Medical Grand Rounds

BACTERIAL COLITIS

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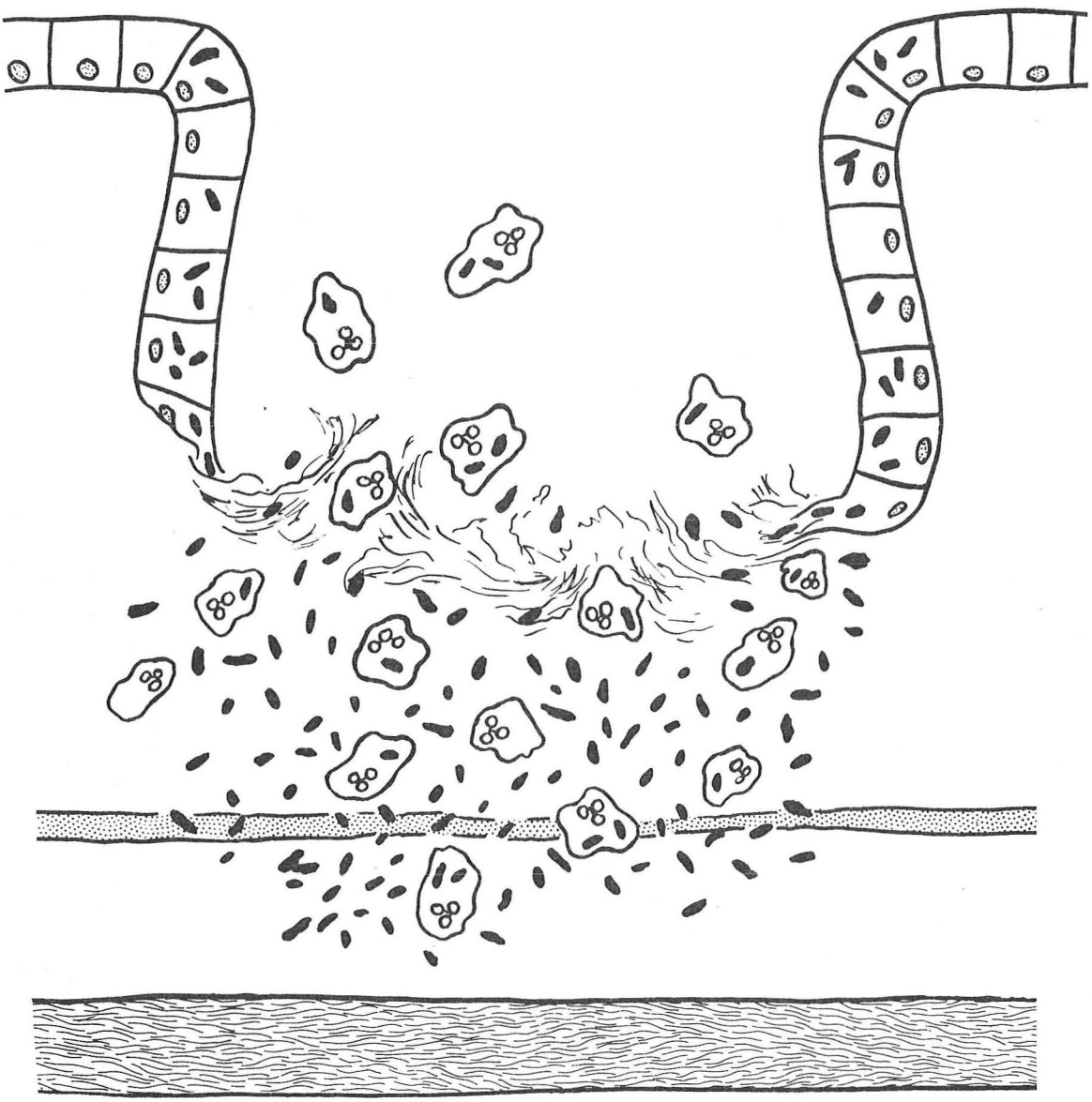

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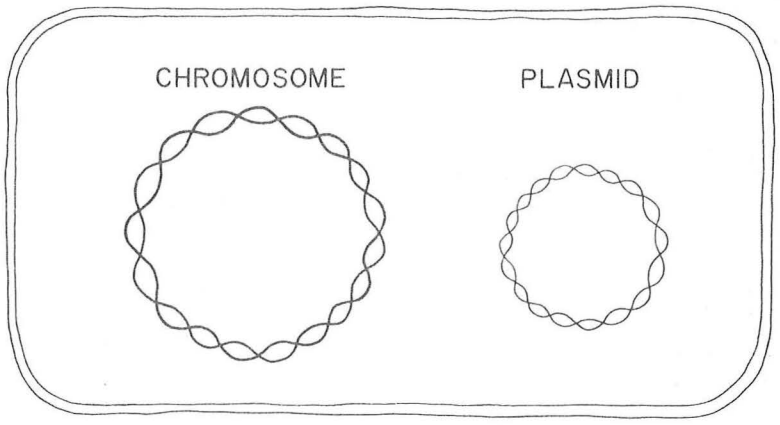
Over the past ten years there has been remarkable progress in understanding many of the mechanisms whereby bacteria produce disease in the human gastrointestinal tract. Many of these advances have come largely as a result of a better understanding of why toxins are produced by certain bacterial species and how the genetic information for the synthesis of these toxins is transported from one bacterium to another. In general, those bacteria that are capable of producing disease of the gastrointestinal tract in man do so because they possess the necessary genetic information to synthesize one or more of three types of virulence factors. These virulence factors include 1) a group of substances that allows the bacterium to adhere to the surface coat of the gastrointestinal tract, 2) specific proteins that either interfere with water and electrolyte absorption in the small bowel or that induce small bowel secretion of water and electrolytes and 3) factors that allow a given bacterium to penetrate the membranes of the intestinal epithelial cell and provoke cell destruction and inflammation. The material presented in this protocol is divided into three broad areas. The first section will review the major findings that have increased our understanding of the molecular biology of enteric bacterial infections. The second section will describe in detail many of the clinical features of the major bacterial illnesses producing acute tissue invasion and clinical colitis. The third section will outline a rational approach to be taken in working up patients who present to the emergency room or to the physician with signs and symptoms consistent with acute inflammatory colon disease.

I. THE MOLECULAR BIOLOGY OF ACUTE BACTERIAL ENTERIC INFECTIONS

1) Review of relevant bacterial genetics:

In classical genetics essentially all of the genetic information necessary for a cell to synthesize products essential for its existence is considered to be encoded on the chromosome of the cell nucleus. More recent work, however, has clearly demonstrated that in bacteria there are a number of other extra-chromosomal genetic elements that are fully capable of autonomous replication in the cell. One of the most important of these structures, with respect to bacterial pathogenicity, is the plasmid. Thus, as shown diagramatically in Fig. 1, a bacterium such as E. Coli may contain two types of genetic information. The chromosome consists of a covalently

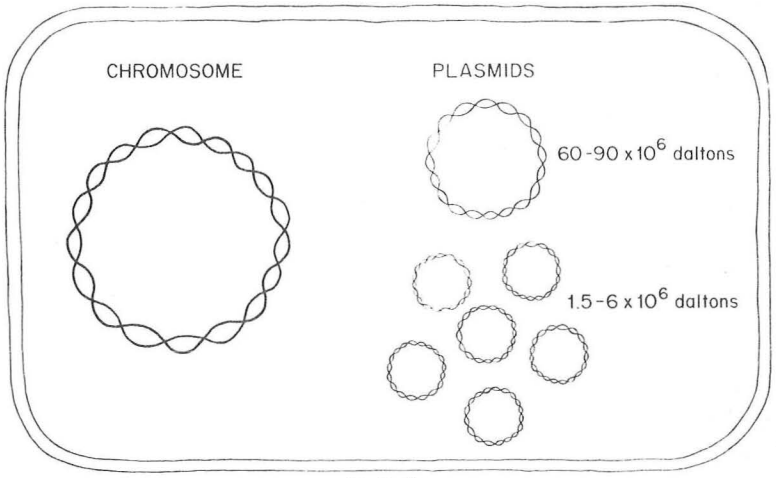
Fig. 1



E. Coli

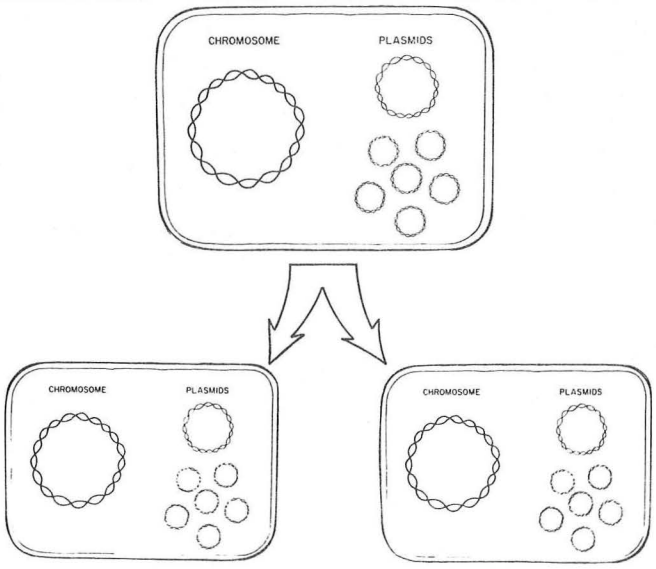
closed circle of duplex DNA that encodes the genetic information necessary for the normal physiological function and survival of the cell. The bacterium may also contain a second genetic element, the plasmid, that also consists of a covalently closed circular molecule of duplex DNA. Such DNA usually encodes genetic information that is considered to be "non-essential" for the function of the cell. However, as will become apparent later in this review, the plasmid appears to encode all, or nearly all, of the genetic information necessary for a given enteric bacterium to act as a pathogen. Not all plasmids are the same size, as shown diagramatically in Fig. 2.

