic and x-ray) and microscopic features in UC and CD are summarized in Table 2.

Table 2 also emphasizes that there can be considerable overlap in the gross findings between UC and CD and that study of microscopic changes provides valuable additional information.

Table 2. Ulcerative Colitis (UC) Versus Crohn's Disease (CD) of Colon: Gross and Microscopic Findings in Active Disease[a]

	UC	CD
GROSS FINDINGS (ENDOSCOPY & X-RAY)		
Total proctocolitis	+++	+
Distal predominance	++++	+
Right colon predominance	0	+++
Rectal sparing (relative)	+	+++
Skip areas	0	++
Punctate lesions	0	++
Ulcerations or erosions	+	++
"Cobblestoned"	+	++
"Atrophic"	+++	+
Pseudopolyps	++	+
MICROSCOPIC FINDINGS (COLORECTAL BIOPSY)		
Nonspecific acute and chronic inflammation		
Diffuse or patchy	++++	++
Focal	0	++
Granulomata	0	+
"Granulomatous features"	+	++
Crypt abscesses	++++	++
Submucosal penetrance	+	++

Key for frequency (estimated) of findings: 0, never seen or very rare; +, uncommon (20%); ++, fairly common (20-50%); +++, common (50-80%); ++++, very common (80%).

[a] Modified from Ref. 1.

Greatest differential diagnostic reliability in distinguishing CD from UC is provided by the presence of granulomas. However, most investigators find that granulomas are detectable in less than 25% of biopsy specimens from patients with CD, and under optimal conditions, granulomas can be found in only about half of all patients. Other commonly seen forms of inflammation in CD are: a) nonspecific acute and chronic inflammation,

sometimes with a prominant admixture of macrophages (granulomatous features) which at times even form indistinct collections suggesting early granulomas, and b) conventional nonspecific acute and chronic inflammation without prominance of macrophages. However, even when the inflammation is of the usual mixed acute and chronic type, another feature, its distribution, can be helpful in differential diagnosis: While either UC or CD may be present when inflammation is diffuse or patchy, when the inflammation is focal, the likelihood of CD is greatly increased (Fig. 2).

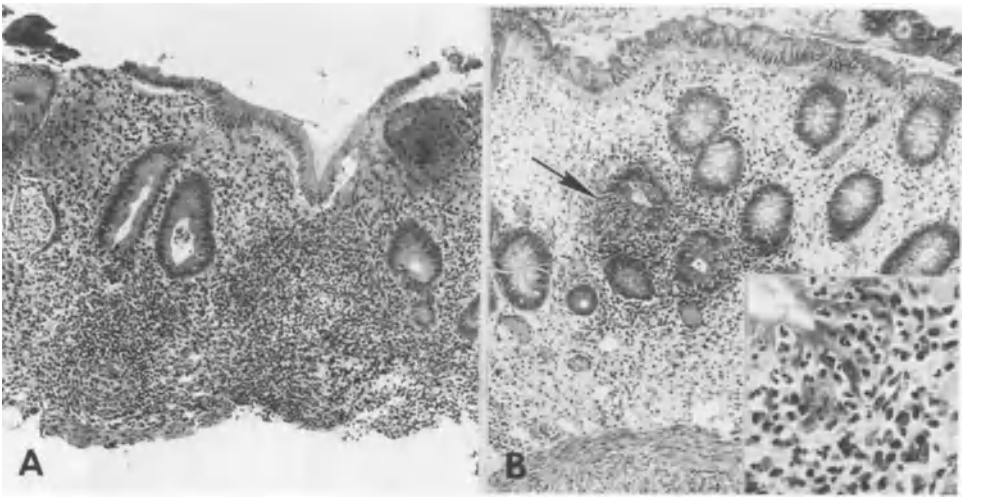

Fig 2. Examples of colo-rectal biopsies from: (A) Ulcerative colitis showing diffuse acute and chronic inflammation; (B) Crohn's disease with a focus of inflammation (Arrow). Inset: Detail of inflammation in (B) demonstrating its non-specific character.

We verified the fact that focal nonspecific inflammation (FNI) favors CD over UC by conducting a blinded study of colo-rectal biopsy material from patients with active ulcerative colitis, Crohn's disease, ulcerative proctitis, and idiopathic IBD of indeterminate type (1). The results strongly favored the association of FNI (with or without granulomatous features) with

CD (Table 3). While FNI was not seen in any of the 14 biopsy specimens from patients with active UC, it occurred in 10 of 23 showing active CD. At the same time, patchy and diffuse inflammation were also noted in CD, emphasizing that those distributions do not exclude CD. The results with ulcerative proctitis and indeterminate colitis (Table 3) additionally stresses the nonspecific character of the distribution.

Table 3. Rectal biopsies in Crohn's disease and ulcerative colitis[a]. Distribution of non-specific acute and chronic inflammation.

Disease	Focal	Patchy	Diffuse	Absent
Crohn's disease	10[b]	4	9	9
Ulcerative colitis	0	2	12	---
Ulcerative proctitis	0	1	1	0
Indeterminate colitis	2	0	4	4

[a] Data modified from ref. 1.
[b] No. of biopsies.

The value of noting FNI was also borne out in a review of all biopsies showing active inflammation from patients with proven or suspected Crohn's disease seen at Johns Hopkins from 1974-78 (2). Among these 237 biopsies, granulomas were discovered in 25%; 28% showed nonspecific inflammation with granulomatous features (no notation being made about distribution); and 48% revealed only non-specific inflammation without associated granulomatous features. However, in 31% of those biopsies with non-specific inflammation without granulomatous features, the lesion was also described as focal. In contrast, focal inflammation was not observed in any patients with active UC studied during the same time period (1974-78). Thus, these results give additional support to FNI as an aid in distinguishing CD from UC.

Many observers have emphasized the focal or irregular reduction in goblet cell mucin which is seen in CD. Such focal loss of mucin is, of course, related to the focal inflammation described above. In our experience, disparity in loss of mucin

from goblet cells can be difficult to assess in biopsies and is more useful for establishing the diagnosis of CD in resected specimens. The small size of biopsies similarly limits the feasibility of using depth of penetration of inflammation, presence and character of ulceration, etc. in distinguishing between CD and UC.

3. PATHOGENESIS AND ETIOLOGY OF IDIOPATHIC IBD

Observations on human tissues: While there is much overlap in the pathologic appearance of CD and UC, as is apparent from the information summarized in Table 2, there is also much support for the concept that the pathologic differences between CD and UC reflect fundamental differences in pathogenesis and etiology. In addition to the obvious gross distributional differences, it is especially noteworthy that CD is commonly widespread in the intestinal tract whereas UC is typically limited to the colon. The focal nonspecific acute and chronic inflammation (FNI) in CD is now well-known to occur in grossly uninvolved areas of the gastrointestinal tract, as are nonspecific lesions with granulomatous features and granulomas. This was well brought out in a study of uninvolved colonic mucosa of CD using an en face technique that greatly increased the amount of mucosa which could be examined (3). The focal inflammatory lesion of CD may also manifest itself as small, almost microscopic ulcerations (aphthoid ulcers) (4).

Minimal or absent gross involvement with microscopic lesions also fits well with the observation that the rectum can be successfully biopsied for evidence of CD even when it is grossly uninvolved (1). Furthermore, occurence of scattered, microscopic foci of active disease in grossly uninvolved intestine is highly relevant to studies which indicate that detecting active CD by frozen section is unreliable (5) and that post-surgical recrudescence of CD is not reduced by resecting additional "uninvolved" intestine (6,7).

Sommers and his colleagues (8) have shown by quantitative approaches that macrophages make up a disproportionally large fraction of the inflammatory cell population in the lamina

propria in CD, whereas this is absent or much less evident in UC. Other cell types have also been studied. The eosinophil is increased in idiopathic inflammatory bowel disease, but UC and CD do not differ in this respect. Basophils may also be increased somewhat, and the suggestion has been made that this could reflect participation of the IgE immune system.

Careful quantitative study of cell type in the focal lesions led Schmitz-Moorman (9) to conclude that active CD begins with nonspecific acute and chronic inflammation followed by increasing numbers of macrophages, and that the same lesion may then proceed to develop into a full-blown granuloma. All such views present as a central notion the idea that the earliest visible evidence of CD resides in the mucosa and consists of the focal nonspecific inflammation, perhaps followed subsequently by prominant macrophages and granuloma formation.

It is possible that in the early (focal nonspecific) lesions we are seeing the most direct evidence of injury by the agent or factors responsible for CD. One can also speculate that the more advanced manifestations of CD such as ulceration, transmural inflammatory lesions, and sinus tracts represent progression of the disease in a secondary fashion, perhaps with other agents such as luminal bacteria being important factors in causing the progression.

As new investigative techniques become available, they have been applied to study of pathological material from patients with Crohn's disease. Transmission electron microscopy in the hands of most investigators has shown only a wide variety of nonspecific alterations relating to injury and inflammation (10, 11, 12).

Scanning electron microscopy studies have beautifully demonstrated the aphthoid ulcer and the associated changes in surrounding mucosa (3). As mentioned above, in addition to macrophages, specialized studies have been done for eosinophils and mast cells in idiopathic inflammatory bowel disease (13).

There is, of course, much circumstantial evidence for involvement of the immune system in idiopathic IBD, and the heavy

infiltration with lymphocytes, plasma cells and macrophages (especially in CD) is consistent with that view. On the other hand, histopathologic studies have contributed little to helping determine the nature of immune involvement in idiopathic IBD. Immunohistochemical studies to demonstrate any abnomalities in the antibody producing cells in the lamina propria have shown shifts in cell population favoring IgM cells (14). Increased IgG cells have also been seen, but it is well known that IgG plasma cells increase in the intestine nonspecifically with injury and inflammation.

Lymphoid nodules, representing gut associated lymphoid tissue (GALT), were found to underlie aphthoid ulcers in the study of Rickert, et al (4). The possibility that aphthoid ulcers, an early manifestation of CD, may be related to GALT is intriguing since GALT is believed to be fundamental to the development of local immunity. Furthermore, it is now recognized that the GALT is accompanied by specialized overlying epithelial cells which are capable of phagocytosis and thus may play a role in delivery of antigenic material from the lumen to GALT. GALT may, for these reasons, offer special advantages as sites in the search for etiologic agents in Crohn's disease.

In summary, morphologic studies of idiopathic IBD using human tissues have helped greatly to define the distinction between UC and CD. Furthermore, such studies do suggest possible pathogenetic mechanisms in IBD even though precise definition of etiology has not been forthcoming.

Animal inoculation studies: In recent years a number of experiments have been conducted in which tissues from patients with idiopathic IBD were used to prepare homogenates and filtrates that were then inoculated into animals (15). Detection and interpretation of histopathologic alterations have been central to these investigations. Mitchell and colleagues found "epithelioid granulomas" in mouse footpads 6-24 months after injecting tissue homogenate from a patient with CD (16). In later studies, similar results were reported after intra-ileal or intravenous (17) injection of homogenates into rabbits. In other

investigations, granulomas were reported in mouse footpads following injection of tissue from patients with UC (18). And in still another series of experiments it was said that inoculation of homogenate from CD into either the footpad or intravenously led to development of granulomatous lesions in the mouse ileum (19). Cohen, et al. observed granulomas in the ileum of rabbits after inoculating homogenate from patients with CD and UC (20).

Taub and colleagues reported that they were able to repeat these experiments with consistent development of granulomas after injecting either CD or UC homogenates as well as controls (21). And Simonowitz, et al. (22) regularly found inflammation and other nonspecific histological findings one year after intracolonic injection of homogenates from CD in rabbits, but granulomatous lesions were never seen. Other researchers such as Heatley and Bolten (23) could not repeat the results described above even when using multiple species and several routes of inoculation.

Due to the efforts of Dr. Warren L. Beekin and the generosity of the investigators(16-22), several selected examples of the histopathologic material from the investigators' published experiments were made available to us in connection with a workship on Infectious Agents in Inflammatory Bowel Disease held in Tarreytown, New York, on November 17, 1978 (15,24). These materials were examined as a single group by us in collaboration with Dr. John Strandberg, a veterinary pathologist at Johns Hopkins. Each slide was independently assessed for amount and character of inflammation. A special search was made for foreign material, including use of polarized light to demonstrate refractile material.

Granulomas or granulomatous reactions were indeed seen at times in both footpads (16 of 18) and ileal loops (6 of 26) from inoculated animals. In many instances, however, especially among the footpads (12 of 18), the granulomata were associated with foreign material. At times, these were small, non-descript bodies that were either refractile or nonrefractile. In other instances there were recognizable hair or bone fragments that

were in all likelihood introduced during the experimental procedures. Among the intestinal specimens the inflammatory reaction, rather than being granulomatous, sometimes consisted of ordinary inflammation or a frank abscess, at times associated with foreign material. In some of the ileal injection experiments, there seemed to be scars which could be accounted for by nonspecific reaction at the injection or biopsy sites.

Of particular interest were the observations that six out of six footpads showing granulomatous inflammation or granulomata in the footpads obtained from the experiments of Taub, et al. (21) demonstrated foreign material consisting of small non-refractile particles in macrophages and giant cells. It is noteworthy that in their original report the interval between injection and development of granulomas was significantly shorter than in experiments of Mitchell and Rees (16). Furthermore, Taub et al observed granuloma formation from injection of filtrates from UC and control as well as from those with CD.

Two slides in the reviewed material corresponded to Figures 2 and 3 from a study by Cave et al (19), figures that were said to show granulomata in the ileum of mice inoculated with homogenate. One was found by us to be a small intestinal lymphosarcoma, the other a leiomyosarcoma of the uterus (25), both common tumors in aging mice. On the other hand, the negative findings of Simonowitz, et al. were seen to be as described in their article, i.e., there was little or no change in the ileum after injection in most specimens, and all changes were nonspecific.

It was clear that contamination by foreign material could have played a significant role in these inoculation experiments, emphasizing the importance of avoiding contamination. At the same time doubts were raised about relevance of the observations with respect to an etiologic agent in the IBD. The slide review also brought out problems of a purely interpretive nature. Yet it is still true that a few granulomas showed no foreign material, and it is not inconceivable that foreign material could be an adjunctive requirement for inducing the experimental lesions.

Future directions: We believe that investigations into the pathogenesis and etiology of idiopathic IBD must be done with careful attention to experimental details and with recognition of the many problems and pitfalls which the investigator faces. To do less is to expend great effort on gaining results that may be meaningless, or, indeed, lead to further confusion and wasted effort.

Investigators should only use source material which comes from patients where UC and CD (or other forms of IBD) have been definitely identified. It is surprising how often the two entities are lumped together for experimental purposes. It is also important to note whether the tissue demonstrated early lesions (and hence were possibly "primary") or late lesions (and hence with greater likelihood they were "secondary"). In addition, careful attention should be given to selecting both normal and diseased controls. Furthermore, diseased controls should include as wide a variety of infectious and other identifiable causes of IBD as possible.

It is also essential that investigators realize that all histological findings in IBD are qualitatively non-specific, i.e., they can all be produced by more than one agent or insult. Furthermore, secondary host responses such as those due to bacterial and tissue-derived products can readily confound results. In animal experiments, lesions that may derive from natural and unrelated disorders in the animals must not be adjudged a response to the inoculated material. Direct demonstration of etiologic agents is exceedingly difficult, and all such findings of which we are aware must be regarded are highly speculative.

On balance, morphological studies have so far made their largest contributions by helping to define UC and CD and by providing a basic method for detection. We feel that these functions are essential and that ignoring them can imperil or invalidate an investigation. However, more precise information will only come from morphologic techniques when combined with other experimental parameters and when methods that add precision

and definition such as histochemical, radioautographic, and immunohistochemical techniques, etc., are used. The studies of Das, et al. (26), in which material from lymph nodes from patients with CD produced lymphomas in nude mice, are a good illustration of how investigative approaches which go beyond conventional morphology may be fruitful in demonstrating etiological agents. Another example of a potentially useful procedure that goes beyond direct injection of tissue homogenates into experimental animals is the study of Cohen, et al. (27) in which an incredibly vigorous granulomatous response has been produced in the cheek tissue of C57Bl/10 mice (but not several other strains) after injecting them with macrophages that had been exposed in vitro to homogenates from idiopathic IBD.

4. CONCLUDING COMMENTS

We want to reiterate our belief that the term "Inflammatory Bowel Disease" should be used in a broad sense to cover known as well as idiopathic forms. In addition to being preferred usage from the standpoint of differential diagnosis, we feel it is conceptually preferable when dealing with investigations into the pathogenesis and etiology of IBD. Study of those forms of IBD where the cause is known should contribute much to our understanding of basic mechanisms of pathogenesis and pathophysiology in these conditions. This process should in turn lead to a more exact definition and understanding of causation in idiopathic IBD by identifying new and appropriate methodology and suggesting relevant questions. These principles are illustrated nicely by the investigation of patients with idiopathic IBD for presence of Clostridium difficile toxin, studies that were originally concerned only with antibiotic colitis(28). Later, related studies led to the demonstration that apparent exacerbations of UC or CD can occur as a result of therapy. Investigations into the entire spectrum of IBD is the approach that that will maximize chances for solving the riddle of idiopathic IBD.

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EPITHELIOID GRANULOMA AND RELATED HISTOLOGIC FEATURES WHICH MAY DIFFERENTIATE CROHN'S DISEASE FROM ULCERATIVE COLITIS IN RECTAL BIOPSIES

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Inflammatory bowel disease of more than several months' duration usually proves chronic and idiopathic rather than acute and self-limited. Nevertheless, the diagnosis is not always clear from the clinical, radiologic and endoscopic findings.

The first problem is encountered in patients in whom it is unclear whether their gastrointestinal illness is organic or functional. In such patients, the finding of non-specific inflammation or granuloma in a rectal biopsy is helpful because it establishes the organic nature of the patient's complaints. Such knowledge is more likely to lead to appropriate treatment. The histologic findings which indicate non-specific inflammation are lamina propria inflammation, intraepithelial crypt abscess, non-specific crypt abscess, isolated giant cell or distorted crypt architecture. Some of these non-specific findings are found in rectal biopsies from all patients with active ulcerative colitis and in 70-75% of patients with active Crohn's disease.

The second problem is to decide which type of idiopathic inflammatory bowel disease the patient has. For all practical purposes, this is a differential diagnosis between Crohn's disease and idiopathic ulcerative colitis. Most of the findings in rectal biopsy in these two diseases are non-specific and indistinguishable from one another. However, the findings of an epithelioid granuloma in a rectal biopsy is very useful in differentiating Crohn's disease from ulcerative colitis in developed countries, where other causes of granuloma are rare. In a retrospective study of 243 rectal biopsies from 90 patients with proven Crohn's disease we found epithelioid granuloma to be the most reliable histopathologic criterion for differentiating Crohn's from ulcerative colitis. If partial serial sections from at least two rectal biopsies (fig. 1) were examined in each patient (minimum of 180

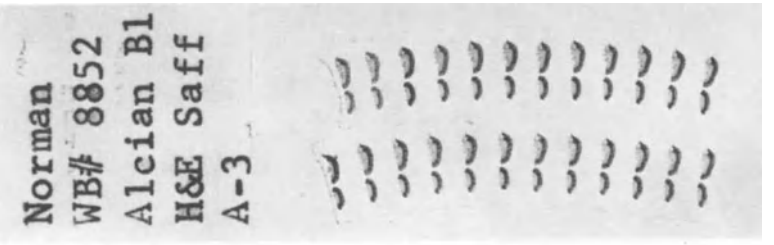

Fig. 1 - Serial sections of two rectal biopsies.

sections), granuloma was found in 28% of patients with Crohn's disease[1]. Diagnostic epithelioid granulomas may be very small and very infrequent in rectal biopsies from patients with Crohn's disease. Sixteen percent are seen in less than six successive 4 µM thick serial sections[1]. It is therefore not surprising that a single routinely processed rectal biopsy is of no help in diagnosing Crohn's disease[2].

In our retrospective review of rectal biopsy[1], we evaluated other findings alleged to favor Crohn's disease over ulcerative colitis, such as: preservation of goblet cell mucus, disproportionate submucosal inflammation and paucity of crypt abscesses. None proved helpful in differentiating Crohn's disease from ulcerative colitis.

In a blinded review of patients without inflammatory bowel disease, we found the following findings in approximately one quarter to one half of this population: a few polymorphonuclear leukocytes in the lamina propria, decreased goblet cell mucus, lamina propria edema or abnormal surface epithelium. Thus, these findings are not diagnostic of inflammatory bowel disease, and may be a variant of normal.

Histologic criteria will be defined because much of the confusion in the pathologic literature on inflammatory bowel disease is semantic. Lamina propria inflammation means increased numbers of round cells or leukocytes in the lamina propria (fig. 2). Round cells, especially plasma cells, are obviously increased when

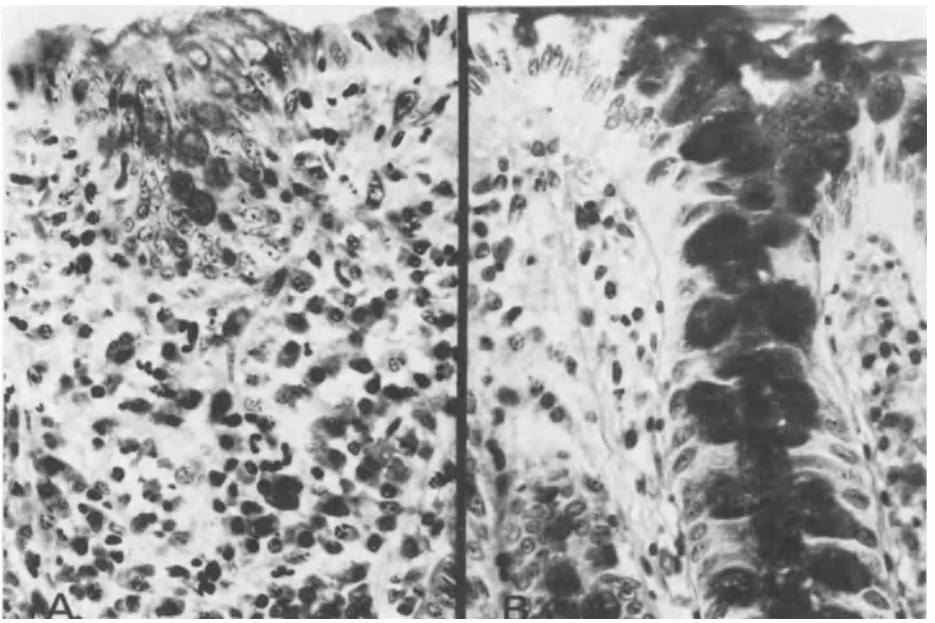

Fig. 2 - A) Inflamed lamina propria. B) Normal lamina propria.

two observers agree on this finding; the normal variation in the number of round cells within the lamina propria is so wide that the increase must be marked to be considered significant. Polymorphonuclear leukocytes within blood vessels or within artifactual areas of hemorrhage are not significant. Furthermore, a rare leukocyte within the lamina propria can be seen normally, but greater numbers are usually indicative of pathology. An intraepithelial crypt abscess is a collection of polymorphonuclear or eosinophilic leukocytes confined to the crypt epithelium; it is probably a precursor of crypt abscess (fig. 3). Non-specific crypt abscess is easy to diagnose when it is obvious and large. Early crypt abscesses are easily missed; they

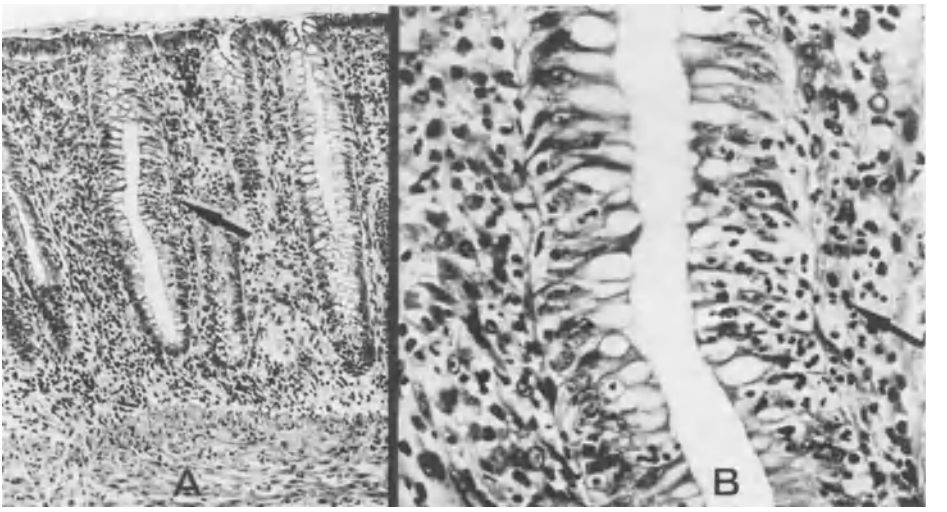

Fig. 3 - Intraepithelial crypt abscess. A) Low power. B) High power.

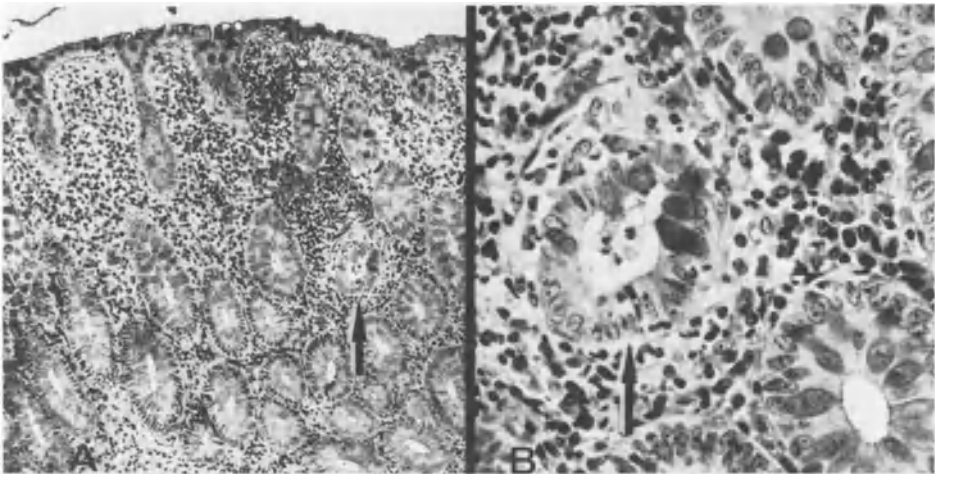

Fig. 4 - Early crypt abscess. A) Low power. B) High power.

consist of a chain of polymorphonuclear or eosinophilic leukocytes extending into the crypt lumen from the lamina propria through the crypt epithelium (fig. 4). The connection between the lamina propria and the crypt lumen may only be apparent after examination of serial sections. Even if the crypt abscess is very small and involves only a few leukocytes, it is clinically significant in our experience. Distorted crypt architecture indicates prior destruction of crypts (fig. 5). The lumenal surface may be villous rather than normally flat; the glands may be dilated, tortuous, branching, shortened or diminished in number. An isolated giant cell is a solitary giant cell without associated epithelioid cells (fig. 6); although it may be more common in patients with Crohn's disease, it is also seen in proven ulcerative colitis and acute self-limited colitis.

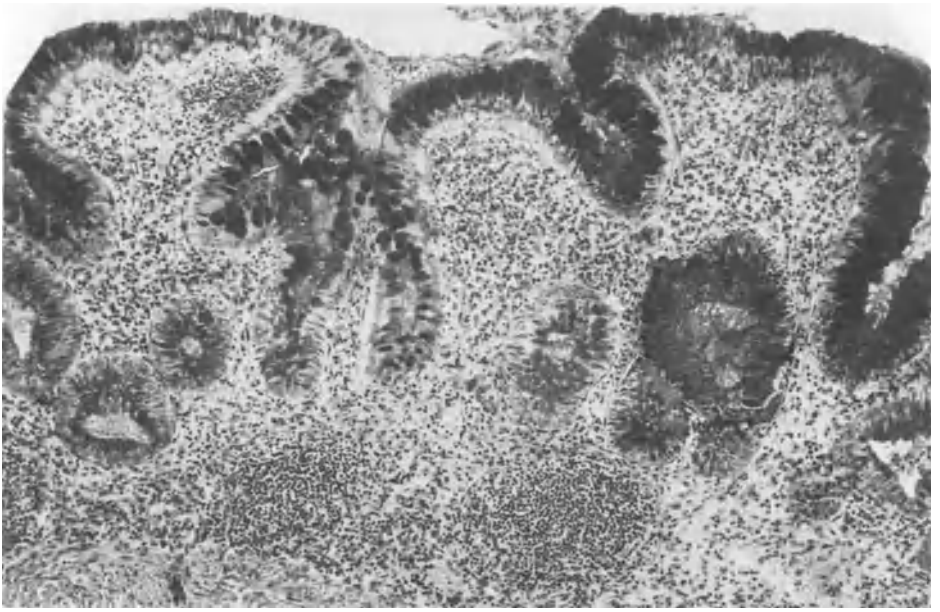

Fig. 5 - Distorted crypt architecture.

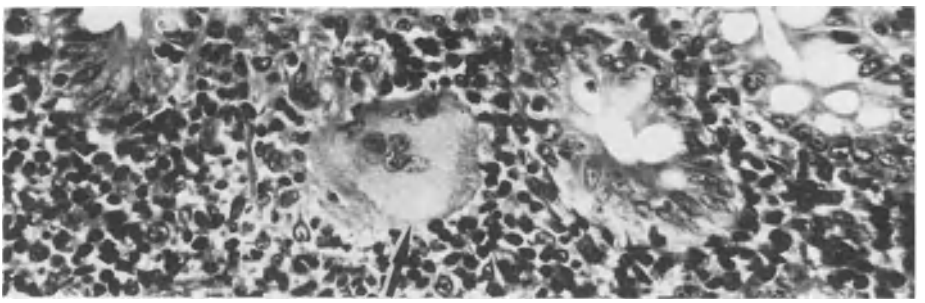

Fig. 6 - Isolated giant cell.

These first five findings are non-specific and are seen in both ulcerative colitis and Crohn's disease. Except for isolated giant cell, these findings tend to be widely distributed in biopsies and can usually be detected in step sections. Isolated giant cell, epithelioid granuloma, granulomatous crypt abscess and possible granuloma precursors such as histiocytic collections and focal mixed inflammation, tend to be extremely focal and often require partial serial sectioning for detection. Nevertheless, 16% of the granulomas found in a retrospective study were greater than 124 µM in diameter and were thus visible in 31 or more successive serial sections[1].

An epithelioid granuloma is a discrete collection of at least three to five epithelioid cells, with or without accompanying giant cells, and without caseation necrosis (fig. 7). Giant cells, if present, contain mutiple nuclei and homogeneous

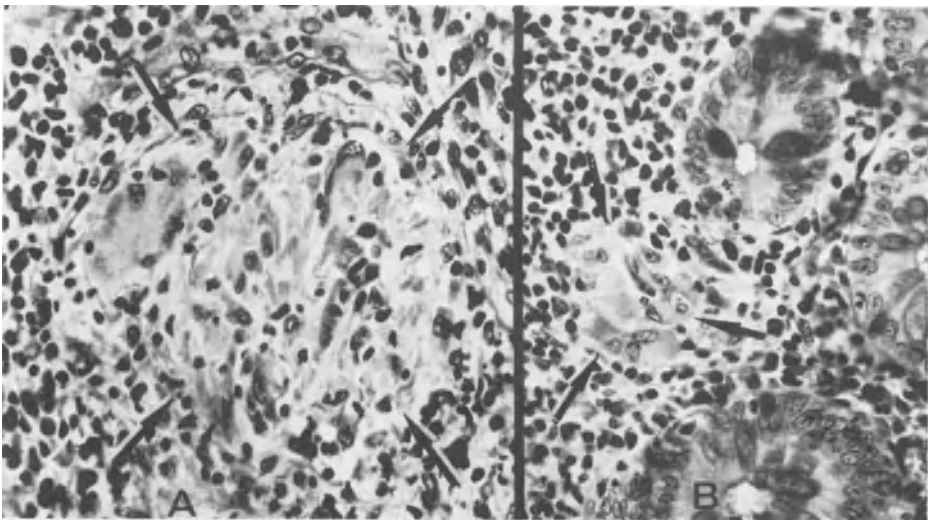

Fig. 7 - Epithelioid granuloma. A) Small granuloma. B) Microgranuloma.

find powdery cytoplasm containing no foreign bodies. A granulomatous crypt abscess is an epithelioid granuloma located in the lamina propria adjacent to the crypt base and extending through the crypt epithelium into the crypt lumen (fig. 8). The associated inflammatory cells are often chronic (lymphocytes and plasma cells) but may be acute (polymorphonuclear and eosinophilic leukocytes). A histiocytic crypt abscess is a crypt abscess containing mostly epithelioid cells and histiocytes mixed with inflammatory cells (fig. 9). It does not have enough organization and recognizable epithelioid cells to be called a granuloma. A histiocytic collection is a group of isolated epithelioid cells or histiocytes buried

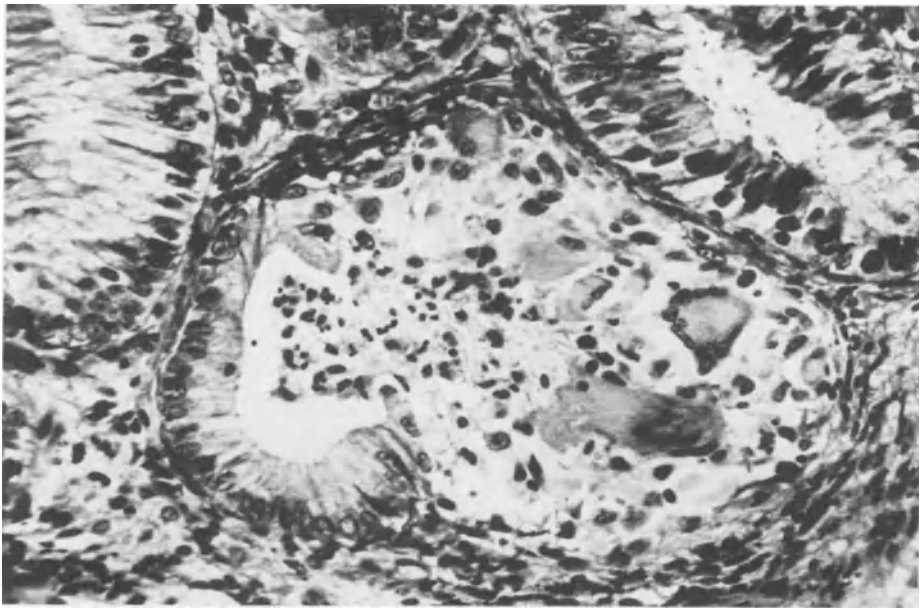

Fig. 8 - Granulomatous crypt abscess.

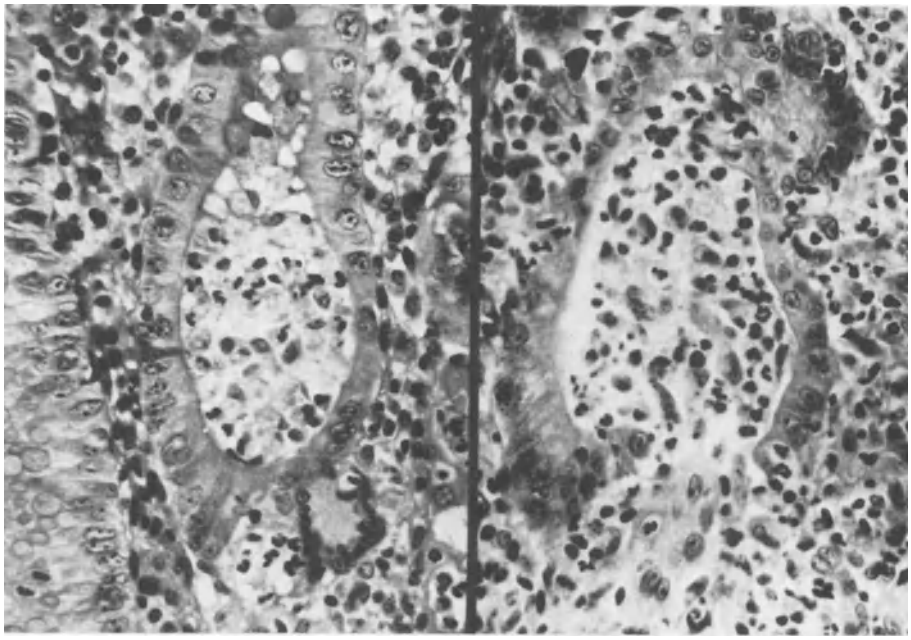

Fig. 9 - Two histiocytic crypt abscesses

within a matrix of chronic and/or acute inflammatory cells (fig. 10). They are not sufficiently organized to be considered an epithelioid granuloma. Focal mixed inflammation[3] is best detected at low power in tangential sections and involves an area the size of one or two crypts. It is a focus of mixed chronic and acute inflammation and may be associated with an early non-specific crypt abscess (fig. 11).

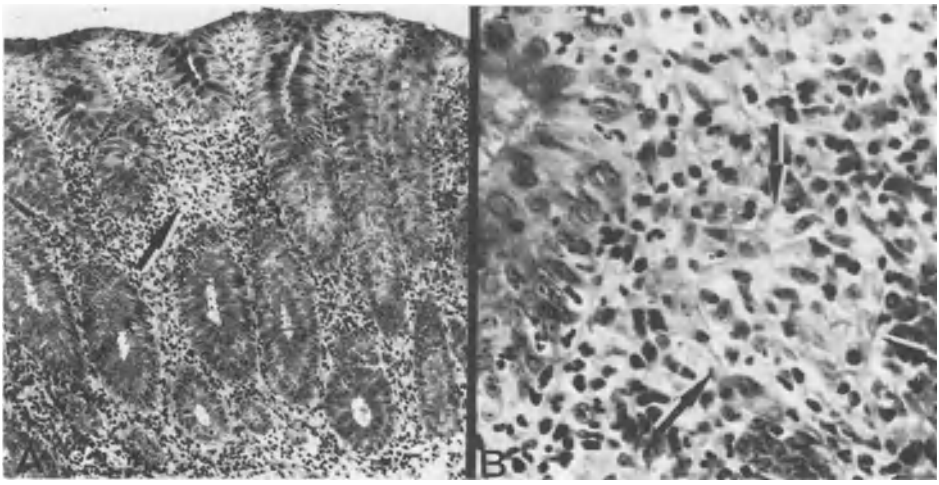

Fig. 10 - Histiocytic collection. A) Lower power. B) High power.

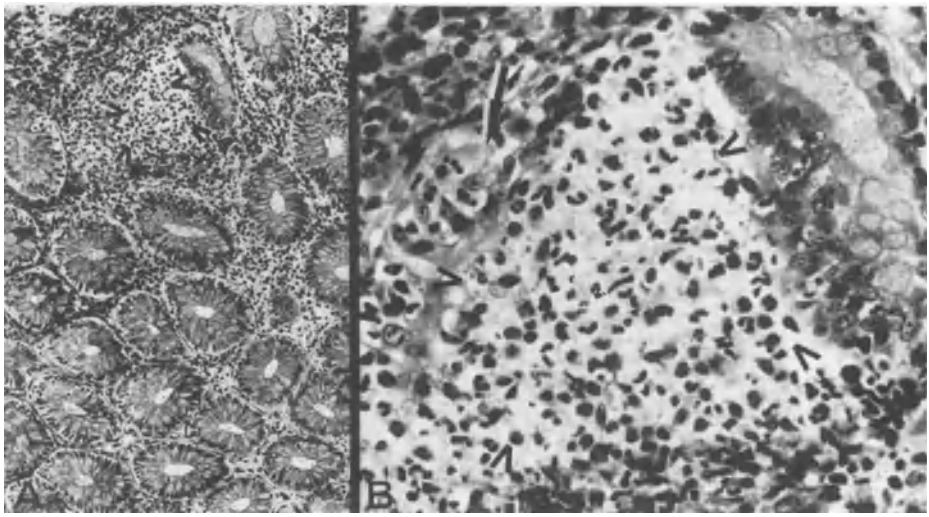

Fig. 11 - Focal mixed inflammation outlined by V markers.
A) Low power. B) High power, arrow = crypt abscess.

In our experience to date, we feel secure in differentiating Crohn's disease from ulcerative colitis only when we find an epithelioid granuloma or a granulomatous crypt abscess. We are involved in long-term follow-up studies to assess the differential diagnostic value of finding a histiocytic crypt abscess, a histiocytic collection or a focus of mixed inflammation. Perhaps one or all of these new criteria will prove reliable and we will be able to make the correct differential diagnosis between Crohn's disease and ulcerative colitis in a higher percentage of cases after examining two suitably processed rectal biopsies.

SUMMARY

Our studies to date indicate that histologic features other than epithelioid granuloma may not help differentiate Crohn's disease from ulcerative colitis. Partial serial sectioning of two rectal biopsies doubles the diagnostic yield for granuloma as compared to a single biopsy. Fewer step sections are adequate to diagnose more diffuse inflammatory findings such as lamina propria inflammation, intraepithelial abscess and non-specific crypt abscess, but partial serial section is needed for the highest accuracy in diagnosing granuloma, granulomatous crypt abscess, histiocytic crypt abscess, histiocytic collection and focal mixed inflammation. The likelihood of finding an epithelioid granuloma in a rectal biopsy is almost as high in normal as in abnormal-appearing rectal mucosa by sigmoidoscopy[1]. Whether the accuracy of differentiating Crohn's disease from ulcerative colitis by rectal biopsy can be further increased by use of other criteria than epithelioid granuloma will require long-term study.

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DYSPLASIA AND CARCINOMA IN INFLAMMATORY BOWEL DISEASE

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Chronic inflammatory conditions often serve as promoting factors for the development of malignant tumors. Within the intestinal tract, enhanced carcinoma formation has been recorded in chronic infections such as tuberculosis and schistosomiasis and especially in cases of inflammatory bowel disease (IBD), including both ulcerative colitis (UC) and Crohn's disease (CD). The dominant precursor lesion of colonic adenocarcinoma in the general population is the adenoma[1,2], but it has been estimated that about 1000 or 1 percent of all new cases of carcinoma in USA arise as a complication of chronic IBD[3]. Although these colitis-related tumors provide only a small fraction, they more commonly afflict younger patients, behave in a more aggressive fashion, and it appears that the large majority can be prevented. The present review will concentrate on the identification of epithelial dysplasia and its use in determining which patients with chronic colitis are more apt to have or develop a carcinoma.

CARCINOMA IN IBD

Incidence of carcinoma

Ulcerative colitis: The group at greatest risk for development of colonic carcinoma are the patients with extensive ulcerative colitis involving at least one-half to two-thirds of the colon and often the entire organ. It has been calculated that 13% of patients with universal colitis will bear tumors, and there is a direct relation of the incidence to the duration of the disease[4]. Whereas 1% or less of the patients at risk with disease in the first decade have carcinoma, the incidence climbs sharply in the ensuing years[5-7]. Thus, it has been noted by Greenstein that the incidences of tumor per patients at risk rises to 4.5% at 15 years, 13% at 20 years and 34% at 30 years[4]. Similarly, Devroede observed an incidence of tumors in 3% of patients with colitis starting in childhood and lasting 10 years and a further increase of 20% for each subsequent decade of disease[8]. This prompted the suggestion that colitis starting at an early age was an added risk factor, but this has not been confirmed by others[4,9]. Rather, it is more likely that these younger patients have

less mortality and other complications requiring earlier surgery and that they remain with disease for longer periods. Thus, it appears that the two most prominent risk factors are the extent and duration of the colitis. It should be noted, however, that although the incidence of carcinoma in the first decade is very low, this period contains the largest group of patients at risk and it has been observed in some series that 12 to 22% of the tumors develop before 10 years[9,10]. It has, therefore, been suggested that even patients with a relatively short duration of colitis must be monitored to some extent to detect neoplasia.

It had been generally claimed that patients with a more limited distribution of ulcerative colitis were not at any significant risk for tumor formation, but Greenstein observed that tumors developed in about 5% of patients with left-sided colitis[4]. In these cases it appeared that a much longer duration of disease was required; all tumors occurred after two decades of colitis, and the average duration was 32 years as compared to 20 years for cases with universal colitis.

Crohn's disease: There is a definite increase in tumor formation in patients with Crohn's disease[11-13,7], but this is much less than observed in ulcerative colitis. Factors contributing to this lesser risk include the very low incidence of small intestinal carcinoma in the general population, the greater likelihood that patients with CD of the colon will have more segmental and therefore less extensive gross involvement, and that these patients more often require earlier surgery for other complications[14]. Therefore, smaller areas of the colon are at risk for shorter intervals in CD. In fact, Gyde was able to identify only 32 patients from a series of 513 with CD who had involvement of the colon lasting more than 10 years[13]. This lowered risk explains the delay in appreciation that patients with CD are more prone than the general population to develop carcinomas both in the small intestine and colon. It has been estimated that patients with CD have a 20-fold increase in tumors[11] and an overall incidence of 2.9 to 3.5%[7,13]. As with UC, there is a direct correlation with duration of disease with most tumors developing after 20 to 30 years of enteritis or colitis. It has also been suggested that the incidence of tumors outside the digestive tract may be increased in patients with CD[7], but this has not been confirmed[13].

Clinicopathologic features of carcinoma

Ulcerative colitis: Compared to standard colonic tumors, adenocarcinomas complicating UC more often affect younger patients, are frequently multicentric, and are more evenly distributed throughout the colon[5,15]. Most tumors display grossly an ulcerative or infiltrative form, and a polypoid type or carcinoma arising

in a prior benign polyp is very uncommon. It is probable that this has contributed to the relatively late presentation and poorer prognosis in most patients, and it is common for the tumors to become clinically evident only at the time of advanced stricture or metastatic disease. Practically all of the tumors arise in areas of chronic colitis, including regions of inactive as well as active disease. An increase in neoplasia has also been noted in the biliary tract, particularly in cases with the complication of sclerosing cholangitis. There appears to be no enhancement of tumors in other parts of the gut or other tissues[7]. Histological examination reveals adenocarcinoma with a highly variable degree of differentiation; of interest is that a high proportion of the tumors are of the mucinous or signet ring cell types[16], similar to that seen in gastric tumors, and this must be appreciated in study of endoscopic biopsies.

Crohn's disease: Compared with carcinoma in UC, the tumors arising in patients with CD may also be multicentric but their distribution is more variable and only about two-thirds occur in areas of definite active disease[7]. Within the colon, the tumors are slightly more prominent in the right portion and about 22% are found in areas without gross colitis[12]. Of cases affecting the small bowel, 40% of the tumors have been noted in excluded segments and most of the remainder in intact regions of chronic enteritis. Furthermore, there appears to be a slight increase in tumors affecting non-diseased portions of the proximal gut including the esophagus and stomach[13]. The finding of tumors in areas without overt inflammatory disease may relate to the tendency for CD to be present in a more diffuse fashion at a microscopic and subclinical level. More tumors arise in older patients compared to UC, probably reflecting the longer duration of active disease required in CD and the more diffuse age distribution in this disorder. The macroscopic and histologic features of the adenocarcinomas are similar to those seen in UC, with the same tendency to ulcerative and infiltrative forms and the presence of the mucinous type[17,18]; carcinoma has also been noted occasionally to involve fistulous tracts.

Clinical Options

Given the strong epidemiologic link between colitis and carcinoma formation, particularly in patients with extensive UC of long duration, it had been thought that prophylactic pan-colectomy after 10 years should be performed. However, this has not been accepted by most patients and physicians, as the disease activity is often relatively mild and infrequent and the majority of cases will not attain a tumor. Unfortunately, the alternative approach of detecting tumors at an early stage has not been accomplished, due in large part to the subtle and infiltrative

nature of the tumors yielding relatively late gross alterations and symptoms. Accordingly a marker that would serve to identify those patients at even greater risk for having or developing carcinoma has been sought. At present, the histologic identification of epithelial dysplasia in endoscopic mucosal biopsies appears to offer the best indicator[10].

DYSPLASIA IN IBD

Definition of dysplasia

It is advocated that the term dysplasia as applied to the intestinal epithelium in IBD be restricted to denote a neoplastic alteration or transformation[19]. This implies that such dysplastic epithelium is not only an indicator of intraepithelial neoplasia but is also capable of direct invasion without further cytologic modification. In practice, the detection of dysplasia in endoscopic mucosal biopsies serves to identify the patient with IBD who has developed at least a benign neoplastic conversion of the intestinal epithelium; furthermore, as demonstrated below, many patients with the more marked degrees of dysplasia have invasive carcinoma. Alternative terms that have been used include precancer, precarcinoma and adenomatous epithelium[20-22]; the more descriptive designation of dysplasia is presently preferred because it embraces a fuller range of morphologic alterations and stresses the neoplastic quality and potential of the epithelial change.

It is strongly advised that the word dysplasia not be used to include the epithelial features that accompany active disease. Thus, "inflammatory or reactive dysplasia" are phrases best omitted; in such cases, it would be preferable to refer to such epithelia as showing just inflammatory changes or a cytologic atypia if there is some doubt. The identification of epithelial dysplasia is at present totally determined by histologic and cytologic criteria applied to mucosal tissue as described in detail below. Attempts to improve the accuracy by the use of cytologic techniques, demonstration of carcinoembryonic antigen in the epithelia or the study of epithelial kinetics in organ culture have not as yet achieved a practical value[10,23,24].

Incidence and significance of dysplasia

Ulcerative colitis: Early studies of the pathology of UC and carcinoma depicted areas of intraepithelial in-situ tumor and dysplasia adjacent to the invasive lesions[25]. But, Morson and Pang[20] are credited with demonstrating that such epithelial features, which they termed precancer, could also be found in flat mucosa away from the gross tumors in patients with extensive colitis. In their initial study, epithelial

dysplasia was revealed in the rectum of all 23 cases with UC and carcinoma; furthermore, of 9 patients with UC and dysplasia on rectal biopsy, subsequent colectomy disclosed invasive carcinoma in 5 cases. These observations have been largely substantiated by numerous studies[15,21,26-29] with some modifications. It has been shown that the lesion of epithelial dysplasia is commonly patchy, may not always be present or readily detected in the rectum and that it may be absent in some cases of UC with carcinoma. Although early studies including one that employed multiple selective sections had revealed dysplasia in the rectum in over 90% of the cases[30], this probably cannot be achieved by random biospy samples and there also may be a recent shift of carcinoma and dysplasia to more proximal locations[31].

Dobbins[10] provided a critical review and compilation of all publications on dysplasia in UC up to 1977, revealing: (1) of patients with extensive UC and carcinoma, dysplasia was present in some part of the colectomy specimen in 88% but in only 66% in the rectum; (2) of patients with UC but no carcinoma, dysplasia could be demonstrated in 13%; (3) biopsies of patients with extensive UC disclosed a significant dysplasia in 5.7% of cases, including 2.4% for those with disease lasting less than 10 years and 19% for cases with longer duration; (4) of cases showing a prominent dysplasia in rectal or colonic mucosal biopsies, subsequent colectomy specimens revealed an invasive carcinoma in about one-third. Additional studies since that review have been supportive of the predictive value of dysplasia in biopsies, with carcinoma revealed at surgery in one-third to over one-half of the cases[6,9,32,33]. It has been demonstrated that epithelial dysplasia may precede the overt development of carcinoma by several years[6]; the mean duration of inflammatory disease in patients with colitis and dysplasia alone is about 14 years as compared to 21 years for cases with carcinoma[9].

All of the investigations to date have dealt with the finding of dysplasia in patients with extensive UC, and there are no data available on its detection and use in cases with left-sided colitis. Although there are much fewer cases of carcinoma in patients with more limited colitis and a longer duration is required, it would seem likely that dysplasia can occur and be used as a successful marker of enhanced risk in this group as well.

Crohn's disease: As the realization that carcinoma may complicate chronic CD has been late, we are just appreciating that there may be an associated dysplasia. In most of the early case reports and reviews of carcinoma in CD, there was only a rare mention of dysplasia[34,35]. But, it has been recently demonstrated that epithelial dysplasia similar to that observed in UC may be seen more often in patients with

CD and carcinoma involving both the colon[36] and small intestine[37]. There have been no large or systematic studies to indicate the incidence and distribution of dysplasia in CD and also no information on the potential value of its detection in biopsies. By analogy with UC, however, it is probable that endoscopc surveillance with biopsy would prove useful in the small group of patients with CD of the colon of longer duration.