Fig. 2



Generally, most bacterial plasmids have been found to fall into two size classes. The smaller plasmids have molecular weights that generally vary from 1.5 to 6 x 10^6 dalton units. The smallest bacterial plasmids that have been described have molecular weights of 1.5 x 10^6 daltons which would contain sufficient genetic information to code, on the average, about 75 x 10^8 daltons of protein: this would be equivalent to approximately two average size proteins. Many other types of plasmids are much larger and have molecular weights varying from approximately 60 to 90 x 10^6 daltons. Obviously, such large DNA molecules would contain sufficient genetic information to direct the manufacture of many specific and different protein molecules. These two classes of plasmids behave somewhat differently during reproduction of the bacterial cell. Generally, a single bacterium contains only one or two copies of the large molecular weight plasmids. During cell reproduction, as shown diagramatically in Fig. 3, the replication of these large plasmids is tightly coordinated with replication

Fig. 3



of the chromosomal DNA. This ensures that each of the daughter cells will receive a copy of the large plasmids. In contrast, the bacterial cell may contain as many as ten to twenty copies of the small molecular weight plasmids. In most cases replication of these small plasmids occurs randomly and is not coordinated to replication of the chromosomal DNA. In this instance, the daughter cells receive copies of the small molecular weight plasmids simply through random distribution during the reproductive process.

It has been clearly demonstrated that DNA elements from one plasmid may be combined with elements of other plasmids or with the bacterial chromosome. Indeed, it is likely that most transmissible plasmids can recombine with the bacterial chromosome but, in most cases, such a recombination is not stable.

A second type of interaction between the different plasmids and between a plasmid and chromosome is illustrated in diagrammatic form in Fig. 4. In some instances a segment of the DNA molecule in a plasmid may undergo

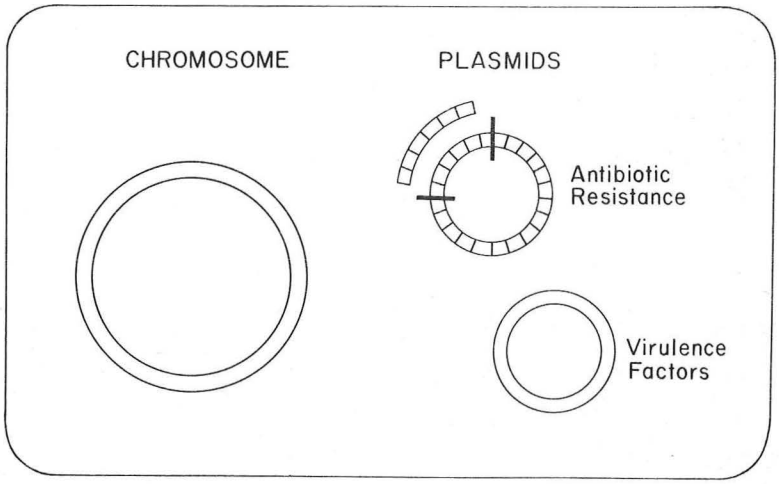


Fig. 4

independent replication. Such an independently replicated DNA segment is referred to as a transposon and this short strand of DNA can then be transferred to other plasmids within the cell or to the DNA chain of the chromosome. Thus, in the example shown in Fig. 4 it is assumed that the

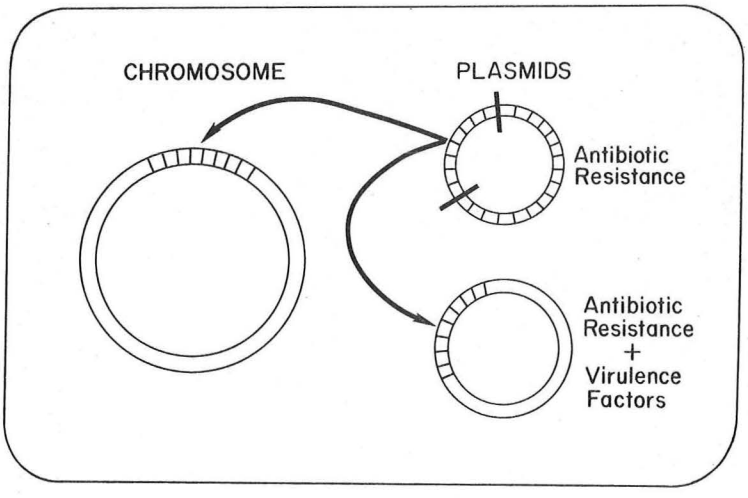
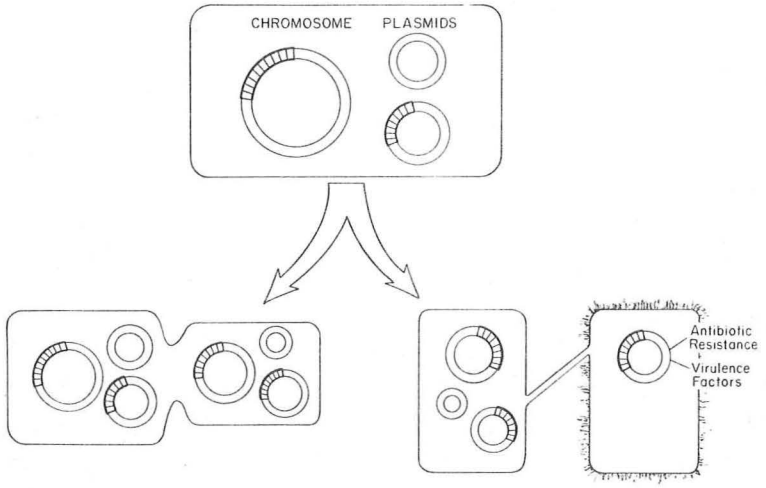


Fig. 5

bacterium contains two types of plasmids one of which is encoded with information to produce antibiotic resistance while the other is encoded with information causing virulence. If that portion of the DNA molecule encoding for antibiotic resistance is independently replicated, then the newly formed transposon can be inserted into either the chromosome or the other type of plasmid as shown in Fig. 5. It should be noted that following this transfer the bacterium now has a plasmid that is encoded with genetic information that would produce both antibiotic resistance and virulence.

These recombined genetic elements can then be passed on to other bacterial cells through two mechanisms. As seen in Fig. 6, the bacterium

Fig. 6

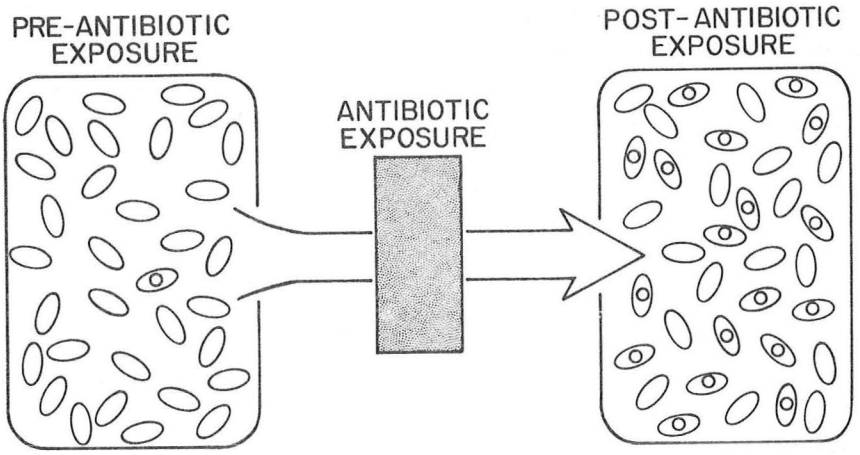


may undergo asexual reproduction through classical fission to produce identical daughter cells. Alternatively, the bacterium may conjugate with a similar or different type of organism and transfer to that organism one or more plasmids. As illustrated diagramatically in Fig. 6, for example, an organism that was neither virulent nor resistant to antibiotics could, through such a mechanism, receive the hybrid plasmid that would render it, and its offspring, both resistant to antibiotics and virulent to the host.