Macroscopic features of dysplasia

Most of the observations have been recorded in cases of UC, but equivalent features have been noted in CD. Yardley described the flat, villous-like mucosal surface corresponding to areas of prominent dysplasia[21]; these are often seen at the edges or overlying an invasive carcinoma but also may be present withut tumor. Its appearance is highly variable and can present as an irregularly polypoid, granular or velvety area, which is often enhanced in specimens after fixation. These areas can usually be distinguished from the more ordinary inflammatory polyps (pseudopolyps), as the latter tend to be small, discrete and have a relatively smooth surface[38]. Exceptionally, there may be very large inflammatory polyps which also exhibit a villous-like surface[39], but careful inspection reveals that this appearance results from an interconnection of strands of mucosal tissue. It should be emphasized that a villous configuration may accompany areas of inflammatory or hyperplastic change[40], and a biopsy must always be performed to delineate a dysplastic lesion.

The appreciation of the macroscopic features has practical value in the gross inspection and detection of dysplasia. Although prominent areas may be visualized with air-contrast radiography, it is unlikely that this modality with its inherent risk of radiation will be routinely employed in monitoring patients. Rather, the gross appearance of the lesion should prove of special assistance in the endoscopic examination and serve to select areas for biopsy. A recent observation suggests that grossly evident areas of dysplasia may be associated with carcinoma at that site in a much higher proportion of cases[31], and biopsy of such lesions revealing even lesser degrees of dysplasia may be sufficient. It has been argued, however, that random biopsy of flat mucosa in the absence of a gross lesion will prove more effective in detecting the earlier, more superficial carcinomas[41]. Furthermore, such gross areas must be sought in the pathologic examination of colectomy specimens.

Histologic interpretation of dysplasia

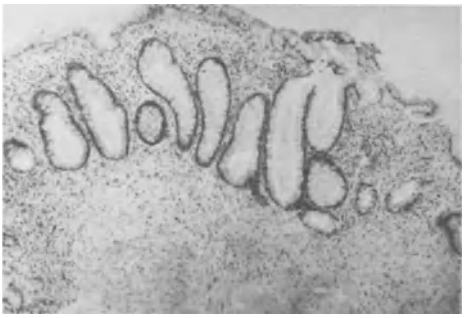
The histologic examination of the mucosal biopsies is at present the crucial step in determining the existence and degree of epithelial dysplasia and the appropriate clinical management. There have been several contributions dealing with the range of morphologic features seen, including in particular the notable publications of Riddell[3,42]. Studies have dealt mainly with cases of UC, but it appears that equivalent characteristics occur in CD as well. But, there has been considerable variation in terminology and descriptions of essential criteria, and it has been difficult to compare the results of the different investigators. To address these issues, a group of pathologists from 10 institutions in USA, England and Sweden have collaborated for the past three years, with the aim of providing a uniform set of definitions, criteria and classification that can be applied in the rating of biopsies for dysplasia. The results have been presented in preliminary form[19], and a monograph with detailed histologic descriptions is in preparation. It is anticipated that this will serve as a standard guide for the morphologic interpretation of mucosal biopsies in IBD.

Classification: Although pathologists are accustomed to identify with precision neoplastic lesions, difficulties in interpretation may arise in biopsies of patients with IBD because of the cumulative acute and chronic effects of the inflammatory process. Thus, even patients in remission with inactive disease may reveal considerable architectural or histologic alterations, characterized by irregular branching of the crypts. When this is combined with the cytologic effects of acute or active disease, including degeneration and regeneration of the epithelial cells, lesions simulating neoplasia in non-colitic patients may be achieved. It is, therefore, exceedingly important that the morphologist be fully aware of the range of inflammatory and reparative effects that may be seen in mucosal tissue in patients with IBD[43]. This caution is reflected in the classification and criteria that have been offered by the IBD-Dysplasia Morphology Study Group[19], and it has been proposed that biopsies be rated as negative, indefinite or positive for dysplasia.

Illustrative Examples: Negative biopsies include normal tissue and the effects of inactive and active disease, without the presence of any areas suspicious of dysplasia. Normal biopsies may be obtained from more proximal colonic portions in patients with less than universal UC, skip areas in cases of CD, and some patients with suspected IBD in whom the diagnosis is not confirmed. Biopsies with inactive disease (Fig. 1) reveal mainly atrophic crypts with variable branching, and the epithelial cells are completely normal or show some residual regeneration. In areas of active or acute disease (Fig. 2), there is typically a much greater degree of degenerative

and regenerative effects in the epithelial cells combined with prominent acute and chronic inflammation in the lamina propria and epithelial region. Compared to normal tissue, there is often prominent stratification and enlargement of the nuclei of the epithelial cells, but this can usually be distinguished from dysplasia by its relatively monomorphic appearance and the lack of hyperchromatism. Special caution should be maintained in analysis of biopsies of pseudopolyps, as these may contain areas with more prominent regeneration. The indefinite category is applied for various technical (including fixative effects, poor quality of sections or staining, and insufficient tissue) and interpratative reasons. The latter is most commonly due to the appearance of epithelial changes or atypia equivalent in structure to lesser degrees of dysplasia but in combination with definite features of active disease such as the presence of many neutrophils or adjacent ulceration (Fig. 3). In such cases, it must be appreciated that the epithelial alterations could still be the result of the active disease, and it is best to avoid an unequivocal diagnosis of dysplasia. For this reason, it is recommended that biopsies of grossly evident active disease should be avoided and samples taken preferentially from more atrophic areas. These biopsies can often be further rated as of a probably inflammatory or neoplastic nature depending on the degree of cytologic atypism, and this has particular utility in further clinical management. The indefinite grade is also employed when there is the appearance of some unusual growth or inflammatory form whose nature is not fully appreciated; this includes some cases with incomplete maturation and prominent basal nuclei[3], especially florid effects of lymphoid follicular inflammation, and hyperplastic-type lesions with relatively slight cellular atypism[19].

The positive category includes all cases in which the diagnosis of dysplasia can be rendered with reasonable certainty, and it is important at present that these cases be further subdivided into low grade and high grade dysplasia for clinical decisions. Low grade dysplasia (Fig. 4), which corresponds to the previously described mild and lesser degrees of moderate dysplasia, is characterized by the presence of epithelial cell nuclei with increased and irregular chromatin, more prominent stratification approaching one-half of the cell and a slight degree of pleomorphism. It is probable that this diagnosis should be made only in the absence of any definite effects of acute or active inflammatory disease to avoid an erroneous evaluation. The designation of high grade dysplasia embraces cases with moderate cytologic atypicality combined with more certain architectural alterations of neoplasia (such as glandular crowding and adenomatous arrangement) and all biopsies showing severe cellular dysplasia including carcinoma in-situ (Fig. 5). There are greater degrees



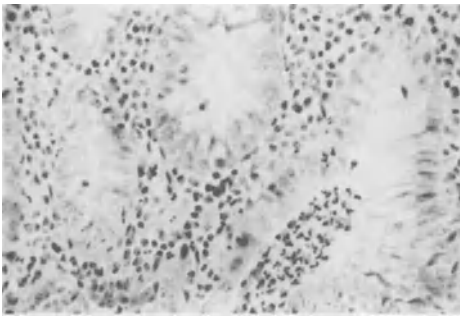
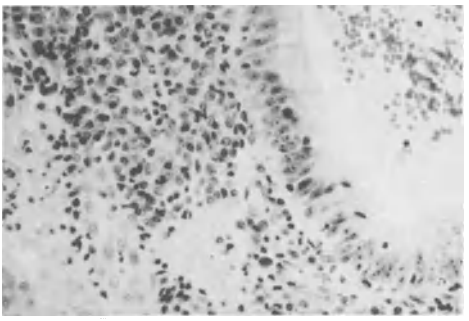


FIGURE 1. (LEFT). Mucosal biopsy of inactive colitis, negative for dysplasia. Short crypts are separated from the muscularis mucosae (bottom), and they appear irregular and show focal branching. There is no cytologic atypism.
FIGURE 2. (RIGHT). Mucosal biopsy of active colitis, negative for dysplasia. There is marked inflammation with crypt abscess (right). Crypt epithelial cells have enlarged nuclei with slight stratification. The nuclear chromatin pattern is fine, and there is no prominent pleomorphism.



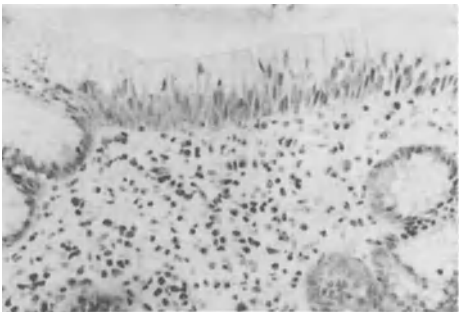


FIGURE 3. (LEFT). Mucosal biopsy, rated as indefinite-probably inflammatory. Inflamed crypt at right. Epithelial cells contain larger nuclei with greater variation in size and shape. But, inflammatory cells are present in epithelial layer, and adjacent region showed erosion.
FIGURE 4. (RIGHT). Mucosal biopsy, positive for low grade (mild) dysplasia. Surface epithelial cells (top) contain elongated nuclei with prominent stratification occupying about one-half of cell length. There is no acute inflammation.

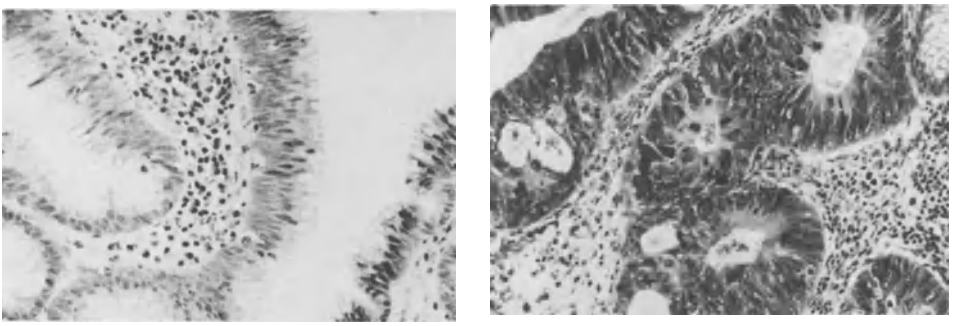


FIGURE 5. Mucosal biopsies, positive for high grade dysplasia. LEFT, example of moderate cytologic dysplasia with most epithelial cells containing enlarged and stratified nuclei filling most of cell length. At low power (not shown), there was tubular arrangement of glands similar to that seen in adenoma. RIGHT, example of severe cytologic dysplasia with marked nuclear hyperchromatism and pleomorphism, and early cribiform development.

of nuclear hyperchromatism, pleomorphism and stratification in these cases; and the villiform surface may be evident particularly in the more severe cases. In all phases of IBD, there may be an associated increase in endocrine cells and the appearance of areas of Paneth cell and pyloric gland metaplasia, and these alterations may be observed also in areas of dysplasia[42]. It should be stressed that the diagnosis of dysplasia is often accomplished by a combination of histologic and cytologic features, as any single alteration may be common to inflammatory disease[6]. Whenever an area of dysplasia is identified, it is important to look as well for any associated carcinoma including tumor that has penetrated the lamina propria (intramucosal carcinoma) or extended into the submucosa (invasive carcinoma).

Future needs

The greatest requirement is the development and adoption of a uniform set of definitions and criteria that can be applied to the evaluation of mucosal biopsies in all phases of IBD. This would provide a ready guide for all morphologic interpretations in clinical practice and permit more rapid accumulation of data in collaborative studies. This effort is being prepared by the IBD-Dysplasia Morphology Study Group[19], and it will be important to test the validity of this system in general practice.

Preliminary observations indicate that the diagnosis in a biopsy of negative for dysplasia is in general accurate and that screening could therefore be achieved by persons familiar with the proposed system. But, it appears that there is an increase in the level of disagreement in biopsies rated as indefinite or positive for dysplasia particularly of the low grade, and it may prove beneficial that such biopsies be subjected to some form of a review.

It is also important to determine in a more systematic fashion the extent to which the gross surface alterations can be used to select biopsy sites and identify the cases with dysplasia. It is possible that an improvement in the endoscopic optics will permit finer visualization of the mucosal surface[44]. More data is clearly needed in all patient groups to allow for more rational recommendations in clinical management, and this is particularly lacking in cases with limited chronic colitis including patients with left-sided UC and with CD of the colon. Further information on the predictive value of dysplasia in relation to its extent should be sought; this includes comparison of cases with dysplasia limited to the more basal portions of the crypts as opposed to the surface epithelium, and the presence of dysplasia in single versus multiple biopsies.

CLINICAL RECOMMENDATIONS

Based on the available data, tentative recommendations for the clinical care of patients with chronic IBD who are prone to develop carcinoma can be provided[6,9,10,19,31,41,44]. Once a patient reaches the point of an established increased risk for tumor, such as 10 years with extensive UC, the choice of prophylactic total colectomy should be offered. If this is declined as is often done, a set program of periodic surveillance with endoscopy and biopsy should be instituted. It must be emphasized that the aim is not to achieve the situation in which all patients have invasive carcinoma, as this would permit the development of many cases with advanced tumors. Rather, the present accomplishment where a biopsy showing high grade dysplasia is predictive of tumor including more superficial types in at least one-third of cases may be sufficient. Further data is needed to determine the potential value of biopsies showing low grade dysplasia, whether the same incidence of tumors but of more early forms may be attained.

1. Monitoring: For patients with extensive UC, this should be begun at 7 to 8 years and certainly by 10 years. Complete colonoscopic examination is favored, and the frequency has been arbitrarily set at an annual basis. Recognizing that tumors may develop at an earlier time, yearly rectal examinations may be indicated prior

to 7 years. Since tumors occur later in patients with left-sided ulcerative colitis and with Crohn's colitis, surveillance might be started at about 15 years after onset of disease in this group. In patients with CD limited to the small intestine, there is no available procedure to visualize and biopsy directly the area and no information to suggest that dysplasia as a marker is present in the colon.

2. Biopsy choice: Endoscopic biopsies should be obtained in all parts of the colon and rectum from stenotic lesions, any villous-appearing or other atypical polyps, perhaps one or two ordinary pseudopolyps, and multiple random areas of the flat mucosa that appear the least inflamed (i.e., atrophic regions). It is imperative that the biopsy samples be labelled for their location to permit reassessment of any suspicious areas.

3. Histologic interpretation: Aside from noting the extent and degree of activity of the IBD, each biopsy should be rated or graded as:

a. Negative for dysplasia, including normal, inactive or active disease.

b. Indefinite, providing the particular technical or interpretative reason. This should be further graded as probably inflammatory, probably dysplastic or uncertain.

c. Positive for dysplasia, indicating if possible if this is of a low-grade or high grade degree.

As indicated above, it may prove useful to permit a review of cases showing indefinite or positive biopsies before further clinical decisions.

4. Clinical response: This should consider whether a grossly suspicious lesion was observed at endoscopy as well as the histologic grading, and the following actions would be recommended[19]:

a. Continue regular surveillance if all biopsies are negative for dysplasia; or rated as indefinite-probably inflammatory in the absence of a gross lesion.

b. Repeat endoscopic examination within a few months and obtain multiple samples from suspicious areas if random biopsy from flat mucosa is rated as indefinite (probably dysplastic or uncertain) or low grade dysplasia; or if biopsy taken from gross lesion is ranked as indefinite of any degree including probably inflammatory.

c. Advise total colectomy if random biopsy from flat mucosa shows high grade dysplasia; or if biopsy of gross lesion reveals low or high grade dysplasia. Colectomy might also be considered if repeated biopsies demonstrate persistence of indefinite-probably dysplastic or low grade dysplasia.

It must be emphasized that these recommendations for patient management are provisional and based on the existing information. With greater standardization

of the procedures used to detect neoplasia in patients with IBD, it is anticipated that more essential data can be derived from combinations of the various investigations. This will permit us to determine with more certainty the timing, frequency and preferred endoscopic method to use in the monitoring of the patients with various degrees of colitis; the relative values of selected and random mucosal biopsies; and the general accuracy and utility of the proferred classification for interpretation of the biopsies.

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ATHYMIC NUDE MICE IN STUDIES OF CROHN'S DISEASE

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INTRODUCTION

Although a variety of infectious agents including bacteria, mycobacteria, L forms of bacteria and viruses have been inconsistently isolated from tissues of patients with Crohn's disease, no suitable animal model is available to investigate these isolates (1-9). Conventional animals when injected with filtrates of Crohn's disease tissue developed granulomas both at the injection site and in the intestinal wall in about 15-20% of the animals at 8 months to 2 years (10).

Athymic T-cell deficient (nu/nu) mice because of their unique immunodeficiency have been successfully used to culture in vivo various infectious organisms including bacteria, viruses, parasites and mycobacteria (11-14). Organisms which could not be grown in conventional animals were shown to grow and express themselves better in nude mice (15,16). In search for a possible transmissible agent related to Crohn's disease, we used nude mice and recently reported production of murine lymphoma in these mice following injection of lymph node (17) or intestinal mucosal filtrates from patients with Crohn's disease (18).

Mice injected with control lymph node filtrates from patients with ulcerative colitis, cholecystitis, sarcoidosis did not produce such lesions. Preliminary results of indirect immunofluorescence of 2 such lymphomas suggested that sera from patients with Crohn's disease contain antibodies which recognize antigen(s) in the murine lymphoma.

MATERIALS AND METHODS

Nude Mouse Colony: A nude mouse colony on a BALB/c background was established and the colony is maintained inside laminar flow units in a separate pathogen-free room with positive Hepa filter system. Mice of age 8-12 weeks were used for the studies.

Tissue Homogenates: The mesenteric lymph nodes or intestinal mucosa from Crohn's disease and control subjects were homogenized, filtered and injected intraperitoneally into the mice (17).

Serum and Serum IgG: Sera from patients with Crohn's disease (symptomatic or in remission), ulcerative colitis (active or in remission), other diarrheal diseases and normal subjects were obtained. Several medical centers and the National Cooperative Crohn's Disease Study Group (courtesy of Dr. J. Singleton, Denver, Colorado) provided the sera. Sera were coded and the diagnosis and clinical activity of the patient's illness was assessed by the simple method of Crohn's Disease Activity (19) and in the case of sera provided by Dr. Singleton, by the Crohn's Disease Activity Index (20).

Serum IgG was separated from two patients with symptomatic Crohn's disease and also from two control subjects by ammonium sulfate precipitation followed by DE-52 column chromatography.

Fluorescein Isothiocyanate Conjugated Antisera: FITC-conjugated rabbit anti-mouse IgG, IgM, Thy 1-2 and C_3 sera were obtained from Cappel Laboratories, Pennsylvania. FITC-conjugated rabbit anti-human IgG and IgM were obtained also from Cappel Laboratories.

Immunofluorescence Studies of Lymphoid Tissue and Kidneys: Indirect immunofluorescence studies were performed on the cryostat sections of lymph nodes and kidneys using the protocol as described earlier (17). Each serum was absorbed initially with normal nude mouse spleen cells followed by absorption with mouse serum proteins bound to sepharose 4B. Appropriate control sera, as well as the control lymphomas, lymph nodes and kidneys,were examined simultaneously. Nude mouse lymph nodes and kidneys were also examined by direct immunofluorescence for presence of mouse IgG, IgM, mouse complement and Thy 1-2 (for lymph nodes only) by direct immunofluorescence.

RESULTS

Sixteen of the 97 mice injected with Crohn's disease filtrates developed lymphadenopathy due to lymphoma (9 mice) or cellular hyperplasia (7 mice) (Fig. 1 A and B). Lymphomas developed 6 weeks to 8 months after injection of filtrates of Crohn's disease tissue from 7 patients. Each patient's material induced at least one lymphoma. A single

lymphoma developed in one of 72 control nude mice 8 months after injection of filtrate of grossly normal human colon mucosa adjacent to a carcinoma.

Immunofluorescent Studies: Lymphomas and hyperplastic lymph nodes were examined by indirect immunofluorescence using sera from patients with Crohn's disease and control subjects. Eighty-two percent of 30 sera from patients with active Crohn's disease showed positive staining to lymphomas produced by Crohn's disease tissue filtrate (Fig. 2). Sixty-five percent of the same sera were also positive to hyperplastic lymph nodes due to plasma cell hyperplasia produced following injection of Crohn's disease tissue filtrates. Seventeen percent of the sera from 22 patients with Crohn's disease in remission recognized an antigen(s) in the lymphomas or hyperplastic lymph nodes. None of the 41 sera from patients with ulcerative colitis whether symptomatic or in remission, 9 patients with other diarrheal diseases, or 19 control subjects stained the lymphomas or hyperplastic lymph nodes (Fig. 2). No sera, including the sera from patients with Crohn's disease, active or in remission, stained the control lymphoma or hyperplastic lymph nodes produced following injection of ulcerative colitis filtrate or normal colon tissue filtrate.

Intensity of immunofluorescence was correlated with the Crohn's Disease Activity Index. Sera from patients with high Crohn's Disease Activity Index, i.e., 246 $\pm$ 23 (mean $\pm$ SEM), demonstrated 2 to 3+ positive immunofluorescence, whereas the sera from patients in remission with

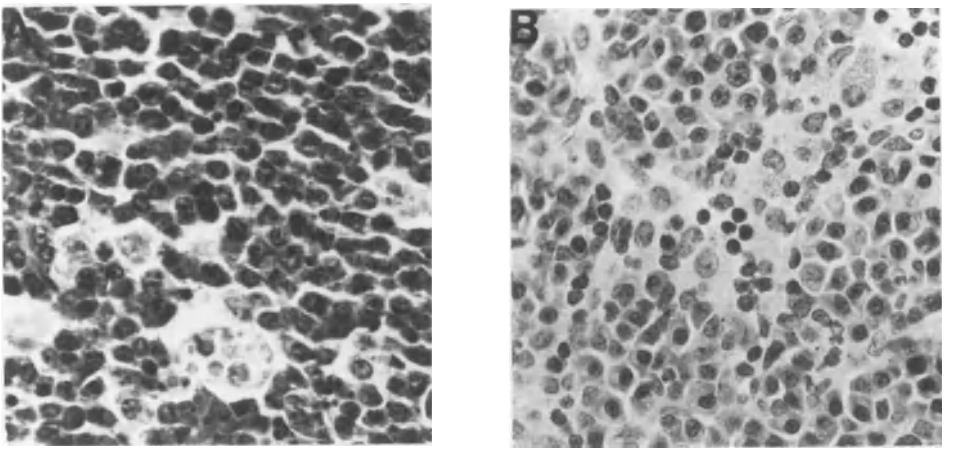

Figure 1: Hematoxylin and eosin. Magnification X 500.
(A) Lymphoma in a nude mouse injected with colon filtrate (.2 μm) from a patient with Crohn's disease. The lymph nodes and spleen were diffusely infiltrated by large neoplastic lymphocyte cells.
(B) Lymph node demonstrating plasma cell hyperplasia in a nude mouse injected with colon filtrate (.2 μm) from a patient with Crohn's disease.

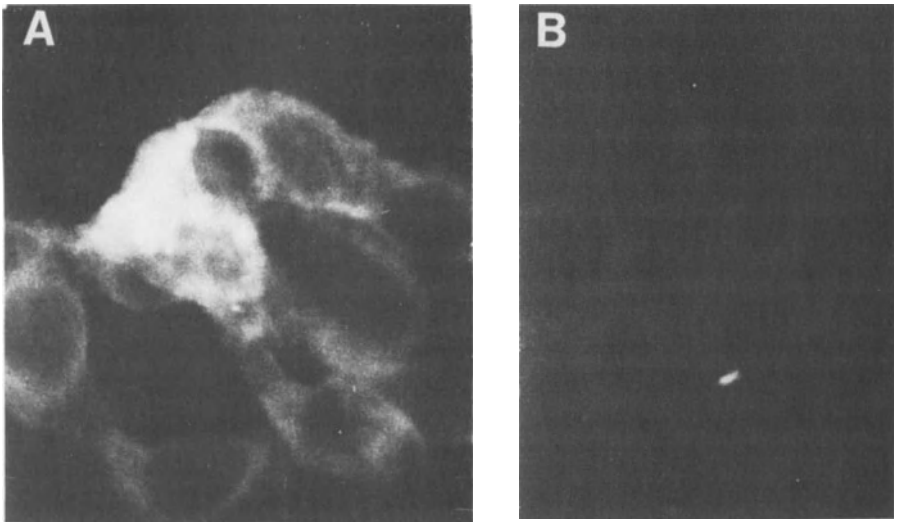

Figure 2: Indirect immunofluorescence of a lymphoma in a nu/nu mouse following injection of lymph node filtrate from a patient with Crohn's disease. Magnification X 400.
(A) Serum from a patient with Crohn's disease resulted in homogenous cytoplasmic staining in clusters of cells.
(B) Serum from a patient with ulcerative colitis showed no staining.

Crohn's Disease Activity Index 130 $\pm$ 22 did not show immunofluorescence. These differences were statistically significant, $p<.007$.

A majority of cells in lymphomas showed positive staining to anti-mouse IgG and IgM (Fig. 3) and was negative to mouse Thy 1-2 and complement. The remaining cells did neither stain with anti-mouse Ig nor with anti-Thy 1-2. Antigenic recognition of Crohn's disease sera was examined in kidneys of mice injected with Crohn's disease tissue filtrates or control filtrates. Glomeruli of 5 of 17 nude mice injected with Crohn's disease tissue filtrate showed positive staining with sera from patients with active Crohn's disease but not with sera from ulcerative colitis patients (Fig. 4). Glomeruli of the same 5 kidneys also revealed immunofluorescence to anti-mouse IgG and IgM suggesting immune complex deposition. Five additional kidneys from mice injected with Crohn's disease tissue filtrates showed positive staining to anti-mouse IgG and IgM. One kidney showed positive staining to anti-C_3 serum. Kidneys from 10 mice injected with ulcerative colitis and 5 mice injected with normal colon tissue filtrate were negative with both Crohn's disease and ulcerative colitis sera.

Absorption Studies: Following absorption of immunoreactive sera from 4 patients with active Crohn's disease with homogenates of colonic mucosa from Crohn's disease patients, immunofluorescent staining disappeared (Fig. 5). However, similar absorption with control colon mucosa

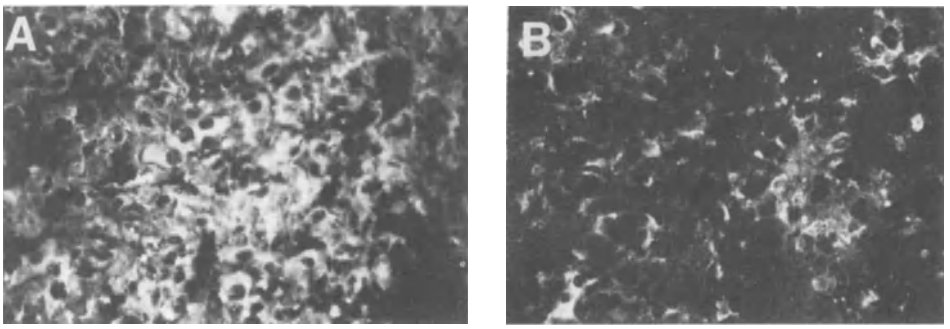

Figure 3: Direct immunofluorescent staining of a lymphoma in a nu/nu mouse following injection of colon filtrate from a patient with Crohn's disease. Magnification X 400.
(A) Anti-mouse IgG gave diffuse intense cytoplasmic staining.
(B) Anti-mouse IgM demonstrated scattered cells with strong cytoplasmic staining throughout the section.

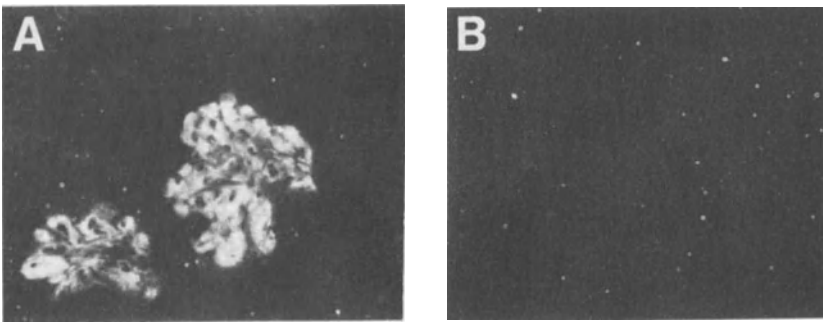

Figure 4: Indirect immunofluorescence of a kidney from a nu/nu mouse injected with lymph node filtrate (.2 μm) from a patient with Crohn's disease. Magnification X 400.
(A) Serum from a patient with active Crohn's disease gave intense glomerular staining.
(B) Staining was completely absent with serum from a patient with active ulcerative colitis.

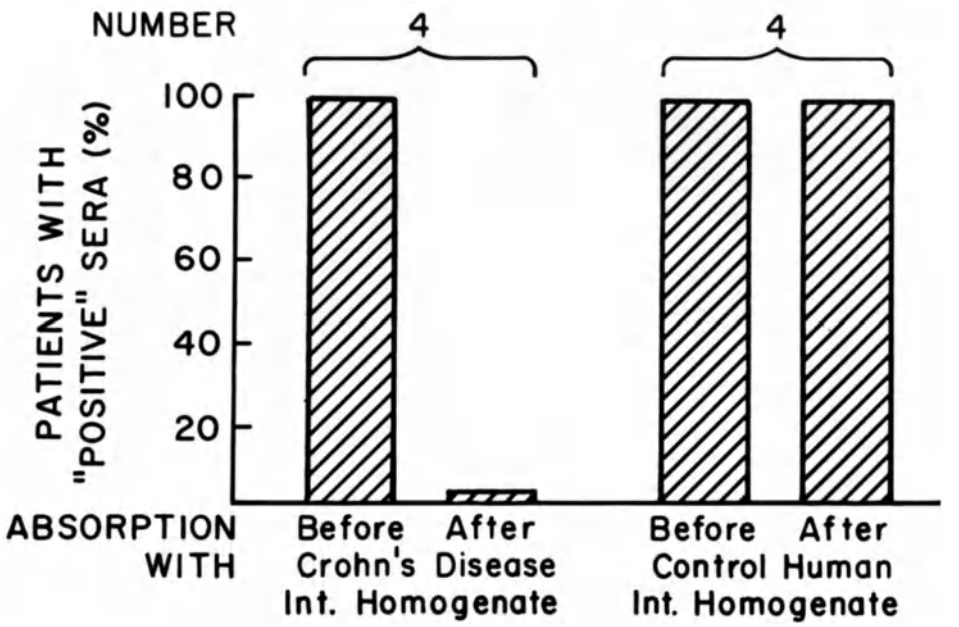

Figure 5: Staining by the indirect immunofluorescent method of induced lymphoma by sera from patients with active Crohn's disease was abolished by incubating sera with tissue from patients with Crohn's disease, but not by incubation with normal tissue.

(normal segments from patients with carcinoma) did not change the staining. In an attempt to identify the mucosal antigen(s) related to specific immunoreactivity, diseased colon from 2 patients with Crohn's disease and 1 with ischemic colitis was minced, homogenized in polytron at 6 X speed in PBS containing 2 mM phenyl methyl sulfonyl fluoride (PMSF) and 0.5% Triton X, centrifuged 18,000 X g for 30 min, filtered (0.2 μm) and the filtrate was passed through a sephadex G-100 column. The elution profile of the filtrate of the mucosa from a patient with Crohn's disease (left side) and the patient with ischemic colitis is shown in Fig. 6. There was no significant difference between them. Fractions were separated as follows: F-1 38-46 ml, F-2 48-60 ml, F-3 72-74 ml, F-4 120-140 ml. Each fraction was concentrated to adjust the protein at about 1 mg/ml and incubated at 37°C with equal volume of immunoreactive Crohn's disease sera which were then used for immunofluorescent studies against murine lymphomas. Additional control experiments were performed by simultaneous incubation of Crohn's disease sera with equal volume of PBS. Immunofluorescence was positive with PBS diluted Crohn's disease sera up to 1:4, but no inhibition of immunofluorescence was observed with any of the 4 fractions. Each fraction was also examined for presence of proteolytic activity which was absent.

Electron Microscopic Studies: Electron microscopic examinations were performed in 5 lymphomas produced following injection of Crohn's disease tissue filtrates

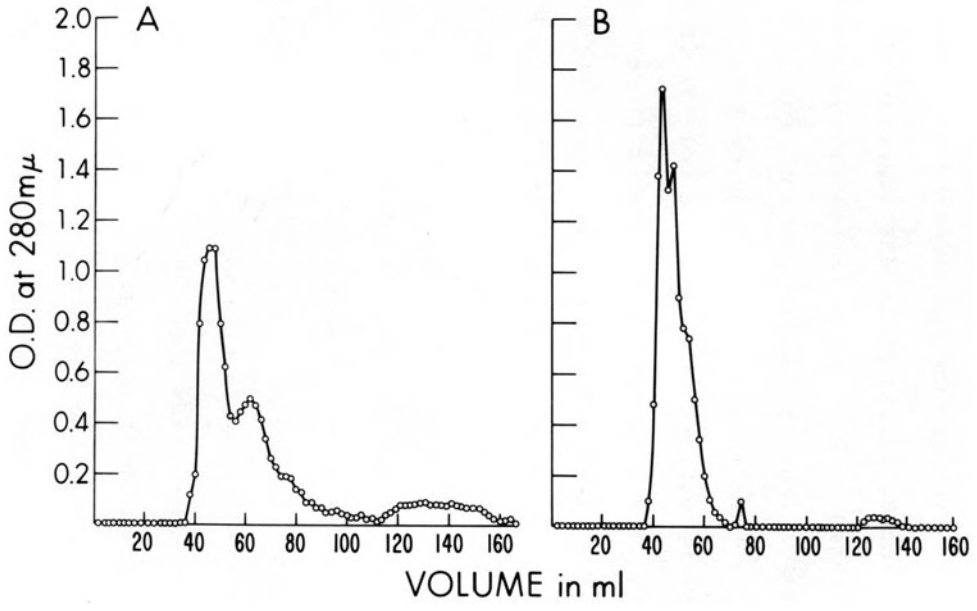

Figure 6: Elution profiles of colonic mucosal homogenates from a patient with Crohn's disease (A) and a patient with ischemic colitis (B). Sephadex G-100 column in PBS; flow rate ~ 0.3 ml/min.

and in one control lymphoma. C-type viral particles were demonstrated in all 6 lymphomas (18). The hyperplastic lymph nodes or normal lymph nodes did not contain C-type viral particles.

DISCUSSION

In search of a suitable animal model to investigate the transmissible agent(s) related to Crohn's disease, we used athymic nude mice (17). Our previous studies demonstrating production of lymphoma in this animal following injection of Crohn's disease lymph node and intestinal filtrate have now been confirmed with additional animals. We have demonstrated in these studies the antigenic recognition of several lymphomas as well as hyperplastic lymph nodes due to plasma cell hyperplasia using a large number of coded sera. About 3/4 of the sera from patients with active Crohn's disease and about 1/5 from patients in remission showed immunofluorescent staining of the lymphomas as well as hyperplastic lymph nodes. None of the control sera from patients with ulcerative colitis or other diarrheal diseases and normal subjects stained the lymph nodes. Control murine lymphoma did not show any staining to sera from patients with Crohn's disease or control subjects.

The nature of the antigen(s) in the lymphomas recognized by Crohn's disease sera is as yet unknown. It is also unclear whether the mechanism of lymphoma production is due to a direct effect of the putative agent associated with Crohn's disease or perhaps by stimulation of endo-

genous murine oncogenic viruses. The viral particles seen by electron microscopy morphologically appears to be murine oncogenic viruses which are present in the lymphomas produced by Crohn's disease tissue filtrate and also in the control lymphoma. The antigen(s) which is recognized by Crohn's disease sera is probably not related to C-type murine viral particles in lymphomas because antigenic recognition of Crohn's disease sera is not present in control murine lymphoma which also contains C-type viral particles. Future studies using immune electron microscopy should reveal the relationship between viral particles and antigenic recognition by Crohn's disease sera. Absorption studies with Crohn's disease tissue homogenate suggest the presence of an antigen(s) in the Crohn's disease tissue. However, the antigen could not be solubilized with 0.5% Triton X. Elution profiles of mucosal filtrates (Fig. 6) in G-100 column were similar to those described by McLaren et al. (21). However, contrary to the presence of cytotoxins in the eluates described by them, none of the peaks showed inhibition of immunofluorescence in the murine lymphoma.

The lymphomas produced in the mice do not seem to be spontaneous lymphoma because tissue filtrate from each of the 7 patients with Crohn's disease produced at least one lymphoma and, moreover, most of the mice were sacrificed within ten months of injection, an age at which spontaneous lymphoma is rare (22).

Hyperplastic lymph nodes produced by Crohn's disease

filtrates contained an antigen(s) recognized by Crohn's disease serum but did not contain any viral particles when examined by electron microscopy. This suggests that the antigen(s) recognized by Crohn's disease sera do not require complete viral particles or are not reacting with the viral antigens.

Since only one injection of the filtrate was given to each mouse, it is unlikely that a non-replicating inducing agent could remain in lymph nodes for months and be present in sufficient quantity to react immunologically with serum from Crohn's disease patients. The presence of an antigen in the lymphoid tissue in the hyperplastic state and in lymphoma state strongly suggest that the antigen is probably replicating in the mice in vivo. Immunoreactivity of the serum could be absorbed by incubation with Crohn's disease tissue. These results suggest that the antigen(s) which is recognized by the sera from patients with Crohn's disease is present both in murine lymphoma and also in Crohn's disease tissue. Future studies are needed to characterize and isolate the antigen present in the murine tissue recognized by the Crohn's disease sera. The studies so far indicate that the nude mice may serve as an exciting model for studies of the etiology of Crohn's disease.

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B. Etiology

INFLAMMATORY BOWEL DISEASE - THE SEARCH FOR AN ETIOLOGY

MARTIN F. KAGNOFF, M.D.

In the session this afternoon we will assess available information on the etiology of inflammatory bowel disease (IBD). Exciting new developments are beginning to point to the etiology of these diseases and our panel will bring you up to date on these new developments. I am optimistic that with the increased intensity of investigation on the etiology of inflammatory bowel diseases coupled with emerging new tools and concepts for studying chronic diseases and inflammatory diseases, we should soon come close to the point where we obtain meaningful answers about what causes IBD.

Much of the new information relevant to the etiology of IBD lies in the area of immunology, microbiology, virology and genetics. This afternoon's panel was selected with that in mind. Thus, Dr. Shorter will focus on what we know currently about the role immune mechanisms play in the etiology of inflammatory bowel disease. Dr. Das will tell us about his exciting new finding of a cytolytic antibody that is present in the serum of patients with ulcerative colitis. Dr. Gorbach will bring us up to date on the possible ways in which one or more bacteria could be involved in causing IBD and Dr. Gitnick will focus on the putative role viruses and/or cytotoxins play in IBD and review the current status of research on transmissible agents in IBD. The session will close with Dr. Onderdonk discussing the experimental model for Carageenan-induced colitis and telling us how experiments using this and different animal models can help yield new information in IBD. By way of introduction to this session, I was asked as moderator to make some general comments on the etiology of IBD and present some background information on how immune mechanisms could be involved in these diseases.

Before proceeding further on a session dealing with the etiology of IBD, I suppose it is logical that we ask the simple question---why is it important to identify the etiology of inflammatory bowel disease? The

answer, simply stated, is that by knowing the etiology of IBD we should be able to develop effective strategies for prevention, effective strategies for early identification of the population at risk for developing IBD, and effective strategies for therapy. To keep the problem of etiology in perspective however, we should keep in mind that effective strategies for disease prevention and treatment sometimes arise in the complete absence of knowing the specific disease etiology. For example, many years before the specific etiologic agent of cholera, the Vibrio cholera, was known, it was known that hygienic measures prevented the development of that disease. Historically, effective treatments for some diseases also have been found before a specific disease etiology was characterized. In the case of several chronic diseases---for example, essential hypertension, coronary artery disease---specific etiologies are unknown. Yet in these diseases the identification of risk factors has helped in the design of prevention strategies, and drugs aimed at lowering blood pressure have helped in the prevention of disease complications. In the case of IBD, such good fortune has not prevailed. There is currently no effective strategy to prevent IBD, there is currently no effective strategy to detect early disease, and treatment falls far short of what any of us would regard as ideal or effective. Thus, it makes sense that no matter how difficult the problem appears, concurrent with appropriate approaches and efforts in other areas, a major effort should continue to identify the etiology of ulcerative colitis and Crohn's disease.

How does one set out to define the etiology of IBD? Where does one start? Where do the clues come from? Does epidemiologic evidence favor an exogenous agent---chemicals polluting our environment, the increased use of birth control pills in women with Crohn's disease, food or food additives, bacteria or bacterial products, viruses? Does laboratory evidence favor a primary immunological abnormality---is IBD an autoimmune disease or are autoimmmune mechanisms important in producing tissue damage once the disease process starts? Does pathologic or physiologic evidence favor vascular or neurogenic factors as the primary cause? Moreover, what is the host background that allows the etiologic cause of ulcerative colitis and Crohn's disease to produce disease? This afternoon's session will attempt to answer these questions and bring us up to date on the state-of-the-art today. Moreover, we will hear whether our panel believes Crohn's disease and ulcerative colitis are caused by one or multiple

etiologic agents or events? Are ulcerative colitis and ulcerative proctitis one or multiple diseases in terms of etiology? For that matter, is Crohn's disease a single disease or several different diseases?

Finally, let me conclude this portion of my introductory remarks with the reminder that a variety of etiologies have been proposed over the years to explain ulcerative colitis and Crohn's disease. New hypotheses have generated tremendous excitement, but as hypotheses have been tested they often have been proven wrong. Should this discourage a vigorous continued effort in the search for an etiology in IBD---in no way. It is only through the development of new ideas and new hypotheses, together with the testing and verification of these hypotheses, with a healthy and realistic degree of skepticism on the part of the investigator and medical community alike, that there is hope for finding the specific etiology of Crohn's disease and ulcerative colitis.

I want to proceed now from philosophy to fact, and perhaps a little speculation. There is little doubt that a body of accumulated evidence supports the notion that immune mechanisms are important in the etiopathogenesis of inflammatory bowel disease---both ulcerative colitis and Crohn's disease. The key question is how major or important a factor are they in these diseases and what is their specific role in producing and perpetuating IBD?

Concepts of the immune system as a complex and sophisticated network of cells that interact with foreign materials, with each other, and with other cells have developed and undergone rapid changes over the past several years (1). The immune system is comprised of lymphoid cells, both circulating and in tissues, that can be activated specifically by antigens or non-specifically by mitogens. Fully 25% of the intestinal mucosa is comprised of lymphoid tissue. When stimulated, lymphoid cells in the intestine or in sites outside in the intestine differentiate and mature to express a variety of effector functions. Lymphoid cells are divided into two main lineages---B-cells and T-cells. B and T-cells themselves are very heterogeneous populations. Lymphoid cells belonging to the B-cell lineage produce antibody when activated. Lymphoid cells belonging to the T-cell lineage, mediate a variety of cell-mediated immune functions after activation. The specific effector functions mediated by T-cells can be of a regulatory nature (i.e., help or suppression of the immune response) or can be of a tissue damaging nature (i.e., killer T-cells).

There is little doubt that immune reactions can produce tissue injury. In IBD a variety of different humoral and cell-mediated immune reactions have been assessed, both in vitro in the laboratory and in patients. Several of these immune reactions have been reported to be present in IBD and have been suggested as potential candidates for the production of tissue injury in these diseases. As shown in figure 1 below, antibody, or antibody coupled to antigen to form an immune complex, together with components of the complement system may cause cell damage. Antibody directed against antigenic determinants that form part of the cell surface, or are attached to the cell surface, can cause cell damage in the presence of normal unsensitized lymphocytes known as "K" cells. This mechanism has been termed antibody-dependent cell-mediated cytotoxicity. Alternatively, T-cells can be activated to become T killer cells and damage target tissues directly. Activated T-cells can release mediators also that attract other damaging cell types into an immune reaction. Natural killer cells---cells that are present in all of us and may belong in the T-cell lineage, can damage target tissues in the absence of apparent antigen activation. Finally, we will hear that other cell types such as macrophages, polymorphonuclear leukocytes, basophils, eosinophils and mast cells may participate in immune reactions that potentially can cause tissue damage in IBD.

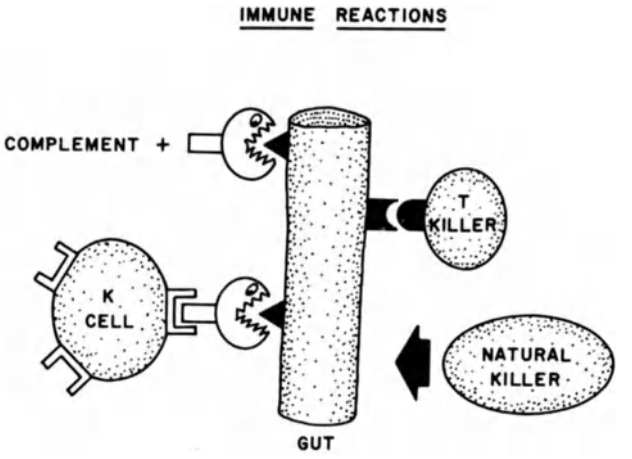

Figure 1. Humoral and cell-mediated immune reactions.

During ongoing immune tissue injury, when looked for carefully, many different immune effector mechanisms as well as a variety of cell types can be present concurrently. Therefore, the important question regarding the role of immune effector mechanisms in IBD is what is the contribution to disease production of any individual immune effector mechanism, and what if any is the major effector mechanism responsible for tissue injury? Answering this question from an experimental approach is clearly complex. It seems possible that a different and perhaps more helpful way to dissect the importance of immune mechanisms in patients with IBD may be to look at the processes involved in the induction limb of the immune response.

I want to say a few words now about the regulation of the immune response and how abnormalities in regulation of the immune response could be involved in IBD. As indicated in Figure 2 below, specific regulatory mechanisms govern the process by which lymphoid cells are activated and immune responses are controlled. These regulatory mechanisms, which are under genetic control, determine the quality and magnitude of an individual's immune response to a given antigen. Thus, marked differences can exist from person to person in the quality and magnitude of the immune response to a given antigen.

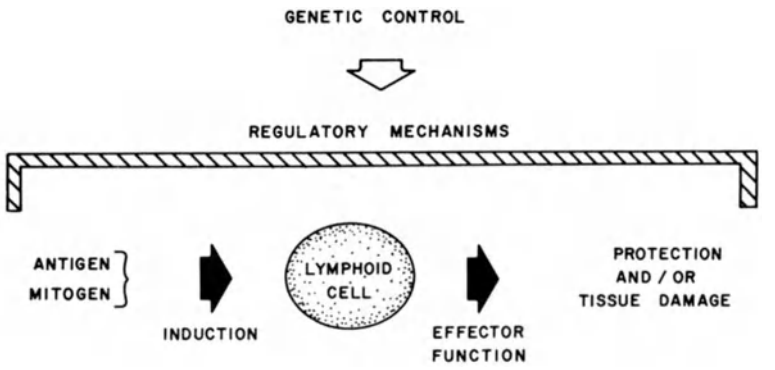

Figure 2. Regulation of the immune response.

Recent studies looking at mechanisms of regulation of the immune response and possible abnormalities of that regulation in IBD have generated considerable interest. The feedback loops and mechanisms involved

in immune regulation are perhaps as detailed and complex as those involved in hormone interactions and regulation in the endocrine system. In recent years it has become evident that subsets of T-cells are particularly active in regulating immune responses. Thus, we now recognize at least two types of helper T-cells that cooperate in the induction of immune responses and increase immune responses as well as several types of suppressor cells that are capable of inhibiting the immune response. There may even be contrasuppressor cells that counteract the suppressive influence of suppressor cells. Thus, one can postulate a variety of ways in which a defect in immune regulation could result in an increased or decreased immune response to a particular agent and play an important role in IBD. In fact, such defects are being postulated and looked for in IBD. For example, it has been proposed that Crohn's disease may be related to decreased suppressor T-cell activity---the problem envisioned in Crohn's disease being an exaggerated immune response to a foreign or self antigen. Alternatively, it has been suggested that an increase in T-cell suppressor activity to one or several antigens in Crohn's disease may result in a selective immunodeficiency. The selective immunodeficiency is envisioned to permit an invasive agent to set up the disease process. These represent only two ways in which possible abnormalities in immune regulation have been postulated to play a role in IBD---many others are certainly possible.

Over the past several years, studies in my laboratory have shown that exposure by the intestinal route to antigen can have a profound influence on antibody and cell -mediated immune responses in sites outside the intestine, in addition to the well-recognized effects on the local intestinal immune system (2-6). Most strikingly, systemic immune responses to many different antigens can be markedly suppressed if the host is fed the antigen being studied before being challenged intravenously or intraperitoneally with the exact same antigen. Inhibition of extraintestinal immune responses after antigen-feeding can be mediated by suppressor T-cells or different types of antibody. Therefore, it seems entirely possible that early intestinal exposure to certain antigens prior to the normal development of such regulatory mechanisms or an abnormality in the regulatory mechanisms involved in governing the relationship between intestinal immune responses and systemic immune responses may be important in IBD.

As I indicated before, regulation of the immune response is under strict genetic control. Therefore, another approach to examine possible

abnormalities in the regulation of the immune response in IBD would involve looking at the genes that govern regulation of the immune response in IBD compared with control patients. This approach has been used already in IBD for certain genetic loci. Thus, genes that map to the major histocompatibility complex, termed HLA in man, seem very important in governing the magnitude and quality of the immune response to a variety of antigens---particularly those antigens that involve T-cell mediated regulation. Thus far, most studies examining differences in HLA antigens between IBD and control patients have not demonstrated significant differences with the exception that patients with IBD and ankylosing spondylitis, like other patients with ankylosing spondylitis have a high incidence of the HLA B27 antigen (7,8). However, the major histocompatibility complex is not the only genetic locus involved in regulating the immune response.

In my laboratory, we have had a particular interest in the influence on immune regulation of genes that map to sites outside the major histocompatibility complex. Our work has focused primarily on the linkage between immune responses and genes that code for immunoglobulin allotypes. The genes coding for immunoglobulin allotype are linked to genes that govern the magnitude and quality of antibody responses to carbohydrate antigens such as those found associated with bacterial cell walls (9,10). We have postulated that perhaps the immune response to certain bacterial cell wall carbohydrates differs in IBD and control patients. Such differences may be relevant to the tissue injury seen in IBD and may be detected by genetic analysis.

In closing, let us for discussion sake make the assumption that an infectious agent is involved in IBD. Normally, the immune response to an infectious agent is designed to protect the host from the adverse effects of that organism. Is it because the nature and quality of the immune response in an individual susceptible to IBD differs from the immune response to the same agent in others, that the potential exists to directly or indirectly induce tissue injury and set up the inflammatory disease process? If the infectious agent is a rare agent, few persons would be expected to be infected and a relatively large proportion of infected persons would develop IBD. Thus, a large proportion of infected persons may share the putative abnormality in immune regulation. On the other hand, IBD may be caused by a common agent---one to which we are all exposed. We then

must ask what unique set of circumstances are present in susceptible individuals to permit the establishment of disease---and how does the immune system play a role in these susceptible individuals? In this case, an abnormality in immune regulation would be predicted to be infrequent.

How does tissue damage result? The immune response activated by an infectious agent could be directed against foreign determinants on the agent---tissue damage being secondary to attack on the agent. Alternatively, the immune response could be directed against determinants on the agent that cross-react with the host's own tissue or against antigenic determinants that arise in the host by virtue of the foreign agent altering the host's own tissue so that it is now recognized as foreign.

Finally, how should we look for the agent? If patients who develop disease are those who develop an exaggerated immune response to the agent, we may not be able to detect the agent in IBD patients as the foreign organism would be masked by or eliminated by the ongoing immune response. Thus, the agent may be more readily detectable in those who do not develop an exaggerated immune response and IBD. The situation becomes even more complex when one considers that it may take a particular combination of agents to produce IBD. Moreover, a variety of microbial agents or host factors may be involved in perpetuating IBD and the inflammatory response once tissue damage has been initiated by a totally different primary etiologic event. I know our speakers this afternoon will address these issues during their talks.

Politics involves the art of compromise. In contrast, science involves the search for the truth. As moderator, I feel that I am on the safest grounds politically to suggest that the etiology of IBD involves one or more microbial, viral or cellular toxic agents activating tissue damaging mechanisms in a genetically susceptible host. Perhaps IBD is one group of diseases where "politics" and "science" will meet!

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IDIOPATHIC INFLAMMATORY BOWEL DISEASE (IBD): A REVIEW OF IMMUNOLOGICAL MECHANISMS*

R.G. SHORTER

INTRODUCTION

While the etiology and pathogenesis of IBD are unknown, a favored hypothesis includes the participation of (1) external agents, possibly microbial, (2) immunological responses in the host, and (3) genetic factors influencing these responses. The purpose of this review is to summarize the immunological evidence that has led to such speculation.

IMMUNOLOGICAL FINDINGS IN IBD

Humoral immunity

Sera of patients with IBD may contain auto-antibodies which react in vitro with antigen(s) in the cytoplasm of colonic epithelial cells[1-10]. However, their presence and titers show no correlation with the site, duration or activity of the disease or the extent of colonic involvement[6,10-13] and they are not cytotoxic in vitro for such epithelial cells[14-16]; furthermore, they are not specific to IBD[6,10,17-19]. In addition, while the intravenous injection of heterologous anti-colon sera into dogs resulted in acute hemorrhagic colitis, no animals developed chronic colonic disease[20,21]. Thus no evidence exists that the deposition of these auto-antibodies in colonic tissues is a pathogenetic mechanism in colonic IBD but, notwithstanding, it is possible that they are not merely a phenomenon secondary to the bowel damage. This concept is supported firstly by the lower incidence of anti-colon antibodies in the sera in other inflammatory diseases of the large bowel[6,17] and, secondly, by the

*This work was supported in part by a grant from the National Foundation for Ileitis and Colitis.

higher incidence of elevated titers of such antibodies in first degree, healthy female relatives of patients with chronic ulcerative colitis (CUC), compared to controls[22]. These latter findings, particularly the family observations, suggest that their ready synthesis in IBD is partly determined by genetic factors. Histocompatibility typing recently has revealed two possible markers for CUC, namely HLA-BW35 and HLA-AW24[23], which if confirmed and extended, would support the concept of a genetic predisposition for this disease and have important immunological implications. In addition, it has been speculated that the presence of HLA-DR2 diminishes the risk of an individual developing either CUC or Crohn's disease (CD)[24]. Furthermore, a strong familial incidence of IBD is well established and the epidemiologic data favor genetic factors to explain this[25].

An important question is: what induces the production of anti-colon antibodies? One answer is that they result from stimuli to the gut-associated lymphoid tissues by a cross-reacting enterobacterial antigen, namely the common antigen of Kunin. This was suggested by the findings that germ-free rat colon or feces share antigenic determinants with a lipopolysaccharide extract of Escherichia coli 014[17,18,26] and that anti-colon antibodies from patients with IBD react with germ-free rat colonic antigen. Apart from somatic O-antigen this extract contains the common antigen of Kunin (CA) which is present in nearly all species of Enterobacteriaceae[27] and cross-reacts with colonic antigen(s). Because CA has greater expression in the lipopolysaccharide extract of E. coli 014 than in those from other enterobacterial strains[28], this serotype of E. coli has been widely used in immunological studies in IBD. In these, the patients showed elevated titers of serum antibodies against the lipopolysaccharide from E. coli 014 compared to controls, but not against those from other coliform serotypes[10,13,17,18]. Similar results also were reported in first degree, healthy female relatives of patients with CUC[22]. Serum antibodies to Kunin antigen, although present in healthy individuals, are markedly higher in titer in IBD[12,29] while, in contrast, antibodies to somatic 014-antigen are detected only rarely[30]. These observations indicate that

a high degree of humoral immunity to CA exists in IBD and that anti-colon antibodies probably are induced by this cross-reacting antigen.

As implied earlier, it cannot be concluded that anti-colon or anti-enterobacterial antibodies are irrelevant to the pathogenesis of IBD. For example, they could be involved in stimulating the inflammatory process in the colonic wall either as constituents of immune complexes or, in cooperation with lymphocytes, through a mechanism of antibody-dependent or 'complex-dependent' cell-mediated cytotoxicity. Circulating antigen-antibody complexes may occur in IBD[31-34], despite some dispute[35], but nothing is known of their antigenic component(s) and they have not been demonstrated in the tissues. However, important indirect evidence for their involvement in the etiopathogenesis of CUC, in conjunction with host hypersensitivity to the common antigen of Kunin, is provided by an animal model. In this the intravenous injection of soluble immune complexes (BSA-anti BSA) induced acute colitis in rabbits whose large bowel mucosa had been irritated by the instillation of dilute formalin[36]. However, chronic colitis, with some similarities to CUC, developed in animals which also had been sensitized to the common antigen of Kunin prior to the injection of the complexes. Sensitization to common antigen alone led neither to acute nor chronic inflammation. Clearly these observations are worthy of pursuit.

Cell-mediated immunity

The results of studies of a variety of cell-mediated immune responses in IBD have been conflicting. These have included skin testing with various standard antigens and with dinitrochlorobenzene, as well as in vitro lymphocyte reactivity to nonspecific mitogens and in mixed lymphocyte cultures. While some claimed impairment of such reactivities in patients with Crohn's disease[37-47], others considered these to be normal[48-52]. Similar testing in CUC has received less attention but most authors agree that the responses are normal in this form of IBD[42,46,48,49-57], although some have claimed impaired reactivities[41,44,45,58-60].

More importantly, despite some discordancy in the findings,

there is evidence that a state of cellular immunity to colonic and to enterobacterial antigens exists in patients with IBD and to a lesser degree in healthy controls[reviewed in ref #61]. Because of cross-reactivity, perhaps the cellular autoimmunity to colonic antigen(s) reflects stimulation by common antigen of Kunin, as suggested for the induction of anti-colon antibodies. Such speculation leads to consideration of the cytotoxicity for colonic epithelial cells (CEC) found in IBD.

Circulating mononuclear cells (MC) from patients either with CUC or Crohn's disease (CD) are cytotoxic in vitro for autologous and allogenic (including fetal) colonic epithelial cells[62-66]. In addition, a high molecular weight factor is present in the sera which confers similar cytotoxic properties to MC from the peripheral blood of healthy controls[66,67], and it was suggested that the active principle is either a cytophilic antibody, possibly an IgM, or an antigen-antibody complex[68]. Mononuclear cells from healthy individuals also become specifically cytotoxic for CEC following incubation in vitro with a lipopolysaccharide extract from E. coli 0119:B14[69], which supports indirectly the observations that Enterobacteriaceae and CEC share antigenic determinants, as does the finding that preliminary exposure of mononuclear cells from patients with IBD to the same bacterial extract abrogated their cytotoxicity for CEC[70]. These results led to a working hypothesis that the gut lesions in IBD result from a hypersensitivity reaction to antigens of enterobacteria normally present in the intestinal lumen of the affected individual and that this hypersensitivity is genetically determined[71]. It was speculated also that the cytotoxicity of circulating MC for colonic epithelium results from antigenic cross-reactivity between enterobacteria and the colonic epithelial cells, and that antibody dependent cell-mediated cytotoxicity (ADCC) is the mechanism involved[68], although alternatively it may represent Natural Killing. While it is unknown if such mechanisms are operative in vivo, this autoimmune cytotoxicity in IBD suggests the involvement of antibodies and cellular immune responses to cross-reacting antigens of Enterobacteriaceae and colonic epithelial cells in the initiation and/or persistence

of the bowel inflammation. Parenthetically, the recent finding of a circulating IgG antibody in patients with CUC which mediates ADCC for a colonic cancer cell line in vitro will be discussed in a presentation which is to follow.

Suppressor cell activity

Hodgson et al[73] demonstrated decreased Concanavalin A-stimulated suppressor T-cell activity in vitro using peripheral blood mononuclear cells from patients with active IBD, and speculated that this might have pathogenetic significance through a mechanism of enhanced autoimmunity, as did Ginsburg et al[74]. However, their findings were not confirmed by Fiocchi et al[75] using either peripheral blood or gut mucosal mononuclear cells (MC) and, recently, these workers claimed that suppressor cell activity was enhanced in intestinal MC isolated from patients with IBD[76]. Nevertheless, Goodacre and Bienenstock[77] noted diminished suppressor cell activity in intestinal MC in Crohn's disease (CD). In some contrast, Elson et al reported that patients with CD may have circulating 'covert' suppressor T cells which can be activated in vitro to inhibit the synthesis of IgM[78] Thus the status of immunoregulatory cell activity in IBD remains to be defined, but this is a promising area for further study because of its importance to the mechanisms of the autoimmune phenomena seen in these diseases.

Gut-associated lymphoid tissue

It is clear that data from observations on peripheral blood mononuclear cells or humoral antibodies may not reflect events taking place in the intestinal wall in IBD. Accordingly, using immunohistochemical techniques, several groups studied small and large intestinal tissues affected by IBD and quantitated the different classes of immunoglobulin-producing immunocytes in the mucosa[reviewed in ref #61]. However, the results were variable and gave no direct support for a primary involvement of local immunologic mechanisms in the etiopathogenesis of these diseases. In addition, while a number of workers have isolated gut mucosal immunocytes and studied some of their functions in vitro[79-85], including suppressor-cell activity and certain cytotoxic properties, the findings also have been controversial

and provide no data either directly to support or refute an etiopathogenic role for mucosal immunity in IBD. It is important to stress that no reports exist which have tested the cytotoxicty of intestinal MC for autologous colonic epithelial cells in IBD. However, this investigative field is in its infancy and future work undoubtedly will prove fruitful in testing immunological hypotheses for the causation and/or chronicity of these diseases.

NEUTROPHILIC AND MONOCYTIC FUNCTIONS

Segal and Loewi[86] suggested that weak, initial, acute inflammatory responses to antigens entering the bowel wall could result in the chronic inflammation seen in CD and showed reduced chemotaxis of neutrophils in this disease compared to healthy controls. Subsequently, Ward speculated that CD is due to a basic defect of phagocytic cells resulting in inadequate degradation of ingested foreign materials, including antigens, penetrating the intestinal mucosa from the bowel lumen[87], and that this might stimulate granuloma formation in a susceptible host. However, others found normal neutrophilic and monocytic chemotaxis in patients with CD and normal neutrophilic but slightly increased monocytic chemotaxis in CUC[88]. Furthermore, Glass and Baker[89] and Chambers and Morson[90], from studies in CD, noted that patients with large numbers of granulomas in the bowel wall had a better prognosis, a finding which would be unexpected if a macrophage defect were the cause of the disease.

It has been claimed that individuals with active IBD have a higher proportion of circulating activated monocytes than healthy controls, and the isolation of an activated monocyte/macrophage population from human colonic mucosa has been reported reviewed in ref #61. In addition, patients with active IBD show greater production and release of acid hydrolases by their peripheral blood monocytes in vitro compared to controls reviewed in ref #61. Thus, it can be speculated that immunological stimuli, resulting in release of lysosomal enzymes from monocytes, might be involved in perpetuating the intestinal inflammation in IBD.

CONCLUSIONS

From this brief review it is apparent that there are tantalizing clues to support a concept that microbial agents (particularly Enterobacteriaceae), host immune responses and related genetic factors have roles in the etiopathogenesis of IBD. It is hoped that further research to test this hypothesis will resolve the enigma of these diseases.

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ULCERATIVE COLITIS: AN AUTOIMMUNE DISEASE?

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INTRODUCTION

Although the etiology of ulcerative colitis remains unknown, it has recently been emphasized that immunologic mechanisms play an important role in the pathogenesis of this disease. Serum from patients with ulcerative colitis contain heterogenous antibodies that react with colon from newborn and adult germ-free rats, human intestinal and gastric mucus cells and Escherichia coli 014 antigen (1-7). Circulating immune complexes occur in patients with ulcerative colitis (8). Predominant IgG-containing immunocytes are observed in the lamina propria (9) and deposition of IgG and complement along the basement membrane of colonic epithelium in patients with ulcerative colitis (10) suggests formation of local immune complexes.

We have isolated a disease-specific tissue-bound IgG from the colonic mucosa of patients with ulcerative colitis and named it "colitis colon-bound antibody" (CCA) (11). CCA recognizes colonic epithelium from other patients with ulcerative colitis as shown by indirect immunofluorescence and radioactive binding studies.

Using sequential elution techniques and processing

the tissues in presence of a protease inhibitor, 2 mM phenyl methyl sulfonyl fluoride (PMSF) reduced the fragmentation of IgG in CCA (12). Intact CCA-IgG was purified using protein A-sepharose 4B affinity chromatography.

This communication summarizes the results of specific binding of purified CCA-IgG to protein(s) in the colonic mucosal extracts of patients with ulcerative colitis. Furthermore, specific cytolytic serum IgG from patients with active ulcerative colitis is demonstrated with an established human colon cancer line as the target cells using antibody dependent cell mediated cytolysis (ADCC) system. Relation of disease state and surgical resection of colon to cytolytic serum IgG has also been examined.

MATERIALS AND METHODS

Extraction and Purification of CCA-IgG: CCA-IgG was extracted from operative specimens of diseased colon of patients with ulcerative colitis as reported earlier (11-12). Intact IgG was separated using protein A Sepharose 4B affinity chromatography.

Purification of Serum IgG: Using conventional techniques, serum IgG was purified from 5 patients with ulcerative colitis and 5 normal subjects with ammonium sulfate precipitation followed by DE-52 column chromatography.

Bindings of Purified CCA-IgG to Colonic Tissue Extracts: To demonstrate formation of immune complexes in vitro, radioimmunoprecipitation experiments were

performed with iodinated colonic mucosal extracts or with iodinated CCA-IgG. Colonic mucosal extracts were prepared by sonication in phosphate buffered saline (0.15 M,pH 7.4) (PBS) (12), concentrated and iodinated by I^{125} Bolton-Hunter reagent (Amersham, Illinois) (13), instead of chloramine-T method. This method avoids denaturation of proteins or decay in their biological activities during iodination and introduces labelled iodine into a free amino group of protein rather than tyrosine residues which may both be present in the specific protein(s) recognized by CCA-IgG. Twenty microliters of iodinated extracts were adjusted to 2 x 10^5 cpm with PBS and incubated at 4^oC overnight with 25 microgram of CCA-IgG or control human IgG. The same volume of 7% polyethylene glycol (PEG) in PBS was added and incubated at 4^oC for another 18 hours. The mixture was precipitated by centrifugation at 25,000 x g for 30 min. at 4^oC, washed twice with 3.5% PEG in PBS and counted by autogamma counter.

CCA-IgG was iodinated with chloramine-T method and 20 microliters of iodinated CCA-IgG or control IgG was adjusted to approximately 2 x 10^5 cpm and incubated with 100 microliters of sonicated colon tissue extracts in PBS. Incubation and separation of the immune complexes by PEG was performed as described above.

ADCC Studies: For ADCC studies, target cells consisted of an established colon cancer cell line, RPMI-4788, and controls were HeLa cells. Cytotoxicity was assessed

by specific release of ^{51}Cr during 4 hours of incubation (14). Effector cells were obtained from normal healthy volunteers by ficoll-hypaque gradient method. Serum and purified serum IgG from patients with ulcerative colitis, symptomatic or in remission, were used as specific antibody. Several samples of sera were also collected before and after surgery from each of five patients with ulcerative colitis who had undergone total colectomy with ileostomy. Sera were obtained from patients with Crohn's disease (symptomatic or in remission), other diarrheal diseases, patients with rheumatoid arthritis, systemic lupus erythematosus and normal healthy subjects. To establish specificity of the serum antibody from patients with ulcerative colitis, absorption experiments were performed with colonic mucosa from patients with ulcerative colitis, Crohn's disease and colon carcinoma and with E. coli 0:14 or 0:11 antigens.

RESULTS

Using different elution techniques (12) and protease inhibitor, e.g., 2 mM phenyl methyl sulfonyl fluoride, tissue-bound IgG was eluted from the colonic mucosa of 12 patients with ulcerative colitis. Intact CCA-IgG was separated from other proteins by protein A Sepharose 4B affinity column. Elution profile of this column is shown in Fig. 1. Acid eluate (second peak) contained intact IgG as shown by double diffusion in agar (Fig. 2), radial immunodiffusion and SDS-polyacrylamide gel electrophoresis.

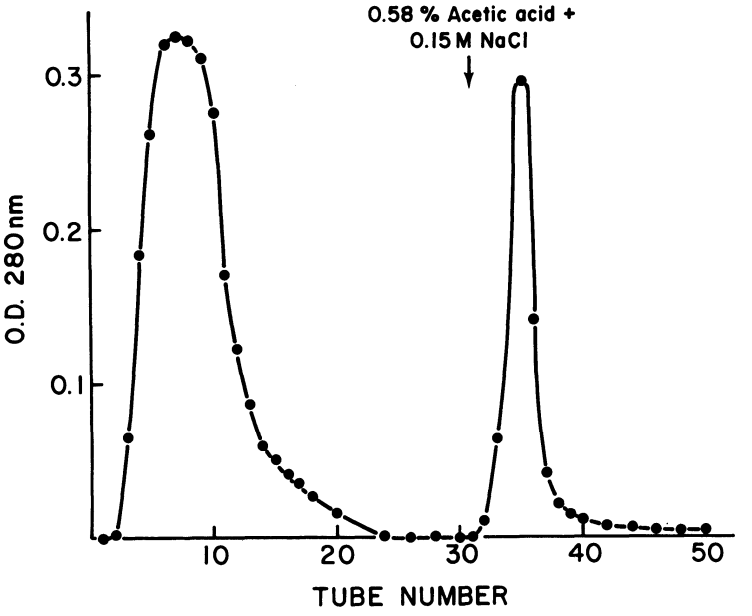

Figure 1. Elution profile of colitis colon eluate on Protein A-Sepharose 4B affinity column. First peak was with phosphate-buffered saline (pH 7.2) and the second peak was obtained with 0.58% acetic acid in 0.15 M sodium chloride. First peak contained fragmented IgG and other unknown proteins, whereas the second peak contained intact 150 K IgG only.

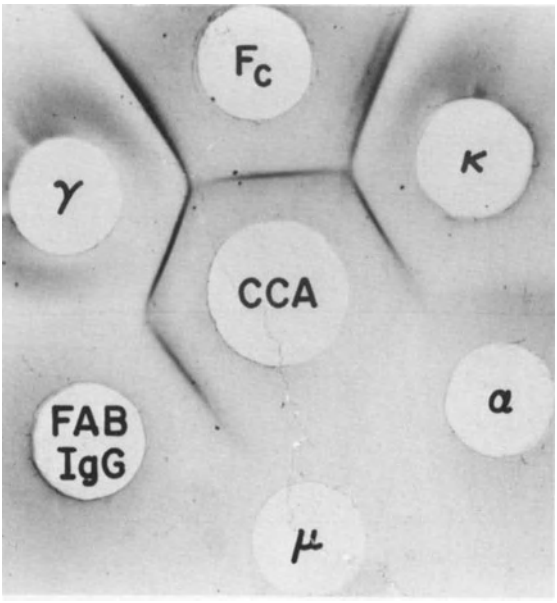

Figure 2. Double diffusion in agar. CCA-colitis colon-bound antibody (second peak protein from Figure 1) at the central well. Peripheral wells contained antisera to γ chain, Fab and Fc fragments of human IgG, Kappa, alpha and mu chains. Precipitation lines between peripheral wells result from components of human Ig in commercial antisera.

Radioimmunoprecipitation experiments with iodinated CCA-IgG and control IgG showed preferential binding of CCA-IgG with the colonic mucosal extracts from patients with ulcerative colitis (Table 1). Using iodinated mucosal extracts from patients with ulcerative colitis, Crohn's disease and normal colon tissue (normal segments of colon from cancer patients), a significantly higher ($p<0.005$) binding was demonstrated between CCA-IgG and ulcerative colitis extracts. The binding of purified CCA-IgG when compared to control IgG was significantly higher ($p<0.025$). Binding of iodinated CCA-IgG to PBS extracts from ulcerative colitis mucosa was also significantly higher than those of control IgG ($p<0.05$).

Recognition of Colonic Bacteria by CCA-IgG: To examine whether CCA-IgG recognizes bacterial proteins, indirect immunofluorescent studies were performed with purified CCA-IgG against E. coli and Bacteroides cultured from feces of two normal subjects, 2 strains of revertant forms of cell-wall deficient Pseudomonas-like organisms obtained from Crohn's disease tissue (a gift from Drs. P.D. Mitchell and K. Parent) and a known Pseudomonas strain cultured from the skin of a patient with burns. No staining was noticed with CCA-IgG.

ADCC Studies: Table 2 summarizes the results of ADCC activity with the sera from patients with ulcerative colitis while symptomatic and in remission, patients with Crohn's dsiease, rheumatoid arthritis, systemic lupus erythematosus and normal controls. Sera (and also

Table 1 *In Vitro* Immune Complex Formation Between CCA-IgG and Colon Extracts

Source of Colon Tissues	No. of Patients	*Percent binding with CCA-IgG Mean ± SEM (range)	Percent binding with Control IgG Mean ± SEM (range)
A. Experiments with Iodinated Colon Extracts			
Ulcerative Colitis	3	29.7 ± 2.04[a] (23-32)	15.43 ± 2.78[b] (10-18)
Crohn's disease	3	10.80 ± 1.48[c] (8-13)	9.43 ± 1.54 (8-12)
Normal[g]	3	12.53 ± 1.50[c] (10-16)	10.80 ± 0.68 (10-12)
B. Experiments with Iodinated Antibody			
Ulcerative colitis	5	3.33 ± 0.92[d] (2.8-5.3)	0.89 ± 0.53[e] (0.3-1.4)
Crohn's disease	6	0.35 ± 0.19[f]	0.39 ± 0.29
Normal[g]	3	0.33 ± 0.15[f]	0.72 ± 0.54

In (A) colonic mucosal extracts were iodinated with Bolton-Hunter reagent and reacted with cold CCA-IgG or myeloma IgG, in (B) experiments were performed with intact ^{125}I CCA-IgG vs. ^{125}I control human IgG. The results are expressed as *Percent binding=(cpm of the precipitate with colon extract - cpm of nonspecific precipitate)/total cpm) X 100. The values in parenthesis show the range among different patients. [a]The values are significantly higher than [b] ($p< 0.025$) and [c] ($p< 0.005$). [d]The values are significantly higher than [e] ($p< 0.05$) and [f] ($p< 0.01$). [g]Normal segments of colon were obtained from patients with colon carcinoma.

purified serum IgG (not shown in Table 2) from patients with symptomatic ulcerative colitis showed significantly higher ADCC activity compared with control sera ($p<0.025$). Two of 17 patients with active ulcerative colitis and 8 of 11 patients with ulcerative colitis in remission showed cytotoxicity in the same range of spontaneous release of ^{51}Cr. However, 3 patients with ulcerative colitis in remission had high ADCC activity similar to the range of patients with active ulcerative colitis. All sera from patients with Crohn's disease, symptomatic or in remission, rheumatoid arthritis, systemic lupus erythematosus and normal subjects had no ADCC activity (Table 2).

Four other patients with ulcerative colitis (not included in Table 2) who have been followed for 2-4 years, had persistently high ADCC activities and they had several relapses and remissions during this period. It is interesting that two of these 4 patients had undergone total colectomy with ileostomy because of refractoriness to medical treatment. Following total colectomy, ADCC activity declined by 3 months. In additional 3 patients, ADCC activities were determined prior to surgery and on several occasions following surgery. Similar results showing a decrease of ADCC following surgery were noted in all 3 patients.

The ADCC activity present in serum from patients with ulcerative colitis could be abolished by absorption of the sera with colonic mucosal homogenates from patients with

Table 2

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) Against RPMI-4788 and HeLa Cells With Sera From Patients with Inflammatory Bowel Disease, Rheumatoid Arthritis, Systemic Lupus Erythematosus and Normal Subjects

		*ADCC % Mean ± SD	
Sera from	No. of Patients	RPMI-4788	HeLa
Ulcerative colitis			
active	17	12.2 ± 6.0	0
remission	11	2.2 ± 2.1	0
Crohn's disease	13	0	0
Rheumatoid arthritis	6	0	0
Systemic lupus erythematosus	2	0	0
Normals	10	0	0

*Spontaneous cell-mediated cytotoxicity without addition of serum was 5.4 ± 2.3 which is subtracted to determine ADCC.

ulcerative colitis, but not with homogenates from patients with Crohn's disease, normal and cancerous segments of colon from patients with colon carcinoma (Fig. 3). Absorption with E. coli 0:14 or 0:11 antigens also did not change ADCC activity of the sera from patients with ulcerative colitis (Table 3). Absorption of the sera with RPMI-4788 abolished ADCC activity but similar absorption with the control HeLa cells did not reduce the ADCC activity.

Further control experiments with aggregated human IgG demonstrated specific Fc receptor binding for ADCC as shown in Table 4. Aggregated IgG in the concentration of 0.4 mg/ml abolished ADCC activity.

DISCUSSION

In vitro immune complex formation studies suggest the presence of a disease-specific protein(s) in the colonic mucosa of patients with ulcerative colitis which is recognized by CCA-IgG purified from colonic mucosa of patients with ulcerative colitis. Specific immune complex formation may occur in vivo in the mucosa as supported by previous morphological studies demonstrating presence of IgG and complement along the basement membrane of colon epithelium from patients with ulcerative colitis (10). Formation of immune complexes can activate monocytes as shown locally by morphological studies (11) and in peripheral circulation of patients with active ulcerative colitis (15). Deposition of immune complexes locally can initiate cell destruction by a complement system or by

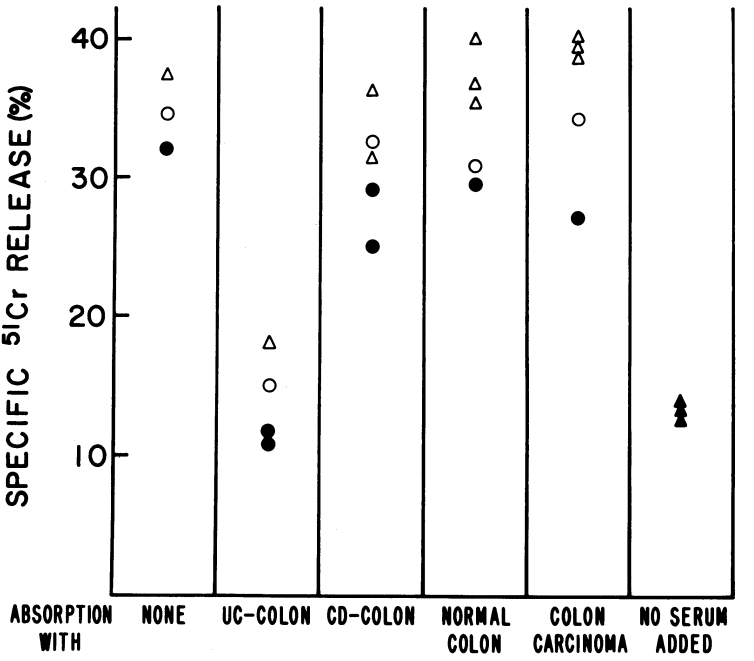

Figure 3. Effect of absorption of 3 sera from patients with ulcerative colitis with colonic mucosal homogenates. Each symbol represents the serum from a single patient. Columns showing the same symbols more than once indicate that the same serum was absorbed on different occasions to examine reproducibility. In the absence of any serum, the spontaneous cell-mediated cytotoxicity (SCMC) was 10-12% (first column from the right side, i.e., closed triangle). Following absorption of the sera with mucosal homogenates of ulcerative colitis colon, the cytotoxicity of each serum decreased to the range of SCMC. Absorption of the sera, with homogenates of colon from Crohn's disease patients, of normal colon or of cancer colon tissue, the cytotoxicity did not decrease.

Table 3

Cytotoxicity of the Sera from 3 Patients with Active Ulcerative Colitis Before and After Absorption with *E. coli* 0:14 and 0:11 Antigens

	CYTOTOXICITY (%)		
Sera from	Before Absorption	Absorption with *E. coli* 0:14	Absorption with *E. coli* 0:11
Ulcerative colitis	29.7 ± 4.4	30.1 ± 4.5	30.6 ± 3.5
Control subjects	8.6	9.0	10.1
No serum added	6.8	9.2	8.4

Table 4

Inhibition of Cytotoxicity Against RPMI-4788 with Ulcerative Colitis Serum by Aggregated Human IgG

Concentrations (mg/ml)	Cytotoxicity (%) with Aggregated IgG	Bovine Serum Albumin
10	4.0	39.4
2	6.0	49.5
0.4	12.5	48.5
0.08	22.5	44.4
0.02	38.1	46.0
0	40.0	
No serum added	10.5	

activation of monocytes or by liberation of lymphokines. Presence of disease-specific CCA-IgG in the mucosa and its recognition of mucosal cell protein(s) strongly suggest such a process. The nature of the putative antigen is not yet known; however, using CCA-IgG, its isolation and characterization might be possible.

The role of CCA-IgG in colon cell destruction is not clear. It is tempting to hypothesize an ADCC-mediated colon cell destruction via CCA-IgG. Serum from patients with ulcerative colitis and purified serum IgG demonstrated ADCC activity against an established human colon cancer cell line. Whether CCA-IgG can cause ADCC is not yet known. It is also unknown as yet whether the antigen in RPMI-4788 recognized by serum and serum IgG of patients with ulcerative colitis related to ADCC is similar to the antigen present in the colonic mucosal extracts of patients with ulcerative colitis recognized by CCA-IgG. Absorption experiments using colonic mucosa from patients with ulcerative colitis, Crohn's disease and carcinoma colon suggest the presence of a specific serum IgG which recognizes an antigen(s) in the colon cancer cell line and in ulcerative colitis colon. Decline of the cytolytic serum antibody following total colectomy also suggests organ specificity of this antibody. It is possible that the serum IgG present in patients with ulcerative colitis related to ADCC activity may be CCA-IgG. Future studies to demonstrate the relationship between the antigen present in the colonic mucosal extract and the colon cancer

cell line and the nature of the two antibodies will explain the pathogenesis of ulcerative colitis. The results of these studies, as well as the studies by previous investigators, support the concept of autoimmunity as the pathogenesis of ulcerative colitis.

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BACTERIAL ETIOLOGY OF INFLAMMATORY BOWEL DISEASE

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Bacteria have been implicated in the pathogenesis of inflammatory bowel disease for several decades, beginning with conventional pathogens such as Shigella and amebae, and moving to the current, more "trendy" bacteria such as L-forms and mycoplasma. It is only natural to consider bacteria as potential culprits in these diseases, since the intestinal mucosa forms the fragile border between the flora of the lumen and the host's tissues. In addition, the gut is threatened on a daily basis by microorganisms from the environment that gain passage through vehicles of food and drink. Besides these ecologic considerations, the histopathologic features of inflammatory bowel disease are strikingly reminiscent of conventional infectious diseases, albeit of an acute and self-limited nature. Ulcerative colitis is not unlike acute bacillary dysentery, while Crohn's disease bears resemblance to intestinal tuberculosis and Yersinia infection.

Normal Flora

The intestinal microflora has specific longitudinal and and cross-sectional distributions that are remarkably stable (Donaldson 1964,1970;Gorbach 1971; Floch 1970,1972). The upper gastrointestinal tract encompassing the stomach, duodenum, jejunum, and upper ileum harbors a sparse microflora composed largely of facultative and anaerobic species derived from the oropharynx. The concentration of microorganisms in the upper bowel is generally less than 10^5/ml.

These organisms are relatively inert metabolically, and this property, in addition to their low numbers, permits normal absorption of dietary foodstuffs.

The lower ileum shows an increase in microbial elements. It occupies a transitional zone between the sparse flora of the upper bowel and the luxuriant populations of the colon. The major change in the ileum is the appearance of gram-negative, enteric bacilli (coliforms) and small numbers of obligate anaerobes. The total concentration of bacteria in the ileum is generally 10^4 to 10^7/gm, although there is considerable variation among normal subjects.

The cecum and large bowel are characterized by stasis and decreased transit time. These features provide a physiochemical environment of low oxidation-reduction potential (Eh), a situation well suited for growth of fastidious anaerobic microorganisms. The low Eh does not arise de novo, but is a complex interaction between the indigenous flora and its niche within the large bowel. For example, the ceca of germ-free mice have an Eh of -49 ± 50 mv, whereas conventional mice with a normal microflora maintain a markedly reduced atmosphere of -236 ± 17 mv. (Maier et al 1972).

Diarrhea may cause changes in the microflora, regardless of the primary etiology (Gorbach 1971). Alterations have been reported in infectious diarrhea due to Shigella, E.coli and V. cholerae, in "nonspecific" diarrhea, in hypolactasic subjects fed lactose, and in diarrhea induced by purging the bowel with isotonic fluid. These changes fall into three major categories:

1. Increase in certain coliform species that are common in the normal flora, viz. Enterobacter, Proteus, Klebsiella, and Pseudomonas. Such strains may gain prevalence while the usual flora of E. coli is suppressed. This can result in a net increase in the total coliform count.
2. Decline in obligate anaerobes. The anaerobic strains which ordinarily are predominant in the fecal flora may actually decline below the coliform count. In

particularly brisk diarrhea such as cholera, obligate anaerobes may fall to very low, even undetectable, concentrations.

3. Retrograde contamination of the upper small bowel by elements of the fecal flora. Colonization of the jejunum by pathogens has important implications in enterotoxin-associated diarrhea. The abnormal flora may persist for several weeks following an acute episode. This abnormality can also be seen in diarrhea induced by saline perfusion of the lower intestine.

Indigenous microflora as the cause of IBD

One of the earliest microorganisms incriminated in ulcerative colitis was the "diplostreptococcus" of Bargen (Bargen 1924), (Table 1). This organism was found in the feces of colitis patients. Intravenous challenge in rabbits reproduced an ulcerative disease of the large bowel. Yet, it was disturbing that similar bacteria from healthy controls produced this pathology in rabbits as well. The theory caved in when other ivestigators found the organism in stools from patients with a variety of unrelated conditions. Even the nomenclature of the organism became unclear as bacterial taxonomy became more sophisticated.

Table 1. Etiologic Agents in IBD
Normal Flora (Trink & Lamont 1981)

Diplostreptococcus (Bargen)
Bacteroides necrophorum (Dack)
Streptococcus (enterococci)
Coliforms
Eubacterium & Peptostreptococcus
Mixed Flora
Dysbiosis

A similar fate befell the ubiquitous Bacteroides necrophorum, brought to prominence by Dack and associates (Dragstedt, Dack, Kirsner 1941). Again, the organism was reported to be more prevalent in colitis patients than in normals, and serum agglutinating antibodies were present in afflicted patients.

The taxonomic problem arose when this organism, or at least similar strains, were found in healthy individuals and in patients with other diseases (Meleney 1941). It is now apparent that these organisms belong to the newly-designated species Fusobacterium necrophorum, a frequent component of the normal flora of the bowel and oropharynx. While present in most healthy individuals it is certainly true that these organisms are capable of producing ulcers when injected into skin or mucuous membrane of experimental animals. However, the thesis has not withstood the test of time, and other workers have been unable to confirm the unique presence of this bacterium in colitis patients.

Among the normal flora components, coliforms are increased in the fecal effluent of patients with ulcerative colitis, especially during periods of relapse (Gorbach et al 1968). Cooke observed that certain E. coli strains in the stools of colitis patients produced necrotoxins, hemolysins, or enterotoxins (Cooke 1967). Such strains also were isolated from healthy individuals, although less frequently. Some workers have found increased numbers and varieties of streptococci, especially enterococci (Cooke 1967, van der Wiel-Korstangie 1975). More recently it has been suggested by van de Merwe and Mol that Eubacterium and Peptostreptococcus species, both elements of the normal microflora, are greatly increased in patients with Crohn's disease (van de Merwe and Mol, 1980). These workers found agglutinating antibodies against these organisms in patients with Crohn's disease but not in those with ulcerative colitis, other diseases, or in healthy controls. Other workers have claimed that the entire microflora is imbalanced, or in a state of "dysbiosis" in IBD patients (Fradkin 1937). However, such theories are more mystical than scientific, and they may be focusing on the "effect" of an altered intestinal milieu, rather than on the "cause" of the disease itself.

From the available data, there is no justification for ascribing etiologic significance to alterations in the balance of the normal flora in ulcerative colitis. The changes that have been described may be secondary to rapid transit through the large bowel, effectively by-passing the normal control mechanisms. It is hazardous to assign a primary role to changes in flora on the basis of simple bacteriologic surveys of fecal microbial populations.

Conventional Pathogens

Table 2. Etiologic Agents in IBD Conventional Pathogens

Shigella
E. coli (invasive)
Yersinia
Campylobacter
Chlamydia
Clostridium difficile
Tuberculosis
Entamoeba histolytica

The pathologic changes caused by virulent Shigella closely mimic those of "idiopathic ulcerative colitis" in the acute period. Because of this similarity and the occasional case of chronic shigellosis, several investigators have attempted to portray ulcerative colitis as a chronic bacillary dysentery in which the infecting Shigella can no longer be cultured (Hurst 1921, Mackie 1932, Felsen 1953).

The advocates of Shigella as an important cause of ulcerative colitis have lost credibility in recent years when the decline in bacillary dysentery in the United States and Europe failed to influence the incidence of idiopathic ulcerative colitis. Furthermore, careful bacteriologic studies of patients with acute ulcerative colitis have failed to recover Shigella in most cases. With the availability of selective culture media and enrichment techniques these organisms should not be overlooked by careful observers.

Entamoeba histolytica has entered the ring as a possible contender for the pathologic agent in ulcerative colitis (Fradkin 1937). There is no doubt that this protozoa causes acute colitis that may lapse into a chronic stage if not adequately treated. However, the pathology of amebiasis is different from that of ulcerative colitis. The lesion in amebiasis is characterized by tissue liquefaction and undermining necrosis with a relative paucity of acute inflammatory cells. This produces the typical "collar-button" appearance on proctoscopic examination. Furthermore, amebae usually can be seen in microscopic sections when examined by experienced observers. It is also apparent that broad-spectrum antibiotics provide rapid relief for the acute symptoms of intestinal amebiasis, although the cyst stage of the parasite may persist. The salutary effect of tetracycline in acute amebiasis has not been reproduced in treating acute ulcerative colitis. Invoking E. histolytica as a causative agent is reminiscent of Elsdon-Dew's classic remark that "Amebiasis is the refuge of the diagnostically destitute."

After reviewing the several microorganisms that have been implicated as "the pathogen" in ulcerative colitis, it is obvious that none has satisfied Koch's postulates of (1) isolation from all cases, (2) growth in pure culture in vitro, and (3) passage of the cultured organism to an appropriate animal model in order to reproduce the pathology. It is intriguing that ulcerative colitis primarily affects the colon, an area that is in intimate contact with a luxuriant microbial population. Furthermore, the microscopic appearance of mucosal ulcerations, crypt abscesses, and abundant inflammatory cells is seen in colitis caused by certain infectious agents, viz. Shigella and E. coli. The conundrum of ulcerative colitis is its self-perpetuating nature, its episodic relapses, and its disappointing response to a variety of antimicrobial agents.

As new microbial pathogens are described, they are tested in the mysterious diseases that remain undiagnosed in our

nosology. Yersinia enterocolytica has been hoisted, and then lowered, with a negative report appearing recently in the literature (Swarbrick, Price et al 1979). Chlamydia, another fashionable microorganism, was initially associated with elevated serologic titers in Crohn's disease, but subsequently it has lost credibility due to three reports showing no association (Swarbrick, Price et al 1979, Taylor-Robinson et al 1979, Munro et al 1979).

Clostridium difficile

The agent of pseudomembranous colitis and certain forms of antibiotic-associated diarrhea (without colitis) has been conclusively identified as Clostridium difficile, a gram-positive, spore-forming, anaerobic rod (Bartlett and Chang 1978, Bartlett, Chang et al 1979). It is found only rarely in the fecal flora of healthy adults, although it can be isolated in stools of neonates. C. difficile was originally isolated from the stools of patients with clindamycin-associated pseudomembranous colitis. The disease was reproduced in a hamster model, either by injecting intracecally the organism itself or the cell-free broth filtrate containing the necrotizing toxin.

Table 3. Clostridium Difficile Toxin in IBD

	Mild	Moderate	Severe
Ulcerative colitis	0/17*	1/10	3/8
Crohn's disease	0/8	1/9	6/7
Total	0/25	2/19	9/15

*Patients with toxin-positive stools/total patients

Trinka and Lamont (1981) reported the presence of C. difficile toxin in the feces of patients with ulcerative colitis and Crohn's disease (Table 3), despite earlier reports to the contrary (Bartlett, Chang Gurwith et al 1978). Toxin-positive stools were found with equal frequency in patients with ulcerative colitis and Crohn's disease, although there

was an apparent increase in the isolation rate from more severe cases. Nine of their patients were treated with vancomycin, and they reported a satisfactory, although somewhat dilatory, improvement with this specific therapy. They specifically mentioned that only 3 of 11 patients with toxin positive stools had received antibiotics in two months prior to testing their stool. In the same issue of Gastroenterology, a contradictory article appeared by Meyers et al (1981). Performing a similar study, they indeed identified the C. difficile toxin in 4 of 44 patients with ulcerative colitis or Crohn's disease (Table 4). However, in each of the toxin-positive cases, both among IBD patients and "diarrhea controls", of which there were five, all had received antibiotics sometime in the preceding six months. These authors concluded that previous antibiotic usage had predisposed these patients to colonization by C. difficile, and they were unable to associate the clinical status of the patient with the presence of this organism.

Table 4. Clostridium Difficile Toxin in IBD (Meyers 1981)

	Toxin-pos. stools/ total patients	No. rec. antibiotics in toxin-pos. group/ total rec. antibiotics 6 months
Ulcerative colitis	3/18	(3/14)
Crohn's disease	1/26	(1/21)
Diarrhea "Controls"	5/21	(5/12)

The issue of C. difficile in IBD raises several important issues, both on a practical level for the treating physician and on a theoretical level for the investigating scientist. There are many problems with implicating this organism in the pathogenesis of IBD. As a starter the laboratory test is difficult to perform. As reported in these papers, the presence of C. difficile toxin was recognized in the feces by a standard tissue culture assay (Chang, Laureman, Bartlett 1979). However, it is known that there may be

non-specific false-positive results, which as yet remain unexplained. The ultimate criterion for diagnosing C. difficile should be isolating the organism itself in the feces by means of a selective culture medium, as well as demonstrating the toxin in the stool (Willey and Bartlett 1979). Unfortunately, both reports of C. difficile in IBD relied on toxin assays in feces, and in neither study was the organism conclusively identified. Epidemiologic features should also be considered in this special group of patients. Antimicrobial drugs are widely used in IBD patients, especially sulfasalazine. Colonization by C. difficile has been associated with a wide variety of antimicrobial agents, including clindamycin, ampicillin, cephalosporins, erythromycin, tetracycline, and metronidazole, as well as sulfonamides such as found in sulfasalazine. Once the organism is acquired, it may remain in the fecal flora with its toxin for up to 9 months after the antibiotic exposure (Bartlett 1981). In some patients this produces relapsing diarrheal episodes, while others live in peaceful symbiosis without symptoms.

On the basis of available data, admittedly incomplete, it would appear that a small percent of patients with IBD may harbor C. difficile, almost certainly related to prior antibiotic use. If the organism or its toxin is identified in the stool, a clinical decision should be made regarding the possible relevance to patient's current symptoms. When exacerbation of diarrheal symptoms is present, it would be justified to treat such individuals with either vancomycin (Tedesco, Markham, Gurwith et al 1978) or bacitracin (Chang, Gorbach and Bartlett et al 1980). It should be pointed out, however, that the response to these antibiotics should be rather prompt, at least within 5 days. Failure to respond during this time frame would indicate that the organism is playing no role in the clinical condition.

Novel Pathogens (Table 5)

Table 5. Etiologic Agents in IBD Novel Forms

Cell-defective variants (L-forms)

-Pseudomonas-like
(Ps. maltophilia) (Parent & Mitchell)

-Mycobacterium
? M. kansasii
(? Mycoplasma) (Burnham et al)

Employing hypertonic culture medium, Parent and Mitchell have reported the isolation of cell-wall defective bacteria, known as L-forms, in homogenized tissue obtained at surgery from patients with Crohn's disease (Parent & Mitchell 1978, 1980). These organisms were isolated from all eight patients with Crohn's disease using small bowel or lymph node tissue, but in none of nine patients with ulcerative colitis or twenty""control" patients with other illnesses. Cell-wall defective organisms should revert to the parent bacterial strain when subcultured on conventional medium. These variants behaved in this manner, reverting to conventional organisms, subsequently identified as Pseudomonas-like Group Va (taxonomy according to the Center for Disease Control). In a recent abstract, these investigators were unable to reproduce disease when the organisms were injected into rabbits (Parent, Mitchell, and Beltaos 1980). It was also noted that all patients in these studies had received preoperative antibiotic bowel preps, and this may be important since L-forms can be induced by the use of antibiotics. The only subsequent reports on the subject have appeared, up to the present time, in abstract form. Graham et al, apparently recovered some type of cell-wall deficient microorganisms from patients with Crohn's disease, but they could not observe reversion to the parent strains (Graham, Yoshimura and Estes 1980). Another abstract by Belsheim et al., reported the isolation of L-forms from tissues of patients with Crohn's disease and ulcerative colitis, but

not from control subjects (Belsheim, Darwish, Watson et al 1980). These L-forms reverted to a variety of coliforms, streptococci, staphylococci, and pseudomonads.

Taking a somewhat different tack, Burnham, Stanford and co-workers (1978) have cultivated mesenteric lymph nodes in Lowenstein-Jensen medium at various temperatures for periods of up to nine months. (This medium is used for culturing mycobacteria such as M. tuberculosis and atypical mycobacteria.) Positive cultures were noted to have a "fine surface growth of organisms which are irregular in shape and stained acid-fast by the Ziehl-Neelsen method". Such positives were found in 33 of 50 patients with Crohn's disease, 11 of 20 with ulcerative colitis, and 2 of 26 controls. However, they have been unable to cause reversion of these cell-wall defective organisms, apparently mycobacteria, to orthodox strains (Stanford 1978). There was a single exception, one of their original patients with Crohn's disease, in whom they isolated Mycobacterium kansasii. On electronmycroscopy they also saw organisms from the original culture that resembled Mycoplasma, although these strains could not be subcultured.

At this stage it is difficult to lend support to the reports of novel microorganisms as etiologic agents in Crohn's disease. Some of these microorganisms are part of the normal flora, and they may be able to colonize diseased tissue more avidly than normal bowel mucosa. We have not had confirmatory evidence to establish the veracity in other laboratories. Then, it is known that antimicrobial agents active against cell-walls can induce L-forms from conventional bacteria in the laboratory. Many of these patients have received such antimicrobial agents either before surgery or in the recent past, and it is conceivable that these drugs were responsible for the presence of cell-wall defective forms. Most importantly, the burden of proof remains on the investigators to fulfill Koch's postulates by isolating the organism in pure culture from afflicted patients and not controls, and then by reproducing the

disease with the cultured strains in appropriate animal models. We await with anticipation, mixed with some skepticism, further reports on these novel microbial forms in IBD.

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CYTOTOXIC INDUCERS IN CROHN'S DISEASE AND ULCERATIVE COLITIS

G.L. GITNICK

The concept that infectious agents might be causative factors for Crohn's disease (CD) was first proposed by Crohn et al (1) and for ulcerative colitis (UC) by Bargen (2). Further impetus to research in this area was provided by the reports of Mitchell and Rees (3) and Cave et al (4) that small (less than 220nm) transmissible agents from human tissues induced changes similar to IBD in mice and rabbits.

Models for IBD

It is of interest that in veterinary medicine, inflammatory bowel diseases similar to those seen in humans have been described. In some cases the cause can be traced to viruses (5), in others to bacteria (6) and in still others to a combination of viruses with bacterial flora (7). The question can be raised as to whether or not such pathogenetic mechanisms are operative in humans.

Feline infectious peritonitis (FIP) is a transmissible disease of cats caused by a coronavirus, a pleomorphic RNA virus, 75nm in diameter, with radiating spikelike projections of its outer envelope (8,9). The virus is heat and ether labile, and phenol resistant. The progressive, nonresponsive, febrile peritonitis caused by this agent was first reported in 1953, and the virus has subsequently been identified as the FIP virus. The peritonitis is serofibrinous, accompanied by large volumes of transudate, and characterized by miliary granulomas of the serosal surfaces of viscera, particularly liver and intestines. In recent years it has been recognized that the virus can affect other systems, and produce granulomatous disease of the lungs, central nervous system, the eyes,

kidneys, liver, or visceral lymph nodes. In this infection, differences in the immune state of the host can result in markedly different disease manifestations (10).

There are a number of veterinary diseases which affect the colon and/or small intestine and produce diseases which resemble the inflammatory bowel diseases of man. Many of the causative organisms have not been described or even sought in human diseases. Among those widely different veterinary pathogens are: the soil algae, Prototheca, which causes an IBD of the large and small intestine of dogs complete with extra-colonic manifestations such as arthritis, iritis, and dermatitis (11); an anaerobe, a species of the genus Clostridium, which produces an ulcerative enteritis of birds (12); the parvoviruses, including the small, stable DNA cryptotrophic virus which causes feline infectious enteritis; an anaerobic spirochete which interacts with one or more gram negative obligate anaerobes to produce an ulcerative colitis of swine (13); and the Mycobacterium johne which produces granulomatous inflammation of the intestine in ruminants (14). Some other interesting diseases which are probably caused by an infectious agent because of favorable responses to antibiotics include canine colitis, which histologically bears a strong resemblance to human ulcerative colitis (15), and canine granulomatous enteritis which resembles Crohn's disease including the presence of granulomas and giant cells (16).

Animal Transmission Studies

As early as 1935, Mones and Sanjaun (17) inoculated crude filtrates obtained from intestinal tissue of patients with inflammatory bowel diseases into rabbits, causing changes similar to those found in UC (see Table 1). Mitchell and Rees (3) transmitted granulomas to the foot pads of mice inoculated with filtrates prepared from CD tissue, but others failed to reproduce this work (18). Cave and associates (19) then reported their ability to inoculate rabbits intraserosally with tissue filtrates from patients with CD or UC and transmit inflammatory changes suggestive of organ specific

TABLE 1

ANIMAL TRANSMISSION STUDIES

1935	Mones & Sanjuan:	Transmitted ulcerative colitis to rabbits
1970	Mitchell & Rees:	Transmitted granulomas to mouse footpads
1973	Bolten, et al:	No transmission to mouse footpads
1973	Cave, et al:	Transmitted granulomas to rabbit ileum
1976	Cave, et al:	Transmitted ulceration to rabbit colon mucosa
1976 1977	Taub, et al: and Donelly, et al:	Control tissues produce granulomas in mouse footpads
1980	Das, et al:	CD filtrates produced lymphomas and splenic antigen in athymic mice
1980	Cohen, et al:	Transmitted granulomas to rabbit ileum
1980	IBD Research Group:	No transmission to rabbit ileum or colon

inflammatory bowel disease. Simonowitz et al (20), in attempting to confirm this work, were not able to transmit granulomas, but observed an inflammatory process in the bowel wall of rabbits inoculated with CD material. We have been unable to confirm this work (21a).

Mouse Transmission Studies

Mitchell and Rees (1970) (3) reported that they were able to induce granulomas in the foot pads of CBA mice by the injection of tissues from a patient with CD, but not by using control tissue. These granulomas evolved slowly over 3 months to 2 years and were present in normal and immunodeficient CBA mice. These findings have been partially confirmed by Taub et al (21) but not by Bolton et al (22) or Heatley et al (23) using different strains of mice. Subsequently, Mitchell and Rees extended their experiments and reported second generation passage of the histological changes. Pretreatment of the homogenates by filtration, autoclaving, irradiation and freezing has shown that the transmissible agent will pass a 220nm filter, withstands freezing, but is destroyed by autoclaving and irradiation. Cave et al (24) demonstrated similar findings in both normal and immunodeficient CBA mice and A_2G inbred mice. Frozen and 220nm filtrates were both effective at inducing granulomas given intraperitoneally or via the foot pads. Both Mitchell and Rees (4) and Cave et al (24) reported systemic spread of the granulomatous process to the intestine. This occurred late, at least 9 months after injection. Taub et al (25) suggested that the process is nonspecific since some of their controls induced similar changes. However, with control inocula these changes occurred early at 25 days and had disappeared in 150 days. Thus, this inflammatory reaction is temporally different and may have a different cause. Cohen et al (26) reported that C57Bl0/J and Balb/C mice were more susceptible to the granuloma inciting agent in CD tissues than CBA mice. He also noted transient granulomas in his control mice, but by 12 months only the CD mice showed persistence of the granulomatous reaction.

Cave et al (24) also noted that A_2G mice injected with UC tissues developed slowly evolving granulomas that could not be differentiated histologically from those induced by CD tissues. The inciting factor passed a 0.2μ filter and withstood freezing. The transmissible agents for CD and UC have been found in ileum, colon and mesenteric lymph nodes. On passage, the agent for CD has been demonstrated in ileum, lymph nodes, foot pads, liver and spleen.