In summary, then, bacteria contain two types of genetic information. The chromosomal DNA encodes for information that is essential for normal physiological functions of the bacterium. In most instances, however, the chromosome is not encoded with information that would allow the bacterium to behave as a human enteric pathogen. Bacteria may also contain a variety of plasmids. The DNA molecules in these plasmids are encoded with genetic information for non-essential cellular functions. These "non-essential functions", however, include the synthesis of products that will allow the bacterium to become a pathogen. The fact that genetic information from one plasmid can be transferred, through the transposon mechanism, to another plasmid has one final implication that is of great importance and that should be emphasized. Consider the situation where a single plasmid has become encoded for the information necessary to impart both virulence and antibotic resistance to the host bacterium, as shown in Fig. 6. Such an organism could exist in a stable population of individuals producing only sporadic cases of gastrointestinal disease. As shown in diagrammatic

fashion in Fig. 7, the number of such pathogenic organisms present in a population would remain relatively small. However, if such a population of individuals were subjected to the widespread use of antibotics

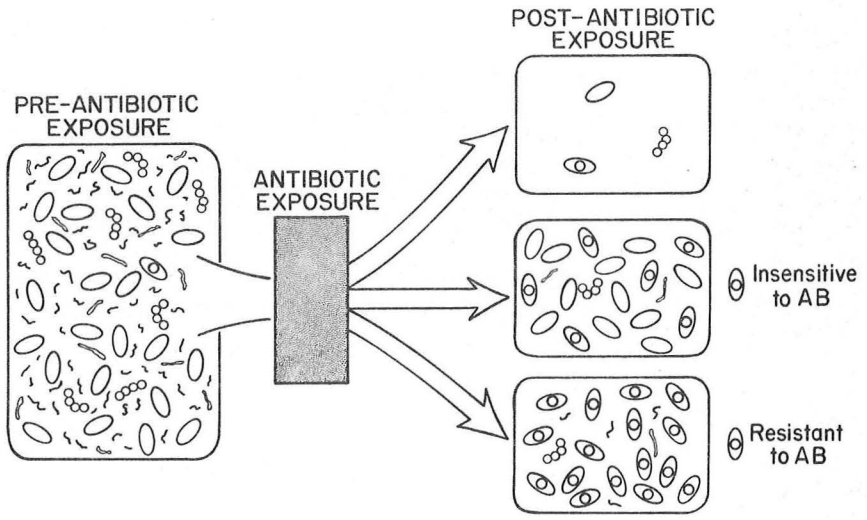
Fig. 7



then there may well be an increase in the numbers of plasmid-bearing organisms since these bacteria would be resistant to the antibotics. In addition, however, since the plasmid also contains the determinates for bacterial virulence, this selection process might well increase the virulence and incidence of the enteric disease. The important point illustrated by this example is that because of the linkage of two plasmid-encoded processes, exposure of the population to antibiotics could lead to a rapid increase in the incidence and severity of a particular enteric syndrome. There are now several reports in the literature that suggest that there has been a marked increase in the frequency and severity of certain gastroenterological disorders synchronously with the appearance of antibiotic resistance in a particular pathogenic organism.

A similar event might also occur in an individual patient. As shown in Fig. 8, for example, the colon of an individual may contain a very small number of organisms carrying a plasmid capable of inducing colitis. The number of such organisms would remain very small because of the suppressor

Fig. 8



substances naturally secreted by the many other, nonpathogenic bacteria normally present in the colonic contents. However, if such an individual were treated with an antibiotic then there might well be massive overgrowth of the pathogen either because it was insensitive to the particular antibiotic administered or because it had acquired resistance to the antibiotic. Such an individual might then manifest a severe colitis due to overgrowth of endogenous pathogen.

2) Functions of bacterial plasmids:

With this brief review of normal plasmid physiology, it is now possible to outline in more detail the type of genetic information that is normally encoded on the plasmid DNA molecules. Again, it should be emphasized, that a bacteria may carry several different kinds of plasmids and so can carry genetic information necessary for the synthesis of a variety of different types of protein molecules. The major types of information carried on plasmids are summarized in Fig. 9. By definition, a plasmid is a self-duplicating, genetic unit. Thus, each plasmid must carry the necessary

PLASMID ENCODED INFORMATION
IN E. Coli

REPLICATION	– Self Duplication
CONJUGATIVE	– Conjugation – Surface Exclusion
AB RESISTANCE	– Inactivating Enzymes
ANTIBIOTICS	– Colicins (E1, E2, E3, etc)
VIRULENCE FACTORS	– Surface Adhesion – Proteins Altering Small Bowel Absorption – Proteins Promoting Tissue Destruction or Invasion

Fig. 9

genetic information to initiate and complete a stable, autonomus replication. A second type of genetic information carried on plasmids has to do with the initiation and completion of the act of conjugation between two bacterium. A plasmid encoded with information for this conjugative function will allow the bacterium to form a "mating pair" through the synthesis of a thread-like appendage, the sex pilus. Once formed, this pilus will allow for the exchange plasmid DNA from the host to the recipient bacterium. By this mechanism, genetic information encoded in plasmids can be rapidly transferred throughout a bacterial population. Some plasmids are also encoded with information for surface exclusion. This genetic information will prevent conjugation from occurring if the recipient bacterium already contains a plasmid with similar or identical genetic information contained in the plasmids of the donor cell.

A third major type of genetic information carried on plasmids is the ability of the bacterium to generate antibiotic resistance. Generally such genetic information is coded for the production of enzymes that can inactivate antibiotics through specific chemical reactions such as acylation

or phosphorylation or for altering the bacterial cell permeability to the drug. In some instances a single plasmid may be encoded with sufficient information to induce bacterial resistance to as many as twelve different antibiotics. A fourth major function of plasmids is to regulate the synthesis of antibiotics by the host organism. In the case of E. coli these antibiotics are referred to as colicins and act to suppress the growth of other types of organisms which may be competing with the E. coli for nutrients in the colon. A number of such colicins have been isolated and characterized.

Finally, and most importantly, it is now apparent that all of the genetic information necessary to make a given enteric bacterium pathogenic is carried on the plasmid. While many such virulence factors have been isolated and identified, these substances can generally be divided into three groups. Thus, the plasmid may encode for substances that 1) promote adhesion of the bacterium to the intestinal lining, 2) that alter small intestinal absorptive or secretory function or 3) that promote tissue invasion and destruction.

3) Plasmid-encoded virulence factors:

A. Surface Adherence Factors

From a variety of studies of different organisms it is apparent that one important factor involved in bacterial virulence is the ability of the organism to adhere to the surface of the small and large intestine. Organisms acquire this ability by synthesizing specialized surface structures that permit adherence. The synthesis of such structures is directed by genetic information carried on the plasmid. The importance of this factor is illustrated by data such as those shown in Fig. 10. If an experimental

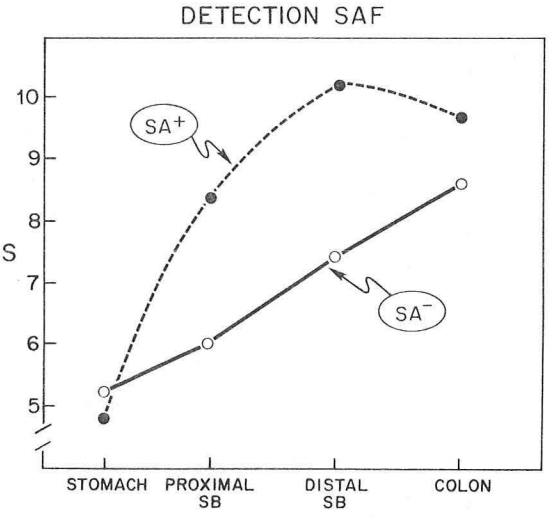


Fig. 10

animal or human subject is administered an enteric organism that lacks surface adherence factor (SA^-) then only relatively small numbers of organisms will subsequently be found in the proximal and distal small intestine. However, if this same organism is infected with plasmids encoded for surface active factors (SA^+) then substantially greater numbers of organisms will be found in the proximal and distal small intestine. One such substance is the K88 antigen of E. coli. When infected with a plasmid carrying this surface adherence factor, the organism has been shown to

synthesize a large filamentous protein containing all of the common amino acids except cysteine-cystine. Such bacteria, as shown in Fig. 11, are covered with a "fur of fine filaments". The filaments of the K88 antigen

SURFACE ADHERENCE FACTOR

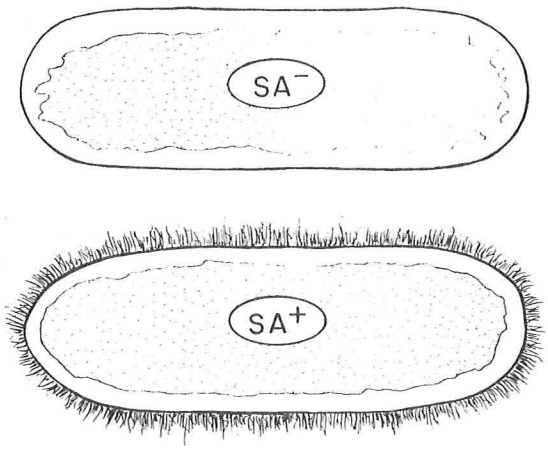


Fig. 11

give the bacteria an adhesive quality that makes them stick to red blood cells and to intestinal epthelial cells. It is apparent from a variety of data in experimental animals and in man that this plasmid-induced surface adherence is a very important factor in allowing a bacterium to behave as a pathogen. In both experimental animals and in human infants E. coli possessing this plasmid will produce mild diarrhea even though no other virulence factor is carried by the bacterium. Furthermore, organisms that produce diarrhea through the elaboration of various enterotoxins also must carry the plasmid encoded for surface adherence factors in order to produce disease in man.

B. Proteins Altering Small Bowel Absorption

A second major way in which plasmids can induce pathogenicity in a given organism is by directing the synthesis of at least two different proteins that are capable of altering fluid and electrolyte balance across the small intestinal mucosa. One of these proteins has a high molecular weight of approximately 84,000 daltons and is inactivated by heating for short periods of time at 60°C. Hence, it is referred to as "heat labile (LT) toxin". As summarized in Fig. 12, extensive investigations indicate that this

HEAT LABILE (LT) TOXIN

- Molecular Wt. 84,000 Daltons
- Elevates Cyclic AMP Levels in Mucosa and in Extraintestinal Tissues

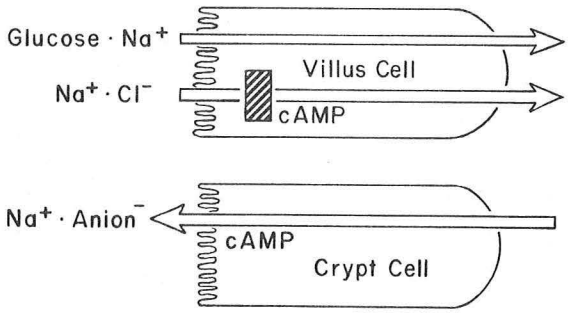


Fig. 12

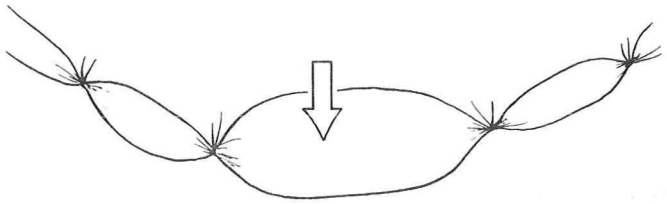
toxin acts by markedly increasing the levels of cyclic AMP within the

intestinal mucosa. This elevation of cAMP has two effects. First, in the mature cells of the upper intestinal villi elevation of the cAMP levels leads to inhibition of coupled sodium and chloride absorption. There is no alteration, however, in the uptake of other nutrients such as glucose, amino acids or other sugars. In the cells of the intestinal crypt elevation of cAMP levels leads to augmented sodium-anion secretion. Thus, heat labile toxin induces the net secretion of an isoosmotic sodium anion solution that will persist even in the face of fasting. The presence of the heat labile toxin (and, therefore, the plasmid containing the genetic information for the synthesis of this protein) can be detected by several indirect means. One method, shown diagrammatically in Fig. 13, is to inject

DETECTION OF LT TOXIN

- Net Fluid Accumulation in Isolated Intestinal Loops

Fig. 13



- Change in Morphology of Tissue Culture Cells, e.g., mouse adrenal cells and hamster ovary cells

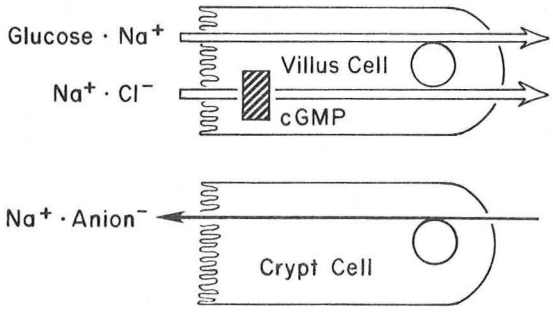
the organisms or cell free filtrates into isolated loops of animal intestine. The presence of labile toxin will be indicated by rapid fluid secretion into the isolated loop. Alternatively, it has also been shown that labile toxin induces marked elevations in cAMP levels in certain tissue culture cells and induces marked morphological alterations. Thus, both mouse adrenal cells and hamster ovary cells can be used to detect, indirectly, the presence of labile toxin production by a given organism.

A second protein produced by pathogenic, enteric bacteria is less well characterized. It has a smaller, and more variable, molecular weight that is variously reported to be in the range of 1900 to 5000 daltons. In contrast to the heat labile toxin, the heat stable toxin does not elevate cyclic AMP levels in the intestinal mucosa (or in isolated tissue culture cells) but does elevate the levels of cyclic GMP. As summarized in Fig. 14, such an elevation of cGMP blocks the coupled absorption of the Na and Cl.

HEAT STABLE (ST) TOXIN

- Molecular Wt. 1,900 – 5,000 Daltons
- Does Not Effect Cyclic AMP Levels in Mucosa
- Does Elevate Cyclic GMP Levels in Mucosa
- The Effect on GMP Levels Occurs in Intestine Only

Fig. 14



However, there is no effect on the secretion of water and electrolytes by the intestinal crypt cells. Hence, the heat stable toxin can be viewed as essentially an antiabsorptive toxin while the heat labile protein should be thought of as essentially a secretory toxin. As a result, heat stable toxin can not be detected by injecting it into an isolated, empty intestinal loop. Rather, the suspected material is usually given into the stomach of a suckling mouse where it will block absorption and lead to distention of the intestine with unabsorbed fluid. There is still considerable uncertainty about the types of plasmids that produce each of these toxins. There are a number of examples of plasmids obtained from E. coli infecting various animals that appear to be encoded for only the stable toxin. Other plasmids, particularly those obtained from human pathogens, appear to encode for both the heat stable and heat labile toxins. It has proven to be impossible, even after repeated passages, to separate the heat stable and heat labile toxin activity that is encoded by these particular plasmids. The association of a single plasmid-DNA species with both stable and labile toxin activity suggests either that two plasmid loci are involved or that the small stable toxin molecule is either an incomplete form or a breakdown product of the larger labile toxin moiety. In any event, the plasmids from human pathogens are relatively uniform in size having molecular weights of approximately 60×10^6 daltons. In contrast, the plasmids obtained from porcine and bovine pathogens are much more variable and have molecular weights between 20 and 80×10^6 daltons.

C. Tissue Invasive Factors

Despite the fact that there are hugh numbers of bacteria in the contents of the lower small intestine and colon, these bacteria usually are unable to penetrate the layers of mucus and the luminal cell membranes of the intestinal epithelial cells. Under certain circumstances bacteria may acquire plasmids that presumably code for specific substances that allow the organism to penetrate these diffusion barriers. Under these circumstances bacteria appear within the cytosolic compartment of the epithelial cells and penetrate into the submucosal and deeper layers of the intestinal wall. This may induce inflammation, ulceration and bleeding from the gastro-intestinal tract. The actual proteins that are encoded by the plasmid that promote such tissue invasion are poorly understood although a number cytotoxins have been described. A number of examples can be cited. One of the classical bacterium that can invade the tissues of the colon and produce an acute colitis is the organism Shigella shigae. Recent studies have shown that this organism produces a toxin that consists of at least two protein molecules having molecular weights of 30,500 and 11,000 daltons. It is likely that the intact toxin consists of one heavy chain and four or five copies of the light chain. As little as 2.5 pg/ml of this purified toxin will kill certain strains of tissue culture cells. Another commonly encountered pathogen capable of inducing acute cell destruction in the colon of the infected individual is the organism Clostridium difficile. This organism has been shown to produce at least two different toxins. The first has a molecular weight of approximately 600,000 daltons. As little as approximately 19 ng/ml will kill a variety of tissue culture cells such as the Chinese Hamster ovary cell. A second toxin has also been obtained from C. difficile that has been designated as an "enterotoxin". This substance, however, is very different from the enterotoxins described

above that induced changes in small bowel ion and water absorption. When injected in small amounts into the cecum of hamsters this semi-purified toxin induces a severe inflammatory reaction. Both the cytotoxin and enterotoxin isolated from C. difficile are inactivated by heating at 56°C for 30 minutes and have similar molecular weights. A number of other apparently different proteins having various toxic properties have been isolated from other enteric pathogenic organisms. However, despite these advances, it is still very unclear how, at the molecular level, these various types of toxins allow a given pathogenic organism to penetrate cell membranes and so induce tissue destruction and inflammation.