Rabbit Transmission Studies

Cave et al (7) employed the New Zealand white rabbit (NZW) as an experimental animal because this animal is large enough to withstand serial biopsy of the intestine. In the initial study, frozen CD tissue homogenates induced slowly evolving granulomas in the ileum, colon, mesenteric lymph nodes and in the liver of some of the recipient rabbits. Control tissues did not incite any changes over the same 9 month period of observation. Subsequently, a larger study, using fresh CD tissue (6 patients), UC tissue (2 patients) and 5 with other diseases was initiated (27). In this study cell-free filtrates (220nm) induced a granulomatous response over 3 to 24 months in some of the recipient animals. Control animals were consistently negative. The rabbits injected with UC homogenates developed slowly evolving round cell infiltrates (28). This was predominantly mucosal in distribution, localized to the cecum and colon, but with rectal sparing. Successful passage of tissues from both the initial CD and UC rabbits followed direct injection of the bowel or IV injection using crude homogenates or cellfree filtrates. The latter route of injection revealed intestinal selectivity for the intestine of the histologic changes, which were similar to those in the first generation of animals. Furthermore, 5 of the CD donors were common to both the mouse and rabbit studies and 3 of these induced lesions in both species.

Simonowitz et al (8) used NZW rabbits and were able to induce a chronic inflammatory response in the colon, ileum and cecum of these animals. Lesions took 12 months to evolve

and were demonstrably different from control animals. They concluded that they had not transmitted chronic Crohn's disease, but they could not exclude the presence of a transmissible agent. Donnelly et al (29) again using NZW rabbits, induced a local inflammatory response with both CD and control tissue homogenates. They used a coarse homogenate. The response in an additional group of animals was abolished by preincubation of the homogenate with ampicillin. Orr et al (30) reported negative results with transmission to NZW rabbits as did Heatley et al (23). The latter group were also unsuccessful using Sprague Dawley rats and guinea pigs.

Virology

While these interesting transmission studies were being undertaken we were seeking agents in tissue filtrates from patients with CD and UC (31) (see Table 2). We reported a statistically significant serologic association of cytomegalovirus with UC. The failure of these studies to demonstrate an association between cytomegalovirus and CD was confirmed by the investigations of Roche and Huang (32), who used hybridization techniques; these investigations, however, did not evaluate the already established association of cytomegalovirus and UC.

Aronson et al (33) reported that biopsies of CD tissue and tissue from some other intestinal disorders produce a cytopathic effect (CPE) on early passage human diploid lung fibroblasts (Wl38). This effect could be passaged. Subsequent characterization suggests that the agents were small RNA viruses that were acid and ether stable, heat stable at $60^{o}C$ for one hour and labile in the presence of magnesium chloride. The agents were found to be pathogenic for newborn CBA mice. Beeken et al (34) extended these observations. Gitnick et al (35) using a different technology which included homogenization and filtration through a 0.2 micron filter showed that a cytopathic substance could be isolated from CD tissues and another material could be isolated from UC tissues (see Table 3). Both were shown to produce CPE in a rabbit ileal cell line

TABLE 2

STUDIES OF VIRUSES AND CYTOTOXINS
IN CROHN'S DISEASE AND ULCERATIVE COLITIS

1962	Schnierson et al:	No viruses found in UC or CD
1973	Farmer & Gitnick:	No specific viral serology in CD. CMV superinfection in UC
1975	Aronson, Beeken, Phillips:	Small RNA virus in CD using WI-38 tissue cultures
1976	Greenberg & Kapikian:	Mycoplasma hyorhinas and SV40 found in Aronson, et al materials
1976	Gitnick et al:	Development of sensitive tissue culture systems for CD CPE and EM studies
1976	Korsmeyer et al:	Anti RNA antibodies in IBD patients and relatives but not in matched controls
1977	Strickland et al:	Lymphocytotoxic antibodies in IBD patients and spouses but not in matched controls
1977	Whorwell, Phillips,Beeken:	Isolation of reovirus-like agent in CD
1977	Riemann et al:	EM of virus-like particles in CD
1977	Cooper et al:	CMV in UC toxic megacolon
1980	Phillpotts et al:	? Tissue factors in CD using Wl38 but no CPE in rabbit ileum or avian tissue culture
1980	IBD research group:	Confirmed UC and CD CPE in each tissue culture system
1981	McLaren and Gitnick:	Heat labile cytotoxin in CD Heat stable cytotoxin in UC and colon CA Heat labile cytotoxin in UC and colon CA

TABLE 3

CPE INDUCERS IN GASTROINTESTINAL DISEASE

	NO.	+CPE
CROHN'S DISEASE		
Ileitis	36	35
Colitis	27	27
Ileocolitis	7	7
ULCERATIVE COLITIS		
Backwash Ileitis	2	2
Colon	27	27
COLON CARCINOMA[+]	27	27
RADIATION ENTERITIS	5	2
NECROTIZING ENTEROCOLITIS	1	1
COLONIC POLYPS BENIGN	2	0
FAMILIAL POLYPOSIS BENIGN	2	1
VOLVULUS	1	0
HIRSCHSPRUNG'S DISEASE	2	0
DIVERTICULITIS	23	1
NORMAL ILEUM	5	0
NORMAL COLON	1	0

[+]Grossly normal intestinal tissue studied

and subsequently were shown to destroy RIF free chick embryo fibroblasts and Peking duck embryo fibroblasts. Neutralization tests employing human sera as well as guinea pig immune sera have suggested that these agents are not immunologically identical. Preliminary electronmicroscopic evidence also suggested differing morphologies. The fact that the cytopathic inducers could be serially passaged made it conceivable that these are either living, replicating agents of a size consistent with a virus or highly concentrated toxic materials. Whorwell et al (36) suggested that the agents were Rotaviruses; however, this was refuted serologically with temporally matched control sera. Phillpotts et al (37) using other methods than previously reported suggested that the cytopathic change was induced by low and middle molecular weight nonviable tissue factors. The nature of these factors was not identified. They did not attempt exact reproduction of the previous reports by using identical techniques used by others. Greenberg et al (38) reported finding a Mycoplasma hyorhinis contaminating some of the cultures used by Aronson et al, but were unable to find Mycoplasma in the cultures reported by Gitnick et al. Reimann et al (39) presented electronmicroscopic evidence of viruslike particles in CD tissue and Dourmashkin et al (40) described a possible precursor to the apthoid ulcer and myxovirus-like budding from the surface of epithelial cells.

Studies by Strickland et al (41) have identified high levels of antibodies to synthetic double stranded RNA and lymphocytotoxic antibodies in the sera of patients with either CD or UC and their unaffected spouses. These studies also revealed high levels of circulating interferon in patients with IBD. Cellular mechanisms of tissue damage in IBD are suggested by several studies which have investigated the in vitro cytotoxic effect of IBD peripheral blood lymphocytes (PBL) to colon epithelial cells in short term culture (42). The cell responsible for this effect is reported to have the characterics of a K-cell (43). Recent studies of circulating nonspecific K-cell activity in IBD have yielded conflicting

results. Increased K-cell activity in CD using a plaque assay was reported by Eckhardt et al (44). In contrast, Britton et al (45) using an antibody dependent cellular cytotoxicity (ADCC) assay with a mouse lymphoma line as a nonspecific target reported insignificant differences between PBL from CD and normal subjects. The possible role of K-cells in the bowel damage of IBD has been further clouded by reports that this cell is absent in lymphoid populations derived from the intestine, both in IBD and in control subjects. The study by Britton et al (45) clearly showed enhanced K-cell activity in mesenteric lymph node cells from patients with CD when compared to disease control mesenteric node cells. Recent work by Chiba et al (46) indicates that a major reason for the apparent lack of K-cell activity reported by other investigators in intestinally derived lymphoid cells is selective deflection of this subpopulation during the prolonged (18 hour) process of lymphoid cell isolation from the gut.

Effector lymphocytes that are responsible for in vitro cytotoxicity against virus infected target cells in humans may include both T-cells (47-49) and K-cells (50-53). Further, interferon has been shown to substantially enhance spontaneous cell mediated cytotoxicity (SCMC) activity against virus infected targets (54). In addition to being potentially important mechanisms of resistance to recovery from viral infection such reactions may also be important in the genesis of chronic tissue damage. For example, the development of chronic hepatitis in patients with hepatitis B infection has been linked to such cytotoxic effects (55). Similar mechanisms could be postulated as contributing to the chronic intestinal damage in IBD.

Thus transmissible cytopathic agents have been described in CD and UC. Although several laboratories have reported the existence of such agents, others have been unable to isolate them. Many problems have existed in extending the initial reports. Among these have been the inability to cultivate the cytopathic materials to high enough titer to

allow proper characterization. The low titer of the cytopathic materials also precluded obtaining adequate electron microscopic photos of these putative agents. Recently one group suggested that the transmissible materials could not be found in tissue culture utilizing modifications of tissue culture systems initially reported but utilizing other systems a cytopathic toxic factor could be identified (37). Subsequently we reported that following inoculation of tissue culture with filtrates from CD and UC 0.2 micron filtrates a transient cytopathic effect (CPE) could be seen in rabbit ileum tissue culture. After this, the tissue culture system recovered and then a definite and extensive CPE developed. In contrast, in chick embryo tissue culture within 48 hours of inoculation extensive CPE developed leading to destruction of the cell sheath and no late CPE inducer could be demonstrated. We further reported that the factor responsible for the early transient CPE in rabbit ileum tissue culture and the early severe CPE in chick embryo tissue culture, was a nonviable toxin unrelated to C. difficile or other clostridial toxins and unrelated to E. coli toxin. However, this finding did not necessarily explain the late extensive CPE which developed in rabbit ileum tissue culture and which was the basis for initial reports of the transmissible CPE inducer derived from CD or UC materials.

Progress in the control of Crohn's disease and ulcerative colitis is unlikely until a better understanding of the pathogenesis of these diseases is achieved. In our ongoing studies seeking the role if any, of infectious agents in the pathogenesis of CD and UC we have recently described the presence of cytotoxins capable of destroying colon, ileal and chick embryo cells while sparing 16 other tissue culture systems (56). These cytotoxins may be of bacterial, viral or tissue origin. If these cytotoxic substances were of tissue origin the narrow range of cell cytotoxicity would be difficult to explain. The selective cytotoxicity for colon, ileum and chick embryo cells while sparing other tissue culture systems would not be characteristic of a general cell toxin. They

are not immunologically related to nor do they have the physical and chemical properties of C.difficile or E.coli toxins.

The inducers of cytopathology which have now been partially characterized and isolated from CD, UC and some control speciments may represent the effector system responsible for the final development of tissue destruction in these diseases. This hypothesis is based on the reproducible finding of these cytopathic inducers in patients with these illnesses and the unique ability of these cytotoxins to destroy only a narrow range of host cells. This range includes colon, ileum or chick embryo cells but excludes most other human and animal tissue systems. The finding of this narrow range of cytotoxicity suggests the possibility that these inducers of cytotoxicity may represent one step in the pathway which leads to tissue destruction in these illnesses. One hypothesis for the development of UC or CD is outlined in Figure 1. It is conceivable that the large and small intestine may react in only a limited number of ways to a variety of insults. A number of inciting agents or combinations of inciting agents may initiate the processes leading to these diseases. These inciting agents may include viruses or viral products, bacteria or bacterial products such as endotoxins, environmental toxins and/or food antigens. In a susceptible host, these may stimulate immune mediation which may lead to the process which eventually produces a cytotoxin that in turn produces cell destruction. Alternatively these cytotoxins may have no pathogenic role in spite of their limited range of cytotoxicity.

FIGURE 1

PATHOGENESIS OF ULCERATIVE COLITIS OR CROHN'S DISEASE

HYPOTHESIS

Inciting agent
Virus or viral product
Bacteria or bacterial product
Environmental Toxin
and/or
Food antigen
↓
Susceptible host
↓
Immune mediation
↓
Cytotoxin Effector
↓
Cell Destruction

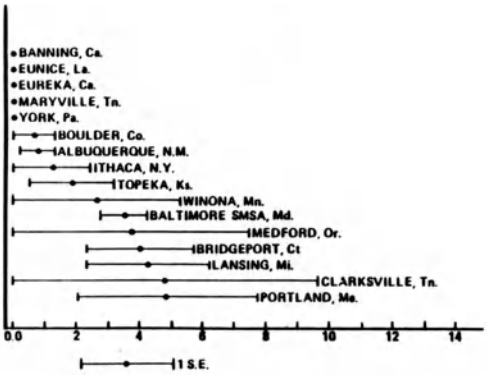

FIGURE 1. ANNUAL AGE ADJUSTED INCIDENCE RATES OF DEFINITE AND PROBABLE CROHN'S DISEASE, PER 100,000 POPULATION, WITH STANDARD ERRORS, BY GEOGRAPHIC AREA, WHITE, PAS AREAS, 1973

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ANIMAL MODELS FOR HUMAN INFLAMMATORY BOWEL DISEASES

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INTRODUCTION

The human large intestine represents a mucosal surface which is constantly exposed to a wide variety of chemical and bacterial stimuli. It is not surprising that inflammatory bowel diseases of the colon (IBD), both acute and chronic, are recognized as important medical problems with a significant morbidity and mortality. Included in this group of diseases are ulcerative colitis, Crohn's disease, neonatal necrotizing enterocolitis and pseudomembranous enterocolitis. Until recently, these four diseases had two features in common. First, all were considered idiopathic since no etiology had been established. The second feature was the occurrence of the characteristic lesions in close proximity to an enormous bacterial population composed of 300 - 400 different species. This second feature has invited speculation regarding the role of intestinal bacteria in the inflammatory process. Despite extensive studies of each disease, only pseudomembranous enterocolitis has been associated with a specific bacterial etiology. This recent success has prompted a renewed interest in the association between the colonic microflora and IBD. In the past, this hypothesis has been pursued along several lines of investigation including: (1) identification of specific bacterial pathogens, (2) "dysbiosis" or alterations of the normal colonic microflora, (3) antimicrobial trials and (4) immunologic response to bacterial antigens.

Because of the difficulty in identifying human populations at risk for prospective study of IBD, animal systems simulating the various disease processes have been sought. This has been a difficult task, since the anatomical differences and occurrence of various bacterial species in animals is often quite different than that found in humans. However, the recent success in determining the etiology of pseudomembranous enterocolitis was partially dependent on the use of an animal model with several features which differed from the disease as noted in

humans. Through the use of an *in vitro* assay system the similarity in etiology between the animal and human disease was established and several important observations noted in experimental animals have proven useful in the diagnosis and treatment of the human disease.

Although the information from experimental models of IBD may not be directly applicable to human IBD, these animal models provide a useful system for experimentation. The ultimate value of any animal model for IBD can only be established once a link to the human disease process has been made.

Table 1. Animal models simulating human inflammatory bowel diseases.

Species	Disease	Inducing Agent	Etiology
Hamster	Pseudomembranous enterocolitis	Antimicrobial agents	*C. difficile*
Guinea pig	Ulcerative colitis	Carrageenan	?
Dog	Ileitis, colitis	None	?
Horse	Colitis	None	Bacterial toxins ?
Pig	Enteritis	None	?
Marmoset	Ulcerative colitis	None	C. fetus ss jejuni ?
Hamster	Proliferative ileitis	Ileal contents from affected animals	Bacteria ?

HAMSTER MODEL FOR PSEUDOMEMBRANOUS ENTEROCOLITIS (ANTIBIOTIC ASSOCIATED COLITIS)

The lethal effects of the antimicrobial agent, lincomycin, for hamsters was first reported by Small in 1968 (1). Administration of lincomycin to these animals was associated with a fatal colitis characacterized by inflammation of the terminal ileum, cecum and colon. Histologic examination revealed vascular congestion, edema, inflammatory cell infiltrates and disruption of the mucosal epithelium. These early studies were largely ignored by clinicians until a number of reports were published detailing the occurrence in human patients of pseudomembranous enterocolitis (PMC) in association with administration of lincomycin or its 7-deoxy derivative, clindamycin (2 - 4). Following these reports incriminating an antimicrobial agent in PMC, studies to define a possible bacterial etiology were begun using the hamster system described by Small. It should be pointed out that there are several important differences

between the disease induced by clindamycin in hamsters and PMC as noted in humans. First, the hamster rarely develops gross evidence of the pseudomembranes which are the hallmark of human PMC. Second, the disease in hamsters appears to most severely effect the terminal ileum and cecum, while the dominant lesions in humans are observed in the distal colon. Finally, the disease induced by clindamycin (and many other antimicrobial agents) in hamsters occurs in virtually all animals and is almost universally fatal. This is in contrast to the relatively small number of humans developing PMC following antimicrobial administration.

The first reported evidence that the disease induced in hamsters with clindamycin was mediated by bacteria was published in 1977 (5). This report presented data which indicated that hamsters could be protected from the fatal colitis if pre-treated with gentamicin and vancomycin, but not with gentamicin alone. Subsequent studies (6) revealed that vancomycin alone was protective. In addition, it was shown that the lethal factor present in hamsters with antibiotic-induced disease could be serially transferred in hamsters and was independent of the antibiotic used to induce disease (6, 7). Isolation of bacterial strains from animals with antibiotic induced colitis yielded one strain which was reported to cause disease when either a broth culture or culture filtrates were injected into the ceca of healthy hamsters. The bacterial strain which caused disease in hamsters was identified as Clostridium difficile.

The critical link between the disease as noted in hamsters and PMC as seen in humans was based on the observations of Larson et al. (8). These investigators cultured stool from several cases of PMC for bacteria, mycoplasma and viruses. None of the cultures yielded a specific pathogen. However, it was noted that a nonviral cytopathic effect occurred in cell cultures innoculated for viral isolation. A similar observation was made by Chang et al. (9), who also reported that the cytopathic effect could be neutralized by specific antisera. In addition, it was shown that an identical cytotoxin was produced by C. difficile in culture and was also detected in cecal contents from hamsters given clindamycin. The importance of the in vitro assay for the cytopathic toxin of C. difficile cannot be overstated. This assay not only provided a crucial link between the animal model and the human disease, but also provided a rapid diagnostic assay for human cases of PMC.

These earlier studies clearly documented the importance of the animal model for PMC in defining the etiology of the human disease. More recent reports (10, 11) have utilized both the hamster model and gnotobiotic mice in an effort to understand the mechanism involved in the development of PMC. It has been shown, for example, that gnotobiotic mice can be associated with C. difficile as a non-fatal system for determining the effect of antimicrobial agents on this bacterial species in vivo. Treatment of C. difficile-associated mice with vancomycin resulted in a decrease in the bacterial viable cell density and amount of cytotoxin present in cecal content. However, a concommitant increase in the numbers of spores present was also seen. Following cessation of vancomycin treatment, both the viable cell density and cytotoxin concentration returned to pre-treatment levels. These experimental studies suggest that C. difficile can survive vancomycin therapy as spores. The human counterpart to these observations may be the relapse of patients treated with vancomycin for PMC after therapy is discontinued. It is evident that there is still a great deal to learn about the mechanisms involved with C. difficile induced PMC. Animal model systems are an essential part of these continuing studies.

CARRAGEENAN MODEL FOR ULCERATIVE COLITIS

In 1969 Watt and Marcus first described the effects of feeding carrageenan to guinea pigs (12). These investigators noted that guinea pigs given solutions of 1% and 5% degraded carrageenan as the sole source of oral fluids developed ulcerations of the cecum and large intestine within 30 days. Gross anatomical studies after sacrifice showed loss of haustra, mucosal granularity, pseudo-polyps and strictures. Microscopic examination of intestinal tissues revealed changes characterized by crypt abscesses, lymphocytic infiltrates and capillary congestion of the lamina propria and superficial portion of the submucosa and atypical epithelial hyperplasia adjacent to areas of ulceration. These histologic findings have been reviewed and confirmed by others (13). It has also been reported that oral carrageenan treatment results in development of colonic lesions in several other animal species (14 - 17). The inducing agent in these studies, carrageenan, is a sulfated polysaccharide obtained from red seaweeds such as Eucheuma spinosum and Chondrus crispus. The natural product has a molecular weight of 100,000 - 800,000 daltons, but can be

broken into depolymerized products of 30,000 daltons by mild acid hydrolysis. The degraded product retains the gel-like and polyanionic qualities of the native material but has greater solubility in aqueous solution.

The toxic effect of carrageenan for animals appears to reflect some local action of carrageenan on the bowel; both native and degraded carrageenan are poorly absorbed and parenteral administration produces no colonic lesions (18, 19). Recent evidence, however, suggests that carrageenan, per se, is not responsible for ulceration of the intestine, since germfree guinea pigs (20) and germfree rats fed carrageenan for extended periods develop no cecal or intestinal ulcerations (I. Hirono, personal communication). More importantly, neither human populations which consume red seaweeds nor patients given large doses of degraded carrageenan develop ulcerative bowel disease (21). Despite the carrageenan dependence of the ulcerative colitis produced in animals, the pathologic changes induced in animals occur predictably and in many respects simulate those noted in human ulcerative colitis. Many authorities have concluded that ulcerative colitis is a multi-factorial disease. The carrageenan model has been used extensively to test several possible contributing factors.

Recent reports (5, 20, 22) have utilized the carrageenan induced ulcerative colitis model in guinea pigs to determine whether the intestinal microflora is a contributing factor in the experimental disease. Quantitative bacteriologic studies of cecal contents during carrageenan treatment showed that major increases in the numbers of coliforms and gram negative anaerobes occurred concommitant with the initially detectable lesions. Treatment of experimental animals with antimicrobial agents prior to and during carrageenan administration showed that aminoglycosides, vancomycin and chloramphenicol were ineffective in altering the disease course. On the other hand, both metronidazole and clindamycin prevented cecal ulcerations in animals given carrageenan (5, 22). These data suggested that obligate anaerobes were essential to the development of cecal ulcerations. However, delay in therapy until ulcers were already present was ineffective in altering the disease course.

Subsequent studies (20) utilized germfree guinea pigs to better define the bacterial populations required for ulceration during carrageenan

treatment. It was found that neither germfree animals nor animals repopulated with a defined microflora, derived from mice, developed cecal ulcerations during carrageenan treatment. In contrast to these results, pools of 10 bacterial isolates derived from guinea pigs with carrageenan induced colitis were capable of promoting the typical lesions during carrageenan treatment. In addition, a single strain of Bacteroides vulgatus isolated from these pools was shown to promote cecal ulceration in germfree guinea pigs in the presence or absence of carrageenan treatment.

Although there is considerable evidence suggesting that bacteria play an essential role in experimental ulcerative colitis, the link between the animal model and the human disease process has not been clearly established. Two areas have been explored in an effort to link the experimental and human disease: (1) efficacy of therapeutic agents in the animal model and (2) common factors between the animal model and human disease which are unique to ulcerative colitis. The therapeutic agent of greatest clinical interest in treating ulcerative colitis is azulfidine. Trials with this agent, given to guinea pigs concommitant with carrageenan administration, resulted in 50% reduction in the number of animals developing cecal ulcerations (23). The use of other anti-inflammatory agents in this model have not been as rewarding, with the exception of other amino salicylate compounds (Onderdonk, unpublished data).

Recent research suggests that increased chemotactic ativity of fecal extracts, a cytopathic effect for cell cultures and increased long chain fatty acid concentrations are common features of both the experimental model and the human disease (20, 24). The specificity of these findings for ulcerative colitis remains to be proven. A continued effort to link the carrageenan induced model for ulcerative colitis to the human disease may provide valuable information regarding the inflammatory process noted in human ulcerative colitis.

NATURALLY OCCURRING ANIMAL DISEASES SIMULATING HUMAN INFLAMMATORY BOWEL DISEASE

A number of animal species have been observed to develop inflammatory bowel diseases similar, in many respects, to that noted in humans. Canine colitis, equine colitis and porcine enteritis all occur spontaneously in a small percentage of animals. Although each of these diseases are quite

interesting and histologically are similar to IBD in humans, these diseases occur unpredictably and cannot be "manufactured" in the laboratory. None of these species currently serves as a useful experimental system. On the other hand, recent reports of an ulcerative colitis-like disease in cotton-top marmosets may prove to be a useful model (25). The cotton-top marmoset is an endangered species and cannot be imported into the United States. Only a few institutions maintain breeding colonies of these animals and it is from these breeding colonies that reports of a wasting disease characterized by colonic ulcerations occurring in a large percentage of animals, crypt abscesses and lymphocytic infiltrates of the lamina propria have been forthcoming. These reports suggest the possibility of a sub-human primate model simulating naturally occurring IBD. There are, however, certain features of the marmoset disease which may preclude its use as a model for human disease. Unlike human ulcerative colitis, the disease in marmosets appears to be associated with the presence of Campylobacter fetus ss jejuni as part of the intestinal microflora. It has also been reported that marmosets with wasting disease can be treated successfully with oral antimicrobial agents.

The spontaneous occurrence of proliferative ileitis in the golden syrian hamster may also serve as a useful experimental model of potential value for studies of IBD. It has been reported that hamsters can spontaneously develop acute and chronic ileitis. Certain colonies seem to be more prone to development of the disease, although the random occurrence of this disease in many animal facilities has been reported. The disease is transmissable from animal to animal by the injection of ileal contents from affected hamsters, confined to the terminal ileum and associated with microorganisms clearly visible within epithelial cells when examined by election microscopy. Histologic changes include hyperplasia of the ileal mucosa with lymphocytic infiltration of the underlying tissues (27). Although the acute, fulminant course of this disease differs from the more chronic enteritis noted in man, the hamster disease can be maintained in the laboratory as an experimental system and may be a useful tool for examining an additional form of IBD.

SUMMARY

Animal systems simulating inflammatory bowel disease in humans are an essential part of the efforts to define an etiology for this group of diseases. However, investigators utilizing these systems should recognize their limitations. Animal systems which simulate human disease often have important anatomic, physiologic and bacteriologic differences from the disease as noted in humans. These differences may be important when observations made in animals are compared to human IBD. Experience, however, suggests that perceived differences between animal models and human disease may only reflect species specific responses to an identical etiologic agent. The most recent evidence that supports this contention are the studies dealing with pseudomembranous enterocolitis. It is also clear, however, that further work with existing animal models and development of better models for inflammatory bowel disease would greatly aid in our basic understanding of the disease process.

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C. Pathogenesis

NEWER CONCEPTS OF IBD EPIDEMIOLOGY

ALBERT I. MENDELOFF, M.D., M.P.H.

I. Today we will consider studies defining the host population in those ways which seem most likely to help us understand why only a percentage of the populations at risk actually develop IBD. We want to know its genetics, its geographical distribution, its dietary background, its socio-cultural experience, its health record prior to contracting IBD, its drug-taking history, and its risks of having certain complications.

Our methodology for determining these factors is far from perfect. We have to make inferences about some of them from such difficult-to-evaluate analyses as responses to medical and surgical treatment. What we have to find out, in short, is (1) in what respects a given population having IBD differs from a population without IBD, (2) why, in a susceptible family or group one person gets CD and another UC, and (3) why, in a cohort of IBD patients some have one type of complication and others have different types of complications.

II. I shall take the moderator's prerogative to present data obtained by our group at Johns Hopkins over the past twenty years. This group includes Drs. Mary Monk, Abraham Lilienfeld, Cedric Garland, and myself.

A. In the period 1960-1963 we carried out a very extensive study of the incidence of IBD in the Baltimore metropolitan area (approximately 2×10^6 population), and reported values for incidence of ulcerative colitis (UC), ulcerative proctitis (UP), and Crohn's Disease (CD) based on the first hospitalization rates. The population was accurately assessed for color, ethnicity, age, sex, and socio-economic class. Extensive interviews were held with each hospitalized patient, hospitalized controls matched for sex, age, and color, persons with other diseases of the lower bowel ("organ controls"), and with a nonhospitalized population living in the same geographical area as the incident case ("neighborhood controls"). These data were reported subsequently (1) (2) (3) (4).

In 1973 we repeated the Baltimore study of the first hospitalization rates for UC, UP, and CD, but without interviews. The comparisons between rates for white males and white females 1963-1973 are seen in Table I, and for non-white males and females 1973 in Table II. The rates for Jewish males vs. non-Jewish males 1963-1973 are shown in Table III. Thus it is seen that in the 10 year interval 1963-1973, the increased risk of developing IBD for Jewish males was of the order 5x, somewhat higher than the 3x risk reported by us in 1963. The Jewish population of Baltimore is approximately 100,000, with an estimated 5% decrease in the decade.

Table I.

Annual First Hospitalization Rates per 100,000 Population for Definite and Probable I.B.D., White, Baltimore, 1963 and 1973

Disease	White males		White females	
	1963	1973	1963	1973
CD GC	2.49	3.65	1.22	3.75
UC UP	3.91	3.92	5.21	5.37

Table II.

Annual First Hospitalization Rates per 100,000 Population for Definite and Probable I.B.D., Nonwhites, Baltimore, 1963 and 1973

Disease	Nonwhite males		Nonwhite females	
	1963	1973	1963	1973
CD GC	0.66	0.83	0.00	1.78
UC UP	1.32	0.84	1.51	3.11

Table III

Estimated Annual First Hospitalization Rates per 100,000 Population for Definite and Probable I.B.D., According to Religion, White Males 20 Years Old and Older, Baltimore, 1963 and 1973

Disease	Jewish males		Other males	
	1963	1973	1963	1973
CD/GC	7.24	20.16	2.04	2.57
UC/UP	9.31	13.44	3.40	3.29

The remarkable rise in CD rates, especially in young white and black females, led us to undertake a study of 15 other localities in the U.S. where we had accurate 1970 census data and could be sure that the hospitals of each locality either were the only hospital serving the population, or included all the hospitals available for the population. The data for these 15 localities proved reassuring, in that the Baltimore data were about median for the entire 15 plus Baltimore (See Figure 1). Age-adjusted incidence rates for white females in Baltimore (Figure 2) showed a marked bimodal peak with extremely high numbers for the age group 20-35 years. In searching for some likely cause to explain this remarkable 10-year increase for these young women, we thought that perhaps the world-wide increase in oral contraceptive use might be a contributing factor. The Royal College of General Practitioners report of 1974 (5) indicated that takers of oral contraceptives were 1.46 more likely to have CD, and 2.06 to have UC, than controls or ex-takers. Since data from western Europe and the UK also indicated rises in CD in young women, this hypothesis deserves some consideration.

Figure 1

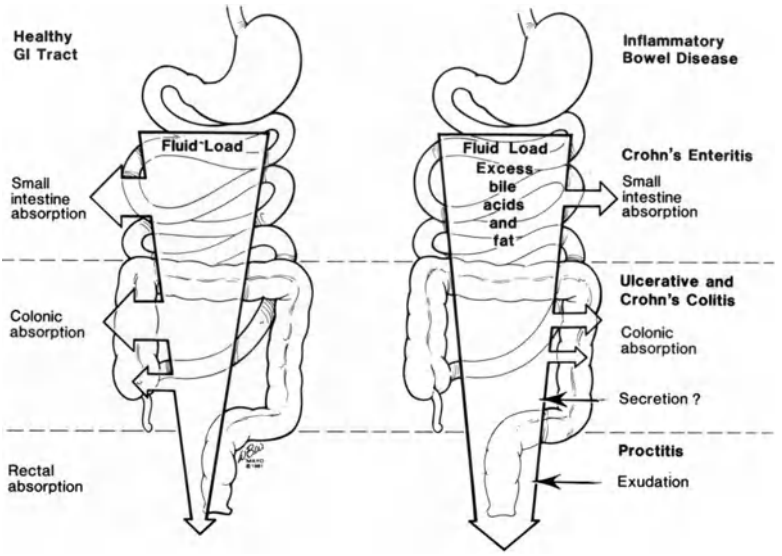

Figure 2

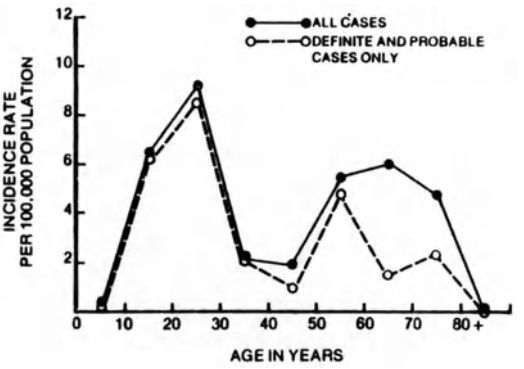

Figure 2 **Annual Age-Specific Incidence Rates per 100,000 Population of Crohn's Disease, White Females, Baltimore SMSA, 1973**

Notwithstanding the striking increase in incidence rates of CD for females, white and black, and increased rates for males as well, in the period 1963-1973, a definite change has apparently occurred in the past 6-7 years. In Scotland Kyle (6) has reported a decreasing rate of increase, as has Hellers in Sweden (7). Our own data, derived from a

familial study in Baltimore from 1977-1979, also shows for the first time an actual decrease in the rates for UC, and a diminution in the rate of increase for CD. There has been some decrease in the use of oral contraceptives during this period but the decreased rates mentioned seem to affect both males and females.

In the course of our study we have also queried CD patients, and age-sex matched hospital and neighborhood controls about their consumption of Corn Flakes and of sweets, both factors attributed by other investigators as being more often eaten by CD patients than by controls. In our study CD patients did not differ from either set of controls in the ingestion of these items.

We believe there has been a slow but progressive increase in the involvement of the large bowel by CD over the past several decades, but anal CD as a major presenting variant has not occurred in the US in contrast to some reports in the UK.

In summary, the epidemiological aspects of IBD may be described as follows: "From the foregoing discussion it is clear that no laboratory, physical, genetic, or psychometric markers exist by which one can define a population at risk to develop IBD. Under such circumstances, either one resorts to yet another attempt to cast a net with mesh fine enough to separate many factors of uncertain significance from a large population, or one determines that one or another characteristic is sufficiently, if not overwhelmingly, impressive to warrant a very intensive study of its frequency in smaller IBD populations and controls. The most striking such features are probably (a) the familial aggregation of cases, and (b) the markedly different attack rates between Jews and coloured races. Although not all reports have supported these data, the Baltimore study in 1960-1963 and its follow-up a decade later demonstrate very clearly the magnitude of the difference in attack rate between American urban Jews in that city and the non-white urban groups. In other countries, e.g. Sweden, where non-whites are not present in appreciable numbers, Jews have been found at increased risk for IBD (8).

It becomes clear, then, especially in view of the genetic data that suggests shared susceptibility genes in families developing IBD, that a major attempt to characterize such families by every means available, and to follow the course of their growth and susceptibility to actual evidence of IBD, should be made at this time. Properly prepared protocols

should also shed light on the possibility of transmissible infectious or other agents in setting in train the complex interaction of the many factors which seem undoubtedly involved in the production of the disease complex we call IBD." ( )

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EPIDEMILOGY OF INFLAMMATORY BOWEL DISEASE. STATE OF THE ART AND ETIOLOGIC INFERENCES

T. GILAT, A. GROSSMAN, Y. BUJANOVER and P. ROZEN

1. INTRODUCTION

Inflammatory bowel disease (IBD) is the most common chronic intestinal bowel disease in western societies. Its etiology is unknown. We are uncertain whether its two components, Ulcerative Colitis (UC) and Crohn's Disease (CD) are single, separate and different diseases or merely represent the extremes of a spectrum.

In such a situation, the data provided by epidemiologic studies may be of help in advancing our understanding of these diseases, their pathogenesis and etiology. The data have recently been accumulating at an accelerating pace and are of considerable interest. Most data in this article are derived from population studies, for reliability and to allow valid comparisons.

2. MATERIAL AND RESULTS

2.1. Incidence

The sharply rising incidence of Crohn's disease in recent decades in many parts of the world is shown in Table 1.

Table 1. Rising incidence of Crohn's disease (Population Studies)

Location	Ref.	Period		Incidence of CD	
		Initial	Last	Initial	Last
Rochester, Minnesota	1	1935/54	1965/75	1.9	6.6
Cardiff, Wales	2	1934	1977	0.2	4.8
Nottingham, England	3	1958/63	1967/72	0.9	2.9
Glasgow, Scotland	4	1961	1970	1.0	2.0
Aberdeen, Scotland	5	1955/61	1962/68	1.7	2.6
Uppsala, Sweden	6	1956/61	1968/73	1.7	5.0
Goteborg, Sweden	7	1951/60	1961/70	1.4	6.3
Malmo, Sweden	8	1958/65	1966/73	3.5	6.0
Stockholm, Sweden	9	1955	1974	1.0	4.8
Basel, Switzerland	10	1960	1969	1.1	2.6
Copenhagen, Denmark	11	1960	1970	1.1	2.5

Hellers (9) and others have raised the possibility that the incidence of CD has reached a plateau in recent years and has stopped rising. At present, there are not sufficient data to answer this question, even for Sweden.

By contrast, the incidence of ulcerative colitis has remained stable during the same time period. (Table 2). There were fewer population studies of UC as compared to CD in this time period. In previous decades, there were suggestions that the incidence of UC was on the rise (13). It may now have reached a plateau.

Table 2. Stable incidence of ulcerative colitis (Population Studies)

Location	Ref.	Period		Incidence of UC
		Initial	Last	
Stockholm, Sweden	9	1955	1974	7.5 stable
Malmo, Sweden	8	1958/65	1966/73	6.4 stable
Copenhagen, Denmark	11	1961	1966	7.3 stable
Tel-Aviv, Israel	12	1961	1970	3.7 stable

Population studies are the most reliable method for estimating the incidence of a disease. Studies of hospitalization rates are less reliable, however they reveal a comparable pattern (Fig. 1). A similar trend is apparent from the study of mortality data (Table 3).

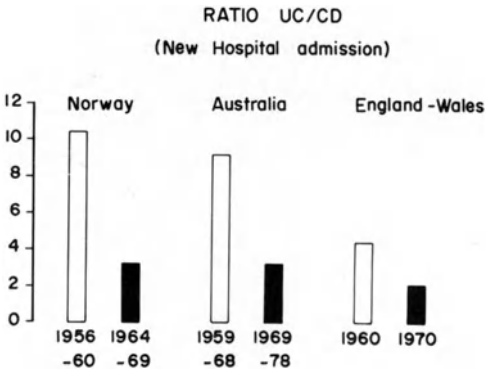

FIGURE 1. First hospitalization rates UC/CD. Changing rate over two time periods in Norway (14), Australia (15) and England and Wales (3).

Table 3. Mortality from UC and CD

Location	Ref.	Periods	Mortality CD $_5$ Cases	Rate/10	Mortality UC $_5$ Cases	Rate/10
England & Wales	2	1950	25		416	
		1975	230		340	
Australia	15	1968/72	43	0.07	167	0.27
		1973/77	84	0.12	141	0.21

2.2. Studies of migrant populations

Ashkenazi Jews. Due to early reports from the U.S.A. of a higher incidence of IBD in Jews (16, 17), they were intensively studied in several countries. Results are shown in Table 4.

Table 4. Incidence of CD in Ashkenazi Jews (Population Studies)

Location	Ref.	Period	Ashenazi Jews	Gen. population
Tel-Aviv	18	1970-76	1.6	1.2
Basel	10	1960-69	2.2	1.6
Cape Town	19	1970-74	2.8	0.6
Baltimore	16	1960-63	7.2	2.5
Stockholm	9	1955-74	10.0	3.0
Malmo	8	1958-73	25.0	4.8

Two major findings are apparent in this table. The incidence of CD in Ashkenazi Jews varies tremendously (more than 10 fold) in various parts of the world. Remarkably, the incidence seems to rise with the incidence of CD in the general population of the area. A different pattern is found when a genetic trait such as primary lactase deficiency is considered. Its prevalence in Ashkenazi Jews in several parts of the world is very similar: Israel - 66%, U.S.A. - 71%, Canada - 69% (20).

The other major finding in Table 3 is the generally higher incidence of CD in Jews than in other Caucasians in the area. The significance of this finding will be discussed later.

Some studies are also available on ulcerative colitis in Jews in various locations. The prevalence of UC in Jews in Tel-Aviv was 37 (per 10^5) (12), while in Johannesburg a world high of 200 (per 10^5) was recorded (21). Here again, we see a very marked variability associated with different geographic conditions.

Developing populations. This group is not as well studied as the Jews.

Almost no exact population studies are available and we are dealing more with impressions than with exact data. Yet a trend seems to be apparent. Ulcerative colitis was very rare in Maoris as compared to the white population of New Zealand. When evaluated by the same method (hospitalization rates) at a later date (22), the incidence has risen markedly. Crohn's disease and ulcerative colitis were almost non-existent in blacks in Cape Town (19), Johannesburg (21), and Kampala (23). With prolonged residence in an urban environment initial cases have begun to appear (21).

2.3. Urban rural differences

These have been sought in several population studies and in general IBD was more frequent in urban than rural residents in the same area and population. This is illustrated in Table 5.

Table 5. CD - Urban-rural differences (Population Studies)

Location	Ref.	Period	Method	Urban	Rural
Wales	24	1967-76	Prevalence	47	34
N. Ireland	25	1966-73	Incidence	3	1
Aberdeen	5	1955-69	Prevalence	49	29
New Zealand	22	1950-66	Hospital rates	119	59
Rochester, Minn	1	1935-75	Incidence	4.9	3
Stockholm	9	1955-74	Incidence	3	3

Comment. Taken together, all the previous data point very strongly to the influence of environmental factors in IBD. A sharp rise in the incidence of a disease within a genetically stable population cannot be explained by hereditary factors. The same applies for marked differences in the incidence of a disease in migrants. The appearance of a hitherto absent disease with changes in life style and habits, as in the developing world, and the urban-rural differences, all point in the same direction. There appear to be factors in the urban industrialized milieu which cause, or predispose to, the development of IBD.

Other epidemiologic data have to be mentioned.

2.4. Sex

The disease is more frequent in females, particularly in CD. This was found in the great majority of population studies (Fig. 2), and in most of them the difference was statistically significant. The cause is unknown.

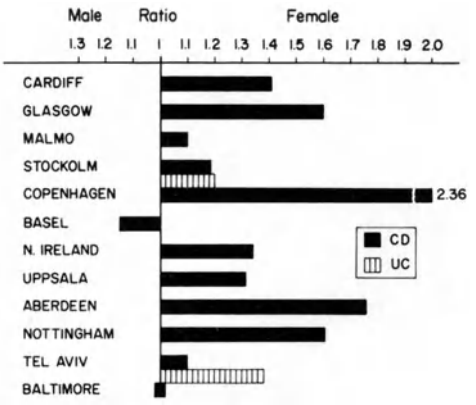

FIGURE 2. Female/male ratio of CD and UC in several population studies.

2.5. Familial Incidence

While in the past less than 5% of patients with IBD were said to have disease in the family (UC or CD), recently tremendously high figures in the 20 - 30% range were reported (Table 6).

Table 6. IBD in families (% of patients having affected relatives)

Location	Ref.	CD	UC
Cleveland	26	35	29
New York	27	20	
Rhode Island	28	22	
Cardiff *	2	10	
Rochester, Minn *	1	10	

* population studies

One wonders whether there might be some bias, in that patients with disease in the family may seek more qualified medical help. However, even in population studies performed in Cardiff (2) and Rochester, Minn. (1) - 10% were found to have disease in the family.

Comment. The family studies plus the previously noted high incidence of the disease in Jews (Table 4) raise the question of genetic influences. Hereditary and environmental factors are not mutually exclusive and the effect of both has been convincingly demonstrated in large bowel cancer. On the other hand, an increased familial incidence does not necessarily prove the existence of genetic factors, and the data in Jews may be sub-

ject to alternative interpretations as discussed further. Thus the question of genetic factors in IBD must still remain open.

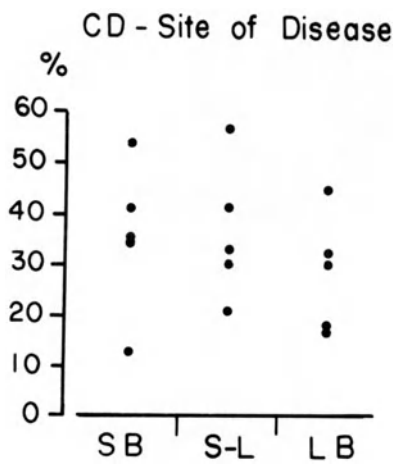

FIGURE 3. CD - Percent of patients having disease in the Small Bowel (SB), Large Bowel (LB) or both (S-L) - in several population studies (Ref. 2, 4, 9, 10, 25).

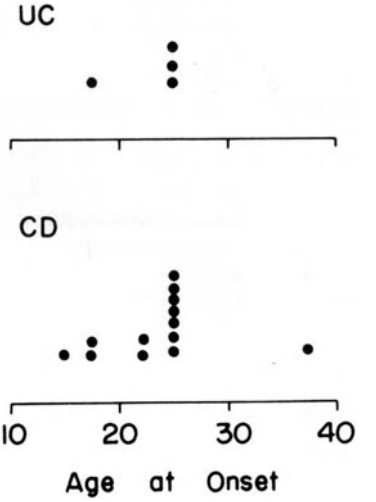

FIGURE 4. The peak age of onset of CD and UC in several studies.

2.6. Site of disease

This has been noted for CD in several population studies (Fig. 3). Considerable variations are apparent in studies from different areas. It may also be recalled that in the OMGE study of colitis which was performed simultaneously in numerous centers and countries, enormous variations were found in the UC/CD ratio (29).

2.7. Age at onset

This shows a remarkable similarity in various studies (Fig. 4). The most frequent age of onset is around age 20-25. Often in the 2nd and more often in the 3rd decade. A small second peak after the 5th decade has been noted in some but not other studies.

3. DISCUSSION

What do these data tell us? There might be genetic influences in the pathogenesis of IBD. The question is as yet unsettled. The evidence for the effect of environmental factors is overwhelming and they are probably

of major importance in the causation of IBD. These putative factors are particularly present in the urban industrialized milieu, and their major influence appears to be in childhood. What might these factors be? Here we have to move from the realm of fact to that of speculation. Bottle feeding (versus nursing), high sugar, and low fiber consumption were found to be associated with IBD in some studies. The evidence to date is insufficient. Interesting data have recently been reported for Hodgkin's disease (30) which may be relevant to IBD. Hodgkin's disease has been shown to be more frequent in children without or with few siblings, less playmates, living in single family homes. In other words, "sheltered" children who have a delayed contact with common infectious agents, namely the EB virus. Similar data have previously been known for Paralytic Poliomyelitis. It is noteworthy that Hodgkin's disease has a peak age of onset in early adult life, is more frequent in Jews and less frequent in blacks in the U.S.A. It all appears "deja vu".

If we assume this analogy, the higher incidence of IBD in Jews might be associated with closer family ties and thus a more sheltered childhood, and not necessarily with genetic factors. IBD may thus be caused by a (rare) agent that is more prevalent in the modern urban environment or, alternatively, the environment may render children more susceptible to a common agent or agents. However, this is all hypothesis and what we need are facts. An international cooperative study involving 18 centers in 10 countries is now underway trying to identify factors in childhood, in the pre-illness period, of patients with IBD which may have a bearing on it's etiology.

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GENETICS AND INFLAMMATORY BOWEL DISEASE

RICHARD B. McCONNELL

The body of published data on which opinions can be based concerning the part played by heredity in inflammatory bowel disease is of very mixed value. No family investigation has been carried out including sigmoidoscopy of all relatives. In few studies has there been physical examination of the relatives or even questioning of each of the relatives. In many the family data has been based merely on the history given by the patients, and it can be assumed that such studies greatly underestimate the number of patients who have one or more relatives who also have inflammatory bowel disease.

DISTRIBUTION OF CASES WITHIN FAMILIES.

In the literature are many reports of two or more cases of inflammatory bowel disease occurring in an isolated family. Only rarely are such reports useful in interpreting the genetic aspects of the condition, as,with a disease which is moderately common in the population,quite large family aggregations can be expected by chance alone, and the main value of these isolated reports is the frequency of the occurrence in the same family of both Crohn's disease and ulcerative colitis.

Of greater value is the detailed single-series data presented by Korelitz (1981). Because they are all from his own patients they give the distribution of intra-familial relationships free from the possible bias of the irregular ascertainment of isolated reports. His 353 index patients all had Crohn's disease and 72 of them (20.4%) had one or more relatives who also suffered with Crohn's disease or ulcerative colitis. The parent-offspring occurrences were slightly more frequent than sib-sib combinations, and the number of affected first cousins, uncles and aunts was much greater than the previous reports in the literature had indicated. Even though Korelitz found as many as 8 affected people in one family, there is nothing in his data to suggest

either dominant or recessive inheritance, nor even of a major gene contribution. They are in keeping with quantitative multifactorial heredity rather than the familial occurrences being due entirely to the common family environment.

The Cleveland Clinic data of Farmer et al (1980) shown in Table I are not quite comparable with those of Korelitz as the single series of 838 inflammatory bowel disease patients had all had an onset of the disease before 21 years of age. It can be seen that the number of affected parents was more than the number of affected sibs but because of the youth of the propositi more sibs may subsequently develop the disease. On the whole, however, these Cleveland data lend support to Korelitz's data and to an interpretation of polygenic inheritance.

Table I: Inflammatory bowel disease among immediate family members of 838 patients with onset of the disease before 21 years of age - 316 ulcerative colitis and 522 Crohn's disease (Farmer et al, 1980).

Affected members	Ulcerative colitis	Crohn's disease
Father-Son	4 (1.3%)	14 (2.7%)
Father-Daughter	8 (2.5%)	6 (1.1%)
Mother-Son	10 (3.2%)	14 (2.7%)
Mother-Daughter	9 (2.8%)	14 (2.7%)
Sibling-Sibling	19 (6%)	39 (7.5%)
Total:	50 (15.8%)	87 (16.6%)

CLINICAL CORRELATIONS WITHIN FAMILIES.

Kemler et al (1980) studied 10 families in which 32 cases of inflammatory bowel disease had developed. They noted that 3 of 4 affected sib-pairs who had identical HLA haplotypes had similar disease patterns. The remaining HLA identical pair had one sib with Crohn's of the small bowel and the other with classical ulcerative colitis. In 6 of the 10 families the affected either all had Crohn's disease or all had ulcerative colitis. In the remaining 4 families both diseases were found.

TWINS.

In Table II are listed the reports of twins up to 1977. It can be seen that amongst the monozygotic twins there is a high concordance rate for Crohn's disease but not for ulcerative colitis. There has been another report of discordance in identical twins in which one had ulcerative colitis (Kemler et al, 1980). It is difficult to imagine a reason why clinicians should report twin pairs when only one is affected by ulcerative colitis more easily than twins when only one is affected by Crohn's disease. Therefore these data support the theory that heredity is more important in Crohn's disease than in ulcerative colitis.

Table II: Reports before 1977 of inflammatory bowel disease in twins

	U.C.	Crohn's	Both
Monozygous Twins:			
Concordant	5	7	0
Discordant	6	1	0
Dizygous Twins	1	2	0

Klein et al (1980) have reported a pair of monozygotic twin boys who developed Crohn's disease affecting the terminal ileum, colon and rectum within eight months of each other when 14 years of age. These authors considered 11 reported sets of twins with Crohn's disease and state that all became symptomatic within 6 years of each other and in seven instances the interval of onset between twins was less than one year. Four pairs had been raised in the same environment but in two pairs they were living apart, one pair for ten years (Morichau-Beauchant et al, 1977).

It is interesting that there has not yet been a report of twins, one with Crohn's disease and the other with ulcerative colitis, even though the two diseases are so often found in sib pairs and in parent-child combinations. This is further strong evidence of the two diseases having a quantitative genetic relationship.

SPOUSES.

Up to 1977 there had been three instances of husband and wife affected, and three more were reported by Mayberry et al (1980), Whorwell et al (1978) and Zetzel (1978). Korelitz was able to add two more instances. It is interesting that sometimes one spouse had Crohn's disease and the other ulcerative colitis. This might be interpreted as indicating that the two conditions have the same exogenous aetiology but

with DR2) and 100 Toronto controls (26%), but in view of the low percentage in this control group, the possibility remains of a negative (protective) association with DR2.

Peña (1980) has excellently reviewed many immunogenetic aspects of inflammatory bowel disease, presenting not only HLA data but also non-HLA B-cell antigen studies. The sera of 23 mothers of patients with Crohn's disease were tested for lymphocyte cytotoxicity and two of them may have recognised B-cell antigens that are primarily associated with Crohn's disease. Pena holds out hope of being able to study T-cell alloantigens.

GENETIC INTERPRETATION.

It seems very probable that Crohn's disease and ulcerative colitis have different environmental causes. If this is so, the likely explanation of the familial association of Crohn's disease and ulcerative colitis is that there is one genotype, with perhaps 10 or 15 genes, which makes people liable to develop inflammatory bowel disease. People who have only a few of these genes are more liable to develop ulcerative colitis; if they have many of these genes (a more complete genotype), the clinical and pathological picture that develops is more likely to be Crohn's disease. This would explain why the relatives of people with Crohn's disease are much more likely to have inflammatory bowel disease than relatives of people with ulcerative colitis. If a patient has a large number of these genes his or her relatives are more likely to have a moderate number of genes than are the relatives of a patient with only a moderate number of them. In our present state of knowledge, this quantitative difference between the Crohn's and colitis genotypes would appear to be the most likely explanation of the familial pattern (McConnell, 1980).