Nevertheless, it is possible by relatively simple tests to detect that a particular organism possesses "tissue invasive factors". In one type of test, for example, an organism can be introduced into the intestine or colon of a test animal. The organisms can then be visualized to have invaded the bowel wall using fluorescent antibodies. A second method is to

Fig. 15

DETECTION OF TIF(s)

- *in vivo* Disease Production
- Invasion of Conjuntival Membrane (Serény Test)
- Hela-Cell Invasion
- Ileal Invasion in Rabbit and GP (Fluorescing AB)
- Morphological Changes in Tissue Culture that are Neutralized by Anti-toxin to C. Sordellii (C. difficile)

incubate bacteria with certain tissue culture cells such as Hela cells. Organisms with tissue invasive properties are able to penetrate the cell membranes and appear within the cytosolic compartment. Another simpler test for tissue invasiveness is to instill the suspected organisms into the conjunctival sac of an experimental animal such as the guinea pig (the Sereny test). Generally, organisms capable of tissue invasion will produce a marked inflammatory reaction in the eye of the experimental animal. Finally, it is also possible to test for specific cytopathic toxins utilizing tissue culture cells. The stool water of patients infected with C. difficile will produce marked morphological changes when incubated with a variety of types of tissue culture cells. This cytopathic activity is neutralized by the addition of specific antitoxin to the stool water samples.

Organisms which possess plasmids encoded for these tissue invasive factors produce characteristic pathological changes when administered to experimental animals or to man. Typically there is destruction of surface epithelium with ulceration, vascular dilation and a marked inflammatory response by the host. Leukocytes often collect in the submucosal tissues (crypt absesses) and collections of fibrin and dead cells collect in

patches on the surface of the inflamed epithelium (pseudomembranes). In some instances where there is marked tissue destruction, such as seen with overgrowth of C. difficile. such pseudomembranes become grossly visible and can be seen through the protosigmoidoscope or colonoscope

4) Disease and Syndromes Produced by Plasmid-encoded Virulence Factors:

From these considerations of the major factors that result in pathogenicity of enteric organisms, it is possible to define three general types of gastrointestinal disease that result from infection with organisms carrying three different types of plasmids. It is of interest that E. Coli is capable of producing all three of these syndromes depending upon which plasmid or set of plasmids a particular population of E. coli carries.

The first clinical syndrome is summarized in Fig. 16 and is characterized by mild, protracted diarrhea due to overgrowth and adherence of the

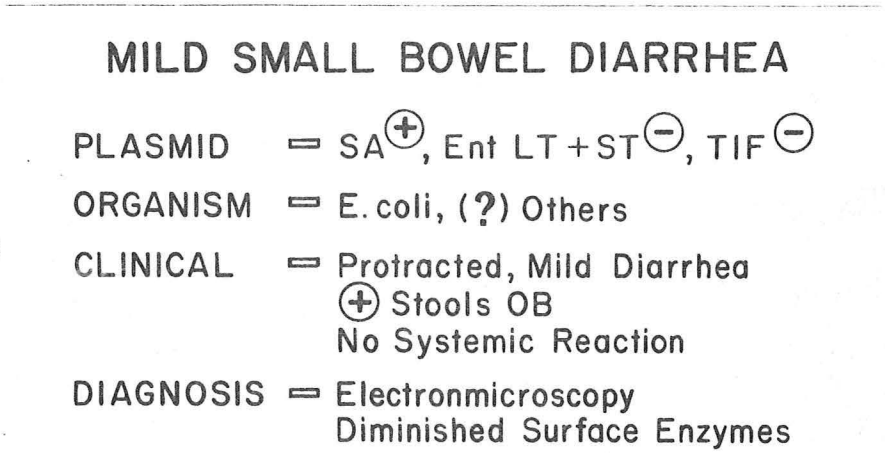


Fig. 16

pathogenic organism in the small intestine. Classically, this disease is produced by an enteropathogenic E. coli. This organism has the capacity to penetrate the intestinal glycocalyx, to become adherent to the mucosal cell surface and to disrupt the microvillous brush border. Such infection produces a mild, protracted diarrhea in infants and in adults and is a well recognized cause of diarrhea in certain animal species such as the rabbit. The stools are occasionally positive for occult blood but there is no systemic reaction such as fever or an elevation of the WBC since there is no evidence of tissue invasion. The offending organism carries plasmids capable of producing surface adherence factors. The organisms, however, do not carry plasmids encoded for either of the enterotoxins or for tissue invasive factors. Thus, this type of E. coli will give a negative Sereny test as well as a negative intestinal loop and suckling mouse test. The diagnosis usually rests on demonstration of bacterial overgrowth in the small intestine, electron microscopy and the demonstration of diminished surface enzyme activities. Thus far, most of this type of disease has been described with overgrowth by an E. coli carrying the SA plasmid. There is no reason to believe, however, that other enteric bacteria could not also produce this disease provided they acquired the appropriate plasmid.

The second clinical syndrome, outlined in Fig. 17, is characterized by the onset of the marked secretory diarrhea and a "cholera-like" syndrome. When this clinical disease is produced by overgrowth of E. coli in the small intestine, the organism is usually referred to as a enterotoxigenic E. coli. This organism must contain two separate plasmids: one plasmid must

"CHOLERA" SYNDROME

PLASMID = SA⊕, Ent LT + ST⊕, TIF⊖

ORGANISM = E.coli, V. cholerae, Salmonella, Others

CLINICAL = Massive Small Bowel Secretory Diarrhea
No Systemic Reaction

DIAGNOSIS = Small Bowel Colonization
Production of LT + ST

Fig. 17

be encoded for surface adherence factors while the other plasmid must be encoded for the production of the heat labile and heat stable enterotoxins. These organisms are negative, however, for plasmids encoded to produce tissue invasive factors. This combination of two types of plasmids allows the organism to overgrow and maintain residence within the small intestine while, at the same time, producing secretory toxin. The patient manifests a very large-volume, isotonic diarrhea but has no evidence of tissue invasion, i.e., no fever or WBC elevation. The diagnosis usually rests with the demonstration of significant E. coli overgrowth in the small intestine and of the demonstration that this E. coli produces enterotoxin, i.e., that the organism is positive in the isolated intestinal loop preparation, the suckling mouse preparation or the mouse adrenal cell or hamster ovary cell tissue culture preparations. While this disease is commonly produced by an enterotoxigenic E. coli, the same combination of plasmids may be found in other organisms such as V. cholerae or various Salmonella. Essentially any enteric organism that is capable of surviving within the small intestine will apparently produce this disease if that organism acquires the plasmids encoding for surface adherence factors and for enterotoxin production.

The third syndrome, as summarized in Fig. 18, is one of acute ulceration and inflammation of the colon, i.e., an acute "colitis". Again, the syndrome may be produced by an E. coli in which case the organism is referred to as an enteroinvasive E. coli. Overgrowth of such an organism in the lower small intestine and colon leads to tissue disruption, ulceration, frequent small-volume diarrhea and the exudation of pus and blood into the feces. There is usually a significant systemic reaction with fever and elevation of the WBC. In order to produce this disease state the organism must carry the plasmid encoded for tissue invasive

factors. Some organisms also will carry the plasmid encoded for the enterotoxins: however, whether or not this plasmid is present does not

ACUTE COLITIS

PLASMID = SA$^{\ominus}$, Ent LT + ST$^{\oplus\ominus}$, TIF$^{\oplus}$

ORGANISM = E.coli, Shigella, Salmonella, Campylobacter, Clostridia, etc.

CLINICAL = Acute, Small Volume Diarrhea, Commonly with Blood and Pus
Systemic Reaction with Fever, ↑WBC

DIAGNOSIS = Establish D_x of Colitis
Identify Organism

Fig. 18

seem to alter the nature of the disease. In addition to the enteroinvasive E. coli, there are a number of other organisms such as Shigella, Salmonella, Campylobacter and Clostridia that may carry this plasmid and may produce a similar clinical picture of acute inflammatory colitis. Generally, the diagnosis is made by the demonstration of an acute colitis with the exudation of pus and the isolation of a organism that is presumed to be the pathogen. Ideally, however, it would be necessary to demonstrate that the isolated organism is indeed capable of tissue invasion, i.e., is positive in the Sereny test.