ANKYLOSING SPONDYLITIS.

It is not certain whether spondylitis is a complication of inflammatory bowel disease or merely an associated disease. The fact that, occasionally the spondylitis develops before the bowel disease makes it difficult to regard it as a complication of the bowel disease. It may be pertinent to the subject that when 47 patients whose only diagnosis was ankylosing spondylitis were investigated by sigmoidoscopy, rectal biopsy and barium enema, 8 had evidence of colitis, and of these 3 had no gastrointestinal symptoms (Jayson et al, 1970). Among the first degree relatives of the 142 Liverpool patients (Lewkonia and McConnell, 1976) there were 3 who had ankylosing spondylitis but no

more probably it has been due to chance.

POSITIVE FAMILY HISTORY.

The numbers of people with a positive family history in reported series of inflammatory bowel disease patients has been very variable, usually between 10 and 20 per cent being found as summarised by Kirsner (1973). Crohn's disease is more often familial than is ulcerative colitis (see Table III). When the relatives are interviewed the percentage found can be expected to be higher than when only the patients are questioned. People can be quite ignorant of the state of health of even their own brothers and sisters in other towns.

Table III: Incidence of positive family history of inflammatory bowel disease in some recent studies.

Authors	Diagnosis	Number of Patients	Positive Family History	
Lewkonia & McConnell (1976)	Ulcerative colitis	103	7	6.9%
	Crohn's disease	39	11	28.2%
Mayberry et al (1980)	Crohn's disease	156	14	9.0%
Farmer et al (1980)	Ulcerative colitis	316	93	29.4%
	Crohn's disease	522	187	35.8%
Korelitz (1981)	Crohn's disease	353	72	20.4%

Amongst the relatives of those with either colitis or Crohn's disease there are found many with the opposite disease. Kirsner (1973) reported that of 103 families with more than one case of inflammatory bowel disease, 31 had both Crohn's disease and ulcerative colitis. Data from the Colon Clinic of the Royal Liverpool Hospital in which the patients were investigated in some detail and the relatives examined showed very much the same as in Chicago (Lewkonia and McConnell, 1976). Amongst the 103 ulcerative colitis patients there were 7 (6.9%) who had inflammatory bowel disease in their relatives, whilst in the 39 Crohn's patients, 11 (28.2%) had affected relatives. Note the higher incidence of positive family history amongst the Crohn's disease patients and again there was a mixture of ulcerative colitis and Crohn's disease in the relatives of patients with both diseases.

Data from the Cleveland Clinic (Farmer, 1980) related only to patients with an onset of disease before the age of 21. Of the 316 ulcerative colitis patients, they found that no less than 29% had a

positive family history of inflammatory bowel disease. In Crohn's disease it was even higher, 35% having a positive family history. This high incidence of positive family history is very much what one finds in early-onset cases of disease in which the genetic basis involves a number of different genes, in other words a polygenic basis. For instance, patients in whom duodenal ulcer develops under the age of 15 have a very high incidence of duodenal ulcer in the mothers and fathers, very often in both sides of the family (Cowan, 1973). Similarly, these young onset inflammatory bowel disease patients at the Cleveland Clinic have a strong familial component.

GENETIC MARKERS.

There have been many attempts to find blood group or other associations with inflammatory bowel disease but none has been established. The distribution of the ABO blood groups and the secretor/non-secretor frequency does not differ from population controls in ulcerative colitis (McConnell, 1966) or Crohn's disease (Biemond et al, 1981).

Reports of abnormal distribution of HLA-A and HLA-B antigens in some series of inflammatory bowel disease patients (Asquith et al, 1974; Biemond et al, 1981; Gleeson et al, 1972; Delpre et al, 1980; Tsuchiya et al, 1977) have not been confirmed in other series (Lewkonia et al, 1974; Thorsby and Lie, 1971; Woodrow et al, 1978, Cohen et al, 1981; Burnham et al, 1981). There certainly is no strong association of either ulcerative colitis or Crohn's disease with any particular A or B antigen comparable for example, with the association of HLA-B8 with coeliac disease.

Kemler et al (1980) have studied 15 sib-pairs with inflammatory bowel disease and found no support for the hypothesis that a gene locus close to the HLA-A or HLA-B loci predisposes to the development of inflammatory bowel disease. Four pairs shared both HLA haplotypes compared with 3.75 expected by chance. Seven pairs shared 1 HLA haplotype compared with 7.5 expected by chance and 4 shared no haplotype compared with 3.75 expected by change.

There have been only a few studies of the HLA-DR antigens in relation to inflammatory bowel disease. Burnham et al (1981) found a significant reduction in the incidence of HLA-DR2 in 142 patients (22.5%) compared with 500 Nottingham controls (35.8%). Cohen et al (1981) did not however find any difference between their 47 Crohn's patients (25.5%

evidence of inflammatory bowel disease clinically or on barium enema examination and sigmoidoscopy. Macrae and Wright (1974) also found ankylosing spondylitis in the relatives of colitics.

This association of spondylitis and bowel disease in families has been found previously (McBride et al, 1963; Haslock et al, 1974). Ankylosing spondylitis is quite strongly familial (Emery and Lawrence 1967) and about 90% of patients are HLA-B27 positive compared with less than 10% of the general population. In the Liverpool series of colitic and Crohn's patients who were HLA tested, all 4 spondylitics had HLA-B27. Of the 5 patients who had HLA-B27 and who did not have spondylitis, 3 were female and, therefore, unlikely to develop the condition. Thus, of the 6 male HLA-B27 bowel disease patients, 4 had already developed ankylosing spondylitis at the time of testing.

The high frequency of ankylosing spondylitis in this small series of HLA-B27 positive inflammatory bowel disease patients (66% of 6 patients) stands in sharp contrast to a prevalence of ankylosing spondylitis of about 5% in HLA-B27 positive people in the general population. This indicates that inflammatory bowel disease is a potent initiating or potentiating factor in the development of ankylosing spondylitis. The latter, therefore, should be regarded as a complication. It may be that there is subclinical but nevertheless active bowel disease in those patients in whom ankylosing spondylitis appears to develop first. The same applies to spondylitic relatives in whom it was not able to demonstrate bowel disease. These rather meager data suggest that by HLA typing all ulcerative colitis and Crohn's disease patients it may be possible to identify the few who have a 66% likelihood of developing ankylosing spondylitis.

SUMMARY

The most striking finding that emerges when one examines the relatives of patients with inflammatory bowel disease is that a number of the relatives of patients with ulcerative colitis have Crohn's disease, and that a number of the relatives of the patients with Crohn's disease have ulcerative colitis. There is a higher proportion of affected relatives when the patients have Crohn's disease than when they have ulcerative colitis. This indicates a stronger hereditary element in Crohn's disease than in ulcerative colitis. The incidence of affected relatives is particularly high when the diseases are of early onset, a

finding in other condition in which there is polygenic inheritance.

The data available at present indicate that genes at several loci are involved in the hereditary predisposition to inflammatory bowel disease. If a few relevant genes are present, the clinical picture that develops is ulcerative colitis; a more complete genotype leads to the development of Crohn's disease. Study of the familial distribution and other genetic aspects of ulcerative colitis and Crohn's disease leads to the conclusion that, at least from the genetic point of view, they should be lumped together as one disease: inflammatory bowel disease.

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POSSIBLE ROLE OF PROSTANOIDS AS MEDIATORS IN THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASE

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1. INTRODUCTION

The etiology of ulcerative colitis and Crohn's disease is still not known. Various themes were suggested: allergic, dietary, infectious, autoimmune, vascular, psychosomatic and others, but none has so far yielded any conclusive answers. Irrespective of the specific etiology, the possibility that prostanoids may have a role as mediators in the inflammatory process and in the alteration in intestinal secretory and absorptive function in IBD was explored in the present study.

Prostanoids are among the principal mediators of the inflammatory response. Prostanoids are found in high concentrations in various tissues during active inflammation (1). Their exogenous administration can produce the cardinal signs of inflammation while the antiinflammatory properties of steroidal (2) and non-steroidal anti-inflammatory agents (3) are ascribed to their inhibition of prostanoid synthesis.

Prostanoids were also suggested to mediate the diarrhea associated with several clinical conditions and that induced experimentally by hormones, laxatives and drugs (4). Diarrhea is also the main side effect when prostanoids are administered for therapeutic purposes (5).

The purpose of the present study was to evaluate prostanoid synthesis by colonic mucosa and peripheral blood mononuclear cells (PBM) in IBD, to test the effect of drugs and to establish the type of cell responsible for their colonic synthesis. The activities of Na-K-ATPase and adenylate cyclase, two enzyme systems involved in intestinal fluid transport, and known to be affected by prostanoids was also determined in order to elucidate their possible role in the pathogenesis of the diarrhea in IBD.

2. MATERIAL AND METHODS

2.1. Patients

Rectal biopsies and venous blood were obtained from patients suffering from active ulcerative colitis and Crohn's disease before treatment and/or during remission maintained by sulfasalazine. For comparison, biopsies and blood were also obtained from healthy subjects without any sign or symptom of gastrointestinal or any other disease. The clinical factors taken into consideration in assessing disease activity were fever, abdominal pain, frequency of defecation and sedimentation rate.

2.2. Organ culture

Colonic biopsies were organ cultured for 24 h as described (6). After the first hour the medium was withdrawn, fresh medium added and the culture continued for 23 h. At the end of the culture the medium was kept at -20°C for up to 2 weeks before prostanoid determination.

2.3. Isolation of peripheral blood mononuclear cells (PBM) and monocytes

PBM were isolated as previously described in detail (7). The cells were separated by a standard Ficoll-Hypaque sedimentation, washed and suspended in RPMI 1640 medium. Monolayers of monocytes were prepared by incubating PBM in multiwell plates for 1 h. The non-adherent cells were removed by repeated pipetting. More than 95% of the adherent cells were monocytes and 0-4% lymphocytes. The adherent cells were cultured for 24 h, the medium was removed and frozen at -20°C and their number counted according to the number of nuclei collected from the well following 8 min incubation with Zap-Oglobin.

2.4. Effect of drugs on prostanoid synthesis by colonic mucosa and PBM

Colonic mucosa or PBM obtained from the same subject were cultured in medium containing either sulfasalazine (100 μg/ml), sulfapyridine (50 μg/ml), 5 aminosalicylic acid (5-ASA) (50 μg/ml), azathioprine (100 μg/ml), flufenamic acid (10 μg/ml), aspirin (100 μg/ml), methyl prednisolone (1 μg/ml), salicylic acid (100 μg/ml) or disodium chromoglycate (50 μg/ml).

In these experiments, prostanoid synthesis by colonic mucosa or PBM cultured in drug-free medium served as control.

2.5. Isolation of intestinal epithelial and inflammatory cells

Isolation of intestinal inflammatory cells was performed according to Bull and Bookman (8). Tissue obtained at surgery from patients with active ulcerative colitis and Crohn's disease was washed in RPMI-1640 containing antibiotics. The mucosa was dissected and incubated with stirring for 15 min at 22°C in $Ca^{++}Mg^{++}$-free Hank's buffer containing 1 mM dithiotheritol to remove adherent mucous. Following a second wash the mucosa was incubated for 90 min at 37° in the same buffer containing EDTA 0.75 mM to dissociate crypt epithelial cells from the tissue. Morphologically 80% of the cells isolated following this incubation were epithelial and the fraction was defined as the epithelial fraction. The mucosa was then incubated for 18 h in RPMI-1640 medium containing antibiotics, 10% pooled human blood AB serum and 0.05 mg/ml collagenase. Suspended mononuclear cells were centrifuged over a layer of Ficoll-Hypaque to remove red cells and debris. For comparison, cells were isolated from normal colonic mucosa obtained at surgery from sites at least 10 cm from areas of disease in patients with carcinoma of the colon. About 80% of the cells isolated were lymphoid cells and the fraction was defined as the lymphoid fraction. The epithelial and lymphoid fractions were cultured for 18 h as described for PBM.

2.6. Prostanoid determination

Prostanoid content in the various cultured mediums was determined by radio-immunoassays: PGE_2 was determined as previously described (9). 6-Keto $PGF_{1\alpha}$, the stable metabolite of PGI_2 and TXB_2 the stable metabolite of TXA_2, were also determined by radio-immunoassays as previously described by us (10).

2.7. Na-K-ATPase activity

Colonic Na-K-ATPase activity was determined according to Tripp et al. (11). Rectal biopsies were homogenized in 1.0 ml of ice-cold Tris HCl buffer (20 mM, pH 7.4) containing 1 mM Na_4EDTA and 1 mM $MgSO_4$.

Total (Mg, Na and K) stimulated ATPase activity was determined by incubating 25 µl of the homogenate with 550 µl of medium containing imidazole buffer (30 mM, pH 6.6) , Na_4EDTA (0.15 mM), $MgCl_2$ (6.5 mM), KCl (13 mM), NaCl (140 mM) and disodium ATP (4.4 mM)for 30 min at 37°C. The incubation was stopped by adding 0.1 ml trichloroacetic acid (25% v/v). The inorganic phosphate liberated to the supernatant following centrifugation (3000 RPM, 15 min) was determined. Mg-ATPase was determined similarly in medium containing 1 mM ouabain from which the KCl was omitted. Na-K-ATPase activity was determined by subtracting the Mg stimulated ATPase from the total (Mg, Na and K) stimulated ATPase activity.

2.8. Adenylate cyclase activity

Rectal biopsies were homogenized in ice-cold Tris HCl buffer (50 mM, pH 7.4) containing $MgCl_2$ (3 mM) (ATP 3 mM), phenyl methyl sulphonyl fluoride (10^{-4} M) and mercaptoethanol (2 mM). Adenylate cyclase activity was determined according to Salomon et al. (12). The homogenate — 20-40 µg protein, was incubated for 10' at 30°C in 0.1 ml of the following reaction mixture: 50 mM MOPS (3-(N-morpholino)propane sulfonic acid, 6 mM $MgCl_2$, 0.2 mM EGTA (ethylene glycol-bis (β-amino ethyl ether)-N-N'-tetraacetic acid), 10 mM KCl, 0.3 mM ATP (Sigma No. A-2383), 1 mM c-AMP, β-mercaptoethanol 2 mM, GTP 0.5 mM, methyl sulphonyl fluoride 10^{-4} M and [α-^{32}P] ATP (40-50 cpm/pmol). The regeneration system consisted of 10 mM phosphoenol pyruvate and pyruvate kinase 100 µg/ml. Basal and NaF (10 mM) stimulated adenylate cyclase activities were determined in all instances.

In some experiments maximal and persistent activation of the adenylate cyclase were obtained by substituting GTP γS (0.1 mM) for GTP in the incubation mixture.

The reaction was stopped by adding a solution containing 0.4 mM c-AMP 4 mM ATP and ^{3}H- c-AMP (5000 cpm) and the tubes were transferred to a boiling water bath for 3'. After centrifugation (3000 RPM for 5 min), the separation of the reaction product, ^{32}P - c-AMP, was achieved by sequential chromatography on Dowex 50 cation exchanger and on neutral alumina.

Protein was determined according to Lowry et al. (13). Statistical evaluation of the data was performed according to the Student's t test for unpaired data.

3. RESULTS

Cultured colonic mucosa obtained from patients with active ulcerative colitis synthesized significantly more PGE_2, 6-Keto $PGF_{1\alpha}$ and TXB_2 than cultured colonic mucosa obtained from normal subjects. The synthesis of all three prostanoids by colonic mucosa obtained from patients in remission was not enhanced (Table 1). PGE_2 and TXB_2 synthesis by cultured

Table 1. Prostanoid synthesis by cultured colonic mucosa

	No. of subjects	PGE_2	6-Keto $PGF_{1\alpha}$	TXB_2
		(ng/mg tissue/23 h; mean ± S.E.)		
Normal subjects	39	11.2 ± 1.0	1.01 ± 0.10	0.46 ± 0.05
Ulcerative colitis				
active	28	21.4 ± 2.0*	1.53 ± 0.15*	1.03 ± 0.14*
remission	18	12 8 ± 1.9	0.76 ± 0.17	0.38 ± 0.12

Colonic mucosa obtained from normal subjects and patients with ulcerative colitis was organ cultured for 23 h. Prostanoid accumulation in the medium was determined by radio-immunoassay.

* Significantly different from normal subjects. $p < 0.01$.

cells of the epithelial fraction obtained from inflamed colonic mucosa of patients with active Crohn's disease was significantly higher than their synthesis by cells isolated from normal mucosa. Only TXB_2 synthesis by cultured epithelial cells isolated from ulcerative colitis patients was enhanced (Table 2). Prostanoid synthesis by cultured lymphoid cells isolated only from Crohn's disease but not from ulcerative colitis patients was several-fold higher than their respective synthesis by lymphoid cells isolated from normal mucosa (Table 2).

Table 2. Prostanoid synthesis by intestinal epithelial and lymphoid cell fractions

	Fraction	Normal mucosa	Crohn's disease	Ulcerative colitis
		(ng/1 × 10^6 cells/18 h; mean ± S.E.)		
PGE_2	Epithelial	0.4 ± 0.1	1.0 ± 0.3**	0.8 ± 0.3
	Lymphoid	1.5 ± 0.3	5.6 ± 1.2*	4.0 ± 2.0
6-Keto-$PGF_{1\alpha}$	Epithelial	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
	Lymphoid	0.3 ± 0.1	3.2 ± 1.9**	1.4 ± 0.7
TXB_2	Epithelial	0.2 ± 0.1	0.8 ± 0.3*	5.4 ± 3.5**
	Lymphoid	0.5 ± 0.1	2.4 ± 1.4**	5.8 ± 4.3

Epithelial and lymphoid cell fractions were isolated from inflamed intestinal mucosa obtained from 4 patients with active Crohn's disease, from 5 patients with ulcerative colitis and from normal mucosa resected from 12 patients with adenocarcinoma of the colon. The cells were cultured for 18 h and prostanoid accumulation in the medium determined by radio-immunoassay.

Significantly different from normal mucosa:
* $p < 0.01$; ** $p < 0.05$.

Only PBM isolated from patients with active Crohn's disease synthesized during 24 h of culture significantly more PGE_2 and TXB_2 than cultured PBM isolated from normal subjects. PGE_2 and TXB_2 synthesis by cultured PBM isolated from patients with active ulcerative colitis was similar to their synthesis by PBM isolated from normal subjects (Table 3).

The addition to the medium of sulfasalazine or 5-aminosalicylic acid inhibited 6-Keto-$PGF_1\alpha$, PGE_2 and TXB_2 synthesis by cultured rectal mucosa. Sulfapyridine inhibited only 6-Keto-$PGF_1\alpha$ and PGE_2 synthesis. TXB_2 synthesis by cultured PBM was not affected by sulfasalazine or its components whereas only 5-aminosalicylic acid inhibited PGE_2 synthesis (Table 4). Prostanoid synthesis by colonic mucosa was also inhibited by

Table 3. Prostanoid synthesis by cultured PBM

	No. of subjects	PGE_2	TXB_2
		(ng/1 × 10^5 cells; mean ± S.E.)	
Normal subjects	18	0.23 ± 0.03	1.04 ± 0.16
Ulcerative colitis			
active	17	0.22 ± 0.03	1.13 ± 0.11
remission	12	0.25 ± 0.04	1.19 ± 0.27
Crohn's disease			
active	15	0.50 ± 0.86*	2.17 ± 0.42**
remission	11	0.17 ± 0.02	0.89 ± 0.13

PBM were separated and cultured for 24 h. Prostanoid accumulation in the medium was determined by radio-immunoassay.

Significantly different from normal subjects:
* $p < 0.01$; ** $p < 0.05$.

aspirin, flufenamic acid, methylprednisolone, and azathioprine. Salicylic acid did not affect the synthesis of all 3 prostanoids, whereas disodium chromoglycate inhibited only 6-Keto-$PGF_{1\alpha}$ and TXB_2 synthesis (Fig. 1).

Na-K-ATPase activity in colonic mucosa obtained from patients with active ulcerative colitis was significantly lower than its activity in colonic mucosa obtained from normal subjects or from patients in remission, respectively (Table 5). In contrast, both basal and NaF stimulated colonic adenylate cyclase activity in patients with active ulcerative colitis was two-fold higher than the respective activity in colonic mucosa obtained from ulcerative colitis patients in remission.

4. DISCUSSION

The results reported in the present study indicate that in active ulcerative colitis, colonic prostanoid synthesis is enhanced. These findings are in accordance with other studies confirming enhanced PGE

Table 4. Effect of sulfasalazine and its constituents on prostanoid synthesis by cultured colonic mucosa and PBM

	Colonic mucosa			PBM	
	6-Keto-$PGF_{1\alpha}$	PGE_2	TXB_2	PGE_2	TXB_2
	(% of synthesis in drug-free medium				
Sulfasalazine 100 μg/ml	64.3 ± 7.2* (10)	64.6 ± 6.2* (10)	28.3 ± 7.3* (10)	170.0 ± 69.0 (5)	104.0 ± 20.0 (5)
5-Amino-salicylic acid 50 μg/ml	56.0 ± 7.8* (13)	44.4 ± 5.9* (28)	59.0 ± 6.8* (8)	71.4 ± 7.9** (6)	74.4 ± 16.2 (6)
Sulfapyridine 50 μg/ml	76.9 ± 7.3* (12)	75.6 ± 6.1* (26)	81.0 ± 17.8 (5)	77.7 ± 11.6 (5)	82.4 ± 10.1 (5)

Rectal biopsies and PBM were obtained from normal subjects and cultured for 24 h in drug-free medium and in medium containing each of the drugs. Prostanoid synthesis in the drug-free medium was considered as 100%. Results are mean ± S.E.

Significantly different from prostanoid synthesis in drug-free medium:
* $p < 0.01$; ** $p < 0.02$.

(14) and PGI_2 (15) synthesis as well as stimulated prostaglandin synthetase activity (16) in active ulcerative colitis. All the prostanoids determined in the present study were found in inflammatory exudates in several other clinical and experimental conditions (1). It is thus suggested that irrespective of the specific etiology, prostanoids may serve as mediators of the local inflammatory response in IBD as well. Recently, lipoxygenase products of arachidonic-acid — the leukotrienes — were shown to be also involved in inflammatory responses (17). When methodology to detect and quantitate these products in minute amounts will be available it will be of interest to evaluate their possible role in the pathogenesis of IBD.

Table 5. Colonic Na-K-ATPase and adenylate cyclase activities

	Na-K-ATPase		Adenylate cyclase		
		(μmole/mg protein/h)	Basal	NaF	
			(μmole cAMP/mg protein/10 min)		
Normal subjects	13	2.6 ± 0.5	20	36.0 ± 2.9	103.0 ± 9.8
Ulcerative colitis					
active	13	0.7 ± 0.1*	12	74.4 ± 13.8*	177.0 ± 24.5*
remission	6	2.9 ± 0.4	9	23.9 ± 2.5	62.5 ± 6.9

Colonic biopsies were obtained from normal subjects and ulcerative colitis patients. Na-K-ATPase and adenylate cyclase activities were determined as described in Material and Methods. Results are mean ± S.E.

* Significantly different from normal subjects and ulcerative colitis in remission. $p < 0.01$.

The cells responsible for the enhanced colonic prostanoid production in IBD were found to be the inflammatory and not the epithelial cells. Both types of cells are capable of synthesizing prostanoids. It therefore appears that in addition to the increase in the number of inflammatory cells in the inflamed mucosa their capacity to synthesize prostanoids is also enhanced.

Tissue inflammatory cells originate in the bone marrow and are recruited from the peripheral blood. Monocytosis was previously reported in active IBD (18). In the present study, prostanoid synthesis by cultured PBM isolated from patients with active Crohn's disease was found to be enhanced. On the other hand, in active ulcerative colitis, in spite of the monocytosis, prostanoid synthesis by cultured PBM was found not to be stimulated. This difference is possibly due to the presence of a separate subset of cells or to the presence of a prostanoid synthesis inhibitor in ulcerative colitis.

Methylprednisolone, sulfasalazine and its components were found in the present study to effectively inhibit colonic prostanoid synthesis.

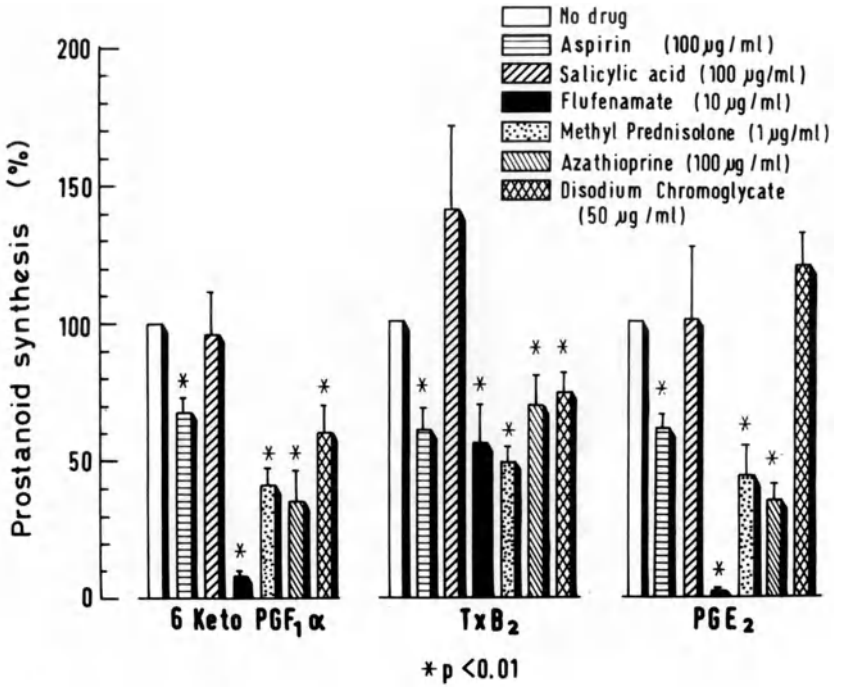

FIGURE 1. Effect of aspirin, salicylic acid, flufenamic acid, methyl prednisolone, azathioprine and disodium chromoglycate on 6-keto$PGF_{1\alpha}$, TXB_2 and PGE_2 accumulation by cultured rectal mucosa. Each subject served as its own control. Results are mean ± S.E. of 5 to 13 cultures performed with each drug.
* Significantly different from the amount accumulated in drug-free medium, $p < 0.01$.

Only 5-aminosalicylic acid was found to significantly inhibit PGE_2 synthesis by cultured PBM. Other reports confirmed the inhibition of colonic prostanoid synthesis by prednisolone (14) and sulfasalazine (16).

Sulfasalazine, but not its components, was also found to inhibit prostanoid breakdown in several organs of several tissues (19). Several small uncontrolled clinical trials failed to demonstrate the efficacy of other nonsteroidal anti-inflammatory agents in the treatment of IBD (20). Other trials, more carefully conducted, managed to show that 5-aminosalicylic

acid, the most potent prostanoid synthesis inhibitor (6), is probably the active component in sulfasalazine (21).

It is thus difficult at present to conclude from the cumulative data whether inhibition of prostanoid synthesis is the mechanism to explain the therapeutic efficacy of steroids and sulfasalazine in IBD. Colonic Na-K-ATPase, the enzyme involved in intestinal electrolyte and water absorption (22) was found to be inhibited in active ulcerative colitis, whereas adenylate cyclase, the effector of various secretory stimuli (23) was found to be stimulated. Decreased absorption and increased secretion can both contribute to net intestinal fluid accumulation, manifested as diarrhea in active IBD. Both enzyme systems are affected by prostanoids. Prostaglandins were shown to inhibit gastric (24) and intestinal Na-K-ATPase (25). Prostanoids also stimulate adenylate cyclase activity (23). The enhanced colonic prostanoid synthesis in active IBD can thus mediate the diarrhea by affecting these two enzyme systems.

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PROSTAGLANDINS AND THE MODE OF ACTION OF SULPHASALAZINE IN ULCERATIVE COLITIS: TWO OPPOSING VIEWPOINTS

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1. INTRODUCTION

The mode of action of sulphasalazine still continues to puzzle gastroenterologists after more than 30 years successful use in the treatment of ulcerative colitis and other inflammatory bowel diseases. Interest in the last few years has focussed on possibl relationships between sulphasalazine and the prostaglandin syster and this review aims to summarise two diametrically opposite viev that have recently been formulated.

Several factors complicate any attempt to analyse the mode of action of sulphasalazine in ulcerative colitis. It is pertinent to note that "ulcerative colitis" is probably not a distinct or unique disease entity, but rather a "heterogenous collection of inflammatory colonic disorders" (Kirsner, 1975; ref. 1). Moreove very few definite conclusions have yet been established concerning its aetiopathogenesis, although several theories (for example of immunological, infective or vascular causes) have been well aired in this volume and elsewhere (2-12). Together, these facts make it hard to focus on the likely targets of therapeutic agents

In common with Crohn's disease (also treated with the same drugs, but in different ways (7,8,13-15,21) and not discussed further here), the natural history of ulcerative colitis is one of spontaneous relapses and remissions (2,7,8) so that the two major therapeutic aims are to induce remission and prevent relapse (Northfield, 1977; ref. 8). As noted below, the principa therapeutic agents for colitis (sulphasalazine and anti-inflammatory steroids such as prednisone) are used differentially for treatment of remission and relapse (8,13): this means that conclusions regarding mechanisms for therapeutic effects in, say,

relapse cannot or should not be extrapolated as explanations for beneficial effects obtained against remission.

A final complication concerning sulphasalazine is the identity of the therapeutically active component. As shown in several studies (16-21), sulphasalazine (see Figure 1 for structure) is broken down in the colon into sulphapyridine and 5-amino-salicylic acid by azo-cleavage due to bacterial action. Splitting of sulphasalazine is reduced in man when growth of the colonic flora is inhibited after ampicillin treatment (22) as well as in germ-free rats (23). This recalls the early development of sulphasalazine by Svartz (20,24): it was hoped that the combination of sulphapyridine (antibacterial) and a salicylic acid derivative (putative anti-inflammatory agent) might produce an effective drug against rheumatoid arthritis. This did not materialise, perhaps because the implicit assumptions concerning the nature of rheumatoid arthritis were incorrect, but good results in ulcerative colitis were reported as early as 1942. Ironically, there has been a revival of interest in sulphasalazine for rheumatoid arthritis as an alternative treatment in patients unresponsive to conventional therapies (25).

2. DRUG TREATMENT OF ULCERATIVE COLITIS

2.1. Prevention of relapse

Sulphasalazine is the mainstay of maintenance therapy for the prevention of relapse in patients recovered from acute episodes or relapses of ulcerative colitis (8,10,13,16,20,21,26). This has been found by several groups and established in controlled double blind trials (27,28). Under these conditions the drug may be taken for long periods of time and is reasonably well tolerated (13,20,21); unacceptable side effects in susceptible patients are probably caused by sulphapyridine which is absorbed after release in the colon (16,17,21). Thus patients showing adverse reactions to sulphasalazine tend to have higher blood levels of sulphapyridine and may be slow acetylators (20,21,29,30); sulphapyridine is acetylated at different rates according to acetylator phenotype (31).

Compared to sulphasalazine, anti-inflammatory steroids are

ineffective for maintaining remission from ulcerative colitis (8,13,20,26,27,32,33); moreover, long-term corticosteroid treatment has side effects and carries well-recognised risks. However these steroids may be indicated for long-term usage in cases of chronic ulcerative colitis in which persistent relapse of established disease has occurred (13,34). Azathioprine, although ineffective on its own (35), may offer opportunity for a reductic in steroid dosage (34).

On the basis of the established pharmacokinetics of sulphasalazine it has recently been proposed that the active therapeuti moiety might be 5-aminosalicylic acid rather than the parent molecule. Some small scale trials on an assortment of patients with ulcerative colitis or Crohn's disease support this view in that results were as good or better with 5-aminosalicylic acid (administered rectally) as with sulphasalazine or placebo (36-38) However, convincing evidence that 5-aminosalicylic acid is as effective as sulphasalazine for maintenance therapy for long-term prevention of relapse is still lacking.

2.2. Induction of remission

The optimum treatment for acute attacks of ulcerative colitis is anti-inflammatory steroids and their dramatic benefits have been documented in a number of studies (8,13,21,26,32,39,40). In severe attacks, Truelove and Jewell (41) recommend addition of an intensive intravenous regimen. Steroids may also be supplemented by sulphasalazine, but there is little evidence that sulphasalazine on its own can effectively control acute ulcerative colitis (8,13,16).

The differences in relative effectiveness of steroids and sulphasalazine for prevention of relapse or treatment of the acute phase suggest that there is likely to be more than one target for the mode of action of these drugs. It may even be a false step to attempt to explain each drug's effects in terms of a single mechanism.

3. PROSTAGLANDINS AND THE GASTROINTESTINAL TRACT

Prostaglandins are produced in all tissues of the gastro-

intestinal tract and have numerous actions on the gut which have been extensively reviewed (42-47). Among the most prominent effects are inhibition of gastric acid secretion, cytoprotection of the mucosal layer (see below), effects on smooth muscle - both excitatory and inhibitory - and inhibition of sodium transport and fluid reabsorption. It now seems likely that the major product of arachidonic acid metabolism in gastrointestinal tissue is prostacyclin (PGI_2); of the "classical" prostaglandins, PGE_2 is generally found in larger quantities than $PGF_{2\alpha}$.

Several roles of prostaglandins in inflammation have been proposed since prostaglandins, especially PGE_2 and prostacyclin, cause vasodilatation, potentiate the algesic and vascular effects of other inflammatory mediators and are found in inflamed tissue (48-52). Moreover, aspirin-like drugs exert their anti-inflammatory and analgesic effects by reducing tissue prostaglandin synthesis (51-54).

Arachidonic acid is also transformed by lipoxygenase enzymes into a series of non-cyclised hydroxylated fatty acids (leukotrienes) which have potent biological actions on smooth muscle and as chemotactic agents (55-58). Leukotrienes are the major products of arachidonate metabolism in leukocytes, and include the slow reacting substance(s) of anaphylaxis (59,50,61), but their formation and actions in the gastrointestinal tract have not yet been established in any great detail.

4. PROSTAGLANDINS, SULPHASALAZINE AND THE ACUTE PHASE OF ULCERATIVE COLITIS

As expected, larger than usual amounts of prostaglandins are found during the acute phase or active ulcerative colitis (see Table 1 for summary) and they may contribute to the characteristic symptoms of diarrhoea, pain and inflammation. However, it is not certain to what extent the prostaglandins are the cause or the consequence of the symptoms. As noted in Table 1, elevated levels of prostaglandin-like material were also found in colons from guinea-pigs with experimental ulcerative colitis (70); an interesting finding in this study was that the apparent amount of prostaglandin synthetase (assayed *in vitro* in terms of

TABLE 1. INCREASED PROSTAGLANDINS IN ULCERATIVE COLITIS

	Source	Reference
Human	Increased bioassayable PGE2 in stools	62
	Increased bioassayable PGE2 in portal venous blood	63
	Increased synthesis in colorectal biopsies from labelled AA	64,65
	Increased urinary PGF2α metabolite by GC/MS	66
	Increased synthesis of immunoassayable PGE2 from colonic biopsies	67
	Increased immunoassayable PGE2 production from peripheral monocytes	68
	Increased immunoassayable PGE2 released into rectal dialysis bags	69
Guinea-pig	(carrageenan colitis)	
	Increased bioassayable PG content of colon/caecum	70
	Increased synthesis of bioassayable PG from colonic microsomes plus AA	70
	Decreased cytosolic breakdown of labelled PGF2α	

AA = arachidonic acid;
GC/MS = gas chromatography/mass spectrometry

microsomal conversion of arachidonic acid) was also elevated and, surprisingly, that amounts of prostaglandin metabolising enzymes were reduced. These adaptive changes show that the amounts of enzymes in the tissues are subject to sensitive metabolic control and this is compatible with a prominent pathophysiological role. It is not known if similar changes occur in man.

Several studies have shown that sulphasalazine is a weak inhibitor of prostaglandin biosynthesis in various assay systems (see Table 2 for summary) and have led to the idea that this explains its therapeutic actions in ulcerative colitis treatment. Sulphasalazine is only of comparable potency to aspirin and much less potent than indomethacin, flurbiprofen or flufenamic acid. 5-aminosalicylic acid is a weaker inhibitor still (except in the study of Sharon et al; ref. 67) and sulphapyridine is inactive.

TABLE 2. PG SYNTHESIS INHIBITION BY SULPHASALAZINE AND ITS COLONIC METABOLITES

Condition	Inhibitory potency (ID50 value in micromolar)			
	SZP	SP	5-ASA	reference compound
A	>250	?inactive	∿250	flufenamic acid 0.07
B	1590	-	potentiates	indomethacin 7.1
C	250	-	-	indomethacin 18
D	470	2900	7070	aspirin 550
E	660	11800	7800	aspirin 500
F	∿1500	inactive	>>2500	indomethacin 0.8-4.0

Details and reference: A. PGE2 synthesis by 'cultured' human rectal mucosa (RIA)(67); B. Prostacyclin production by rat colonic fragments (RIA)(71); C. Labelled PG formation by human rectal mucosa homogenates (72); D. Bioassayable PG production by bovine seminal vesicle microsomes (73); E. Tone of isolated stomach fundus strip preparation (73); F. Bioassayable PG production by various microsomal preparations (74,75).

At first sight the idea that sulphasalazine benefits ulcerative colitis by inhibiting prostaglandin synthesis is attractive in view of the evidence that synthesis is elevated in the acute phase. However, there are a number of reservations which preclude acceptance of this hypothesis that sulphasalazine acts in the colon as an aspirin-like drug:

(i) As noted above, sulphasalazine is of doubtful efficacy in the treatment of the acute phase.

(ii) Sulphasalazine is a more potent inhibitor of prostaglandin breakdown than of synthesis (see below); thus the effect of treatment in vivo may be to enhance rather than depress prostaglandin levels.

(iii) Sulphasalazine does not possess anti-inflammatory actions or other properties characteristic of the aspirin-like non-steroidal anti-inflammatory drugs.

(iv) Treatment of ulcerative colitis patients or those in remission with aspirin-like drugs of low (salicylate) to high anti-prostaglandin synthetase inhibitory potency (indomethacin, flurbiprofen) has not given favourable results, i.e. the

treatments have exacerbated rather than improved symptoms despite in some cases reducing prostaglandin generation (76-79). In fact, aspirin-like drugs are well known as mucosal irritants and their tendency to produce gastric and duodenal ulcers in both man and experimental animals has been extensively documented although the mechanisms are far from clear (43,45-47, 80-82).

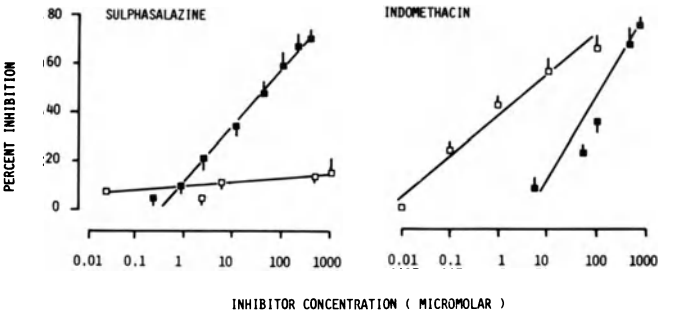
(v) In general, aspirin-like drugs have not proved useful for the treatment of other common types of diarrhoea, although benefit has been claimed in the treatment of radiation-induced diarrhoea (83,85) and that associated with medullary carcinoma of the thyroid (84).

(vi) It has yet to be shown that concentrations of sulphasalazine high enough to inhibit prostaglandin synthesis effectively (~250-1000 μM, see Table 2) can be achieved in the colon after therapeutic doses.

However, it is interesting that anti-inflammatory steroids, which are well established as effective therapeutic agents in the acute phase, may well act to reduce colonic synthesis of prostaglandins and related substances. In intact cells and whole organs (but not in broken cell systems) corticosteroids reduce the synthesis and release of prostaglandins - and presumably leukotrienes - by inhibiting the phospholipase-catalysed release of the necessary arachidonate substrate (86-89) The effect is indirect and blocked by inhibitors of RNA and protein synthesis (90-92) and is due to the generation of a peptide phospholipase inhibitor which has been dubbed "macrocortin" (93) or "lipomodulin" (94). This may explain why corticosteroids are more powerful anti-inflammatory agents than aspirin-like drugs which only inhibit the cyclooxygenase pathway, thereby reducing formation of prostaglandins, prostacyclin and thromboxanes. Moreover, the latter inhibition of arachidonate utilisation might even enhance leukotriene formation if the substrate is redirected to the lipoxygenase pathways (95,96). Such an explanation might account for the greater clinical effectiveness of steroids in acute colitis, although several other powerful actions of glucocorticoids on immune mechanisms and leukocyte metabolism must also be considered.

5. SULPHASALAZINE AS INHIBITOR OF PROSTAGLANDIN BREAKDOWN: MODE OF ACTION FOR PREVENTION OF RELAPSE?

Detailed studies in our laboratory have shown that sulphasalazine and closely related analogues inhibit the inactivation of classical prostaglandins in cell-free homogenates of organs from several species (74,75,97). As shown in Figure 1, much lower concentrations are needed than for synthesis inhibition (50 percent inhibition of breakdown is obtained with ∿50 μM); this contrasts with indomethacin which also inhibits both



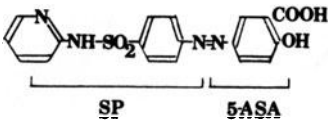


FIGURE 1. Structure of sulphasalazine showing 5-aminosalicylic acid and sulphapyridine moieties and inhibition by sulphasalazine and indomethacin of prostaglandin synthesis (□) and breakdown (■) in rabbit colon microsomal and cytosolic preparations respectively. Points show mean ± s.e.m., n = 3-6.

synthesis and inactivation, but with high selectivity against synthesis (Figure 1). We have extended these studies to show that sulphasalazine also inhibits prostaglandin inactivation in the perfused lung, in the anaesthetised rat and in rat colon ex vivo (but not in kidney or lung) after oral administration of therapeutic doses (74,97,98). Other workers have found that sulphasalazine is a potent inhibitor of prostaglandin breakdown in human colonic homogenates (99). The colonic metabolites 5-aminosalicylic acid and sulphapyridine were inactive as

inhibitors of prostaglandin breakdown (74,75,98,99).

Our interpretation is that sulphasalazine inhibits the first and most important enzyme in the degradative pathway of prostaglandins, that is prostaglandin 15-hydroxydehydrogenase (PGDH). We have recently found that sulphasalazine inhibits purified PGDH from bovine lung and human placenta (100). The inhibition appears to be uncompetitive and is optimal in homo-analogues in which the carboxyl group of the salicylic acid is replaced by $-CH_2COOH$. Moreover, sulphasalazine does not inhibit other enzymes concerned with prostaglandin breakdown, e.g. 9-hydroxydehydrogenase or Δ-13 reductase (74).

Prostaglandins which are oxidised by PGDH to their 15-keto equivalents lose biological activity, so the implication from our studies is that sulphasalazine therapy might potentiate prostaglandin actions in the colon consequent to reduced degradation. We thus postulate that sulphasalazine potentiates the cyto-protective and anti-ulcer effects of prostaglandins in colonic mucosa by a local action on the enzymes of prostaglandin inactivation.

There is now accumulating evidence that prostaglandins fulfil a cyto-protective function in the mucosa of the stomach (and perhaps colon) and that ulceration may result from a prostaglandin deficiency (43,45-47,101-103). This is particularly so in the case of the stomach, since prostaglandins have been shown to promote healing of several types of ulcers and to protect against trauma, both in experimental animals and in man (43,45-47,104,105). The mechanisms responsible for cyto-protection are not yet clear but they may include direct microvascular dilation, enhancement of mucus secretion or reduction of acid secretion.

In the context of this theory, it is also of relevance that Peskar and colleagues (106) have shown that carbenoxolone - a drug of proven value in the therapy of gastric and duodenal ulcers - selectively inhibits human gastric mucosal PG inactivation. In recent studies we have found that carbenoxolone inhibits purified PGDH preparations (100) as well as prostaglandin breakdown in supernatants from rabbit colon and other organs but does not inhibit prostaglandin synthesis (106a).

These studies also suggest to us that drugs which selectively inhibit prostaglandin degradation rather than synthesis form a definite pharmacological type with anti-ulcer properties (see ref. 107 for discussion).

As stated, we speculate that during maintenance therapy sulphasalazine may help to normalise a prostaglandin deficiency which might otherwise increase the tendency to ischaemia and therefore favour erosion and ulceration. This situation could be termed the 'pre-disease' or 'metastable' state and it is of interest that Hultén and coworkers (108) using an isotope wash-out technique have shown that colonic blood flow was 'slightly or significantly below that in controls" in patients with chronic mild ulcerative colitis and "normal or even reduced in long-standing quiescent or inactive ulcerative colitis" (in contrast to "large increases" in the mucosal layers of patients with severe colitis). A "pre-disease" state in which microvascular abnormalities precede the development of ulcers has also been proposed in the guinea-pig model of ulcerative colitis (J. Watt, personal communication); it is noteworthy that therapy with sulphasalazine is more effective in this animal model than prednisolone or azathioprine in preventing the development of ulcerous lesions (109).

Our theory for the mode of action of sulphasalazine has proved controversial. Clearly, further studies of the effects of sulphasalazine and its metabolites on both prostaglandin synthesis and breakdown would be worthwhile. The colonic levels of these drugs should be measured carefully after oral dosing in man. Further, effort should be directed towards a careful evaluation of the pathophysiology of the two phases of ulcerative colitis (if they can be distinguished), with special regard to their differing management using drugs with widely differing effects on the prostaglandin system. The analogue homosulphasalazine, which is a more potent and selective inhibitor of prostaglandin breakdown with little activity on prostaglandin synthetase (97), may also play an important part in such studies and might be worth testing clinically. If it were more potent in vivo, this may reduce adverse side effects since the amount of sulphapyridine released

after bacterial cleavage would be less.

6. 5-AMINOSALICYLIC ACID AS A COFACTOR FOR PROSTACYCLIN SYNTHESIS

A completely new dimension to the problem of sulphasalazine's mode of action is provided by results of our recent studies on prostacyclin synthesis by colonic fragments *in vitro* (110). We wished to extend our previous results to an intact cell system; thus small pieces of intact rat caecum or human colonic mucosa (20-75 mg) were incubated *in vitro* for 10-30 minutes after a preincubation period at 4°C to allow drug penetration. The synthesis and release of prostaglandins into the supernatant were measured by radioimmunoassay.

In both rat and human colon, there is release of more 6-keto $PGF_{1\alpha}$ (the stable breakdown product of prostacyclin) than $PGF_{2\alpha}$, with even smaller amounts of PGE_2. Figure 2 shows that release of prostacyclin from rat caecum (measured as

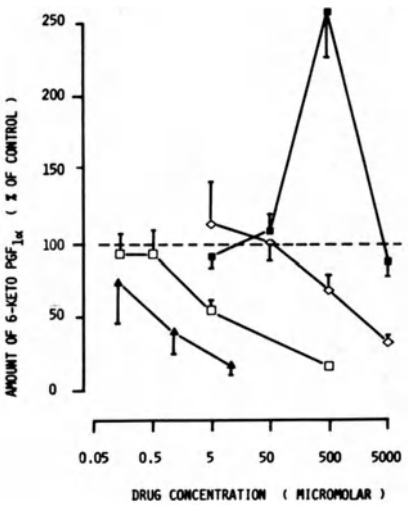


FIGURE 2. Release of prostacyclin from rat caecal fragments and its inhibition by flurbiprofen (▲), indomethacin (□) and sulphasalazine (◇). 5-aminosalicylic acid (■) enhances release. Prostacyclin measured by radioimmunoassay as 6-keto $PGF_{1\alpha}$. Points show mean ± s.e.m., n = 6 or 15 (5-ASA).

immunoreactive 6-keto $PGF_{1\alpha}$) is inhibited dose-dependently by flurbiprofen, indomethacin and sulphasalazine (ID_{50} values quoted in Table 2). Note that sulphasalazine is a weak inhibitor as explained above.

Much to our surprise 5-aminosalicylic acid did not inhibit release but potentiated it at 500 μM. We propose that 5-aminosalicylic acid must be acting as a cofactor for colonic prostacyclin synthesis, and we have observed similar results in human colonic mucosa and rabbit caecum (data not shown). Release of $PGF_{2\alpha}$ is also enhanced; that of PGE_2 is slightly increased but in any case is very low. Similar enhancement of prostacyclin release is obtained using phenol, hydroquinone and adrenaline, and these are well known as cofactors for prostaglandin synthetase action in other systems (111-113). Like 5-aminosalicylic acid they spontaneously oxidise in air giving brown solutions, are phenolic in structure and can trap free radicals. We postulate that the mechanism of 5-aminosalicylic acid is to scavenge free radicals (produced during conversion of endoperoxide PGE_2 to PGH_2) which otherwise cause self-inactivation of the cyclo-oxygenase component of prostaglandin synthetase (114). The biphasic effect of 5-aminosalicylic acid (and hydroquinone, not shown) is probably due to excessive trapping of activated oxygen species, therefore depriving the reaction of its substrate; inhibition at high cofactor concentration has been observed previously (111).

The implication is that *in vivo* 5-aminosalicylic acid (released by bacterial cleavage of sulphasalazine) might enhance colonic prostaglandin output, except if concentrations become very high, and therefore contribute to local colonic cytoprotection and vascular integrity. This is therefore a second mechanism which suggests that sulphasalazine therapy might raise prostaglandin levels.

These experiments should provide a fresh impetus for further studies and debate concerning this most interesting drug.

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PATHOGENESIS OF DIARRHEA IN INFLAMMATORY BOWEL DISEASE

S. F. PHILLIPS, M.D.

A precise description of the pathogenic mechanisms of diarrhea in inflammatory bowel disease is impossible, for the gaps in our understanding of the physiology of absorption and transit in the small and large intestines are prominent. Given this shaky foundation, all that is certain is that mucosal inflammation must derange these still obscure, normal mechanisms quite variably. Moreover, the clinical spectrum of inflammatory bowel disease is quite wide, anatomically and qualitatively, and it is necessary to distinguish clearly several major categories. The important divisions should perhaps be 1) disease confined to the colon, 2) disease of the small intestine, and 3) diarrhea after proctocolectomy and ileostomy.

Throughout, the major clinical features of diarrhea should be kept in mind. Increased frequency of stooling is most common but this index has wide inter-individual variations and disturbances from normal can be interpreted confidently only for that individual. Consistency of stool is also important clinically but its lack of quantification relegates it to being of subjective interest only. Rectal discomfort or the presence of unusual constituents of the stool (blood, mucus, undigested food) may accompany diarrhea but are nonspecific features. Fecal weight is perhaps the most objective index and will receive the most attention; however, even this has limitations

when weight is used as a single index. In Western societies normal stool weight has an upper limit of 200-300 gram/day.

1. Diarrhea in the Colitis of Inflammatory Bowel Disease.

Colitic disease should include chronic ulcerative colitis and Crohn's disease of the colon but should also be subdivided into proctosigmoiditis, or other localized forms of inflammation, and colitis with total or near total involvement of the large intestine.

Table 1. Fecal weight in inflammatory bowel disease

	n=	Range	Mean ± SEM	References
CUC (severe)	9	100-1500 g/d	620	Smiddy (1950)[1]
CUC (severe)	14	140-870	384 ± 49	Caprilli (1978)[2]
CUC	25	100-1500	347 ± 77	Mayo Series[3]
CUC	10	-	560 ± 63	Breuer (1981)[4]
CUC	13	-	474 ± 60	Breuer (1981)[4]
PROCTOSIGMOIDITIS	8	-	234 ± 50	Breuer (1981)[4]
ACTINIC PROCTITIS	5	100-725	303 ± 125	Mayo Series[3]

Table 1 gives results from seven series in which total fecal weight per day was recorded from patients with colitis. When the colon is involved extensively, and especially when the disease is of moderate to marked severity, fecal output varies widely but is generally about twice the normal value in health (up to 300 grams daily). On the other hand, when disease is confined to the rectum, total daily weights are at about the upper limit of normal for health. These findings in proctitis support our clinical impressions. Thus, it should be noted that in the best descriptions of idiopathic proc-

tocolitis, by the group from St. Mark's Hospital (5), constipation or normal bowel habits were found in approximately two-thirds of patients. Indeed, a syndrome of "fecal stasis in inflammatory bowel disease" has been described (6). Further, it has been shown that the potassium to sodium ratio in stools from patients with proctosigmoiditis is approximately normal or very slightly elevated (4). When elevated this reflected an increase in potassium loss, which is probably related to fecal mucus. The data can be interpreted as supporting the essentially normal composition of stools in proctosigmoiditis.

Thus, localized rectal disease might best be considered primarily as an exudative process. Although studies are not available to support the hypothesis that the functions of the small intestine and proximal colon are normal in proctosigmoiditis; this seems a reasonable proposal. On the other hand, exudation of blood and mucus is consistent with our clinical findings. An interesting speculation is that the sensitivity of the rectum to its contents may be increased in proctitis. This could be due to decreased compliance of the rectal segment in proctitis (7), and might well have the effect of contributing to the frequency of defecation and the rectal discomfort noted by these patients.

On the other hand, the available evidence supports a somewhat different hypothesis for diarrhea when the colonic involvement is more extensive. Under these circumstances, although small intestinal function may be normal, there appears to be a decreased absorption by the colon of sodium, chloride and water. These functions of diseased colon have been studied _in vivo_ and _in vitro_; both approaches yield

results most consistent with a primary defect in the absorption of sodium, chloride and water.

Table 2. Water and electrolyte absorption in colitis

	H_2O	Na^+	K^+	Cl^-	References
CUC (n=6)	↓	↓	Normal	-	Duthie (1964)[8]
CUC (n=4)	↓	↓	Secretion	-	Harris (1970)[9]
Crohn's (n=8)	↓	↓	-	↓	Head (1969)[10]
Pre vs post*	NC	NC	-	NC	
Proctitis (n=10)	-	↓	-	-	Rask-Madsen (1973)[11]

*See text for details, NC = no change

Duthie's experiments (8) employed segments of diseased colon studied at laparotomy, the other reports are of perfusion studies in unoperated bowel. In general, a decreased absorption of sodium was the most consistent finding and water usually followed sodium. Chloride was absorbed poorly in one report. These results give little indication of a net secretory state for sodium, although potassium was secreted in one set of experiments. It is of interest that one set of observations was on patients with Crohn's disease of the colon before and after defunctioning ileostomy (10). Although there was clinical improvement in the colitis after diverting ileostomy, there was no improvement in the ability to absorb electrolytes and water.

Observations on colitis tissue <u>in vitro</u> (Table 3) are more limited (12,13). Hawker and Turnberg (12) showed reduced mucosal to serosal (M → S) fluxes of sodium and chloride but there was little or no change in the serosal to mucosal (S → M) movements of these ions; potassium flux was normal. Archampong (13) showed an increased sero-

sal to mucosal flux of sodium, however the mucosal to serosal flux was also increased. Overall, these results are inconclusive but there appears to be no overwhelming evidence to support an active process of secretion.

Table 3. Electrolyte transport in colitis studies in vitro

	Sodium		Chloride		Potassium		Ref.
	M→S	S→M	M→S	S→M	M→S	S→M	
Crohn's (n=3)*	↓ ↓	normal	↓	normal	normal	normal	12
CUC (n=7)*	↓ ↓	normal	↓	normal	normal	normal	12
Crohn's (n=4)	↑	↑ ↑	-	-	-	-	13
CUC (n=4)	↑	↑ ↑	-	-	-	-	13

*Healthy controls vs untreated, $p < 0.001$
Healthy controls vs steroid treated, N.S.

Breuer (4) has drawn attention to the difference between fecal compositions in patients with Crohn's or ulcerative colitis. Although one must be careful not to overinterpret observations on total fecal composition, these findings are of note in that the osmotic gap in stools is much larger for Crohn's disease than for ulcerative colitis. In other words, the sum of concentrations of sodium, potassium and chloride accounted for a greater proportion of the total osmolality of stool water in ulcerative colitis than it did in Crohn's colitis. The most likely cause of this osmotic gap in Crohn's colitis is that organic anions predominate in Crohn's disease, when it might be anticipated that absorption of carbohydrate in the small intestine is less complete than usual. On the other hand, the osmotic gap being lower in ulcerative colitis indicates that the osmolality can be largely

accounted for by sodium, potassium, and chloride; this finding is consistent with a proposal that in ulcerative colitis the major abnormality is a failure of sodium and chloride absorption.

Attention has been directed by other participants to potential mediators of electrolyte and fluid secretion by the colon in states of colitis. Thus, prostaglandin production by colonic mucosa is increased in colitis and prostanoids have been implicated in the diarrhea of inflammatory bowel disease as well as in the inflammatory process, per se. Prostaglandins are able to influence electrolyte transport by colonic epithelium in vitro (14) and the possibility that prostaglandins play a role in diarrhea remains attractive, but unproven. However, as described above, there is no strong evidence of a secretory state in the colon affected by inflammatory bowel disease. Also not discussed in detail here are the potential effects of secondary bacterial infections in patients with inflammatory bowel disease (Clostridium difficile, Campylobacter). Again, the hypotheses are attractive but they must remain at this point sub judice.

2. Disease of the Small Intestine

The major effects of inflammatory disease in the small intestine should be considered with or without the presence of associated colitis; moreover, it is easiest to combine the consequences of severe ileal disease in the unoperated bowel with the consequences of ileal resection, a surgical procedure which is required frequently in patients with Crohn's disease.

The work of Hoffman need not be recounted in detail here (15). In summary, two major causes of diarrhea after ileal resection

can be identified. One is induced primarily by malabsorbed bile acids and the other by malabsorbed fat. When the ileal resection is small, hepatic synthesis of bile acids is sufficient to compensate for their increased fecal losses and the luminal concentrations of bile acids are maintained within the micellar range. When steatorrhea is present it is of mild degree, but the excess of bile acids which enters the colon impairs electrolyte and water absorption (16). Thus, the term "bile acid diarrhea" has been applied to this circumstance (15). On the other hand, when the ileal resection is extensive, hepatic compensation for wastage of bile acids is incomplete and the concentration of bile acids in the lumen is too low for adequate micellar solubilization of fat. This aggravates steatorrhea and, in these circumstances, it is thought that the malabsorbed fat is responsible primarily for diarrhea. Excessive amounts of fatty acids in the colon are known to impair electrolyte and water absorption (16). Consistent with these proposed pathogenic mechanisms are the therapeutic observations that a reduction in the dietary intake of long chain fats will reduce the severity of diarrhea in the second instance whereas a sequestrant of bile acids (cholestyramine or aluminum hydroxide) is needed for effective therapy of bile acid diarrhea.

These concepts are important as a basis for our understanding of the mechanisms of diarrhea but they require some modification in the light of newer information. One relevant report (17), emphasizes the role of the colon in the diarrhea that follows ileal resection. These investigators reasoned that variable portions of the large bowel are removed in association with resection of the ileum and they examined independently the role of missing segments of the small

and large intestines. The amount of colon removed was an important determinant of the severity of diarrhea, whereas the length of ileal resection was not. On the other hand, fecal excretion of fat was correlated well with the length of ileum removed but not with the proportion of the colon resected. These findings were confirmed and extended in a subsequent report (18).

A second important determinant of bile acid diarrhea, additive to the length of ileal resection, has now been identified. Thus, intracolonic pH is known to influence markedly the solubility of secretory bile acids (19). Since the potential of dihydroxy bile acids to impair sodium, chloride and water absorption in the colon must depend on their ability to enter the aqueous phase, factors that modify the solubility of bile acids might well have an important influence on the propensity of bile acids to provoke diarrhea. Fromm's group (19) showed that the aqueous concentrations of deoxycholic and chenodeoxycholic acids are related to fecal pH; the higher the intraluminal pH the more dihydroxy bile acids pass into solution and the more should be their secretory potential.

A final point relates to the absorptive capacity of the small intestine. Residual or recurrent Crohn's disease after ileal resection, or extensive disease of the proximal bowel preoperatively must be anticipated to reduce absorption of all components of chyme, water, electrolytes and constituents of the diet.

Can these observations be linked together? It is possible that short chain fatty acids (volatile fatty acids, VFA's) might be such a link, since they are important determinants of colonic function (20).

These organic anions are generated by the anaerobic bacterial metabolism of carbohydrate and their important role in veterinary physiology has been well established for many years. However, the importance of short chain fatty acids in human physiology has been appreciated only more recently. However, it is known that these anions can be well absorbed from the human small and large intestine and that their presence in the lumen facilitates the reabsorption of sodium ions from the colon. Moreover, being acidic radicals, their generation in the lumen will tend to lower the intraluminal pH. Thus, colonic resection and rapid transit through the remaining large intestine might be anticipated to reduce the opportunities for bacterial degradation of unabsorbed carbohydrate. The net result of this paucity of organic anions might well be a reduced absorption of electrolytes and water; moreover, the tendency for a less acidic intraluminal environment might facilitate the solubilization of secretory bile acids and hence the diarrheogenic effects of bile acids.

Thus, Cummings (17) showed that the greater the length of colon resected, the lesser the luminal concentration of organic anions. Under these circumstances, there was a parallel increase in the sodium and chloride concentration of stools, supporting the probability that sodium and chloride were poorly absorbed concurrent with incomplete generation of organic anions. Not surprisingly, transit time was also reduced when the amount of colon resected was larger.

3. Diarrhea after Ileostomy

Increased volumes of effluent from conventional ileostomies is a

common and practical clinical problem. It is important to exclude mechanical complications of abdominal operations and the possibility of recurrent inflammatory bowel disease must be kept in mind. Moreover, patients with ileostomies are subject to other causes of small intestinal diarrhea. Indeed, the absence of a colon makes them particularly sensitive to sodium and water depletion. Thus, diarrheal diseases that are well tolerated by those with an intact colon, for instance lactase deficiency or giardiasis, may produce severe fluid imbalance in the ileostomist.

Hill (21) has demonstrated quite clearly that the terminal 20 cm of ileum is most important in the adaptive process that occurs after colectomy. Loss of even this short segment is enough to impair sodium, chloride and water absorption and larger than usual stomal losses must then be anticipated. Ileostomy diarrhea may also be a presenting manifestation of mild steatorrhea and in most instances this reflects also the absence of a relatively short segment of ileum removed at the time of proctocolectomy. In practice, this point is often overlooked, but a reduction of the dietary intake of fat may have dramatic benefits.

Some special problems apply to the continent ileostomy. We (22) examined fecal volumes in a group of healthy subjects, disease controls with well functioning Brooke ileostomies and unselected patients with Kock ileal pouches. We found that 30% of the continent group had laboratory evidence of mild malabsorption. This was most evident by an excessive fecal volume (greater than 1000 ml/24 hr) but the majority of these patients also had mild but definite steatorrhea and a few had impaired absorption of vitamin B_{12}. Most patients were

asymptomatic, other than having noticed the need for more frequent emptying of the pouch, as a result of increased fecal volumes. We found a significant increase in total bacterial counts and in numbers of anaerobic bacteria in the jejunum of continent patients with diarrhea (23). The increased jejunal flora were not fecal in nature but were qualitatively of the upper gut variety. Moreover, we found no difference in the bacterial counts of stomal effluents among patients with continent or conventional ileostomies.

We treated those patients with malabsorption and diarrhea with a course of metronidazole (250 mg t.i.d. for 7-10 days) and noted a decrease in anaerobic flora in the jejunum. More important, output from the ileostomy decreased, as did fecal fat; those with abnormal Schilling tests also showed an improvement towards normality. Thus, we feel that some patients with continent ileostomy have bacterial overgrowth of the small intestine and that this can produce mild malabsorption and diarrhea.

In summary, diarrhea in inflammatory bowel disease has many potential causes. Disease of the small intestine will lead to malabsorption and unabsorbed carbohydrates, electrolytes and water will comprise a larger load to the distal bowel. Should this load exceed an unknown critical volume, the reserve functions of the ileum and colon could be overwhelmed. Diarrhea from small intestinal disease will certainly be contributed to by excess bile acids and/or fat that enter the large intestine. Colonic resection will add another variable. Thus, the simple concepts of "bile acid" and "fatty acid" diarrhea need modification. When disease is confined to the large intestine, impaired colonic absorption of sodium and chloride is the

most consistent finding. There appears to be little need to propose a specific mechanism of secretion under these circumstances. The pathogenesis of diarrhea in localized proctitis, when in fact diarrhea is present, might well be largely on a basis of exudation.

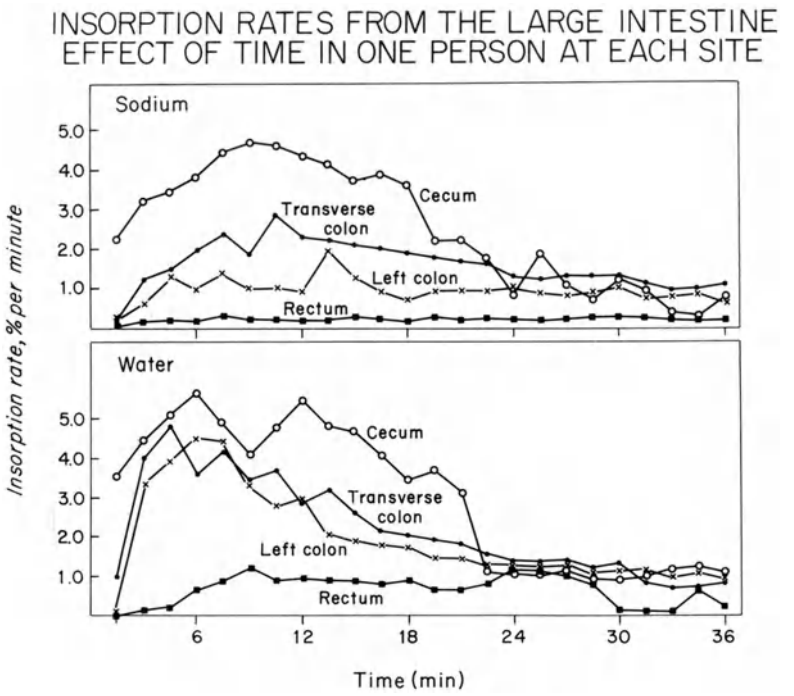

FIGURE 1. Summary of potential mechanisms of pathophysiology for diarrhea in inflammatory bowel diseases. Normal functions illustrated on the left, disorders that could produce excess stool bulk on the right. (Reproduced with permission from "Inflammatory Bowel Disease" edited by RN Allan, MRB Keighley, CF Hawkins and J Alexander-Williams, Churchill Livingstone.)

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PSYCHIATRIC ILLNESS AND INFLAMMATORY BOWEL DISEASE

D. H. ALPERS

Previous studies of the association between inflammatory bowel disease and psychiatric illness have generated mixed results, and the findings have been hard to evaluate because of methodological problems in both ascertainment and evaluation of subjects. Most studies have allowed the introduction of selection bias by using patients referred for study, for treatment, or who were self selected, rather than by using a consecutive series. Precision of medical and psychiatric evaluation has often been faulty. Moreover, there have been few attempts to look at the relationship between ulcerative colitis or Crohn's disease and psychiatric disorder other than a simple association. It is not known, for example, if Crohn's or ulcerative colitis patients with a diagnosable psychiatric illness differ from others in terms of the severity or natural history of their gastrointestinal involvement. Finally, the suggestion that psychologically stressful life events may precipitate symptoms is complicated by methodological problems of such magnitude as to make the available studies uninterpretable. This report will discuss two areas; problems with methodology and the evidence (including our own) relating psychiatric illness to inflammatory bowel disease. The difficulty in using this information to support a psychiatric etiology for these diseases will also be stressed.

Methodological problems

Latimer has reviewed 20 studies of Crohn's disease (1) and found only three which utilized consecutive cases (2-4). Gerbert has reviewed 16 studies reporting a relationship between Crohn's disease or ulcerative colitis and psychological variables (5). All studies were retrospective, and only 5 used controls (and these mostly healthy controls). The psychological methods used were mostly poorly defined and only four detailed even adequate methodology (2-4,6). Gerbert concluded that there is a strong association between psychopathology, stressful life events, personality factors, and Crohn's disease. Engels has utilized various psychometric tests to elaborate a theory involving a loss and dependency as important in the pathogenesis or continuation of symptoms in patients with ulcerative colitis (7).

Several methods have been used in these many studies, most of them allowing prejudice on the part of the investigator. The studies are all retrospective in the sense that the data were obtained retrospectively. This approach can easily lead to possible distortion of misinterpretation of past events. After all, in ideal psychiatric studies the data are not obtained retrospectively even though they must be gathered after the fact. The proper control group should be matched for sex and age, since these variables may affect life events and the response to them. Nonetheless, none of these studies have used disease controls, and only a few used even healthy subject controls. In most, if not all, of the studies the investigator was aware of the medical diagnosis and presumably the theory to be tested.

Regarding evaluation, Weiner (8) has noted that the diagnosis of Crohn's disease may be difficult and has critized previous investigators for failure to identify subject populations clearly. Precision of the psychiatric assessment can also be faulted. Frequently the method of psychiatric evaluation is unstated, or referred to as interview, chart review, or psychological testing (5). It is rare that the criteria for the psychiatric diagnoses used are specified. Among the four

studies noted above which Latimer and Gerbert found to have used the consecutive method of selection (2-4,6), none indicates how their psychiatric diagnoses were derived. Like others, these authors used general terms such as dependency needs, emotional disturbance, and hysterical traits to describe psychiatric findings in their populations.

The problems of the relationship of stressful life events to the onset of illness in inflammatory bowel disease have been discussed for some time without much resolution (9,10). Both positive (9) and negative (10) events have been claimed to start a series of physiological reactions in the gastrointestinal tract leading in some way to the onset of disease or the exacerbation of symptoms. Bockus (11) claimed that the "most predominant personality traits (of Crohn's disease) were anxiety and emotional immaturity" and set the stage for the prejudices of the next generation. His observations were, however, not supported by any validated scientific method.

In most if not all of the studies linking stress to inflammatory bowel disease the reporting of events and illnesses were done by the patient, and the hypotheses being tested were clear to the subjects. The importance of a supervised and guided interview cannot be stressed too greatly. The meaning subjects attach to words in the interview questions and to events in their lives differ greatly, and it cannot be assumed that any cohort of patients will respond similarly. No guidelines were provided to the subjects reporting life events as to the type of events or illness expected to be reported. Thus, the correlations are based only on patient recall. Even so, in all studies the magnitude of the relationships is small. For example, in one study (12) life stress scores in ulcerative colitis in females were 1.6 vs 2.07 in controls and were considered the same, but in irritable bowel syndrome (2.18) they were considered higher. The groups studied differed in age, sex, and other demographic variables, and so varied in the frequency of reporting and readjustment required by each life event. Finally, since the disease group usually included only those who sought treatment and could be identified

retrospectively, those who self select may have personality characteristics (e.g. anxiety) leading to selection by a psychiatry clinic or study group.

Two major models have been suggested for linking life stress and disease (13). In the first model, stress elicits a series of coping efforts which increase sensitivity to symptoms and lead to increased treatment seeking behavior. In the second model, an underlying personality type is involved which tends to report life changes or is hypersensitive to these events, without necessarily feeling stress, but which leads to increased treatment seeking behavior. With either model, an increased diagnosis of illness can result, not necessarily reflecting an increased disease incidence.

Therefore, any investigator wishing to study the relationship between stressful life events and the onset or exacerbation of illness must face a series of theoretical problems, which seem to this author difficult to resolve with present methodology. First, life events may alter illness behavior, but not the true incidence of the illness under study. Any conclusions will then pertain only to this self referred group. Second, only undesirable events or changes in life are stressed, yet no change might have an effect when change might have been expected. Such "events" presently are unreported. Finally, the reaction to the life event may be more important than the event itself, and will be related to the personality traits of the subject. Thus, a study of life events should be accompanied by a careful psychiatric evaluation if the events are to be properly interpreted. These concerns are germaine not only to the researcher but also to the practitioner, who is likely to make fallacious associations based on an incomplete history and in one subject. It is worth remembering that some members of the psychiatric community also have grave doubts about the usefulness of studying life events in relationship to onset of disease. Wershow and Reinhardt have written (14): "One might suggest a moritorium on papers employing the schedule of recent experiences and similar instruments. The point has been amply made that some relationship exists between change in life-ways,

let alone stress, and illness. However, the relationship is a weak one. Some people become ill or are hospitalized and. . . no discernable changes in the life have occurred. Others meet life change in other ways, some withdraw into sleep, or leave the field in other ways . . . trying to force the data into a stronger position than our favorite hypotheses warrant will only lead us further down the current cul-de-sac."

Within the past few years increasing attention has been given to objectivity of diagnosis in psychiatry. Attempts have been made to define explicit diagnostic criteria (15,16) and to develop structured psychiatric interviews which are diagnostically based (17-19). Increasing attention has been paid to the reliability (19-21) and the validity (15,22,23) of psychiatric diagnosis. These methods have been shown to compare favorably in terms of reliability to diagnostic studies such as EKG's and radiologic procedures (19,24). We have examined the associations of diagnosable psychiatric disorder and inflammatory bowel disease using a consecutive sample, age and sex matched controls with chronic illness, careful gastroenterological assessment, and psychiatric interview of known reliability (19) and concurrent validity (22) and explicit psychiatric diagnostic criteria (15).

Psychiatric illness in inflammatory bowel disease

Fifty patients were enrolled in each of three study groups. The controls were age and sex matched to the ulcerative colitis patients, but were very close to the Crohn's disease patients in these variables. Thirty percent of the controls had a diagnosable psychiatric illness, compared with 26% for ulcerative colitis (25) and 52% for Crohn's disease. The latter value is statistically different from controls (X_2, $p < 0.05$). In addition, the score for obsessional symptoms was higher in Crohn's disease patients ($p < .05$). Higher levels of obsessional symptoms were also found in ulcerative colitis patients, but there was not a monotonic relationship between severity of ulcerative colitis and the number of obsessional symptoms reported. The most common illness in

all three groups was depression. In the ulcerative colitis group, only two other diseases were found, anxiety neurosis and alcoholism.

The finding of no increased incidence of psychiatric disease in ulcerative colitis is consistent with recent studies (12,26-29), only one of which (28) used definable diagnostic criteria, but with a smaller sample size. One study found no difference in life stress events between ulcerative colitis patients and controls (12). However, the controls were nonhospitalized persons, not age and sex matched, and the data was gathered in a home interview by lay observers, without psychiatric evaluation of the patient. Another study found no more psychopathology in colitis patients compared with normals (29), although the controls probably included patients with irritable bowel syndrome known to have a high incidence of psychiatric disorders (30).

In the largest series (2) concluding that there was a correlation between the severity of emotional and bowel problems, the diagnoses were largely taken from charts (only 38% were interviewed for the study), but the controls were retrospective and there were no matched control subjects. The emotional process of patient dependency and helplessness proposed by Engel is based on case studies alone (31). While case studies can be very useful for the individual, it is difficult to make conclusions from them concerning groups of patients.

The lack of association between the prevalence of psychiatric disease and ulcerative colitis does not disprove a relationship between psychological processes and ulcerative colitis. We have examined only a specific set of psychiatric diagnoses and personality measures. There may be others not studied by us or others which would differentiate between ulcerative colitis and other chronic medical illness. Moreover, psychiatric problems when detected in an individual with ulcerative colitis should no more be ignored than in any patient. The task for the gastroenterologist is to make the proper psychiatric assessment.

We cannot explain the association of psychiatric disease and Crohn's disease. However, we have some confidence that it is not artifact in that the sample was consecutive, each subject was personally examined using an interview of known reliability and validity, and the diagnostic criteria were specific. Our finding of an excess of depressive disorders and obsessional features confirm the findings of previous, less stringent, studies. Sheffield (4), Whybrow (32), and Gazzard (33) found rates of depression in their samples from 32-38%,compared with 36% in our sample.

There seems to be no evidence of a causal relationship between Crohn's disease and psychiatric illness. The severity of the two disorders was independent of each other. Moreover, the onset of psychiatric illness preceded the onset of Crohn's disease exactly as often as the reverse. The occurrence of this association has important implications for the patient. Only about one third of our patients had a psychiatric diagnosis made prior to the study. Depression is an illness which typically responds promptly to outpatient chemotherapy. This fact may be important in the management of patients with Crohn's disease.

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D. Management

Introduction

Henry D. Janowitz, M.D.

Granted our complete ignorance of the cause and thus of the cure of Inflammatory Bowel Disease, it is doubtful whether we can do more than treat the complications of this enigmatic group of diseases. Yet we must manage our patients hoping to improve their clinical condition and perhaps to influence their disorders.

D. G. Tytgot has summarized his vast experience on the place of colonoscopy in the treatment of these patients. I would only add that microscopic and gross pathology are complementary.

J. W. Singleton, in his usual concise fashion, has brought together all the relevant controlled trials of drugs right up to the time of the symposium. This assembly of information is of outstanding value.

Mr. J. Alexander-Williams, in his usual provacative and witty fashion, has presented a persuasive conservative approach to the surgical management of IBD. His more innovative concept of treating strictures in Crohn's disease by plasty needs more of his and others' experience before this moderator is persuaded.

The experience of the Mount Sinai Medical School with the continent ileostomy was clearly put forth by Dr. Irwin Gelernt. The place of this operation, whose advantage over the conventional Brooke type ileostomy is purely cosmetic, in the long-term life history of the patient needs obviously longer-term follow-up.

Discussion at the symposium considered the rectal mucosal stripping operation with ileal pull-through preserving rectal and anal musculature as yet highly experimental, and the place of a rectal ileal pouch in this type of operation yet to be established.

I. H. Rosenberg's presentation of the usefulness of total parenteral nutrition is sober, critical and useful in outlining its value and clear

limitations. His oral presentation touched on the role of enteral forms of nutritional support.

From M. E. Ament's discussion of the problems of management of IBD in children, the moderator would emphasize the areas where the management of Crohn's disease and ulcerative colitis in little people is somewhat different from that in big people.

COLONOSCOPY IN INFLAMMATORY BOWEL DISEASE

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Colonoscopy is an established procedure in the evaluation of patients with inflammatory bowel disease (IBD). The combination of direct inspection of the mucosa and the capability of taking multiple biopsies unquestionably improves the diagnostic possibilities. Despite the frequent combination of certain mucosal abnormalities with specific types of IBD, few of these endoscopic changes however, are diagnostically specific and overlap occurs regularly. This is mainly explained by a restricted reaction pattern to damage, of which the basic elements consist of edematous swelling, erythema, coating by mucoid or purulent exsudate, tiny superficial to serpiginous, linear or deep epithelial necrosis and ulceration, nodular disorganization, pseudopolyp formation, retraction, stricturing etc. The colonoscopist is often able to detect the presence of damage and inflammation, but regularly needs histology to specify the exact type of IBD.

The purpose of this review is to highlight the role and value of colonoscopy in IBD

A. Preparation for colonoscopy

Various methods have been used for colonic preparation such as 24- to 48 hours liquid diet and judicious use of laxatives. If the patient has less than 3 bowel movements per day, 150 to 300 ml of magnesium citrate, 30 to 60 ml of castor oil, 15 to 30 g of magnesium sulfate plus 10 to 20 mg bisacodyl, or the equivalent amount of another cathartic may be used. Prior to the procedure, one or two large volume enemas are given till the return is clear. Patients with more active IBD should only be prepared with liquid diet and enemas. We prefer whole gut irrigation (Levy et al. 1976) which is usually well tolerated even in rather ill

patients. Occasionally a very ill patient with UC is unable to tolerate the procedure due to vomiting. We have the distinct impression that many patients with severe colitis improve clinically after such cleansing of the colon. Whether the lavage solution containing mainly sodium sulfate (Davis et al. 1980) will be equally effective and well toleraqed in IBD needs investigation.

Preparation for ileoscopy via an ileostomy only requires a liquid diet for 24 hours prior to the procedure with fasting for 12 hours before the endoscopy. Neither purgatives nor enemas are necessary.

Many colonoscopists prefer not to use premedication because the procedure is often quite easy and passing the sigmoid usually creates no major problem. When stricturing or fixation of the colon is present, the procedure may be painful, necessiting the use of diazepam and/or I.V. pethidine or other opiates, and when indicated a smaller caliber endoscope. At the end of the examination the patient can be given naloxone (0.4 mg Narcan I.V. or I.M.) which immediately reverses the opiate effects.

B. Detection and differentiation of various forms of IBD

Despite the absense of truly specific endoscopic features, the compilation of various abnormalities usually allows an accurate endoscopic diagnosis (Waye 1977, 1980; Williams and Waye 1978; Hogan et al. 1980). More recently particular attention has been given to the endoscopic spectrum of the various forms of infectious colitis and the spectrum of ischemic damage. Consideration of previous local or systemic therapy is essential, because this may obscure or interfere with the usual characteristic findings.

The main differential still centers around ulcerative colitis (UC) and Crohn's disease (CD). Despite increased experience over the last years, there still remains a minority (less than 10%) where this differential is difficult if not impossible or where the diagnosis changes back and forth over the years.

B.1. Ulcerative colitis

At the start is should be stated that there is no advantage in performing colonoscopy in acute UC when the diagnosis can be made by history

and proctoscopic examination. However, repeated examinations, whenever necessary in very ill patients are more easily tolerated using fiberendoscopic equipment. Furthermore, one should realise that there is no unique macroscopic mucosal abnormality, which is pathognomonic for the endoscopic diagnosis of UC.

Active UC usually inflames the mucosa in a continuous symmetric fashion. The colitis may extend throughout the whole colon or may involve only the left side. Characteristically the rectal mucosa is affected. The occurence of spontaneous rectal sparing in UC is rare but if corticosteroid enemas have been used, apparent sparing is quite common and should be kept in mind since sigmoidoscopy is then not representative.

The earliest endoscopic abnormality in UC is a loss of the normally visible vascular pattern. In addition the mucosa is usually uniformally erythematous. A fine granular appearance of the mucosal surface is quite typical. The mucosa is often extremely friable to the slightest trauma. Purulent exudates may be visible as tiny punctate flecks or as circumscribed, yellowish plaques. Characteristically ulcers, when present, tend to be discrete, occuring in areas of grossly inflamed mucosa. Only when UC is severe, the surface epithelium seems to be melting away and can become extensively eroded, forming longitudinal or confluent areas of ulceration, which may occasionally spare the rectum.

Postinflammatory pseudopolyps are often seen in UC. Pseudopolyps may vary in appearance from isolated normal colored mucosal bumps to rounded cherry-red spheres, or myriads of clustered finger-like mucosal projections. Such long filamentous pseudopolyps, especially those arising in grape-like clusters, are quite characteristic for UC. The surface is usually smooth and glistening and a yellowish-white exudate often covers the top. Occasionally pseudopolyps may reach large dimensions and cause some narrowing of the lumen.

Backwash ileitis is characterized by rather uniform generalized erythema, edema and friability of the terminal ileum usually without ulceration.

Differential points between UC and CD are summarized in table 1.

Table 1. Differential Diagnosis between ulcerative Colitis and Crohn's Disease.

	UC	CD
Continuous involvement	always	exceptional
Distribution	symmetric	asymmetric
Patchyness	absent	frequent
Rectal involvement	almost always	often absent
Vascular pattern	blurred or lost	often normal
Erythema	characteristic	less pronounced
Edema (Blunting of septa)	present	present
Friability	common	uncommon
Spontaneous petechiae	common	rare
Profuse bleeding	common	rare
Granularity (fine/coarse)	common	less common
Cobblestoning	absent	characteristic
Superficial - small ulceration	occasional	frequent
Large (> 1 cm) ulceration	severe disease	common
Deep - longitudinal ulceration	rare	common
Linear ulceration	rare	common
Serpiginous ulceration	rare	common
Aphthoid ulceration	absent	characteristic
Mucosa surrounding ulcer	abnormal	normal
Bridging	occurs	occurs
Pseudopolyps	occur	occur

B.2. Crohn's disease

Colonoscopy starts with anal inspection and examination. The macroscopic appearance of (peri)-anal lesions such as fistulas and fissures is often by itself highly suggestive of CD.

Occasionally the only colonoscopic abnormality is some diffuse mucosal reddening or some mild generalized friability, more or less indistinguishable from that seen in UC. In other patients the mucosa has a smooth and somewhat pale atrophic appearance. Sometimes zones of redder color alternate with patches of a peculiar whitish opaqueness.

An important and well known feature in CD is discontinuity of the mucosal lesions, with segments of normal looking bowel, interspersed between abnormal areas or involvement of one wall with normality of the opposite or adjacent wall.

A characteristic differentiating feature in CD is that epithelial defects may occur in completely normal looking mucosa, whether the ulcerations are small or large. Presumably in the early stages and at

some distance from more severe lesions, tiny rounded aphthoid-like structures, a few mm in diameter, may be seen in otherwise normal appearing mucosa. They usually occur in crops but may be scattered throughout the colon as isolated lesions. These tiny rounded ulcers have slightly raised swollen margins with a greyish or yellowish crater. It may well be that such aphthoid ulcers develop from multiple small well circumscribed raised erythematous plaques, measuring a few mm and surrounded by normal intact mucosa (Watier et al. 1980). The glistening appearance of the mucosa is unbroken in contrast with that of aphthoid ulcers where a tiny crater, sometimes hemorrhagic, is seen in the midst of the lesion. Between the tiny aphthoid ulcers and the very large, deep longitudinal railroadtrack ulcers, there is a scala of intermediate forms, varying considerably in shape and depth. Some ulcers are serpiginous in appearance and manifest a tendency to longitudinal alignment. Most ulcers are sharply outlined, abruptly surrounded by normal or only slightly raised mucosa. Linear ulcers often run parallel to the longitudinal axis of the colon and are considered more characteristic for CD than for UC (Cook and Dixon, 1973). Such linear ulcers can easily be visualized in smooth even mucosa, but more difficult in coarse nodular mucosa.

Often the interhaustral septae are thickened, blunted, interrupted or even absent, especially in a gut segment with diminished distensibility. Occasionally there is a striking predilection for ulcers to cluster in and around areas of septal convergence and fusion.

Cobblestoning with a rough irregular nodular mucosal pattern, with or without intersecting linear depressions or ulcerations is quite characteristic though not pathognomonic for CD (Williams and Waye, 1978). In contrast with the appearance of pseudopolyps the base of cobblestones is wider than their height. Associated edema and fibrosis frequently compromise the colonic lumen.

Mucosal bridging is formed by the confluence of adjacent deep ulcerations, undermining a strip of surface mucosa.

Fibrotic stricturing is common in CD. Often the stricture is somewhat irregular with evidence of inflammation and focal epithelial destruction in the adjacent mucosal lining.

The appearance of fistulous communications is usually characterized by focal marked edema and erythema, surrounding the fistulous opening, which is often not readily apparent.

Whenever possible, inspection of the terminal ileum should be attempted. Ulceration of the ileocecal valve alone can be sufficient to make a correct diagnosis, even if the endoscope cannot be passed into the terminal ileum. Discrete mucosal ulcers in the terminal ileum are rather characteristic for CD. Since IBD not infrequently affects younger patients, it should be realized that nodular lymphoid hyperplasia of the terminal ileum is a normal finding. The presence of these non-friable excrescencies in the ileal mucosa should not be confused with the cobblestoning of chronic CD or with polyposis.

B.3. Infectious (entero) colitis

Several acute and chronic infectious diseases, involving the colon, may mimic the features of IBD.

In amebiasis, the mucosal appearance may vary from diffuse edema, granularity and friability resembling UC to a more classic appearance demonstrating ulcers, sometimes discrete, with undermined edges surrounded by fairly normal looking mucosa. The ulcers may be covered with a yellow-white exudate. Occasionally the ulcers are rather deep and punched out in appearance, usually surrounded with a small rim of marked erythema.

Endoscopic findings in shigellosis may vary from unusually intense mucosal erythema and hyperemia with much adherent mucus to marked hyperemia, some friability and evensuperficial ulceration, suggestive for UC. Although the mucosa may have a magenta hue, the striking friability encountered in UC is usually absent. Also in shigellosis the lesions may be patchy, including aphthoid erosions, presumably occuring in patients infected with less violent strains.

Endoscopic findings in salmonellosis vary from relatively minor abnormalities such as mucosal edema, hyperemia and loss of vascular pattern to diffusely hyperemic granular mucosa with friability and petechial hemorrhages mimicking UC. Discrete ulcers are usually only seen in the more proximal colon and not distally. In severe cases the markedly edematous mucosa may be covered a greenish necrotic slough, reminiscent

of antibiotic associated colitis.

Yersinia enterocolitis may be associated with uniformly edematous, erythematous and friable mucosa, consistent with UC. Usually however, the lesions are focal and consist mainly of aphthoid ulcers or small punched out ulcers, surrounded by areas showing a normal vascular pattern, resembling CD.

Also in campylobacter colitis, the endoscopic findings vary. Usually zones of edema, patchy erythema and friability alternate with areas of more or less normal mucosa. Occasionally however, there is obvious granularity, mucopurulent exudate formation and spontaneous bleeding, which may be indistinguishable from UC. Gross ulceration is usually absent. Also in campylobacter the focal nature of the inflammation is increasingly recognized, in particular the presence of tiny aphthoid erosions.

Tuberculosis may cause ulcers, strictures and skip lesions, similar to CD. The most common area of involvement is the ileocecal region. The coecum and ascending colon may be narrowed and contracted. The transverse colon may also be involved with ulceration and at times irregular constriction. The involved segment is usually shorter compared to CD. The mucosa may be erythematous and edematous with superficial and deep ulcerations, often with raised, indurated margins. As in CD, ulcers may be surrounded by normal mucosa. Also cobblestoning, segmental involvement and linear ulcers are likewise seen. At times a hypertrophic ulcerated fleshy mass, resembling a carcinoma may be seen, especially in the coecal area.

In schistosomiasis hyperemia, edema, granularity and friability may be present. In addition punctate hemorrhages, shallow ulcerations and multiple polypoid lesions may be observed.

B.4. Ischemic colitis

Acute ischemia of the colon, which is rarely examined, may show a characteristic dark blue or violaceous appearance. In a very rare instance the purple-black discoloration of gangrenous mucosa is obvious. Usually there is impressive edematous swelling and occasionally hemorrhagic bullae may be observed. In other patients one sees small red spots in a

pale colonic mucosa. After a few days the initial stage is followed by disruption of the mucosa with linear or trough-like ulcerations, inflammatory pseudopolyps and luminal narrowing. The necrotic lesions are sometimes covered by a yellow-greyish adherent plaque. The involved colon is sharply demarcated at both ends but erythema and tiny ulcerations may occasionally be separated from the involved segment by a few cm. In particular this ulcerative stage may show considerable similarity with CD and UC, causing confusion. In contrast to ischemia, the changes of UC are maximal and most frequent in the rectum and not around the splenic flexure area, the lesions usually progress to shortening and uniform narrowing and the ulceration is often much finer. Pseudopolyposis however, may look identical in both conditions. Characteristic features in CD are the asymmetrical patchy discontinuous distribution of the lesions and a characteristic tendency to stricturing and fistula formation. In the recovery phase of ischemia, the edema gradually disappears and re-epithelialisation of the denuded surfaces occurs. A smooth stricture may develop with slight pinkness and some loss of the normal vascular pattern as the only visible abnormality.

B.5. Antibiotic-associated colitis - Pseudomembranous colitis

Antibiotic-associated colitis has become a major clinical consideration in the differential diagnosis of IBD, particularly if the condition presents without the characteristically raised whitish plaques attached to the mucosal surfase or when the rectum is not involved (Burbige and Radigan, 1981). Pseudomembranous colitis is readily identified by the presence of elevated yellowish plaques on a non-friable mucosal surface. Such raised, sharply demarcated plaques may vary from pinhead to several centimeters in width. Bleeding may occur when the plaques are removed. In more severe cases, in addition to the plaques, edema, erythema and friability with punctate hemorrhages may be seen. Rarely there is extensive sloughing of the necrotic mucosa.

B.6. Diverticular disease

Diverticular disease may be associated with reddening and petechiae of the folds in the thickened distorted sigmoid colon. This may progress to maroon discoloration of the tips of one or several folds. Such hyperemic folds may occasionally ooze blood. In rare occasions the reddened

folds may actually appear polypoid. The gradual decrease in the intensity of the erythema from tip to base, merging into normal mucosa should lead to a correct diagnosis. In addition such polyp-like folds usually have a wide base.

B.7. Radiation colitis

During the acute stage, rectosigmoidoscopy may reveal edematous dusky mucosa and erythema, rather difficult to distinguish from UC. Severe damage leads to friability and mucosal ulceration. At the late stage, sigmoidoscopy or distal colonoscopy may reveal a granular, at times friable mucosa with multiple characteristic telangiectasias, which may be most prominent adjacent to ulcerations. Rectal stricturing may also be present.

B.8. Behçet's syndrome

In Behçet's syndrome the rectum is usually normal or may demonstrate discrete ulcers, surrounded by normal adjacent mucosa, quite suggestive of CD. On colonoscopy one may find diffuse mucosal thickening and spread-out punched out ulcers.

B.9. Colonoscopic biopsies

Colonoscopic biopsies are usually taken with a standard 7 Fr forceps with round cups or with oval jaws, with or without central spike. Such biopsies are usually small and superficial and contain only mucosa and fragments of muscularis mucosa and occasionally a small part of the submucosa. The size of such biopsies is much smaller compared to standard rectal biopsies. Recently a biopsy forceps became available with much larger cups (figure 1), to be used with a colonoscope with a large biopsy channel. The cup volume of this forceps is about $10mm^3$.
The majority of colonoscopic biopsies are taken in order to differentiate between UC and CD. Mucosal ulceration, fibrinopurulent exudate, infiltration of the mucosa by inflammatory cells, crypt abscesses and lymphoid aggregates are nonspecific findings. Although there are other criteria such as discontinuity,disproportion and transmural extension of the inflammatory reaction, marked lymphoid hyperplasia, fissuring ulceration, lymphangiectasy etc., which orient towards CD, the detection of focal granulomas or microgranulomas (Rotterdam et al. 1977) remains of paramount

importance in diagnosing CD (Chambers and Morson, 1980).

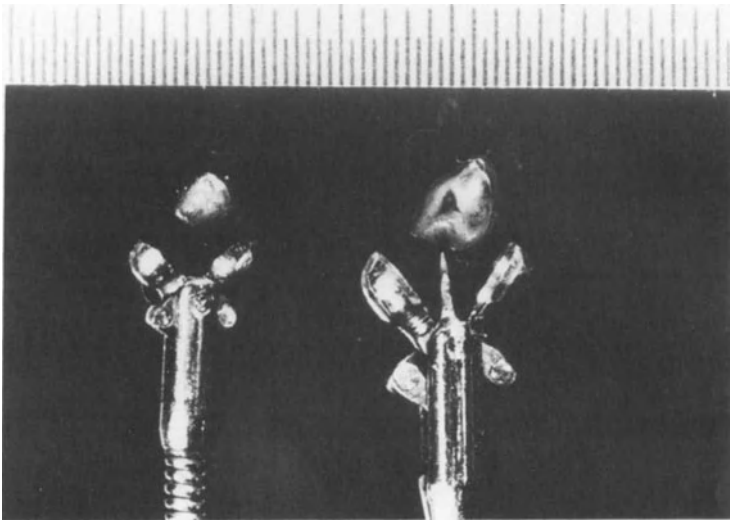

Figure 1. Standard and large (Ø 3.3 mm) biopsy forceps for colonoscopic biopsy.

The histological diagnosis of CD obtained via colonoscopic biopsies varies substantially (Table 2). This is explained, to some extent, by the number of sections examined. Whether the use of a larger colonoscopic biopsy forceps will increase the yield of granulomas needs further investigation. Initial experience suggests that greater amounts of tissue can be sampled without apparent enhanced blood loss or risk of perforation. Moreover the easiness with which such biopsies can be oriented improves the accuracy and completeness of the histological evaluation.

C. Determination of extent, distribution and severity of IBD

It is common experience that radiology may underestimate the extent of mucosal involvement in UC. According to Das et al. (1974), half the patients with ulcerative proctitis have microscopic evidence of colonic inflammation extending up to the splenic flexure area. Colonoscopists should not forget that occasionally normal looking mucosa with intact vascular pattern may show evidence of quite extensive chronic inflammation in the lamina propria. After topical treatment, the sigmoidoscopic appearance of the rectal mucosa may appear quite normal, despite

unequivocal edema, erythema and friability in the more proximal colon, occasionally explaining the puzzling ongoing bloodtinged diarrhea in UC.

Table 2. Histological diagnosis of Crohn's disease via colonoscopic and rectal biopsies.

Colonoscopic biopsies	Number of patients	Number of biopsies	% with obvious colonic involvement	% Crohn histology	% with granulomas
Meuwissen et al. 1976	15		100	87	33
Lux et al. 1978	67		100		27
Geboes et al. 1978	75	(≥ 5)	80	10	6
Hogan et al. 1980	17	(≥ 6)		53	
Waye, 1980					6
Schipper & Tytgat, 1981	300	(≥10)		50	30
Rectal biopsies					
Dyer et al. 1970	79		78		23
Rotterdam et al.* 1977	99		62		13
Hill et al. 1979	349		68	15	15
Surawicz et al. 1981	90				28
Iliffe & Owen, 1981	35		54	28	15

* normal sigmoidoscopy

When evaluating patients with fistulae between small bowel and colon, it may be important to determine whether sich fistulous communication is a manifestation of small bowel disease alone or whether the large bowel is also involved. When fistulae cause acute angulation in the large bowel especially the sigmoid colon, it may be necessary to use a small caliber endoscope to pass beyond the diseased segment for proper inspection and biopsy. Precise definition of colonic involvement is imperative in CD when surgery is considered. Surgeons are reluctant to cut across or to leave behind areas of gross abnormality, inspite of there being no or only few data with respect to the question whether or not an anastomosis through an area of ulceration increases surgical morbidity or hastens recurrence of disease (Kåresen et al. 1981).

D. Evaluation of the value of different therapeutic regimens

Frequent inspection of the mucosal lesions in patients with severe attacks of UC during intensive medical therapy (usually without prior bowel preparation) may improve the accuracy of clinical evaluation and may be helpful in deciding whether or not the medical therapy should be continued. Colonoscopy is also increasingly used to evaluate different

therapeutical regimens, especially for research purposes. During such studies it became apparent that the characteristic continuous and symmetrical involvement of the colon in UC may become less striking upon anti-inflammatory treatment. Colonoscopy is increasingly used in many centers to assess the therapeutic response in CD, especially because the improvement in clinical symptomatology not always parallels the endoscopic evolution. It is our policy to titrate the medical therapy not so much upon the clinical evolution but mainly upon the objective endoscopical changes. Aphthoid lesions in Crohn's disease may occasionally disappear in a few weeks, but in other patients they will persist for a long time. Even large extensive ulcerations in CD may occasionally heal upon medical therapy, leaving behind a somewhat depressed area, covered with atrophic mucosa.

E. Screening for malignancy or premalignant changes in UC

Colonoscopy has been suggested as a means of cancerscreening in patients with long-standing UC (Cook and Golligher, 1975; Lennard-Jones et al. 1977; Yardley et al. 1979; Butt et al. 1980). As cancers occuring in UC tend to be infiltrating rather than exofytic, they may not be readily apparent endoscopically (Crowson et al.1976; Katz et al., 1977). Rather then identifying cancer itself, colonoscopy has been proposed as a way of identifying patients particularly at risk by looking for histologic evidence of severe dysplasia or precancerous mucosal changes (Morson and Pang 1967; Riddell and Morson 1979) which appear to carry a 30 to 40% likelihood of cancer being found at subsequent colectomy (Dobbins 1977).

Biopsies should be taken preferentially from thickened areas of mucosa, from slightly elevated areas (Yardley et al. 1979), from areas that are velvety in appearance or from areas with minor roughness of the surface, which may be made evident through the reflections of light on the irregular surface. Blackstone et al. (1981) recommend to also consider the gross appearance of suspicious areas. In particular polypoid lesions with features which differ from those of other surrounding pseudopolyps, should be biopsied or removed. Such polypoid structures may have a more irregular and more friable surface mucosa or may be larger and of firmer consistency. When no suspicious areas are visible endoscopically, biopsies should be obtained with increments of 10 cm throughout the colon, so that a total of 8 to 9 biopsy sites are covered. It is indeed not uncommon to

find dysplasia in relatively normal or atrophic looking mucosa.Although it has been found that a high proportion of patients with dysplasia in colonoscopic biopsies also have dysplasia in sigmoidoscopic biopsies (Riddell 1976; Nugent et al. 1979) most experts would agree that it is desirable to obtain multiple biopsy samples from various parts of the colon. How often colonoscopic biopsies are required is a matter of opinion, logistics, patient acceptability and results of previous examinations. Annual colonoscopy in patients with a history of chronic UC, of 10 or more years duration, with involvement of the whole colon is increasingly being practised in larger centers. The procedure may be performed more often in a few patients with suspicious findings on previous colonoscopy (Williams and Waye 1978; Yardley et al. 1979). Others, with normal findings may perhaps have an annual rectal biopsy and colonoscopy every two years. Because the liability to carcinoma in patients with colitis confined to the left half of the colon is only slightly increased, a special follow-up program for these patients is probably not indicated (Butt et al. 1980). In addition to the patients with extensive longstanding colitis, also the patients treated by colectomy and ileorectal anastomosis, should be screened annually for dysplastic changes in the rectal remnant.

Even when severe dysplasia is found, authorities differ on whether or not to recommend colectomy. For some, the mere presence of dysplasia, severe of even moderate, constitutes an indication for colectomy (Nugent et al. 1979). Others would advise surgery only for severe dysplasia, found at different sites within the colon or rectum and preferably on sequential examinations (Lennard-Jones et al. 1977; Dobbins 1977; Butt et al. 1980). Blackstone et al. (1981) consider the presence of dysplasia associated lesions or masses, especially single polypoid masses, a strong indication for colectomy.

F. Evaluation of the nature of a stricture

Colonoscopy is indicated to assess the nature of a stricture and helps to determine whether or not surgery is indicated. The standard 18 mm colonoscope passes most strictures without much difficulty. One should refrain from using excessive force, if passage becomes difficult, in order to avoid fracturing a fibrotic rigid colonic wall. In such instancies a small caliber endoscope should be tried if passing the stricture is

considered necessary.

Careful study of the nature and appearance of the mucosal surface and determination of the presence of active inflammation within the narrowed zone is essential (Hunt et al. 1975). Smooth inactive looking strictures usually indicate a benign background. Strictures caused by active inflammation reveal the narrowed segment to be lined by ulcerated edematous and friable mucosa. Malignancy should be suspected when the stricture is unyielding to gentle pressure, when the stricture has a rigid, abrupt, shelf-like distal edge or when the stricture is excessively friable or covered with an irregular heaping mucosa. Multiple biopsies should be taken from the edges and within the stricture, together with brush cytology when the stricture cannot easily be passed.

If an endoscope cannot be passed, not even a small caliber one, the patients requires surgery as well for mechanical reasons as for the possibility of an underlying malignancy.

G. Assesment of postinflammatory polypoid lesions

Colonoscopy may occasionally be indicated in UC for histologic characterisation of filling defects, which may be caused by somewhat unusual, often large and irregular polypoid lesions. In a rare instance chronic bloodloss may be caused by giant pseudopolyps. Such pseudopolyps are easily removed via polypectomy for proper histological examination. In addition any suspicious polypoid defect, which differs in size, mucosal architecture and coloration from the surrounding pseudopolyps in patients with long-standing colitis should be biopsied or removed.

Occasionally localized tumor-simulating defects may be observed in the colon in patients with CD. Colonoscopy is helpfull in elucidating the true nature of such lesions, usually made up of clusters of polypoid or nodular masses.

H. Localisation of the site of hematochezia

Massive hematochezia may occasionally occur in IBD, especially in CD, because of its tendency to cause large excavating ulcers, which can erode large submucosal vessels. Colonoscopy is usually rewarding in identifying the bleeding deeply ulcerated segment and helpful in planning subsequent

surgery.

In a rare instance, pseudopolyps may be identified as the bleeding source. Care should be taken when trying to remove such polyps especially if the surrounding mucosa is heavily inflamed, because of slow healing of the coagulation site and prolonged oozing from the inflamed margins.

I. Evaluation of the peri-anastomotic site after resection

Radiological detection of early recurrence at the level of the anastomosis is notoriously difficult. Colonoscopy is often helpful in identifying superficial or deeper ulceration at the anastomotic site, often continuing for a few centimeter more proximally. In addition multiple biopsies may be useful in elucidating the nature of the recurrent inflammation.

J. Evaluation of stomal dysfunction

The cause of ileostomy dysfunction, either mechanical obstruction or recurrence, can often be identified by endoscopy, using a small caliber fiberendoscope. The stoma itself and the proximal ileal mucosa can be closely scrutinized and biopsied. Careful insertion of the instrument usually allows evaluation of about 50 cm of terminal ileum. In case of ileostomy dysfunction due to recurrent disease endoscopy may furnish important information for the surgeon regarding the proximal demarcation of the inflammatory proces.