5) Interaction of the Different Plasmids in the Same or Different Organisms:

It is apparent from these considerations that the particular disease syndrome produced by infection with a given enteric pathogen is determined primarily by the plasmid that the pathogen carries rather than by the organism itself. For example, the syndrome of a secretory diarrhea can be produced by a number of different organisms provided that they carry the appropriate plasmids. Similarly, an invasive colitis also will be produced by a variety of organisms carrying the appropriate plasmid. The current concepts concerning the relationship between plasmids, enteric bacteria and the production of disease is summarized in Fig. 19. Over many

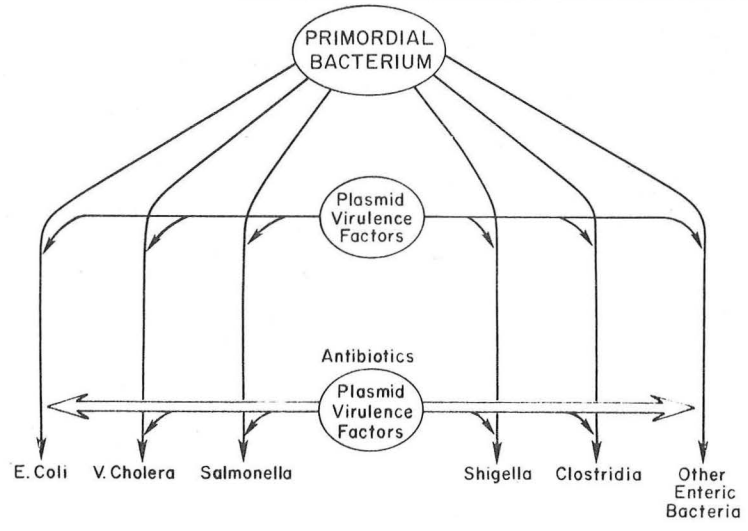


Fig. 19

millions of years there evolved distinctive classes of enteric bacteria with metabolic characteristics that allowed them to survive within the gastrointestinal tract of animals and man. The genetic information that encoded for these essential metabolic characteristics that allowed survival was carried primarily on the DNA molecules of the bacterial chromosomes. In addition, certain of these bacteria were infected with DNA molecules carried in the plasmids which, in some cases, encoded for proteins that were detrimental to the host and produced disease. Presumably the acquisition of such information must have had some evolutionary advantage for the organism that acquired this new genetic information.

Presumably, over many thousands of years, the same plasmid was acquired by several different types of organisms. For example, it is the current opinion of many molecular biologists that both E. coli and V. cholera acquired a plasmid encoded for the synthesis of the enterotoxins. Thus, today a cholera-like syndrome is seen with infection with either enterotoxigenic E. coli or with V. cholera. Another point shown in Fig. 19 is the serious possibility that in modern times the widespread use of antibiotics may lead to the more rapid dissemination of plasmids carrying virulence factors to many types of enteric organisms. This would occur, as discussed above, because of the coupling of genetic information encoded for drug resistance and virulence factors on the same plasmid. Finally, recently published data has clarified the interactions that occur between different types of plasmids when carried by the same organism. As summarized in Fig. 20 an organism carrying no plasmids encoded for virulence factors or encoded only for the production of enterotoxins (Ent LT + ST) will produce no disease in an infected individual. Only if the organism carries the plasmids encoded for surface adherence and for enterotoxin production will secretory diarrhea result. Similarly, as shown in the lower portion of Fig. 20, organisms carrying plasmids encoded for tissue invasive factors are capable of producing acute colitis. If the plasmid for enterotoxin production is inserted

Fig. 20

PLASMID			DISEASE
SURFACE ADHERENCE	Ent LT + ST	TISSUE INVASIVE FACTOR	
⊖	⊖	⊖	None
⊖	⊕	⊖	None
⊕	⊖	⊖	Mild SB Diarrhea
⊕	⊕	⊖	Severe SB Diarrhea
⊖	⊖	⊕	Severe Acute Colitis
⊖	⊕	⊕	Severe Acute Colitis
⊖	⊕	⊖	None

into such bacteria there is essentially no change in the type of clinical disease produced. However, there are now examples of bacteria carrying all three types of plasmids. In this situation there is the induction of a severe secretory state and tissue invasion in the infected animal or individual.

6) Identification of Plasmids Carrying Virulence Factors in Patients Infected with Bacterial Pathogens:

From the clinical point of view, it is obviously extremely important to be able to identify by rapid and inexpensive means the bacterial causes of a particular syndrome seen in an individual patient. In the past, this has generally been done by attempting to culture a presumably pathogenic organism from the stool or gastrointestinal tract contents. From the foregoing discussion, however, it should be apparent that this method of diagnosing acute infections of the gastrointestinal tract is inadequate and, at times, may be misleading. For example, there are no culture techniques available that would allow one to distinguish an E. coli capable of producing disease from a nonpathogenic E. coli. Furthermore, if one isolates an organism such as Salmonella or Shigella from the stool, it does not necessarily follow that this organism caused the disease. Ideally the diagnosis of a particular syndrome of enteric disease should rest with the identification of the plasmids or their products that are known to be capable of producing a particular syndrome.

From the Discussion above it is apparent that most currently available methods for carrying out such a diagnosis rest with the identification of the products of a particular plasmid. Thus, the presence of the plasmid encoded for adherence factor must be detected by morphological changes in the organism and by the ability of such organisms to adhere to cell surfaces. The presence of the plasmid encoded for enterotoxins must be identified using isolated loops of rabbit intestine or the suckling mouse model. Alternatively, changes in the morphology of adrenal cells or ovary cells in culture may be used to identify the presence of these toxins. The presence of tissue invasive factors requires the utilization of the Sereny test or other methods for detecting tissue invasion. All of these methods are expensive and time consuming, and require highly specialized facilities in research laboratories. These methods are not generally available to a community hospital.

It is now apparent, however, that in the very near future much more direct diagnostic procedures will become available. These methods do not rely upon identification of the gene product but, rather, detect directly the presence of the disease-producing plasmids. These methods are based upon the ability of molecular biologists to isolate the individual plasmids, to purify and cleave the plasmid DNA molecule and to synthesize DNA chains that are complementary to the DNA chains of the plasmid through the use of DNA polymerase. In practice, stool samples from a patient would be dried onto a piece of filter paper as shown in diagrammatic form in Fig. 21. The filter paper would then be floated on

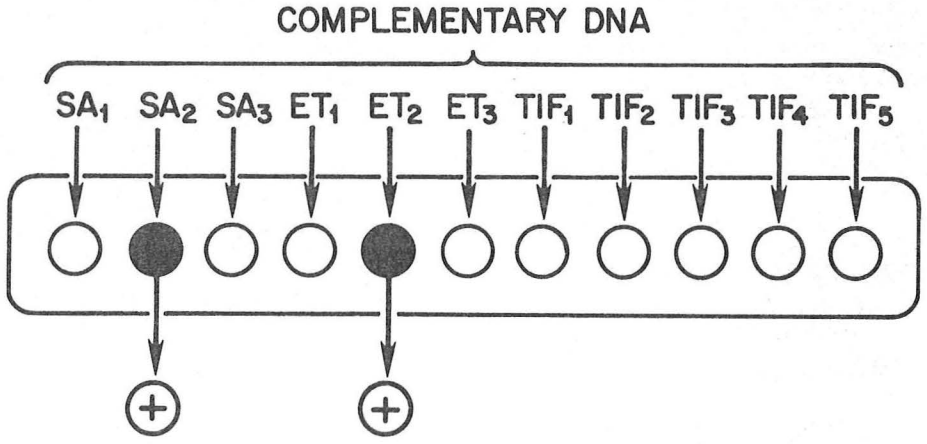


Fig. 21

sodium hydroxide solution to lyse the bacterial cells and to bind the plasmid DNA molecules to the filter paper. The stool specimens are then overlaid with a series of different, specific complemenatry DNA molecules labeled with either radioactivity or a moiety that will fluoresce under ultraviolet light. The plasmid DNA bound to the filter paper would interact only with its specific complementary DNA molecule. The filter paper would then be washed and binding of the complementary DNA molecules to the stool specimens would be determined by an appropriate means. Thus, as shown in the example in Fig. 21 a given stool specimen might be positive for a plasmid encoded for one particular surface adherence factor and for another plasmid encoded for one particular enterotoxin. Such a test would require only a few hours to perform and would give the physician highly specific information as to the nature of the disease.

II. SPECIFIC TYPES OF BACTERIAL COLITIS

In the past, classical bacillary dysentery, i.e., invasion of the colon by bacteria, was thought to be largely caused by infection with Shigella dysenteriae resulting in the disease known as Shigellosis. More recent work, however, has clearly identified a number of other bacteria that are capable of tissue invasion and that cause a clinical picture that is essentially indistinguishable from that produced by infection with Shigella. The most important of these organisms are outlined in the table shown in Fig. 22. Recent data published largely in the European literature,

Fig. 22

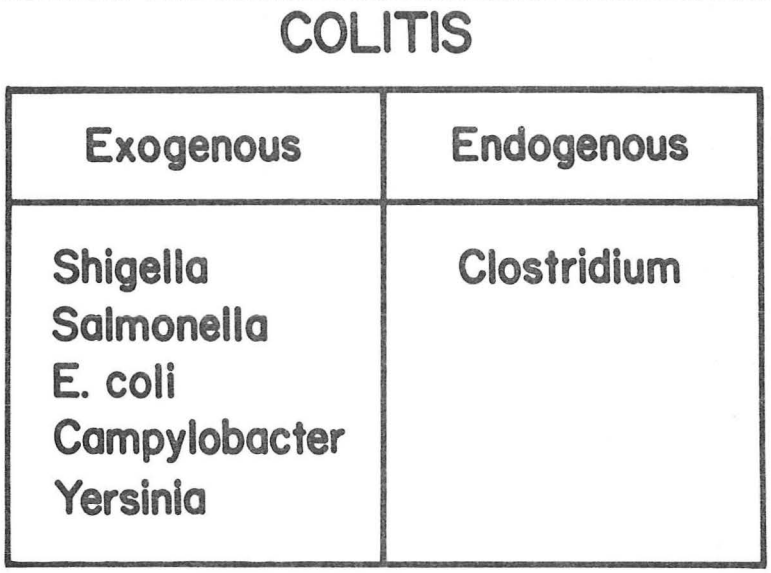
TISSUE INVASIVE ORGANISMS

Terminal Ileitis	Yersinia
Entero-Colitis	Salmonella Campylobacter
Colitis	Shigella Salmonella E. coli (enteroinvasive) Campylobacter Yersinia Clostridium

but also reported in the United States, has indicated that Yersinia is capable of producing at least three different enteric syndromes in man. It may cause mysenteric adenitis, particularly in children. It may invade the tissues of the distal ileum producing fever and right lower quadrant pain. Such individuals are often operated on with the diagnosis of acute appendicitis but are found to have marked inflammation and thickening of the terminal ileum. Less frequently, Yersinia has also been shown to be capable of invading the colon and producing an acute colitis with ulceration and pus formation. Infection with Salmonella classically produces the symptoms of an enteritis. Patients frequently experience

cramping, periumbical pain, vomiting and a large-volume diarrhea. More recently, campylobacter infection has been recognized to produce a similar clinical syndrome. Finally, a number of different organisms have now been described that are capable of invading the tissues of the colon and produce a syndrome of acute bacterial colitis that is essentially indistinguishable from the syndrome produced by infection with classical Shigella. The most important of these bacterial agents include Salmonella, enteroinvasive E. coli, campylobacter, Yersinia and clostridium. As illustrated in Fig. 23, it is useful to divide these organisms that are capable of producing acute colitis into two different groups. The

Fig. 23



first group, entitled "Exogenous" include those organisms in which the pathogen is acquired from the external environment. This generally takes place through contamination of water, food or other animal products with the pathogenic organism that had been excreted in the feces of the carrier animal or man. In contrast, endogenous colitis arises from the overgrowth of the pathogen normally carried within the contents of the colon. For example, it is likely that Clostridium difficile is a normal inhabitant of the colon whose growth is suppressed by the normal bacterial flora. When this flora is markedly reduced by the administration of antibiotics, this organism may overgrow and produce a severe colitis.

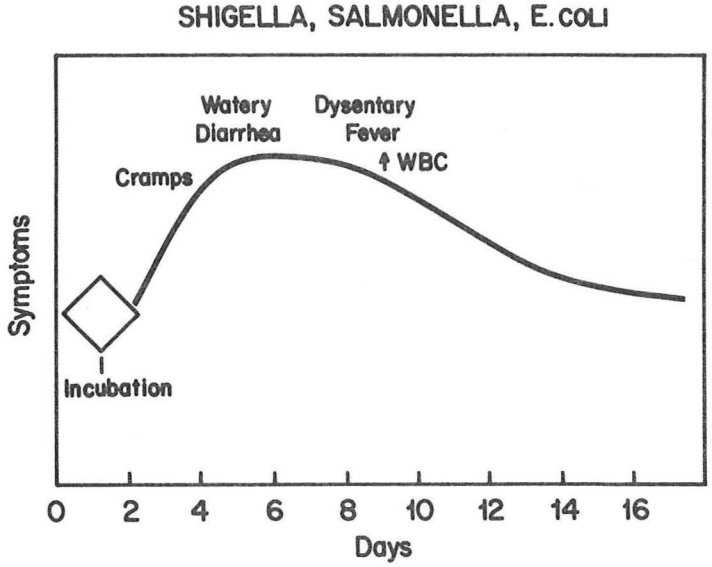
In the following sections a detailed description is given of the two most recently described organisms that produce acute colitis, i.e., campylobacter and clostridium. In addition, however, a brief description also is presented of the typical findings in amoebic colitis and in Crohn's disease and ulcerative colitis since it is usually necessary to differentiate these syndromes during the work up of patients presenting with acute inflammatory bowel disease.

1) Colitis due to Shigella, Salmonella and enteroinvasive E. coli:

The classical features of acute bacillary dysentery caused by infection with Shigella are well known and are well described in the standard textbooks. The clinical features of this disease, therefore, will be discussed only briefly in this protocol and are shown diagrammatically in

Fig. 24. The infection is acquired through the intake of food or water contaminated with feces from a carrier source. Following an incubation

Fig. 24



period as short as 12 hours, but more commonly several days, the onset of the disease is heralded by abdominal cramping pain. The clinical course may vary from a very mild diarrheal illness to an acute severe disease that ends in death. Usually, however, the cramping pain is rapidly followed by the onset of a fairly large-volume diarrhea that lasts for a few hours to several days. This gradually evolves into the clinical picture of dysentery with frequent, small-volume stools often containing mucus, pus and blood and evidence of tissue invasion with the onset of fever and an elevation in the WBC. Sigmoidoscopic examination at this time usually reveals a mucosa which is friable, ulcerated and exuding pus cells. If a barium enema were performed (which should not be done) one would find extensive ulceration of the colonic mucosa that was indistinguishable from the picture of acute ulcerative colitis. There is significant volume depletion and loss of potassium, and there may be significant blood loss. Rarely the disease may be fulminating and result in perforation of the colon and death. Much more commonly, however, the disease is of only mild or moderate severity and is usually self-limited with an average duration of only 7-10 days. Following symptomatic recovery, individuals may continue to shed Shigella in the stool: however, there is probably no such disease as "chronic symptomatic Shigellosis". Any patient who has a chronic ulcerative colitis and is excreting Shigella probably has one of the underlying idiopathic colities (i.e., chronic ulcerative colitis or Crohn's disease) and is simply a carrier of Shigella.

Recent data has clearly established that both Salmonella and tissue invasive E. coli are capable of producing an identical clinical syndrome. Classically, infection with a Salmonella produces symptoms primarily related to an enteritis. The patient commonly has vomiting, periumbical pain and a large-volume diarrhea. Less commonly, however, the Salmonella species apparently is carrying the plasmids for tissue invasive factors and produces an acute dysentery syndrome indistinguishable from Shigellosis. Similarly, tissue invasive E. coli will produce colonic invasion, crypt absesses, mucosal ulceration and bleeding. All three of these organisms,

i.e., Shigella, tissue invasive Salmonella and tissue invasive E. coli, will give a positive Sereny test when inoculated into the guinea pig eye. The diagnosis of these illnesses usually is dependent upon the isolation of a Shigella or Salmonella from the stool of a patient with the appropriate clinical findings. In order to establish that the disease is due to infection with E. coli, it would be necessary to demonstrate that the organism is tissue invasive.