Some authorities advise endoscopy of the continent ileostomy whenever problems occur (Waye 1978, 1980). Direct inspection of the inverted nipple, which is normally responsable for continence, can be accomplished by making a U-turn maneuver within the ileal pouch. Shortening or asymmetry of the nipple or deviation due to adhesive bands with fixation in an awkward position or presence of fistulas are usually easily identified and occasionally corrected endoscopically.

K. Contra indications and complications

In severely ill patients with acute colitis or with evidence of peritoneal irritation, colonoscopy is absolutely contraindicated. Since toxic dilatation of the colon is not infrequently associated with localized or sealed bowel leak or imminent perforation, the idea of endoscopic deflation of the colon should be regarded as dangerous. As a matter of fact some endoscopists have tried to decompress a toxic dilated colon

however without much success.

The overall incidence of colonoscopic complications in IBD is comparable to that seen in other situations. There is no evidence in the literature that the complication rate is higher. Occasionally colonoscopy may be followed by a temperature rise. Recently we observed a patient with rather severe UC in whom colonoscopy was complicated by high fever and cutaneous pustule formation. Presumably repetitive compression of the colonic wall, bearing micro-abcesses and puss collections during the procedure lead to pyema and peripheral cutaneous spread. Upon antibiotic treatment the cutaneous lesions rapidly regressed.

CONCLUDING REMARKS

From reviewing the literature, one gets the impression that judicious use of colonoscopy in IBD will increasingly contribute to improve the diagnostic accuracy and both medical and surgical patient management. Furthermore colonoscopy may prove indispensible, mainly because of its capability of providing proper tissue at any level of the colon, preferably from untreated patients, and by allowing detailed inspection of (early) mucosal alterations, which ultimately may be helpful in unraveling the pathogenesis of IBD.

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DRUG THERAPY OF INFLAMMATORY BOWEL DISEASE

J.W. SINGLETON

Drug therapy in inflammatory bowel disease is of widespread interest for several reasons. First, because no definitive medical therapy exists for either Crohn's disease or ulcerative colitis, patients and their physicians remain ultimately frustrated with all available medical therapy. But drug therapy claims attention and controversy beyond that frustration because every clinician is an experimenter who makes first hand experimental observations of the course of every patient. Inevitably clinicians develop strong opinions from the success or failure of treatments for which they bear the responsibility. Rueful experience has repeatedly proven however that there are so many variables acting upon the course of ulcerative colitis or Crohn's disease in addition to the variables of drug choice and dose, that observations from single cases or even ten or fifteen cases may be grossly misleading. Realizing this, our English colleagues, notably Drs. Truelove, Avery Jones, and Lennard-Jones, beginning almost thirty years ago,[1] took advantage of their uniquely large and cooperative groups of patients to study in statistically valid controlled trials the effect of drugs in inflammatory bowel disease. Largely due to their efforts, the results of properly controlled clinical trials have come to be accepted as the best guide to drug therapy. Despite their expense, difficulty and complexity such studies remain essential for the ultimate evaluation of medical, and perhaps also surgical, therapies.

STEROIDS

Adrenocorticosteroids are the best documented and most generally accepted drugs for control of the inflammatory process in both ulcerative colitis and Crohn's disease.

Topical steroids, applied as suppositories, intrarectal foam, small volume enemas, or by slow intrarectal drip have been shown in satisfactory controlled trials[2-5] to be superior to placebo for ulcerative colitis. No studies of

topical steroids have been done in Crohn's disease, but local steroid applications by one or more of the above means are thought by most experienced clinicians to be helpful for rectal Crohn's disease. Since the effect of topical steroids probably depends mostly upon contact with the inflamed mucosa, physical form, administered volume, time of administration and positioning after administration may all be varied to suit the distribution of inflammation in the bowel. No controlled trials of topical therapy of extensive or universal colitis have been reported and it is reasonble to restrict use of local steroids to relatively distal disease. Topical steroids do not prevent relapse of ulcerative proctitis after remission.[2]

Oral steroids have been studied in many controlled trials in ulcerative colitis and a few in Crohn's disease. Good evidence exists that 40 mg of prednisone daily is the optimal oral dose for moderately active ulcerative colitis,[6] and that once-a-day dosage is as effective as divided doses, with less likelihood of steroid side effects.[7] Oral steroids, either cortisone 50 mg twice daily or prednisone 15 mg daily, are not effective in prevention of relapse once a remission has occurred.[8,9] In the 10 to 15 percent of patients who never achieve satisfactory remission of ulcerative colitis, chronic low-dose prednisone may be useful, but this indication has not been studied in a controlled trial.

The single controlled trial of oral prednisone in active Crohn's disease[10] showed that it was clearly superior to placebo, thus confirming its use by most, but not all[11] experienced I B D clinicians. Daily doses of 15 to 60 mg, depending upon the degree of activity of disease, were used in this trial. Several controlled trials of long-term prophylactic use of steroids in quiescent Crohn's disease have found these drugs not to be useful.[10,12] Similarly, prednisone has not prevented recurrence of disease following surgery.[10,13] More than half of Crohn's disease patients, once started on steroids, continue to require them to keep the disease quiescent. In such patients the lowest possible dose should be used, an every-other-day schedule should be attempted to minimize side effects, and addition of azathioprine may be useful (see below).

Parenteral steroids are required for control of severe ulcerative colitis. The balance of evidence indicates that ACTH is not superior to hydrocortisone[14] and prednisolone is probably superior to either because of its lesser mineralocorticoid effect. Hospitalization, bowel rest by nil per os or nasogastric suction, intravenous fluids, electrolytes and blood, and usually broad spectrum

antibiotic coverage are all added to parenteral steroids for treatment of severe or fulminant colitis by the great majority of experienced clinicians.[15] A similar regimen is employed with severe Crohn's disease, again on the basis of experience, without controlled-trial support.

SULFASALAZINE (SALAZOPYRIN, AZULFIDINE^{R})

Sulfasalazine by mouth in doses of 4-6 grams per day has been shown to be useful for mild to moderately active ulcerative colitis, both for disease limited to the rectum or rectosigmoid and for more extensive disease.[16] However, the combination of oral prednisone and steroid enemas was superior to sulfasalazine.[17] Sulfasalazine has no role in treatment of severe or fulminant colitis. The best established indication for this drug is in prophylaxis of exacerbations, once remission has been obtained. Sulfasalazine, 2 grams per day, was significantly superior to placebo in preventing flare-up in a definitive trial[18] and a subsequent study indicated that a prophylactic effect is evident even beyond a year of quiescence.[19]

Three prospective double-blind controlled trials support the use of sulfasalazine in active symptomatic Crohn's disease of mild to moderate severity.[10,20,21] Results of one of these[10] suggested that it was especially effective in disease involving the colon and less so for disease confined to the small bowel. The most recent study,[21] involving only 26 patients, found it effective in small bowel disease as well, and clinical experience confirms that small bowel lesions do on occasion respond to sulfasalazine. The impossibility of oral administration in severely symptomatic Crohn's disease precludes its use in this setting. Several controlled trials have been conducted to study sulfasalazine as prophylaxis against reactivation of Crohn's disease[10,22] or recurrence after surgery.[10,13,23] None of these has demonstrated such an effect, and the "trend in favor" of sulfasalazine in one study[23] is balanced by the trend in favor of placebo in another.[10] A large careful study of sulfasalazine as adjunct to prednisone in treatment of active Crohn's disease also failed to show a beneficial effect of sulfasalazine and calculated the chance of having missed such an effect as less than 5%.[24]

The moiety of sulfasalazine active in ulcerative colitis appears to be the 5-aminosalicylic acid[25,26] which appears to exert a local therapeutic effect upon the diseased bowel when released from the sulfapyridine moiety by bacterial hydrolysis.[27] The occurrence of toxic side-effects correlates with blood levels of the absorbed sulfapyridine.[28] Thus efforts are underway

by at least two pharmaceutical companies to find a non-toxic carrier to deliver 5-aminosalicylic acid to the distal bowel. Sulfasalazine enemas are available outside the United States and have been shown effective in ulcerative proctitis.[25,26] Toxicity of sulfasalazine for spermatogenesis in humans and experimental animals is now documented by several reports[29,30] and may limit long-term use of the drug in young men. Finally sulfasalazine does substantially alter the bacterial flora of the colon in some patients. It has been implicated as a predisposing factor in exacerbations of Crohn's disease caused by colonization of the bowel by Clostridium difficile.[32]

IMMUNOSUPPRESIVE AGENTS: AZATHIOPRINE AND 6-MERCAPTOPURINE

Azathioprine was tested in both ulcerative colitis and Crohn's disease in the late 1960's and early 1970's in a number of small controlled trials. In acute ulcerative colitis it was tested as an adjunct to prednisone, showed little or no benefit,[33] and has been abandoned by most clinicians. A controlled trial has suggested an effect of the drug in preventing flare-up of ulcerative colitis in patients who have had more than one flare-up.[33] Another suggests it to be a useful adjunct to prednisone in chronically active colitis,[34] but neither was definitive.

Both positive and negative results were obtained in early trials of azathioprine in Crohn's disease and on the basis of the positive trials it was adopted by some English clinicians as a useful adjunct to prednisone. A more recent study from two London Hospitals unequivocally showed azathioprine to have a prophylactic effect against recrudescence of active disease in a select group of patients who had responded to treatment of active disease with the combination of prednisone and azathioprine and had then been symptom-free on azathioprine alone for six months.[35] However a large controlled trial of azathioprine alone compared with placebo in active Crohn's disease failed to show a statistically significant benefit, although a trend was noted in favor of azathioprine.[10] No subgroup of patients could be identified as especially responsive to azathioprine. Many clinicians remain convinced that azathioprine is useful for Crohn's disease[36,37] and their conviction was strengthened by a controlled study showing that the metabolite of azathioprine, 6-mercaptopurine, was statistically significantly superior to placebo in patients resistant to therapy with prednisone or sulfasalazine alone.[38] Further controlled trials of azathioprine or 6-mercaptopurine in Crohn's disease are needed before these drugs are accepted as standard therapy because each has substantial toxicity[39] and is oncogenic[40] and teratogenic.[41]

At this time the use of azathioprine should be limited to patients with disease resistant to steroid therapy, and possibly also as an adjunct to steroids in patients with chronically active disease, in whom azathioprine may allow dose reduction or discontinuance of steroids.

ANTIBIOTICS

Broad spectrum antibiotics, including coverage for strict anaerobes, are used in fulminant colitis to decrease the likelihood of sepsis and peritonitis resulting from the abnormally fragile colon. This indication for antibiotic therapy in ulcerative colitis or Crohn's disease has never been tested in a controlled fashion. Metronidazole, an antibiotic especially effective against strict anaerobes, has been tested in Crohn's disease in two trials. In the smaller, earlier, trial involving only 22 patients metronidazole or placebo was given in addition to sulfasalazine or prednisone and no benefit was noted.[42] A second, larger trial, comparing metronidazole with sulfasalazine is yet unpublished but is said to show approximately equivalent therapeutic effects of the two drugs in patients with active Crohn's disease.[43] In another uncontrolled series of patients, anaerobic bowel flora markedly decreased during metronidazole treatment in those patients whose symptoms improved.[44] Metronidazole has been enthusiastically recommended for treatment of the perianal complications of Crohn's disease as a result of uncontrolled observations in 17 patients, 15 of whom experienced healing of perianal fistulae and abscesses during one to 12 months of continuous administration at a dose of 20 mg per kilogram body weight per day.[45] A careful controlled trial of metronidazole for perianal Crohn's disease is sorely needed.

Long-term administration of broad spectrum antibiotics to patients with chronically active Crohn's disease is used by some experienced clinicians[46,47] but no controlled trials have been reported. Those most frequently given in this way are tetracycline, ampicillin, and trimethoprim-sulfamethoxazole. It should be noted that patients whose Crohn's disease has flared in association with Clostridium difficile colonization of the bowel usually gave a history of recent prior antibiotic treatment.[32]

NEWER DRUGS IN INFLAMMATORY BOWEL DISEASE

Disodium cromoglycate has been thoroughly evaluated in both ulcerative colitis and Crohn's disease. Administered orally it was not effective in either disease, neither in treatment of active disease or in prophylaxis of

flare-ups. However it has been found to be superior to placebo in ulcerative proctitis when given as an enema.[48]

Several agents which stimulate cellular immunity have been evaluated for therapeutic action in Crohn's disease. Levamisole, an antihelminthic which stimulates cellular immunity, was no better than placebo in two small controlled trials[49,50] and is quite toxic. Transfer factor, when tested in a controlled trial[51] proved no better than placebo. Similarly BCG oral administration was disappointing in a controlled trial.[52]

Antilymphocyte globulin was evaluated in active ulcerative colitis as an adjunct to conventional steroid therapy, but found to be of no benefit when compared with placebo.[53] Interferon,[54] superoxide dysmutase[55] and high doses of vitamin A[56] have each recently been reported to benefit Crohn's disease, but none has been critically evaluated. Following the demonstration that patients with ulcerative colitis have elevated levels of prostaglandin E in rectal mucosa, stool and blood, inhibitors of prostaglandin synthesis, such as indomethacin[57] and flurbiprofen[58] were given in uncontrolled studies but were ineffective. It now appears that sulfasalazine inhibits both synthesis[57] and degradation[59] of prostaglandins in the rectal mucosa, raising the possibility that the increased levels of prostaglandins in active ulcerative colitis might be exerting a beneficial cytoprotective effect. This area greatly needs clarification.

SUMMARY

Adrenocorticosteroids and sulfasalazine are established as useful drugs for treatment of both ulcerative colitis and Crohn's disease. Controversy exists concerning the value of every other drug reported to benefit these diseases. Carefully controlled prospective clinical trials are the primary tool for resolving these controversies.

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TOTAL PARENTERAL NUTRITION (TPN) IN INFLAMMATORY BOWEL DISEASE

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Since the development of nutritional techniques that permit total alimentation by a parenteral route, patients with severe disease and impairment of a gastrointestinal tract have been among the most common recipients of this form of therapy. In gastrointestinal disorders the rationale for using intensive nutritional support therapy is two fold. One, to restore or maintain adequate body nutrient composition in patients who cannot achieve normal nutrient status by dietary means, and two, to permit bowel rest and enhanced healing in patients who are receiving nothing by mouth and need nutritional support by an intravenous route for maintenance of nutritional status.

Table 1.

REPORTED PREVALENCE OF NUTRITIONAL DEFICIENCIES IN INFLAMMATORY BOWEL DISEASE

Deficiency	Crohn's Disease (%)	Ulcerative Colitis (%)
Weight loss	65-75	18-62
Hypoalbuminemia	25-80	25-50
Intestinal protein loss	75	NS
Negative nitrogen balance	69	NS
Anemia	60-80	66
Iron deficiency	39	81
Vitamin B12 deficiency	48	5
Folic acid deficiency	67	70
Calcium deficiency	13	NS
Magnesium deficiency	14-33	NS
Potassium deficiency	6-20	NS
Vitamin A deficiency	11	–
Vitamin C deficiency	NS	–
Vitamin D deficiency	75	NS
Vitamin K deficiency	NS	–
Zinc deficiency	40-50	NS
Copper deficiency	NS	–
Metabolic bone disease	NS	NS

NS = Reported but incidence Not Stated

Adapted from Driscoll RH Jr, Rosenberg IH: Total parenteral nutrition in inflammatory bowel disease. *Med Clin North Am* 62:185-201, 1978.

It is not surprising therefore that patients with severe inflammatory bowel disease, particularly Crohn's disease, were among the early group treated with TPN. Such patients have been documented to have a high degree of nutritional deficiency (Table 1) including evidence of calorie and protein deficits as well as deficiencies of micronutrients such as iron, folic acid, zinc and other vitamins. The causes of these nutritional deficiencies which have such a prevalence in inflammatory bowel disease are multiple but most important is the decreased oral intake which results from anorexia and abdominal pain and diarrhea while increased gastrointestinal losses, malabsorption and the increased nutritional requirements induced by fever and inflammation are also important. Thus the first rationale for TPN therapy in patients with inflammatory bowel disease is clearly present since many such patients cannot meet or maintain their nutritional requirements in the face of a limited oral intake. The second rationale has to do with the common use of total bowel rest therapy, i.e., the complete elimination of oral intake is commonly used to control symptomatic exacerbations of inflammatory bowel disease. The theoretical basis for bowel rest or a nothing-by-mouth regimen is that symptoms can be relieved and healing can be promoted by diminishing gastrointestinal and pancreatic secretions, decreasing intestinal motility, diminishing bacterial flora in the gut, and lessening the metabolic requirements of the affected gastrointestinal tract for continuing secretion and absorption of oral nutrients. Clinical experience indicates that diarrhea and abdominal pain can be lessened or alleviated in many patients by avoidance of oral intake. Parenteral nutritional maintainance is the only way that nutritional status can be maintained in such patients who would otherwise undergo continuing nutritional deterioration if maintained only on dextrose, water and electrolytes by peripheral vein.

It will be the purpose of this paper to review the published experience with TPN in patients with inflammatory bowel disease along with the experience at the University of Chicago for the past decade and to make certain recommendations about the role of TPN in the management of patients with Crohn's disease and ulcerative colitis. Our approach to the decision making regarding the use of TPN will be summarized.

TPN IN CROHN'S DISEASE

From the results of many studies of patients with severe and symptomatic Crohn's disease reasonable expectations of TPN with bowel rest include:

1) weight gain and positive nitrogen balance in almost all patients, if sufficient calories and protein are given; 2) at least temporary remission of symptoms in 60-70% of Crohn's patients and a few, 5-15%, prolonged remission; 3) active disease of varying severity will continue in the remainder (30-40%); and 4) about half will avoid expected surgery during the episode for which they are being treated (1). Reports from several centers published since the topic of TPN in Crohn's disease was reviewed (2) are discussed below.

Active Crohn's disease without fistulae

Table 2.

TOTAL PARENTERAL NUTRITION IN CROHN'S DISEASE

Primary Therapy for Failure of Medical Treatment (in nonfistulous disease)

Series	No. of Patients	Successful Nutritional Repletion	Clinical Remission (in Hospital)	Full Remission or Readily Managed Ambulatory (≥3 Months)
Reilly et al, Fischer et al[3,4]	21	ND	14	ND
Anderson, Boyce[5]	4	4	4	1
Cohen et al[6]	3	3	2	2
Franklin, Grand[7]	2	2	1	ND
Marshall[8]	2	2	2	2
Vogel et al[9]	8	8	8	3
Greenberg, Jeejeebhoy[10]	29	ND	26	22
Elson et al[1]	16	16	12	7
TOTALS	85	35 (100%)	69 (81%)	37 (61%)

ND=Not Detailed

In a nonrandomized prospective trial of TPN, all 20 patients with Crohn's disease gained weight and a positive clinical response occurred in 65%, with relief of pain and diarrhea among the most consistent results (1). Three patients (15) sustained a prolonged total remission after hospitalization, and five (25%) had a partial remission. Further, only 10 of 16 patients anticipated to require surgery actually had had operations in the 16-month follow-up, and those who had surgery did exceptionally well, as none had post-operative complications. Results from eight other uncontrolled trials of parenteral therapy in nonfistulous Crohn's disease (3-10) are similar: nutritional repletion was successful in all patients, 81% had a clinical remission in hospital, and 16% either had a full remission or were readily managed on an ambulatory basis for longer than three months (Table 2). In the first controlled prospective trial of parenteral nutrition in active colitis, including a small number of patients with Crohn's colitis, complete bowel rest with TPN was considered to have no primary therapeutic effect (Dickinson et al, 1980). However, because of some limitations in study design and the small number of Crohn's patients,

these results must be considered inconclusive in respect to Crohn's colitis.

Fistulae in Crohn's disease

Table 3.
PRIMARY THERAPY FOR FISTULAS IN CROHN'S DISEASE

Series	No. of Fistulae and Patients	Successful Nutritional Repletion	No. of Fistulae Healed (in Hospital)	Long-term Closure (≥3 Months)
Reilly et al [3]	2/2	ND	0	0
Anderson, Boyce[5]	2/2	2	1	0
Cohen et al [6]	1/1	1	1	1
Vogel et al [9]	2/2	2	1	1
Eisenberg et al [12]	20/18	ND	5	4
MacFadyen, Dudrick [13]	31/23	ND	17	ND
Greenberg, Jeejeebhoy [10]	14/14	ND	7	7
Elson et al [1]	5/5	5	1	1
TOTALS	77/67	—	33 (43%)	14 (30%)

ND=Not Detailed

Early reports that TPN was effective in the management of intestinal fistulas gave hope that it would be of help in this difficult and frustrating complication of Crohn's disease. In our study (1), only one of five fistula patients had lasting benefit from TPN, and that patient had a postoperative enterocutaneous fistula which may not have been directly related to active Crohn's disease. None of our patients with fistulas had obstruction, abscess or any other structural reason for treatment failure. Eisenberg et al (12) had a similar experience (Table 3): only two of 18 Crohn's patients with fistulas, treated with TPN, avoided surgery. In contrast, MacFadyen et al (13) evaluated their experience with TPN in the treatment of Crohn's fistulas. Their series included 31 fistulas in 23 patients studied; of these, 16 fistulas were present in 13 patients with small-bowel disease, and 15 fistulas were present in 10 patients with large-bowel disease. The spontaneous closure rate of small-bowel fistulas was 75%, whereas only 30% closed spontaneously in large-bowel disease. Unfortunately, follow-up of these patients was not given. Greenberg et al (10) found spontaneous closure of Crohn's fistulas in six of seven patients receiving TPN and prednisone, but observed only one closure in seven patients receiving TPN alone. Again, follow-up data were not supplied.

Clearly, better definition of type of fistula, longer follow-up, and information on associated treatment are necessary to establish the true role of nutrition support, with bowel rest, in the management of fistula in Crohn's.

Growth retardation

Table 4.

NUTRITIONAL THERAPY FOR GROWTH RETARDATION IN CROHN'S DISEASE

Series	Therapy	No. of Patients	Weight Gain	Linear Growth	Follow-Up
Layden et al[15]	TPN	4	4	4	4-14 months
Morin et al[16]	Enteral (tube)	4	4	4	3-12 months
Strobel et al[17]	Home TPN	17	17	14	ND
Kelts et al[18]	TPN & enteral	7	7	7	1-3 years
Kirschner et al[19]	Intensive oral nutrition including formula supplements	7	7	7	1-4 years

ND = Not Detailed

Retarded skeletal growth and delayed onset of puberty accompany inflammatory bowel disease in over 20% of young patients, particularly those with Crohn's disease (14). A causal relationship with a single deficiency has not been established; however, a pronounced deficit in caloric intake is regularly observed (14). Many patients limit their dietary intake in response to abdominal pain and diarrhea.

To restore skeletal growth, nutritional requirements must be met. Sulfasalazine or corticosteroid therapy, preferably administered on an alternate-day schedule, may provide sufficient relief of symptoms, allowing adequate dietary intake for growth to be restored. Oral formula supplements are often required to meet calorie-protein goals. Patients who fail to respond to a carefully selected oral regimen may require intensive nutritional support by enteral tube or TPN.

Available data on the treatment of growth retardation in Crohn's disease patients indicate that adequate nutritional intake - using TPN (15) or oral feedings supplemented with parenteral nutrition (18) - may re-establish skeletal growth when intensive oral therapy fails (19)(Table 4).

Short bowel syndrome

The availability of TPN therapy has changed the management of and prognosis for the severe short bowel syndrome to a major extent. In the acute stage after abdominal catastrophe and major intestinal resection, TPN is useful for the maintenance of fluid and electrolyte homeostasis and prevention of nutritional deterioration. After the initial postoperative and hypersecretory period, TPN is often helpful in the transition from intravenous to oral management. Often, this transition progresses from TPN to a combination of parenteral and enteral nutrition, and finally to oral support.

Some patients with Crohn's disease cannot maintain adequate nutrition orally because of the extensive loss of bowel or the presence of continuing disease. For such patients, continuous TPN support may mean the difference between life and death following progressive inanition. These patients, as well as some with uncontrollable short bowel syndrome or intractable but unresectable Crohn's disease, are most likely to be managed by a home regimen of TPN.

Home total parenteral nutrition

In patients who are destined to need intravenous nutrition support for protracted periods and in some, indefinitely, particularly those with inadequate intestinal length for nutritional maintainance, TPN may be employed at home for prolonged periods (22). An experienced team must be available to supervise the administration and to monitor progress and to provide safeguards. In such home programs TPN infusions are usually administered overnight through a permanent catheter inserted in a large central vein and provided with a heparin lock. Several studies indicate that selected patients with severe malnutrition resulting from extensive and permanent loss of GI function can maintain a normal body weight and achieve social rehabilitation on home therapy. In a group of patients with severe short bowel syndrome, extensive Crohn's disease, and other gastrointestinal conditions home therapy has now been maintained for several years with good results (22). In other studies the use of TPN at home for several weeks or months may limit the requirement for protracted periods of time in the hospital (17).

TPN IN ULCERATIVE COLITIS

Table 5.

TOTAL PARENTERAL NUTRITION IN ULCERATIVE COLITIS

Primary Therapy for Failure of Medical Treatment

Series	No. of Patients	Nutritional Repletion	Clinical Remission (in Hospital)	Full Remission or Readily Managed Ambulatory (>3 Months)
Reilly et al, Fischer et al [3,4]	11	7	1	ND
Cohen et al [6]	1	ND	0	–
Stanchev [20]	10	9	4	ND
Franklin, Grand [21]	5	5	2	1
Vogel et al [9]	1	1	1	1
Elson et al [1]	10	9	4	3
Dickinson et al [11]	13	ND	6*	ND
TOTALS	51	31 (61%)	18 (35%)	–

ND=Not Detailed

In ulcerative colitis TPN may be expected to achieve temporary remission in 33% of patients; long-term remission occurs less often (Table 5). Though the total number of patients with ulcerative colitis treated with TPN and total bowel rest is small, it appears that this form is limited as a primary modality of treatment. TPN appears to have little influence on refractory patients with active disease, and rarely can TPN avert colectomy. On the other hand, TPN is useful to prepare the malnourished patient for surgery, and, possibly, to supply postoperative nutritional support. When used for these purposes, TPN provides a valuable adjunct in the management of patients with ulcerative colitis.

MODES OF TPN THERAPY

Whether administered by the peripheral or central route TPN should be planned and administered by an experienced team consisting of physician, nurse, pharmacist, and dietitian. Techniques of solution preparation, catheter insertion and administration have been described in several reports (2). Prior to or upon initiation of therapy the patient's fluid electrolyte and acid base balance should be corrected. Transfusion of blood or albumin may be indicated. In central TPN hypertonic dextrose, amino acids, intravenous fat and other nutrients are administered through a central vein usually via subclavian catheter. Central TPN can provide up to and above 4000 kcal/day.

In some patients peripheral vein TPN can be employed. This consists also of the administration of amino acids, dextrose, electrolytes, vitamins and fat. Such regimens can provide between roughly 1400 and 2000 kcal/day with a major calorie source being the fat emulsions. Although peripheral TPN solutions can rarely maintain positive calorie and protein balance in any but the smallest patients, they can provide supportive therapy for patients with limited nutritional deficits. Such a regimen is convenient for short periods of up to two weeks to avoid the additional risks and demands of central vein TPN. Obviously patients must have good peripheral veins and must be able to tolerate the volume of fluid to be infused.

Once instituted it is important that the nutritional and metabolic progress of the patients be monitored carefully. With careful attention to safety and monitoring it is possible to keep the complications of TPN to a low level.

SUMMARY

The patients in whom TPN has been employed represent a severe test for any therapy. Many had been referred with complex and difficult management problems.

Despite the positive nutritional response in virtually all patients, a positive clinical response occurred less often. The symptomatic results among the Crohn's patients are consistent with the tendency of some of these patients to respond to bowel rest with or without TPN. To determine how much of this clinical response was due to TPN is a matter of continuing research interest.

TPN appears to be useful in the rehabilitation of some severely malnourished patients prior to surgery. Most patients going to surgery in the Crohn's group did exceptionally well postoperatively. This experience lends weight to a common but unproved claim that one of the most beneficial roles of TPN in Crohn's disease is in preparation for surgery. In surprising contrast, six of the seven ulcerative colitis patients undergoing surgery in the Elson study had postoperative complications. After colectomy, four of these six required a second laparatomy. Other workers have found the same benign postoperative course in ulcerative colitis patients as in Crohn's disease. One explanation may lie in the fulminant course in many ulcerative colitis patients. This technique clearly does not prevent colectomy in fulminant ulcerative colitis and delaying the operation while it is tried may even be detrimental.

At present we consider TPN to be indicated in the therapy of Crohn's disease in the following situations: 1) for the patient who fails to respond to maximal medical therapy and in whom surgery is to be avoided if possible; 2) to prepare the nutritionally depleted patient for surgery and, in some, for maintenance through the postoperative period; 3) following major intestinal resections for Crohn's disease resulting in short bowel syndrome; and 4) for selected patients with growth retardation not responding to oral therapy (14). Primary therapy of Crohn's fistulas with TPN remains controversial. TPN appears to be less useful in patients with ulcerative colitis and is probably only beneficial in those patients who are nutritionally depleted and require surgery. TPN does not appear to be indicated in fulminant ulcerative colitis, nor in the nutritionally replete patient admitted for elective colectomy. In patients between these extremes, the risk and expense have to be individually weighed against the spectrum of

possible but not always predictable benefits.

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Ulcerative Colitis and Crohn's Disease in Children

M.E. Ament

Ulcerative colitis and Crohn's disease have been increasingly recognized as causes of chronic abdominal pain and diarrhea in children. During the past decade, the proportion of cases seen between these two conditions appear to be nearly equal. Prior to the last decade, most cases of inflammatory disease in the pediatric age group were attributed to ulcerative colitis.

Most people who care for children with these two conditions believe misdiagnosis in the past could account for the change in the frequency of diagnosis in these two conditions.

Ulcerative colitis may have a variable course in terms of its severity in the pediatric population just as it does in the adult. Its onset may be insidious or abrupt. The symptoms may range from just a small amount of rectal bleeding with normal stools to fulminant diarrhea with colonic hemorrhage and prostration. Ulcerative colitis in the pediatric population may be classified into mild, moderate, or severe disease.

Mild ulcerative colitis is the least common form found in children. It is characterized by less than three stools per day, absence of anemia, normal serum protein, absence of fever and systemic symptoms. Eighty percent of those with the mild form have the condition confined to the rectum or left colon. One-fifth of those with mild disease have diffuse involvement of the colon. The extraintestinal manifestations of ulcerative colitis are similar to those seen in severe disease. They include: 1) arthritis and arthralgia; 2) skin lesions, such as erythema nodosum and pyoderma gangrenosum; 3) hepatic dysfunction; 4) iritis, and 5) thrombophlebitis. The extraintestinal manifestations of the disease are not different from those seen in moderate to severe disease and they occur independent of as well as during active attacks.

of colitis.

One-fifth of those patients classified as having mild disease may progress disease may progress on to have moderate or severe disease. The extraintestinal manifestations of mild disease may present weeks to months before symptoms and signs of colitis appear. Rectal bleeding may occur in the absence of diarrhea. Patients with mild disease may have frequent small stools without the appearance of gross blood. The immediate mortality in mild disease is nil.

Moderate Ulcerative Colitis. Sixty-percent of all pediatric patients are classified as having moderate disease. Diarrhea is a major symptom of those with moderate disease. They typically have four to five stools per day, and the stools contain gross blood. They typically have crampy abdominal pain, intermittent low-grade fever usually less than $39^{o}C$, and have cramps relieved by defecation. They have intervals of anorexia and weight loss. Patients with moderate disease may progress to severe disease. These patients dramatically respond to the use of corticosteroids.

Characteristics of Severe Ulcerative Colitis. These patients typically have large volumes of diarrhea and have a fall in the hemoglobin concentration. They are typically hypoalbuminemic and have high fevers as much as $40^{o}C$. They typically have tachycardia, rapid weight loss, marked elevation of sedimentation rate, and steady abdominal pain and tenderness. This is the rarest form of the disease and it is sometimes associated with sudden onset of symptoms with rapid progression to serious illness. Their diarrhea is often profuse and very bloody. They have constant rectal bleeding and tenesmus. They often become weak and dehydrated because of the fluid and protein losses from the colon. Because of their blood loss they often become hypotensive and weak and have an associated tachycardia, both with a fever and from fluid losses. Patients with severe ulcerative colitis often develop tender abdomens which become tympanitic. Patients like this have to be carefully monitored for evidence of perforation. Patients with severe or fulminant ulcerative colitis typically have aphthous ulcerations in their mouth and often develop white blood counts of greater than 20,000 along with their anemia. Because of the protein losses from their GI tracts, they develop pretibial edema.

Natural History of Ulcerative Colitis in Children. Sixty to seventy-five percent have intermittent attacks of symptoms. Four to

ten percent have one attack with no subsequent attacks for a decade. Five to fifteen percent never gain a remission.

The differential diagnosis of ulcerative colitis includes: 1) infectious colitis; 2) irritable bowel syndrome; 3) post-antibiotic colitis; 5) hemorrhoids; 6) anal fissure; 7) rectal polyps; 8) carcinoma of the colon; 9) factitious proctitis.

Diagnosis of ulcerative colitis is made by the exclusion of infectious colitities. Patients must have three stools which are negative for Shigella, Salmonella, Campylobacter infections and three stools which are negative for the presence of Entamoeba histolytica. Patients who have been on antibiotics within a period of six weeks of the onset of the diarrhea must be cultured for the presence of Clostridium difficile. Their stools must be tested for this particular bacteria and its toxin. Blood tests must be done to rule out the presence of amoebiasis, even if the stools are examined. The counter-immuno-electrophoresis test must be done to screen for amoebiasis. In addition, at proctosigmoidoscopic examination, direct wipings of the rectal mucosa should be done and placed in Schaudinn's reagent to look for the trophozoites in wet mounts. Patients who have change in frequency of bowel movements and have either gross or microscopic presence of blood in their stools should have a proctosigmoidoscopic examination and rectal biopsy done to exclude or prove the diagnosis of ulcerative colitis.

Proctosigmoidoscopic examination and rectal biopsy must always precede doing a barium enema examination. At proctosigmoidoscopic examination, the endoscopist must look for the following: 1) the presence or absence of ramifying mucosal blood vessels; 2) granular appearance of the mucosa; 3) blunting of the rectal valves; 4) induced friability with cotton mucosal swabs. Infants and children are best examined with the use of sedation in order to avoid movement when the examination is done. Movement of the patient during the examination can result in trauma to the mucosa and false positive evaluation of the mucosa. Proctoscopic examination needs to be done looking for changes beginning in the most distal rectum first as one moves proximally. Patients with ulcerative colitis most typically have changes beginning in the most distal rectum. Most patients with ulcerative colitis have disease which begins in the rectum.

Occasional cases of what appear to be typical ulcerative colitis have patchy changes in the rectum or appear to have rectal sparing.

Even areas that appear to be normal to the naked eye should be biopsied to look for microscopic evidence of colitis. The presence of ulcers seen grossly at proctosigmoidoscopic examination is typical of Crohn's disease, amoebiasis, and Campylobacter infections. Gross ulcers are not typically seen in ulcerative colitis.

Rectal biopsies should always be done at the time of proctosigmoidoscopic examination to confirm the visualization of the mucosa.

Barium enema examination should only be done 24-48 hours after a proctosigmoidoscopic examination is completed.

It is best for patients to have a double contrast barium enema in order to look at the details of the mucosa most carefully. Patients who have fever, abdominal pain and tenderness should not have a barium enema examination, since this could worsen their symptoms and could, in severe cases, result in perforation of the colon.

The intensity of the mucosal disease at proctosigmoidoscopy usually parallels the severity of attack. Radiographic evaluation does not always provide a reliable estimate of the severity of the disease. The rectal biopsy confirms the presence of inflammation of the colonic mucosa. It establishes the diagnosis in cases with normal proctosigmoidoscopic examination or when the examination is equivocal. The biopsies may show trophozoites of Entamoeba histolytica.

The role of colonoscopy in ulcerative colitis is unknown. It is rarely necessary. It should be done in cases in which the rectum is minimally involved and standard single contrast barium enema examination is normal. Colonoscopy can define the nature and the extent of the disease. One can never use colonoscopy in moderate to severe colitis. Its major role is in surveillance for cancer, which does not play a major role in the pediatric population.

The barium enema changes in ulcerative colitis in the pediatric population are no different from those in adults. Typically, one sees minute ulcerations along the edges of the bowel, usually involving the rectum and spreading proximally. The changes, however, are not specific. The post-evacuation film usually shows denudation of the mucosa and disappearance of a reticulated pattern. Cotton button ulcers may be seen along the colon. Pseudopolyps may also be visualized in both

acute and chronic disease.

One should also evaluate the presacral space to look for an increase in its diameter. Shortening, loss of haustra, and narrowing of the colon are all indications of chronic colitis.

The barium enema may help to differentiate ulcerative colitis from Crohn's disease, but the only specific changes are the presence of fistulas and abscesses which are specific indications of Crohn's disease. Otherwise, all the changes seen are common to both diseases. The severe disease always involves the entire colon, but universal colitis is present in 20% of cases. Loss of haustra markings, foreshortening, and narrowing of the colon indicate the chronicity and severity.

Extraintestinal Manifestations of Ulcerative Colitis. Twenty percent of pediatric patients develop arthritis and it usually appears at the time of onset of the bowel symptoms. The arthritis typically involves the large joints and is migratory. It may also be monoarticular. The joint cartilages and bony appositions are usually unaffected. Patients with the arthritis of ulcerative colitis usually have the serum negative for rheumatoid factor. It usually subsides with the control of the colitis.

Skin Lesions in Ulcerative Colitis. Erythema nodosum occurs in 3% of all pediatric patients with ulcerative colitis. It is more commonly seen in female children than males. It usually develops during the exacerbation of the disease. Pyoderma gangrenosum occurs in from 1 to 4% of all pediatric patients with ulcerative colitis. The lesions are often severe and respond only to the use of large dose corticosteroid therapy. Aphthous ulcerations of the mouth occur in 10% of pediatric patients with ulcerative colitis.

Ocular Lesions. Iritis is the most common eye lesion seen in ulcerative colitis in pediatric patients but it is extremely rare and occurs in less than 1% of all pediatric patients. Episcleritis is less common. Iritis is usually seen in conjunction with arthritis.

Renal Disease in Ulcerative Colitis. Nephrolithiasis and urolithiasis may develop in patients with ulcerative colitis who have severe diarrhea and have marginal fluid balance. It is uncommon in most pediatric patients.

Hepatobiliary Disease. Twenty percent of all pediatric patients with ulcerative colitis show a non-specific elevation of the alkaline

phosphatase. This correlates with the presence of pericholangitis in liver biopsies. Jaundice in the form of a hepatitis picture occurs in rare cases of ulcerative colitis in the pediatric population and may be the presenting manifestation of the disease, but this is highly unusual. Less than 2% of all pediatric patients may present with a picture of chronic active liver disease which may precede the onset of their colitic picture. Sclerosing cholangitis may be the initial manifestation of ulcerative colitis, but is an extremely rare occurrence in the pediatric population. Less than one percent of pediatric patients have this serious manifestation of this disease.

Medical Treatment of Ulcerative Colitis. Medical treatment of ulcerative colitis in the pediatric age group is not truly different from that in adults. For those who have mild disease, there are three choices: 1) rectal steroid enemas; 2) oral prednisone or prednisolone; or 3) the use of salazopyrine. Patients who are treated with corticosteroids have the greatest chance of gaining a clinical remission as compared to those in whom salazopyrine is chosen.

Some physicians may prefer to use oral corticosteroid therapy because it is non-invasive and the advantages of using it compared to rectal steroids may be significant. The long-term effects of introducing steroid enemas into the rectum of a child have not been evaluated, but because of their potential for creating psychological problems, we more commonly refrain from using them. Although there may be greater absorption from using oral versus rectal steroids, the absorption of the latter may be considerable.

For moderate inflammatory bowel disease, there really are only two choices: oral or intravenous corticosteroids, and for severe disease there is only one choice: hospitalization, placement of an intravenous line, delivery of intravenous corticosteroids, and parenteral nutrition. For those patients who receive prednisone, the dosage is 1.5 mg/kg/day given in four divided doses. For those who receive methylprednisolone, the dosage is 1.2 mg/kg/day given in four divided doses. For rectal corticosteroids, the dosage is 50-100 mg hydrocortisone hemisuccinate given twice per day. The maximal dosages of all these medications are used for a period of two weeks. If the patient fails to gain either a complete or a partial remission by two weeks, it is unlikely the patient is going to respond to this therapy. If at the end of the two weeks

the patient has entered into a complete clinical remission, then the dosage of the oral corticosteroids is gradually reduced by 1/8 of the total initial dosage per week until the medication is discontinued. For those patients who have received rectal corticosteroids, the dosage is reduced by 1/2 for the next two weeks. If a patient has gained a full clinical remission,with the use of intravenous corticosteroids, the patient is then changed to oral corticosteroids at a comparable dosage and a gradual process of reduction of the dosage by 1/8 of the total initial dosage per day is used. In patients who are initially treated for mild disease with Azulfidine or Salazopyrine, the dosage is 50 mg/kg/day.

Maintenance Therapy. For patients who enter a full remission with the use of corticosteroids, Salazopyrine is introduced as the drug to maintain remission when the dosage of corticosteroids is reduced to half the maximal dosage. This is because most patients on corticosteroid therapy cannot be maintained in full remission when the corticosteroid dosage is reduced below this level.

Seventy-five percent of all pediatric patients will gain a full remission in fourteen days. An additional 15% will gain partial remission in fourteen days. Ninety percent of the patients will gain a full or partial remission at the end of one month. Patients who fail to have a major clinical improvement by fourteen days of treatment are unlikely to gain a remission by continuation of this therapy. Approximately 10% of patients with moderate to severe ulcerative colitis do not gain a remission from therapy.

Some patients who have disease confined only to the rectum fail to respond to any treatment.

The use of Salazopyrine to maintain patients in chronic remission with ulcerative colitis is three times more effective than using placebo. Patients should be continued on Salazopyrine for as long as the patient remains in remission.

Surgery in Ulcerative Colitis. Several factors may enter into making a decision for surgery:: 1) What is the functional capacity of the patient? 2) What is the amount of systemic corticosteroids necessary to maintain the patient at a functioning level? The indications for colectomy in ulcerative colitis are: 1) suspected or proven coronic perforation; 2) toxic megacolon; 3) incapacity

created by the disease; 4) the severity, duration, and extent of the disease.

Some patients with severe ulcerative colitis prove to be unresponsive to medical management. Those patients should definitely undergo early surgery. Those patients who have uncontrolled colonic hemorrhage and require blood replacement equal to half or more of their blood volume within 24 hours should undergo surgery. Patients who have shown gross retardation for more than one year and have been unresponsive or only partially refractory to medical management should undergo surgery. Patients who have either intractable uveitis or intractable pyroderma gangrenosum should also be considered for surgery.

Most patients with ulcerative colitis in the pediatric age group require colectomy for their disease either because of growth failure secondary to the continuous activity of the disease and the need for continuous corticosteroid therapy or because the disease proves to be intractable.

Most patients with ulcerative colitis in the pediatric age group undergo surgery within five years of the onset of their disease.

Other Treatment. There is no specific diet for patients who have ulcerative colitis. Lactose free diets should only be applied to those patients with a known history of milk intolerance prior to the onset of their ulcerative colitis. Patients can develop secondary lactase deficiency and lactose intolerance if they become malnourished from the disease.

There is no evidence that keeping the patient on a milk-free diet really alters the course of the disease. There is no evidence that putting the patient on a high fiber diet alters the course of the disease.

The use of drugs to alter the frequency of bowel movements such as diphenoxalate hydrochloride and loperamide and codeine are contraindicated in ulcerative colitis because they may give the patient a toxin megacolon-like syndrome. The use of bulking agents in patients with ulcerative colitis has no benefit other than to give the patients a more formed stool, which does not in any way reduce the inflammatory changes in the colon.

Surgery in Ulcerative Colitis. The major advances in ulcerative colitis have come in the form of the surgery available. The standard

total coleetomy and ileostomy for patients with ulcerative colitis has proven life-saving over the past two to three decades.

Despite the life-saving measure of an ileostomy in patients with ulcerative colitis, many patients and many physicians do not recommend surgery when it may be indicated because of the presumed psychological effects such an operation may have on their patients. Many patients fear the social consequences which may come from wearing an ileostomy. There are real fears of being able to find and hold a mate by teenagers who have colectomies and ileostomies.

There really are no good studies to show what the consequences are of having an ileostomy on a long-term basis. There have been no objective studies which have been done to evaluate what occurs to patients with ulcerative colitis who have had an ileostomy and must wear an ileostomy appliance.

There is no question that this surgery has made a major difference in the outcome of patients with ulcerative colitis. Patients with ulcerative colitis who have growth retardation of two or more years and have not undergone pubertal changes, benefit most in terms of growth and development from having a colectomy and ileostomy.

Patients who are pubertal and have such surgery gain least in terms of growth.

Total colectomy and ileostomy can completely reverse all the patient's symptoms and turn patients who have been invalids into functioning individuals.

In the past five years, there have been two major advances in the surgery available for patients with ulcerative colitis in the pediatric age group. One is the development of the Koch pouch, or the internal ileostomy and the second is the development of the endomucosal rectal pull-through with ileal reservoir. These two operations have enabled patients who were highly fearful of having surgery for ulcerative colitis to undergo it.

In the case of the former, it allows the patient to not have an external ileostomy appliance, although the individual must still empty the internal pouch several times per day. The endomucosal rectal pull-through with ileal reservoir has been done in over eighteen patients at our institution. Patients who have this procedure have all been continent and have anywhere from two to eight bowel movements per 24 hours.

Some of these patients have been reluctant to have surgery until this patient was offered to them. Should this operation fail these patients would go back to having a standard ileostomy or could have a coat pouch fashioned.

We are currently recommending endomucosal rectal pull-through procedure and ileal reservoir for all of our pediatric patients with ulcerative colitis.

CROHN'S DISEASE

Crohn's disease, which is a chronic inflammatory condition which may involve all or any part of the gastrointestinal tract from mouth to anus, has been found to occur with almost equal frequency as ulcerative colitis in the pediatric patient.

Although patients with Crohn's disease have been described from the first year of life, most cases of Crohn's disease do not become clinically apparent until the end of the first decade.

The commonest clinical symptoms of Crohn's disease in the pediatric population are: diarrhea, abdominal pain, and fever. Diarrhea occurs in 90% of patients and is typically characterized by being watery. Patients with diarrhea may have a change in the consistency of the stool as well as a change in the frequency. Bloody diarrhea is uncommon in Crohn's disease.

A small number of patients with Crohn's disease may present with steatorrhea because of the extensive involvement of the jejunum and ileum. Abdominal pain is another major symptom in the disease.

The abdominal pain seen in Crohn's disease is of several types. The most commonly associated pain occurs postprandially and although it may occur in the right lower quadrant, it is frequently referred to the midepigastric or the periumbilical area. Patients who have postprandial abdominal pain in the midepigastric or periumbilical area should have an upper gastrointestinal and small bowel series to look for evidence of Crohn's disease if no lesions are found in the stomach and duodenum.

Other patterns of pain associated with Crohn's disease are a chronic pain which appears to be uninfluenced by eating or activity. This type of pain may be localized to the area of bowel involvement.

Fever in Crohn's disease is typically low-grade and seldom exceeds

39°C. Patients with Crohn's disease where the fever is higher usually are developing fistulas or intra-abdominal abscesses.

The physical signs of Crohn's disease are truly non-specific. The abdominal examination may elicit tenderness confined to the quadrant in which the Crohn's disease is most prominent. Tenderness is typically most often found in the right lower quadrant, the area in which the terminal ileum and cecum are found. However, tenderness can be elicited in almost any quadrant because the pain may be referred and the disease can also involve any segment of the small intestine or colon.

Patients with Crohn's disease sometimes manifest with perianal disease as its initial manifestation. The unexpected findings of perianal fistula or development of unexplained hemorrhoids in an asymptomatic patient may herald the development of Crohn's disease by as much as three years.

Fissures in Crohn's disease typically develop in the ventral and lateral aspects of the anal canal. Fissures that develop dorsally are typical of anyone who strains at stool.

Patients with Crohn's disease may manifest clubbing of the fingers and toes. This is typically seen in those individuals who have extensive and severe involvement of their colons. Patients with Crohn's disease typically have more profound growth failure and are typically more underweight than those individuals with ulcerative colitis. Patients with Crohn's disease may present as their initial manifestation with failure to grow and undergo sexual maturation. These individuals frequently have minimal or no apparent gastrointestinal symptoms.

The extraintestinal manifestations of Crohn's disease are similar to those seen in ulcerative colitis and will not be discussed further. The frequency with which they occur is similar or somewhat less than in patients with ulcerative colitis. Diagnosis of Crohn's disease depends in part on an accurate history and physical examination and certain specific laboratory tests and examinations. The patients who present with chronic diarrhea and are suspected of having Crohn's disease should have at least three stools examined for enteric pathogens and three stools examined for Entamoeba histolytica, trophozoites, and cysts.

Stools also must be examined for the presence of Giardia lamblia to exclude this parasitic agent which can cause malabsorption and

diarrhea. Following obtaining stool cultures, and examination for ova and parasites, patients with the possible diagnosis of Crohn's disease should undergo proctosigmoidoscopic examination. The perianal area should be examined for the presence of fistulas, fissures, and skin tags. Examination of the rectal mucosa may show no evidence of disease or may show patchy areas of spontaneous friability and loss of vascular pattern. Two ulcers may be seen in the rectal or sigmoid mucosa. Their presence means either the presence of Crohn's disease, amoebiasis, or Campylobacter.

Patchy induced or spontaneous friability is characteristic of Crohn's disease. However, some patients with ulcerative colitis may also have this patchy distribution. Rectal biopsies should always be taken at the time of the proctosigmoidoscopic examination to confirm the mucosal findings seen at proctosigmoidoscopic examination. The only specific findings on rectal biopsy typical of Crohn's disease would be the presence of granulomas and giant cells. Patchy changes in the mucosa, although typical of Crohn's disease, are not specific for it. Crypt abscesses may be seen in both diseases but are more common in ulcerative colitis. Although patchy changes are more characteristic of Crohn's disease, they are not diagnostic.

After a proctosigmoidoscopic examination and biopsy, the next study which should be undertaken is a barium enema. This should not be done for 24-48 hours after the proctosigmoidoscopic examination. A double contrast barium enema should be done to exclude the presence or absence of Crohn's disease beyond the rectosigmoid area.

There are only a few changes specific for Crohn's disease in the barium enema, and they include: 1) fistula formation, and 2) abscess formation. The barium enema is the best way to visualize the cecum and the terminal ileum.

Since most patients with Crohn's disease have terminal ileum and right colonic involvement, this is the best choice of studies to do first.

Once barium has been cleared from the colon and terminal ileum, an upper gastrointestinal small bowel series should be done to look for the presence of Crohn's disease in the small intestine. Findings are most typically confined to the terminal ileum or the terminal ileum and right colon. The changes one looks for include: 1) loss of mucosal pattern

in the involved area; 2) narrowing of the lumen of the bowel; 3) fistula formation; 4) edema, and 5) abscess formation.

Other Laboratory Studies. Patients with Crohn's disease should have CBC, total proteins, and albumin determined as well as liver function tests. Patients with Crohn's disease typically have hypoproteinemia and hypoalbuminemia. These occur either secondary to protein loss through the diseased mucosa and/or secondary to malnutrition from decreased appetite and intake. Twenty-five percent of patients with Crohn's disease in the pediatric population will show some evidence of abnormalities in their alkaline phosphatase determinations.

Treatment. Approach to treatment in patients with Crohn's disease is not dissimilar from that used in the treatment of ulcerative colitis. The major way in which treatment differs in the two is the fact that surgery may not be curative in patients with Crohn's disease. Furthermore, patients with Crohn's disease should not be offered a Koch pouch or endorectal mucosal pull-through for extensive colonic disease. Patients with Crohn's disease who have severe and incapacitating colonic disease requiring surgery may have an end ileostomy performed or an ileal rectal anastomosis if the rectum is spared.

Patients with Crohn's disease may have severe growth failure. This is due to a combination of decreased caloric and nutrient intake as a manifestation of the disease. The poor caloric and nutrient intake is in part secondary to the systemic effect of the disease as well as due to the crampy abdominal pain which occurred as part of the inflammatory changes present within the intestine.

Several studies have been done in patients with Crohn's disease substantiating the poor caloric and protein intake of patients with this illness. Several studies have documented that patients with Crohn's disease on the average take 60-80% of their required calories for age and sex. Similar studies have documented that patients with Crohn's disease take in suboptimal amounts of protein.

Extensive endocrinological studies done by a number of investigators have failed to show any consistent endocrinological abnormality in patients with Crohn's disease. Recently, certain investigators have shown that somatomedins, a group of polypeptide substances with growth hormone-like properties, may be diminished in patients with Crohn's disease. Patients with Crohn's disease, however, have been shown to have normal release of growth hormone during sleep

and when stimulated. However, somatomedins have been shown to be depressed.

The deficiency of these substances may in part be responsible for the delayed growth in patients with Crohn's disease. Several investigators have shown that patients with Crohn's disease can successfully gain weight and grow despite the presence of active disease when they were supplied with their nutrients either by means of parenteral nutrition or enterally by means of nasogastric tubes or through gastrostomy feedings. All these studies indicate that prolonged intervals of time in which the nutrients are provided are required in order for patients to begin to grow. A minimum of 3 months of nutritional support during which patients receive 125-150% of their basal needs are necessary to bring about a growth spurt.

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Surgical Management

John Alexander-Williams

Whether or not Crohn's disease and ulcerative colitis are different parts of the spectrum of the same disease or separate diseases, the principles governing their surgical management are essentially different. Therefore, the two diseases will be discussed separately.

CROHN'S DISEASE

General Principles

While most of the life-long management of patients with Crohn's disease is in the hands of the gastroenterologist it is usually the surgeon who is called upon to make the most dramatic and often life-saving acts. The satisfaction of surgeons in their frequent dramatic successes should be tempered by the realisation that almost all deaths due to Crohn's disease occur as a result of an operative complication.

In planning the strategy of the surgical approach to Crohn's disease it is essential to remember some important general principles.

Crohn's disease is a pan-enteric disease with focal exacerbations and with intermittent activity throughout the patient's life. It follows, therefore, that it is impossible to cure Crohn's disease by surgical excision.

The surgeon must realise that, as yet, it is impossible to cure Crohn's disease,it can only be managed.

In the overall management of the life-time problem of Crohn's disease the surgeon is likely to be needed on more than one occasion to manage certain specific complications that can be treated in no other way.

If the first principle is true then there is no such thing as recurrence of Crohn's disease, it is simply a recrudescence in a different site.

The Aims of Surgery

Operative intervention is required in Crohn's disease for one or more of the following specific reasons:

i) to release pus under tension,
ii) to remove, relieve or bypass areas of stenosis sufficiently severe to cause symptoms,
iii) to treat fistulas associated with recrudescence of active Crohn's disease; usually associated with stenosis,
iv) to prevent acute or chronic blood loss and, rarely,
v) to treat an impending perforation (toxic dilatation) or an established perforation.

As well managed patients with Crohn's disease usually live a normal span of life, they are likely to be under medical and surgical management for 40 or 50 years. As the risk of recrudescence of the disease, sufficiently severe to warrant treatment, is approximately 6 per cent a year, many patients will require four or five operations in their life time. Therefore, the surgeon should never try to cure all the disease at the first operation; he should treat only the specific complication that has occurred and he should not feel that the so called recurrence is an indictment of his surgical prowess. To rid the patient of all possible risk of recurrence is a practical impossibility.

Having defined principles and aims it is then possible to formulate some simple surgical rules:

Rule 1 When you operate only deal with the specific complication that has provided the indication for the operation.

Rule 2 Do not take out bowel just because it is affected by Crohn's disease.

Rule 3 To deal with the complication, do not take out more bowel than you have to.

Rule 4 Even in a defunctioned segment try not to leave stenosed bowel behind.

Rule 5 Take every step to make the operation as safe as possible.

Specific Rules

The specific indications for, and techniques of, surgical management differ in different parts of the alimentary tract. Although several parts of the tract may be affected simultaneously, they are usually affected to a different extent and there is usually only one part in which there is an indication for operation intervention. Therefore, the specific rules will be described under the different anatomical headings.

1. Gastro-duodenum

These organs are usually involved simultaneously although the duodenum is more likely to produce the first clinical manifestation, if only because it is the narrower part of the bowel. Gastro-duodenal Crohn's disease usually occurs in association with disease elsewhere in the alimentary tract although, rarely, it is the first manifestation.

The complications of gastro-duodenal Crohn's disease that require surgical intervention are bleeding and stenosis or both.

It is often difficult to differentiate Crohn's ulceration from peptic ulceration on endoscopy or biopsy. Useful additional diagnostic tests are acid secretory studies and the therapeutic response to cimetidine.

As it is not feasible to resect the duodenum, this is the one area of the bowel in which bypass is the preferred treatment; the best results appear to follow gastrojejunostomy (1). As the patients are usually young and may have gastric hypersecretion secondary to extensive small bowel resection, it is usually wise to add a vagotomy. Selective vagotomy has theoretical advantages in that it is less likely to provoke diarrhoea and this is what I aim to do. It is important to be certain that there are no distal stenoses present in the jejunum; these have been the cause of failure in two of our patients. Diarrhoea

after the operation has been a problem in some of our patients but there are, of course, many other factors responsible for diarrhoea in Crohn's disease.

2. Jejunum and ileum

Although the disease most commonly affects the terminal ileum there are often multiple foci of activity throughout the small bowel. When operating for recurrent incomplete obstruction it is often difficult to tell which of the multiple lesions is responsible for the symptoms.

Symptoms of obstruction are the commonest indications for surgical intervention in Crohn's disease of the small bowel; these are almost always sub-acute and, initially, intermittent. Weight loss is often marked because the patient is afraid of provoking symptoms by eating or is anorexic.

Abscess or fistula are the next commonest indications for surgical intervention. Their causes are essentially the same and are usually associated with some degree of stenosis.

In our series of over 700 major operations for Crohn's disease, acute bleeding from the small bowel has been the principal indication for operation in 4 and free perforation into the peritoneal cavity has been the principal indication in 8 (2).

When operating for obstructive symptoms surgeons now realise that it is best to avoid bypass, either in continuity or with exclusion. Bypass should be avoided because of the dangers of abscess and fistula arising at the site of the retained active stenotic disease, of perforation proximal to the stenosis or of the late development of carcinoma (3). There is less agreement among surgeons about how much of the bowel to resect. A number of recent studies have shown that the risk of recrudescence, necessitating further surgical treatment, is no greater in those patients whose resection did not go beyond the limits of diseased activity than in those in whom totally normal bowel was anastomosed (4). Furthermore, our policy of resecting only the stenotic segment has led us to anastomose bowel in which there is obvious macroscopic evidence of ulceration; we have found that this can be done safely. This question will be considered in greater detail in a following section on research.

Small bowel fistulas The most obvious and dramatic of the small bowel fistulas are the entero-cutaneous. In a recent review of 46 entero-cutaneous fistulas treated on our unit, 8 were post-operative following an anastomotic breakdown and not associated with residual or recurrent active Crohn's disease. Seven of these healed spontaneously with enteral or parenteral nutritional support. Thirty nine patients had fistulas arising from areas of recrudescence of Crohn's dssease; most associated with some degree of stenosis due to fibrosis or an associated abscess. None of these healed spontaneously or on medical management; all but two who died healed after surgical treatment, which included resection of the diseased bowel from which the fistula was arising (5).

In our series there were 33 entero-enteric fistulas, almost all of them produced no specific symptoms; many were found incidentally and some only after resection. The entero-enteric fistulas were not in themselves an indication for operation but, as they were usually associated with some stenosis of the bowel, it was the stenosis that was the indication for surgical intervention (6).

In our experience fistulas between the small bowel and urinary system are uncommon but are usually an indication for surgical treatment. Treatment usually consists of resection of the underlying bowel, with no specific treatment to the bladder apart from simple suture.

Abscesses Superficial abscesses, which usually present in the scar of previous laparotomies, require incision and drainage. Most, if not all, subsequently become fistulas and usually require definitive surgical treatment as soon as the patient's condition improves after drainage. It is important to remember that patients whose general condition fails to improve dramatically after the drainage of the superficial abscess may have an associated deep abscess with so tortuous and fibrotic a connection that it does not drain freely. Scanning by ultra-sound, X-ray or isotopes may reveal the associated deep asscess (7).

Deep abseesses detected before or during laparotomy have a similar pathology to that described under the section of fistula. There is almost invariably fibrosis and partial obstruction of the bowel associated with the deep ulcer that led to the abscess. The best form of treatment is to isolate and resect that part of the bowel from which the deep abscess is coming. In my experience this is always possible

although prolonged meticulous dissection is often required before all the loops of bowel are separated. Fortunately, the dissection of matted loops of the bowel in Crohn's disease is much easier than it is with other diseases such as diverticular disease or malignancy.

Large Bowel

Large bowel Crohn's disease is often associated and confluent with concomitant small bowel disease; in this case it is often the small bowel disease that dictates the need for surgical intervention.

The indications for surgical treatment in large bowel Crohn's disease are quite different from the indications in small bowel disease. Stenosis, abscess and fistula can occur but are much less common; the indications for surgical intervention are more likely to be chronic ill health with loss of blood and protein, growth retardation or socially incapacitating diarrhoea (8). An exception to this general rule is Crohn's disease of the rectum which is subject to fistulas and stenosis and which will be discussed below with perianal Crohn's disease. Rarely, acute fulminating Crohn's colitis behaves in a manner similar to fulminating ulcerative colitis with toxic dilatation and, rarely, perforation. The management is essentially the same as ulcerative colitis described below. It is usually failure of medical treatment that is the principal indication for surgery; unlike the indications for small bowel Crohn's disease when it is usually a specific fibrotic complication.

In our experience total Crohn's colitis responds less well to medical therapy than does ulcerative colitis and continued ulceration with bleeding and diarrhoea often forces us to perform a total colectomy. During the past 30 years the pattern of surgery for inflammatory bowel disease in Birmingham has altered dramatically. Thirty years ago we were having to perform colectomy frequently for ulcerative colitis and rarely for Crohn's colitis, whereas now the number of colectomies performed for Crohn's colitis outnumbers those for ulcerative colitis by 4 to 1. This change could reflect a change in referral pattern or a change in the natural history of the disease but is more likely to be related to the relative efficacy of medical treatment.

Operation The principal problems in making a decision about the surgical treatment of large bowel Crohn's disease is to decide how much bowel

should be removed.

When the large bowel disease is in continuity with terminal ileal Crohn's disease the large bowel disease is usually causing little trouble and the minimum should be removed. We have found that it is possible to perform an anastomosis quite safely to the large bowel even if it contains ulceration, provided there is no stenosis.

When a small segment of the colon is involved in Crohn's disease the resultant diarrhoea and blood loss is rarely sufficient to indicate surgical intervention and, therefore, the question of segmental colectomy rarely arises.

The question that is often difficult to answer is whether to perform a total colectomy and an ileo-rectal anastomosis or a proctocolectomy. As Crohn's disease sometimes spares the rectum completely, a total colectomy and ileo-rectal anastomosis is more often feasible in Crohn's colitis than it is in ulcerative colitis where the rectum is usually involved. We have recently reviewed our experience with 128 patients in Leiden, Holland and Birmingham, England. At 10 years after the operation more than half the patients still have a functioning ileo-rectal anastomosis and were very satisfied with their bowel frequency of 4 or 5 stools per day (9). It is rather more difficult to decide what to do for the patient who has minimally involved rectum in Crohn's disease with some fibrosis or in the patient with active perianal Crohn's disease. Some of the future developments in the assessment of patients with this condition will be discussed in the next section under research.

Our experience has taught us that minor degrees of perianal disease do not preclude a successful ileo-rectal anastomosis (10).

Rectal and Perianal Crohn's Disease

Perianal Crohn's disease is very common even in patients whose principal manifestation of the disease is in the small bowel. We found that approximately 50% of all our patients with Crohn's disease had some perianal manifestations; most were simply oedematous skin tags or small, chronic painless fissures (11).

Rarely, we have seen fulminating perianal disease that almost qualifies for the description "malignant". Fortunately, they are rare; none of them have ever developed in patients under review in our own clinic, all have been referred to us with advanced disease. We have

seen many hundred patients with perianal disease, 109 of whom we followed carefully for 10 years. Most patients had relatively little trouble 10 years later and some had even had spontaneous cure of a fistula. Perianal disease was the principal indication for proctectomy in only 11 patients.

In the majority of our patients perianal disease is painless; it's presence is often unknown to the patient and sometimes even to the gastroenterologist unless he has an enquiring mind and an enquiring finger! (11).

Anal fistulas in Crohn's disease frequently follow episodes of perianal sepsis. Once the spesis is drained the fistula often gives remarkably little trouble and sometimes the fistula track appears to heal completely; an uncommon finding in anal fistulas not associated with Crohn's disease. Many fistulas in Crohn's disease have occurred from infection in anal crypts or anal glands but some are caused by deep ulceration in the upper anal canal or rectum and these invariably give high anal fistulas. In the female, they may give recto-vaginal fistulas. We advocate conservative or minimal surgical therapy such as simple drainage of pus. As a cautionary note to this conservative doctrine I have to record that we have seen one patient develop an adeno-carcinoma in a recto-vaginal fistulous track that had been present for more than 10 years (12).

Most of the patients with Crohn's disease that we have seen with faecal incontinence have resulted from aggressive surgical treatment of perianal sepsis or fistulas.

Recto-vaginal fistulas associated with rectal ulceration also should be left alone if they are causing little trouble. However, if they are associated with severe rectal disease they are sometimes an indication for proctectomy. We have closed, successfully, one large recto-vaginal fistula by a conventional 3 layer closure while the patient had a deverting stoma. We have also used proximal stoma diversion in some patients with a persistently discharging ischio-rectal abscess A temporary proximal stoma is always worth attempting as on two occasions we have been able to restore intestinal continuity without impaired continence or recurrent sepsis.

Another difficult problem associated with perianal or low rectal Crohn's disease is the presence of a stricture. Some low strictures

have followed ill-advised anal operations, such as haemorrhoidectomy, but others have arisen spontaneously as a result of chronic ano-rectal ulceration.

I am always surprised by our ability to manage rectal or strictures conservatively. Even very long tortuous strictures that will not even admit the examiners little finger, can be treated by very gentle gradual dilatation, often repeated twice or thrice under general anaesthesia. By such treamment I have produced a functioning and painless anal canal and I continue to manage conservatively some patients that I first saw with an anal stricture ten or more years ago. These were patients with such severe symptoms that they had been advised to have a proctectomy because the physician thought the anus so destroyed by Crohn's disease.

The essential message in the management of ano-rectal Crohn's disease is that the surgeon should be as conservative as possible

ULCERATIVE COLITIS

Ulcerative colitis is a disease that is confined to the mucosa of the large bowel only. It is usually controlled readily by drug therapy. Surgical intervention is required only for one or more of 3 reasons:

i) fulminating exacerbations with risk of perforation or severe bleeding,

ii) chronic diarrhoea, with or without malnutrition, sufficient to make life less acceptable than life with an ileostomy or,

iii) the presence or serious risk of malignancy.

1. Fulminating Colitis

This usually takes the form of toxic megacolon but rarely is associated with perfuse bleeding. I should emphasize that we now see this rarely, and never in patients under regular medical supervision on our unit. We see it only in patients who are referred into our unit with toxic megacolon. The indications for surgical treatment are the absence of objective signs of improvement with a reasonable period of intensive medical therapy. A reasonable period is usually interpreted as 72 hours (13).

Our treatment of choice is an emergency total colectomy with end ileostomy and a mucus fistula of the upper rectum to the lower part of the midline wound. The only exception to this rule is when there is severe bleeding rectal disease, when we would perform a proctocolectomy.

In the last ten years we have seen no patients in whom we could not perform a total colectomy because of localised perforations and so have had no occasion to perform the operation of diversion ileostomy and decompression colostomies (14).

Our reasons for retaining the rectum in emergency colectomy is that it makes it a lesser operation and that, in some patients with fulminating colitis, there is relative rectal sparing. In some patients we have been able to perform an ileo-rectal anastomosis subsequently.

2. Elective Operations for Ulcerative Colitis are usually advised because of chronic continuous disease with chronic ill health or repeated sub-acute exacerbations interferring with normal life. Sometimes the indication is for intractable diarrhoea and urgency in a patient who is otherwise not particularly unwell. Many patients who continue under supervision in our inflammatory bowel disease clinic come to learn from other patients how good life can be with an ileostomy. Many patients make their own decision that the time has come to change the life of a chronic colitic for the fuller life of an ileostomist.

The type of operation performed depends on both the symptoms and on the health of the rectal mucosa. Although I am keen to perform ileo-rectal anastomosis whenever possible for ulcerative colitis I find that I can do this in less than 10% of the patients that we have submitted for surgical treatment. Usually, the rectum is too severely diseased or the patient has intractable diarrhoea and urgency and will not accept the prospect of an ileo-rectal anastomosis. In the past 30 years we have performed 450 operations for ulcerative colitis of which only 34 have involved a primary or secondary ileo-rectal anastomosis. Eight had to have the rectum removed subsequently and only nineteen patients have been followed for more than 6 years with a functioning ileo-rectal anastomosis; most of these have more than 3 stools a day. For a variety of reasons, some inexplicable, our experience is different from that of other centres (15). Our standard operation for ulcerative colitis is a pan-proctocolectomy with a conventional end ileostomy. Because of our limited experience, so far, with continent pouch operations we never perform these as a primary procedure.

3. Risk of Malignant Disease In the past we have performed ileo-rectal anastomosis in patients with chronic, relatively inactive disease of more than 10 years duration in whom it was felt that the risk of malignant

change was high. We then considered the risk to be so great that it would be much better to have the large bowel reduced in size to reduce the risk and so that the remainder could be kept under review by simple rigid sigmoidoscopy. Although some of these operations were highly successful this policy has been abandoned since we understand more about the risk of malignancy and have colonoscopy available for surveillance (16). The philosophy of the management of Crohn's disease and ulcerative colitis is a perfect example of collaboration not competition between internist and surgeon. Neither can, nor should try to, function in isoloation.

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E. New Directions for Future Research

INFLAMMATORY BOWEL DISEASE - NEW DIRECTIONS FOR FUTURE RESEARCH

JOSEPH B. KIRSNER

The etiology and the pathogenesis of ulcerative colitis and Crohn's disease of the small and/or large intestine remain obscure, despite the increasing familiarity with these disorders. The similar clinical features and course of IBD everywhere in the world, despite differing ethnic populations, environmental circumstances, dietary habits and socio-cultural customs and the rising incidence of Crohn's disease, apparently world-wide, suggests the involvement of environmental agents (microbial, dietary, pollutants, etc.). Attempts to clarify the nature of ulcerative colitis and Crohn's disease on the basis of discernible tissue and cellular reactions, as determined by light, scanning and electron microscopy and histochemical and immunological reactions are informative and undoubtedly significant, but their interpretation is limited by questions as to the constancy and specificity of the observed changes, by the influences of anti-bacterial, adrenocortical steroids and other therapeutic agents, nutritional state, by individual differences in host response, and by the likelihood that both the small and the large intestine have a limited repertoire of tissue responses regardless of the inciting etiologic mechanism. Multiple granulomas in the resected bowel wall and rectal biopsies are helpful in the diagnosis of Crohn's disease, but are absent in 40% of patients; and, once present, they may disappear; their pathogenetic significance remains unclear, but probably is limited. An important future need is a universally acceptable histopathological "check list" to facilitate the comprehensive consistent examination of IBD tissues, with the ultimate objective of unifying terminology and facilitating the more accurate diagnosis of IBD. The study of mediators of tissue injury in IBD should clarify the nature of the process, even though unlikely to resolve etiology and pathogenesis. In this regard, studies of prostanoid synthesis and the effects of sulfasalazine are of interest but present evidence does not yet establish inhibition of prostaglandin E_2 production as the mechanism of the beneficial effect of sulfasalazine in ulcerative colitis. The alter-

nate concept of a cytoprotective effect of prostanoids deserves further consideration in view of the known cytoprotective effects of prostaglandins in the upper gastrointestinal tract. The large number and the variety of the local and systemic complications of IBD are unique to these disorders. Since they almost invariably are secondary to the bowel inflammation, they direct attention to the diseased bowel as the likely source of various systemic disease. More detailed study of the systemic complications of jejuno-ileal bypass operations could be investigated in appropriate animal models; such observations should produce useful information regarding human IBD.

More directly, numerous experimental attempts to reproduce human IBD by diverse procedures thus far have failed. A variety of enteric and colonic inflammatory bowel diseases are found among various animal species, including an ileitis in pigs, a granulomatous enteritis in horses, and a colitis in Boxer dogs, but these "natural" animal states have not been investigated thoroughly and their possible relationship to human inflammatory bowel disease is not known. The carrageenan model emphasizes the role of microbial agents and possibly immune responses. However, the experimental conditions are so far removed from the practical circumstances of human IBD as to limit clinical applicability of the experimental findings. Nevertheless, the lack of a suitable animal model of ulcerative colitis or Crohn's disease has handicapped investigations of the IBD problem; justifying the continued search.

Psychogenic disturbances, though clinically important in the course of IBD remain to be clarified, as to their specificity, their patho-physiological effects upon the bowel and their capacity for inducing a tissue reaction such as IBD. This approach now is more attractive in view of the recently demonstrated distribution of endocrine, paracrine and neurocrine cells and opioid peptides in both the neuraxis and gastrointestinal system, secreting the same or similar peptide messengers. The observations presented on this aspect of IBD re-emphasize the importance of careful structuring of psychiatrically-directed clinical studies, to avoid over-emphasis or misinterpretations, appropriate pharmacological, endocrine and biochemical measurements as concomitantly.

Microbiological causes thus far have eluded extensive investigations. Currently, intestinal anaerobes, Yersinia enterocolitica, pathogenic forms of E. coli, and bacterial endotoxins and other metabolites are of interest. Continuing search for "new" microbial agents in IBD is desirable in view of

the recent identification of campylobacter, aeromones hydrophilia and Yersinia as causes of enterocolitis. Studies of the apparent "transmissability" of Crohn's disease (and ulcerative colitis) initially suggesting a viral etiology, now are directed towards low molecular weight cytotoxins whose relevance remains in question. The systemic distribution of granulomas (face, muscle, bone, epiglottis) noted very occasionally in Crohn's disease patients is of interest in this connection. Substantial gaps remain in our knowledge of the gut microflora, the intestinal defenses against microbial injury, including the role of the gastrointestinal secretory immune system (e.g. IgA) and the gut lymphoid apparatus.

Consideration of immunological mechanisms in IBD includes experimental immunological attempts to reproduce either ulcerative colitis or Crohn's disease (thus far unsuccessful); the non-specific colon "autoantibodies", circulating antigen-antibody immune complexes; cell-mediated immune responses including the respective roles of T and B lymphocytes, including suppressor cells, the possible significance of lymphocyte cytotoxicity for colonic epithelial cells, the possible role of the gastrointestinal lymphoid tissue including intra-epithelial lymphocytes, and the gastrointestinal secretory immune system, especially secretory IgA in the evolution of the IBD tissue reaction. Present evidence, demonstrating the local accumulation of immunoglobulins and various components of complement (Clq, C_3, for example) in addition to B-lymphocytes, though yet incomplete, suggests an immunologically-mediated reaction in the bowel wall, both in Crohn's disease and ulcerative colitis; probably as an epiphenomenon, yet contributing secondarily to the tissue reaction.

The diminished response of circulating lymphocytes to various mitogens, especially in Crohn's disease but occasionally also in ulcerative colitis, has suggested to some observers the presence of an immunodeficiency state in Crohn's disease, but the evidence is inconclusive. Lymphocyte responses are variable; and reactions may be diminished in a wide variety of circumstances including under-nutrition, trace metal deficiencies (e.g. zinc), advancing age, operations such as cholecystectomy and also by inhibitory factors circulating in the blood of patients with IBD, perhaps aggregated immunoglobulins or antigen/antibody complexes.

Possible genetic influences, facilitating the development of IBD, including immune defects (e.g. selective immunoglobulin or complement deficiency) associated with particular histocompatibility haplotypes (e.g. C_2

deficiency associated with the $A_{10}B_{18}$ haplotype); in addition to the already emphasized association between ankylosing spondylitis and the HLA-B_{27} haplotype and a genetically-mediated disorder of immune regulation deserve more study. With regard to "familial IBD", the possible role of a common and prolonged exposure of families living in the same household to an environmental agent (possibly a "slow virus") is suggested by the increased incidence of lymphocytotoxic antibodies (LCA) among some IBD families; however, the evidence for such a mechanism remains insufficient.

A potentially useful hypothesis involves the combination of external agents (dietary, microbial antigens) and genetically-mediated host responses; wherein ulcerative colitis and Crohn's disease are considered prototypes of a single disease entity; with multiple intermediate reactions. Sensitization of the host to bacterial and other antigens presumably occurs early in life. Enterobacterial antigens, for example, reach the gut lymphoid tissues before maturation of the gastrointestinal immune apparatus. Subsequent injury to the intestine (e.g. an enteric infection, drugs, ischemia) increases intestinal permeability to various antigens, precipitating an immunulogical inflammation in the bowel wall. The validity of such a concept of course, requires further investigation, but coordinates three attractive pathogenetic mechanisms: genetic vulnerability, the involvement of an external agent and an altered immune response.

The clinically-oriented suggestions for further investigation of IBD appropriately focus upon the IBD patient and re-emphasize the many potentially productive clinical areas for further research: the natural history of IBD, its epidemiology, course, complications and response to various medical and surgical therapies. In turn, these recommendations direct attention to the need for more uniform approaches to morphological, clinical, epidemiological, laboratory and therapeutic aspects; the need for generally acceptable definitions of ulcerative colitis and Crohn's disease; and for clinically practical yet clarifying guidelines as to the severity, activity and prognosis of IBD; investigative requirements which, though widely appreciated, have yet to be fully met.

In conclusion, though the etiology and the pathogenesis of ulcerative colitis and of Crohn's disease remain elusive, the "non-specific" inflammatory bowel diseases have merged as important clinical problems requiring better understanding and management. They also serve as "Rosetta Stones", facilitating clarification of both gastrointestinal and non-gastrointestinal

disorders, through the application of basic scientific knowledge, increasingly sophisticated study of the immunological features of the IBD tissue reaction, in the study of the local and systemic problems, and in the investigation of host defenses and genetic-immunlogical interactions. With this broad research "roadmap" significant advances can be anticipated in the not too distant future; progress which hopefully will clarify the nature of non-specific inflammatory bowel disease and resolve a most challenging medical problem.

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NEW DIRECTIONS FOR MEDICAL RESEARCH IN INFLAMMATORY BOWEL DISEASE

DAVID B. SACHAR M.D.

A. The Human Experimental Model

This section of the Symposium--new directions for future research-- is separated into medical, surgical, and experimental aspects. Yet this separation is actually somewhat arbitrary and even artificial. In fact, these three categories of medical, surgical, and experimental research often converge. One example of this convergence is the study of the ideal human experimental model for Crohn's disease -- namely, the postoperative patient.

Postoperative patients represent the clinical investigator's dream. They constitute a population of patients in whom we can predict with nearly mathematical precision a rate of disease recurrence of approximately 17% a year (1), producing by five years of follow-up a cumulative recurrence rate of about 50%, depending upon the clinical and anatomic patterns of underlying disease (2,3)

This postoperative population therefore represents a natural laboratory, not only for assessing those clinical and pathological features that may influence the rates of postoperative recurrence, but especially for unraveling the significance of those microbial and immunologic aberrations sometimes associated with inflammatory bowel disease. Longitudinal studies of intestinal microbial population and of cellular and humoral immune status, performed on a large cohort of postoperative patients and correlated with changes in their clinical conditions, should help unravel some of the cart-versus-horse dilemmas surrounding the multitude of immunobacteriologic abnormalities described in ileitis and colitis.

Even if it should turn out in studies of this nature that immune derangements are secondary manifestations of disease, rather than primary etiologic mechanisms, the fact remains that immunologic techniques still hold promise in unlocking etiologic mysteries New methodologies for antigen identification in immune complexes, for example, have yet to be extensively applied to inflammatory bowel disease. Yet for all this, immunology does not seem to be as certain a key to solving the problems of ileitis and colitis as it did fifteen to twenty years ago. In the 1960's, the scientific community was prepared to place most of its hopes for solution of these problems on the burgeoning science of immunology. Today, if we were asked which biomedical discipline offered the brightest hopes for solving the puzzles of inflammatory bowel disease, many of us might vote instead for epidemiology.

B. Epidemiologic Tools

These days, we tend to think of inflammatory bowel disease research in terms of immunoglobulin production by gut mucosal lymphocytes, or suppressor cell activity as measured in mixed lymphocyte culture. But it might be time to take a step back from the microscope to apply some of the older tools of epidemiology. Here we see Crohn's disease behaving in many population centers like a classical epidemic disease, emerging in relatively modern times and doubling in incidence with each passing decade. Why don't we mount more of our traditional responses to a challenge of this nature? Why shouldn't epidemiology intelligence officers move into the high-risk areas and gather data on everything from dietary habits to patterns of dental care, from drinking water supplies tc brands of toothpaste, from food additives to cooking utensils?

Just as the immunologist and microbiologist have a natural laboratory in the postoperative patient population, so too do the epidemiologist and geneticist have a natural laboratory: the family. At least 15% of ileitis and colitis patients have close relatives with inflammatory bowel disease (4). These families need to be more throroughly exploited for studies of genetic markers (especially at the HLA-D locus) to identify hereditary influences. Immunologists are also getting back into the picture by studying

families for serologic and other immunologic markers of environmental pathogens.

C. Natural History

In the laboratory of the postoperative patient population, and in the laboratory of the family, we have seen that the boundaries between medical, surgical, and experimental research tend to melt away. The same phenomenon applies to the laboratory of the indibidual patient, in whom the natural history of disease may also provide clues to pathogenesis. Three examples may suffice to make the point.

First, the "missing links" between underlying inflammatory bowel disease and extraintestinal manifestations must hold some valuable pathogenetic clues. Recent evidence linking the so-called "colitis-related" manifestations(5) to circulating immune complexes is suggestive but still tenuous. Even the clinical dogma that such complications as pyoderma gangrenosum parallel the acute inflammatory activity in the bowel have come into question(6). Meddical, surgical, and etiologic issues all converge, therefore, in such simple problems as trying to determine the influence of bowel resection on the subsequent course of pyoderma gangrenosum.

A second "missing link" needs to be found to connect inflammatory bowel disease with the associated complication, sclerosing cholangitis. More than half the cases of primary sclerosing cholangitis currently recognized in large series appear to be associated with ulcerative and perhaps Crohn's colitis (7). Up until recently, sclerosing cholangitis was appreciated only as a very late complication of inflammatory bowel disease, often not appearing until a decade or two after colectomy. We are now, however, on the verge of a new understanding of sclerosing cholangitis as comprising a pathophysiologic spectrum. This spectrum extends all the way from the elevated alkaline phosphatase which we used to shrug off as "pericholangitis", to advanced bile duct inflammation with obstruction and sometimes even malignant transformation. The triple goals of medical, surgical, and etiologic research may therefore be furthered by initiating earlier studies of the extrahepatic biliary tree in colitis patients, utilizing the newer tools of retrograde cholangiography.

The third and most familiar "missing link" in studies of natural history also stands at the intersection of medical, surgical, and experimental research. I refer to the association between inflammatory bowel disease and cancer. Other sections of this Symposium are devoted in detail to this vital issue, but I would like to offer two very brief predictions. The first is that in cases of similar disease duration and anatomic extent, Crohn's colitis and ulcerative colitis may ultimately prove to carry the same potential for colorectal carcinoma(8). The second prediction is that given the selection biases inherent in retrospective hospital studies, the future development of reliable information about cancer risk in IBD will have to depend upon longterm prospective studies in defined catchment areas(9).

D. Medical Therapy

There are at least four promising avenues for future research in medical therapy.

1. Clinical testing of existing drugs. Several currently utilized drugs merit further clinical study. Three examples come immediately to mind. First are antimetabolites of the azathioprine family. The National Cooperative Crohn's Disease Study has demonstrated that the short-term use of azathioprine as a single drug is only slightly better than placebo, but not statistically significantly better, in lowering the Crohn's Disease Activity Index in a group of 136 patients(10). Another study of 83 patients at Mount Sinai and Lenox Hill Hospitals in New York City, however, has shown that the long-term use of 6-mercaptopurine, as a supplement to conventional therapy, is unequivocally better than placebo in healing fistulae($p<0.01$), in allowing reduction of steroid dose ($p<0.001$), and in improving general well-being ($p<0.0001$)(11). The precise role of immunosuppressive drugs in practical therapy must therefore be better defined. In particular, the time is now ripe to determine, by prospective clinical trials, whether the early use of azathioprine in combination with prednisone will have any substantial benefits over prednisone alone in the routine treatment of active Crohn's disease. Indeed, such a multicenter cooperative trial is already in preparation under the experienced guidance of Dr. John Singleton.

A second obvious example of the need for randomized trials of existing drugs is in establishing the role of metronidazole. This drug today occupies a niche in the treatment of Crohn's disease rather analogous to that occupied by immunosuppresive drugs 15 years ago. The possible benefits of 6-mercaptopurine and azathioprine were first suggested by uncontrolled reports similar to those recently emerging for metronidazole(12). In view of the colossal difficulties of any drug evaluation in chronic and complicated illnesses like Crohn's disease (13), it might be prudent for prospective trials to narrow their focus to specific disease manifestations--in the case of metronidazole, for example, concentrating particularly on the control of perineal fistulae.

A third need for randomized clinical trials is in the use of antibiotics. Uncontrolled reports have already appeared (14), and an extensive "underground" use of ampicillin and tetracycline and other broad spectrum antibiotics has developed, but a controlled, prospective therapeutic trial is badly needed.

2. Pharmacologic modification of current agents. Besides the further testing of existing drugs, a second important avenue for medical research is the pharmacologic modification of current agents so as to improve therapeutic ratios. Two examples are appropriate. First, sulfasalazine can be improved. Evidence is accumulating that intraluminal 5-aminosalicylate might be the therapeutically active moiety of the sulfasalazine molecule, while the toxic effects are primarily attributable to the sulfapyridine component. Since this latter component might serve only as a vehicle for the delivery of 5-aminosalicylate to the lower GI tract, it seems inevitable that new sulfasalazine analogues will soon be developed in which the sulfapyridine is replaced by a more innocuous substance. By the same token, analogues could be designed with a weaker molecular bond, so as to

permit the release of the 5-aminosalicylate somewhat more proximally in the GI tract, perhaps by enzymatic action from bacbacteria as lower concentrations than those achieved only in the colon. In this way, the new agent might have greater efficacy than sulfasalazine in Crohn's disease of the small bowel. Similarly, poorly absorbable salicylate analogues for oral administration as well as preparations of 5-aminosalicylate for rectal instillation will be logical developments in the very near future (15,16).

A second opportunity for pharmacologic modification resides in the area of topical steroid instillations. Substantial fractions of steroid enemas and foams are absorbed from the colon and may produce undesirable systemic effects. Surely we will soon be seeing new enema preparations of corticosteroids that are active topically but poorly absorbed systemically.

3. Development of novel pharmacologic approaches. Besides clinical trials and pharmacologic manipulations of existing drugs, a third crucial avenue for future research will be the development and application of new approaches to medical therapy. Dr. Rachmilewitz and his colleagues are already pointing the way along one such path. They are spotlighting the role of locally produced prostaglandins as mediators of inflammation. Perhaps prostaglandin -inhibitors (of which 5-aminosalicylate is one representative) should be more thoroughly explored for their therapeutic potential in inflammatory bowel disease. Conversely, exogenously administered prostaglandins might be studied further for cytoprotective actions in the lower intestine analogous to their effects in the stomach.

Another form of treatment which has been explored only tentatively to date is immunopotentiation. This approach to therapy is based on the observation that many patients with IBD appear to have defective cellular immunity (17), suggesting that correction of the immunologic deficits might thus enhance resistance to the etiologic agent(s) of the disease. I am, however, skeptical of this approach for two reasons. First, I believe that the immune derangements of IBD are more likely to be secondary manifestations of the underlying pathologic process than primary

factors in disease susceptibility (18,19). The second reason for my lack of confidence in immunostimulation therapy is that in most clinical trials it has failed to work. Neither BCG nor transfer factor nor levamisole has acquired a particularly impressive therapeutic track record (20). At Mount Sinai, we just have completed a three-year double-blind palcebo-controlled study of levamisole in 19 patients with Crohn's disease; we are in the process now of breaking the codes, but have seen nothing yet to persuade us that this form of treatment is effective.

4. Dietary therapy. According to the popular nutritional press, if we would only prescribe the proper diet for our patients, there would no longer be any ileitis or colitis for us to contend with. The problem, alas, is not that simple, but dietary therapy still merits investigation. There are three general approaches to dietary research. The first is to study broad dietary modifications, such as restriction of refined carbohydrate, addition of supplementary fiber, substitution of elemental diets, or even total parenteral nutrition. This approach is the hardest of all. Problems of patient selection, randomization, compliance, control diets, blinding, bias, and assessment of long-term outcome are so formidable as to be almost prohibitive. After 20 years, for example, controversy still rages as fierecely as ever concerning the effectiveness of dietary modification for the reduction of cardiovascular mortality. Is dietary research likely to be any more conclusive in the slippery field of IBD? After decades of fussing over even such simple questions as whether or not to let our colitis patients drink milk, are we on any sounder footing now than we were 20 years ago? How much more frustrating will it be to try to prove the merits of one or another total dietary prescription? Nonetheless, the effort could succeed. Two essential prerequisites for success are (1) equally strict enforcement of test and control diets (as opposed to the comparison of test versus ad lib diets), and (2) standardized criteria for the assessment of efficacy. We will return to this latter point at the conclusion of this section, because it has application to all aspects of therapeutic research.

A second approach to dietary therapy is the use of specific dietary supplements, such as particular vitamins, minerals, or medium-chain triglycerides. The techniques and mechanics of this type of research are really no different from those utilized in other clinical trials. The particular benefits of zinc or magnesium, vitamin A or MCT oil, or other nutritional supplements can thus be studied in the same ways by which we study metronidazole of sulfasalazine or other conventional medications.

Finally, there should be room for innovative approaches to diet just as to drugs. Are there bile salt-binding substances more palatable and convenient than cholestyramine? Are there compounds that can bind dietary oxalate in the gut and thus prevent enteric hyperoxaluria? Are there hydrolytic enzymes we can add to foods to break down indigestible dietary components and reduce the frequency of obstructive episodes? Food is a sufficiently important part of life that it deserves some attention in planning the medical management of patients with IBD.

E. Assessment of Efficacy

There is as yet no in vitro or animal model of inflammatory bowel disease sufficiently well established to serve as a test system for the evaluation of therapeutic agents. Neither the granuloma-transmission model, nor the fetal gut organ-culture model which we are using at Mount Sinai, nor the various natural and experimental animal disease models are yet close enough to human ileitis and colitis to provide reliable therapeutic assay systems. For at least the foreseeable future, therefore, I believe that we are tied to clinical trials, with all their headaches and pitfalls. Central to any form of clinical trial is a method of measuring clinical response. If we are studying agents to prevent hyperoxaluria, we can simply measure urinary oxalate. If we are studying MCT or other food supplements to increase body weight, we can--among other things--weigh the patients. But if we are studying the effects of medical treatment on the overall course of the disease, what do we measure then?

This is not the place in which to address the whole thorny question of assessment of disease activity; this issue has been more thoroughly explored in other fora (21). However, we must recognize at least three broad categories of clinical measurement to be considered in any therapeutic studies. One is an activity index. Second is a patient's and physician's global assessment of well-being. Third is the progress toward specifically identified goals of therapy for each individual patient: decrease in steroid dose, closure of fistulae, reduction of diarrhea or bleeding, increase in height or weight, elevation of hemoglobin or serum albumin, lessening of analgesic requirement, etc. psychosocial considerations should also be included in this catalogue of therapeutic goals(22).Whatever measurements are chosen, the point is that not every patient can be fitted into the Procrustean bed of a single activity index; individual treatment goals should be considered in each case. After all, this is the way we practice medicine, so why shouldn't it also be the way we conduct medical research?

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New Directions for Future Research:Surgical/Clinical

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In the management of inflammatory bowel disease the surgeon has one important weakness - he is dangerous. Although the surgeon has the ability to make the most dramatic curative or palliative moves in the overall management of the disease he should be aware that he can inflict the most harm to the patient; almost all deaths in inflammatory bowel disease can occur because of post-operative complications. The goal for the surgeon is to become less dangerous and his most important research endeavours should be directed towards this end.

Let us consider why surgical treatment is dangerous. The principal reasons are:

1) operations are followed by septic complications,
2) intestinal anastomoses may break down,
3) the site of the anastomosis seems to predispose to recrudescence of Crohn's disease,
4) the surgeon may eventually remove too much small bowel in Crohn's disease and,
5) in performing a proctocolectomy the surgeon removes the patient's continence.

Our research endeavours should be directed towards reducing or abolishing these risks.

1. RESEARCH INTO THE PREVENTION OF SEPTIC COMPLICATIONS

All operations on the alimentary tract are prone to septic complications resulting from contamination of the tissues by organisms found in the lumen of the gut. Normally, these risks are minimal after operations on the upper gastrointestinal tract and maximum when the colon is operated upon.

The risks in Crohn's disease are particularly great because:

i) the presence of strictures may predispose to the proliferation of faecal organisms in the proximal obstructed bowel. The jejunum, which normally contains relatively few organisms, can become as heavily contaminated as the colon (1),

ii) the essential pathological feature of Crohn's disease is a breach of the mucosal integrity with crypt abscesses or abscesses penetrating the wall of the bowel. Therefore, when operating on this disease the risk of contamination is not only from opening the lumen but also from dissecting the surrounding tissues and possibly the lymph nodes.

iii) the risk of infection is greater in patients compromised by malnutrition or by drug therapy that affects the immune response; both aggravating factors are common in inflammatory bowel disease.

Recent endeavours to diminish the risk of infection after intestinal operations have concentrated on the following methods:

i) cleansing the bowel and faecal material,

ii) reducing luminal organisms and,

iii) providing adequate tissue levels of appropriate antibiotics to prevent the proliferation of liberated organisms.

There has been much prospective research work on the prevention of infection in patients having large bowel operations for malignant disease. So far there has been relatively little work on patients with inflammatory bowel disease (2). The particular reasons that have inhibited prospective research endeavours in inflammatory bowel disease are the fear that mechanical bowel preparation may be dangerous and the absence of precise knowledge of the organisms that inhabit the lumen and the tissues. Some physicians have felt that mechanical bowel preparation by enemas, purgation or ortho-grade lavage are contraindicated in inflammatory bowel disease because of the dangers of exacerbating the disease or perforating a damaged bowel. We believe that many of these risks are grossly exaggerated and we are accumulating evidence to indicate that mechanical preparation can be undertaken safely in inflammatory bowel disease, prospective research projects are being undertaken.

Obstruction by fibrotic complications means that there is a similar concentration of luminal organisms in the small as well as in the large bowel. It is not known, as yet, what are the infective risks produced by operating into the inflamed and oedematous tissues beside the gut. Accurate quantitate bacterial studies of these tissues and of lymph nodes has to be undertaken.

Single dose or 24 hour prophylactic antibiotic therapy appears to be sufficient to decrease the risk of infection in operations on the bowel, apart from inflammatory bowel disease. However, our studies have indicated that a similar short prophylaxis is inadequate in the prevention of a high rate of infection after operations on inflammatory bowel disease. We need to know how long antibiotic prophylaxis should be continued to reduce significantly the infective risk of inflammatory bowel disease, what antibiotics should be used and for how long.

We need to know what tissue levels of antibiotics are reached in patients with inflammatory bowel disease and whether this is different in different stages of inflammation. We would like to know whether the acute tissue inflammatory process found in these diseases has any affect on the antibiotic concentration or clearance of antibiotics. Are special antibiotics needed in prophylacis or therapy in inflammatory bowel disease?

2. RESEARCH INTO THE PREVENTION OF ANASTOMOTIC BREAKDOWN

The most serious intra-abdominal complication of operations for Crohn's disease is anastomotic breakdown. The factors that might be responsible for a predisposition to anastomotic breakdown in this disease are:

i) the nature of the disease itself,
ii) sepsis,
iii) poor blood supply,
iv) malnutrition.

1) We will see, from evidence presented later, that bowel affected by Crohn's disease apparently has a good blood supply, strong fibrous tissue to take sutures and it appears to heal well. There seems to be no indication that Crohn's disease itself hinders healing of anastomoses.

2) Sepsis undoubtedly has an affect on healing anywhere in the body and adequate experimental evidence exists to show that sepsis impairs

the healing of intestinal anastomoses.

3) The failure of an intestinal anastomosis is sometimes attributed to poor blood supply, particularly so in the colon. One of the problems peculiar to operations in Crohn's disease is that the very oedematous, thickened mesentery of the gut makes it easy to damage the blood supply during dissection. The damage may be greater if the surgeon attempts to remove enlarged lymph nodes. The thickening of the mesentery also makes it difficult to tell whether there is good arterial pulsation up to the limit of resection. The surgeon often has to rely on the colour of the bowel and the presence of arterial bleeding on the cut edges to make a judgement as to whether it is safe to anastomose.

Experimental research in Dr Carey's department in Columbus, Ohio, U.S.A. has shown that the local application of Doppler ultra-sound enables the surgeon to predict accurately the risk of anastomotic failure. Unfortunately, it is difficult to apply this principal to a prospective clinical study in inflammatory bowel disease because of the difficulty of standardizing the other detrimental factors such as sepsis and malnutrition. It would be interesting to know whether the application of intra-operative Doppler studies would decrease the anastomotic dehiscence rate of intestinal anastomoses. Unfortunately, I cannot see how we could make such a clinical experiment "blind". However, it is a study that warrants consideration.

4) I believe that malnutrition is the most important factor mitigating against primary healing of intestinal anastomoses. Fortunately, at the moment in our clinical practice anastomotic breakdown is a rare event. However, two recent incidences of leakage have occurred in patients who had an anastomosis when their serum albumin was 24 and 25 gms per litre respectively. Some surgeons maintain that, at this level of hypo-albuminaemia an anastomosis should not be attempted in inflammatory bowel disease but, rather a stoma should be created until the patient is well nourished and then the anastomosis performed secondarily. A useful prospective study would be to document anastomotic failure in patients who have to have an acute emergency resection while they are malnourished and to see whether the overall complication rate is higher; time to total recovery is longer if a primary anastomosis is made than if the anastomosis is delayed with the patient having a diversion stoma until nutritional deficiencies are corrected.

Another valuable study would be to randomise the treatment of patients who need an elective resection and are malnourished, allocating them either to receive enteral or parenteral nutrition for a period or to have immediate resection and anastomosis. The problem with such a prospective study is that in order to achieve sufficient numbers it would need to be a multi-centre trial and it would be difficult to standardise the other factors affecting the risk. If such a study is not performed we may find ourselves in the time consuming and expensive position of having to conform with the dictum of those who maintain that it is negligent not to restore adequate nutrition before performing anastomoses. Certainly, commercial pressure will be on the side of such a dictum. Preliminary work from our unit has suggested that pre-operative weight has no impact on the risk of post-operative complications (3).

3. RESEARCH INTO ANASTOMOTIC PREDILECTION

Recrudescence or apparent new activity of Crohn's disease is particularly common imediately proximal to an intestinal anastomosis. Such an observation is so common-place as hardly to need challenging. However, the unequivocal scientific figures and measurements to prove that it is a fact not a myth have not yet appeared. If we accept what appears to be obvious, then we need to ask the following questions :

i) is it related to the blood supply at the site of anastomosis,

ii) is the physical trauma of the passage of intestinal contents through a narrow segment or,

iii) is it due to the relative hold-up of faecal material at the site of the anastomosis enabling the intra-luminal damaging agent to spend longer in contact with the susceptible mucosa?

1. We have conducted angiographic and micro-angiographic studies of suture line recurrences in patients requiring reoperation for Crohn's disease. Within the limits of our measurement technique it appeared that the blood supply at the site of recrudescence proximal to the stenosis was as great or greater than that of an affected bowel. From these crude studies it did not seem that diminished tissue oxygenation or perfusion is an important factor in our quest. However, it is possible that we could devise more efficient methods of measurement to reinvestigate this question.

2. To investigate the question of the possibilities of physical trauma at the narrow section of the anastomosis or hold-up of faecal content we could consider applying clinical trials and sophisticated physical measurement I would like to institute prospective randomised multi-centre clinical trials to compare the results in a group of patients who have an intestinal anastomosis performed end-to-end, by an agreed standard technique, with a group having some modification of a side-to-side technique, possibly with staples. Such a study would need to be documented carefully and followed for at least five years; by which time a cure for Crohn's disease could have been discovered and operations made obsolete!

3. Measurement of the rate of progress of food through the alimentary tract and the relative time at which the bolus was held up in various parts of the bowel can possibly be made with the aid of isotopes and external scanning. I envisage a test being developed to monitor the passage of radio active peanuts through the alimentary tract in patients who have had a resection for Crohn's disease. On a prospective basis we could attempt to delineate segments of relative hold-up to see whether these are areas with a predeliction for recrudescence. This would also be a long-term study but it could possibly breath new life into the flagging peanut industry!

4. RESEARCH INTO THE PREVENTION OF UNNECESSARY RESECTION

Patients with Crohn's disease usually need more than one operation during the course of their life and a few have so many operations that they eventually become short of functioning small bowel with consequent disorders of absorption. Although this fear dominates the mind of some conservative gastroenterologists it is surprising how rarely this presents a serious clinical problem; even in a centre such as ours with an aggressive surgical policy. We have a patient whom we inherited when he had already had ten operations; he has now had 17! At the last laparotomy he had only about 60 cms remaining from the duodeno-jejuno flexure to his end stoma. Even with so short a gut he is still able to work most of the time, although not surprisingly there are some intermittent nutritional and electrolyte problems.

Despite the fact that patients can lead a relatively normal life with much of their small bowel resected it is obviously a desirable goal to preserve as much bowel as possible. This is a particularly important

goal in a disease that runs a continuous chronic course and may need many operative interventions. Let us then pursue the following logical argument:

i) if we accept that we have to operate in Crohn's disease principally for stenotic complications and,

ii) if we accept that we should take away the minimum of tissue,

iii) can anastomose safely areas of the bowel even if they are ulcerated and

iv) if we accept that it is a bad principal to leave behind stenotic areas in the bowel -

then we could consider basing the surgical treatment of Crohn's disease on simple mechanistic principles similar to those used by vascular surgeons in dealing with athero-sclerotic lesions of large vessels.

It might be possible to simply overcome the stenotic segment by a longitudinal incision and transverse suturing of the gut as in a pyloroplasty for duodenal ulcer disease (Fig. 1). I have tried this manoeuvre on 15 separate occasions for simple short strictures associated with Crohn's disease, particularly with skip lesions proximal to a major lesion. It was a simple procedure to sew the gut with a single layer of continuous absorbable suture material (Fig. 2). So far the results have been entirely satisfactory. Spured by this success I have also attempted the same manoeuvre in a patient with stenoses of more than 10 cms long (Fig. 3). Under these circumstances a simple transverse suturing, as in a Heinecke-Miculicz pyloroplasty is not feasible; the stenosed bowel had to be sutured in a manner more like a Finney pyloroplasty (Fig. 4).

Having established that this technique is technically feasible and having had no complications from the pilot study, I think that the time has come to plan a prospective clinical comparative study, in patients with small bowel Crohn's disease, comparing non-excisional stenosis-relieving surgery with conventional excisional surgery.

5. RESEARCH IN ULCERATIVE COLITIS

In addition to measures to help decrease the general risks of excisional surgery, which are the same as they are in Crohn's disease, the particular field for improvement in the surgical management of ulcerative colitis is directed towards the restoration or maintenance of continence. The research and development associated with the continent pouch ileostomy

concept has been described in detail already. Much remains to be done in the perfection of the techniques for the simple creation of a nipple valve that will not extrude. There is room for development in the technique of creating a continent perineal pouch, using the musculature of the lower rectum and the anal sphincters to maintain continence. I believe that this field of research will be one of gradual development and innovation and will not involve scientific trials.

I think that it is unlikely that any form of artificial sphincter will be able to be created to control the output from a conventional ileostomy or an ileostomy with a simple pouch but no nipple valve. I do not think that it is likely that we will see much development in synthetic implantable valves or magnetic devices to give pouch continence. Also I think that it is unlikely that there will be any place for the development of techniques for mucosal grafting that will enable the rectal mucosa in ulcerative colitis to be replaced by small bowel mucosa.

In summary, I believe that the future trends in the surgical management of inflammatory bowel disease will mostly be centred around the surgical management of Crohn's disease. Operations will become much safer and ill and malnourished patients will be better able to be prepared for surgical intervention. I believe that we should move into an era of minimal repairative surgery, principally directed towards overcoming stenosis. I think that in the next 10 years we will have moved away totally and finally from the concept that the surgeon can cure Crohn's disease by taking it out.

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