2) Acute colitis due to Campylobacter:

Only fairly recently has it been recognized that Campylobacter is capable of producing an acute invasive colitis. In the past, this organism has been largely overlooked because of the highly specialized culture techniques that are required to isolate and identify the organism. Table I summarizes the results in eight separate series in which appropriate cultures were carried out. In asymptomatic children or adults this

Table I

Location	*Patient population*	*No. of subjects*	*No. of positive cultures (%)*
Brussels	Hospitalized children with diarrhea	800	41 (5%)
	Hospitalized adults with diarrhea	100	4 (4%)
	Asymptomatic, all ages	1,000	13 (1.3%)
Worcester, England	Children and adults with diarrhea	803	57 (7.1%)
	Asymptomatic controls	194	0
Manchester, England	Sporadic cases of diarrhea	182	14 (7.6%)
	Asymptomatic controls	60	1 (1.6%)
Surrey, England	Patients with diarrhea	330	19 (5.8%)
	Patients without diarrhea	120	1 (1.6%)
Edinburgh, Scotland	Patients with diarrhea	196	17 (8.7%)
	Asymptomatic controls	50	0
Hereford, England	Patients with acute gastroenteritis	280	39 (13.9%)
	Asymptomatic controls	156	1 (0.6%)
Rwanda, Africa	Patients with diarrhea (outpatients, patients with measles, inpatients)	204	22 (11%)
	Patients without diarrhea	58	0
Denver	Patients with diarrhea	532	27 (5.1%)
	Asymptomatic controls	81	0

organism usually cannot be grown from the stool. In these various series a Campylobacter was grown out of control subjects in only 0.5% of the cases. In contrast, in unselected adults and children presenting to the physician with acute diarrhea, a Campylobacter was grown from the stool in approximately 8% of the cases. If these data are correct, then Campylobacter

Fig. 25

514 Patients from Denver with Diarrhea	
Campylobacter fetus	26 (5.1%)
Salmonella	19 (3.7%)
Shigella	13 (2.5%)

would represent the single most common identifiable bacterial cause of diarrhea in an unselected patient population. This has been confirmed in one series from Denver as summarized in Fig. 25. Clearly, this organism must now be considered to be a major cause of acute enterocolitis.

Table II summarizes the classification and characteristics of this organism. Campylobacter is a thin, spirally curved, gram-negative rod approximately 0.2 to 0.8 μm wide. Originally the organism was included with the vibrio species because of morphological similarities to Vibrio cholerae. However, there are a number of biochemical differences which clearly differentiate Campylobacter from Vibrio cholerae.

Table II

Classification of the genus Campylobacter

	Taxonomic classification synonyms according to:				
Bergey's Manual[5]	*Veron and Chatelain*[4]	*King*[6]	*Florent*[8] *or Jones*[7]	*Ecology*	*Disease states*
I. *Campylobacter fetus* ss. *fetus*	*C. fetus* ss. *venerealis*	*Vibrio fetus*	*V. fetus* ss. *venerealis* (Florent[8])	Not normal flora; found in bovine semen, preputial fluid, cervical mucus; will not grow in human or animal GI tract	Enzootic abortion and sterility in cattle; venereal transmission; not associated with human disease
	C. fetus ss. *venerealis* biotype *intermedius*				
Campylobacter fetus ss. *intestinalis*	*C. fetus* ss. *fetus*	*Vibrio fetus*	*V. fetus* ss. *intestinalis* (Florent[8])	Not normal flora; found in placentas and gastric contents of aborted ovine and bovine fetuses, in bile, GU, and GI tracts. Will grow in human and animal GI tracts	Abortion in sheep; sporadic abortion in cattle; oral transmission; usual cause of systemic human disease
Campylobacter fetus ss. *jejuni*	*C. jejuni, C. coli* (?)	"Related vibrios"	*Vibrio jejuni* (Jones[7])	Normal GI flora in swine, sheep, cattle, goats, chickens, turkeys, wild birds. Will grow in human and animal GI tracts	Abortion in sheep; enteritis in heifers, calves; avian vibrionic hepatitis (poultry); usual cause of human enteritis, occasional sepsis
II. *Campylobacter sputorum* ss. *suptorum*	*C. sputorum*	*Vibrio sputorum*	–	Normal human oral flora (in gingival crevices)	None described; ? human saprophyte
Campylobacter sputorum ss. *bubulus*	*C. bubulus*	*Vibrio bubulus*	–	Normal GU flora in sheep and cattle found in vaginal and preputial mucus, in semen	None described; ? bovine saprophyte
III. *Campylobacter fecalis*	–	–	–	Found in sheep feces, bovine vagina, and semen	None described

The organism is a well recognized cause of acute diarrheal disease in cows, calves and poultry. As with the other exogeneous enteric pathogens, this organism is usually acquired through the intake of contaminated

material. There may be person-to-person spread of the disease. Not uncommonly there is a history of exposure to animals and to live or butchered poultry. In other outbreaks contact with dogs with diarrhea or the intake of contaminated water has been implicated as the source of the infection. The incubation period, while poorly characterized, has been estimated to be in the range of 2 to 10 days. The onset of the illness is usually heralded by abdominal pain and fever. Diarrhea follows shortly thereafter and, initially, is of large volume and foul smelling. However, after 1 to 3 days of diarrhea a picture of typical dysentery develops. The patient has frequent, small-volume stools and commonly notices the passage of blood and mucus with the bowel movements. Generally, vomiting and marked dehydration are uncommon. Proctoscopic examination shows typical erythema and ulceration of the colonic mucosa and crypt absesses are apparent in the colonic biopsies. This organism does not produce enterotoxins but behaves in a manner entirely analogous to the clinical behavior of Sigella. Presumably, therefore, the organism is carrying plasmids encoded for tissue invasiveness. Infection with Campylobacter, as in the case of Shigella infection, is apparently self-limited and usually lasts from 3 to 15 days. However, there are case reports of relapsing diarrhea over a period of as long as 3, 5 and 9 months. If true, this is an important clinical feature that should be emphasized since such cases might conceivably be confused with chronic idiopathic ulcerative colitis. The organism appears to respond to the administration of erythomycin but appropriate, blinded studies have apparently not been carried out.

3) Acute Colitis Due To Clostridium difficile:

A great deal of new information has become available over the past few years regarding the pathophysiology of acute colitis associated with overgrowth of C. difficile in the colon. This syndrome may represent an "endogenous" form of colitis since it arises from overgrowth of an organism that is probably normally present within the human gastrointestinal tract, at least in some individuals. Alternatively, it may be acquired from the external environment. The onset of the disease usually follows the administration of antibiotics and is characterized by a variable clinical picture of dysentery.Examination of the colon commonly reveals ulceration, inflammation and the presence of pseudomembranes. In the literature the disease is often referred to as "clindamycin colitis", "antibiotic-associated colitis" or "pseudomembranous colitis".

Ever since the advent of the use of antibiotics it has been recognized that individuals may develop diarrhea. In some patients a very severe syndrome develops in which there is gross ulceration of the colon and lower small intestine, evidence of inflammation and severe systemic symptoms. In the past, this clinical syndrome has been attributed to staphylococcal infection and in the old literature is commonly referred to as "staphylococcal enterocolitis". However, with the evolution of culturing techniques for clostridium, it has become apparent that most, if not all, cases of acute enterocolitis seen following administration of antibiotics is due to overgrowth of clostridium difficile.

In recent publications over the past few years there has been an emphasis on the relationship between the administration of clindamycin and

TABLE III

IMPACT OF ORAL ANTIMICROBIALS ON THE FECAL FLORA

Antimicrobial	Fecal levels,*µg/g	Flora changes
Ampicillin	0-250	Decreased streptococci and gram-positive anaerobes; ingrowth of resistant coliforms.
Cefalexin	?	Decreased gram-positive aerobic and anaerobic cocci.
Chloramphenicol	3-11	Modest suppression of coliforms (effect on anaerobes not evaluated).
Clindamycin	100-500	Marked suppression of anaerobes; increased coliforms, yeast, and enterococci.
Erythromycin	1000-3000	Marked suppression of anaerobes (effect on aerobes not evaluated)
Lincomycin	1000-9000	Marked suppression of anaerobes.
Tetracycline	500-2000	Variable; some ingrowth of resistant coliforms.

*Fecal levels of biologically active drug with oral administration in usual recommended doses.

the development of clostridial colitis. However, it is now apparent that while ampicillin and clindamycin are probably the most common antibiotics associated with this lesion, essentially any antibiotic may be associated with overgrowth in the colon of this organism. A number of experimental animals, and particularly the hamster, will develop clostridial overgrowth when challenged with small doses of a variety of antibiotics. Thus, in both the experimental animal and in patients, the syndrome of clostridial colitis can be produced by at least the following group of antibiotics: ampicillin, clindamycin, gentamicin, cephalotin, cefazolin, cephalexin, erythromycin, chloramphenicol, amoxicillin and nafcillin.

It is clear from the clinical behavior of this disease that the organism must possess the ability to invade the tissues of the lower small bowel and colon. To date, two separate toxins are known to be produced by C. difficile. The first to be described is referred to as a "cytotoxin". This protein is heat labile and acid labile. The molecular weight has been reported from several different laboratories to vary between 100,000 to 600,000 daltons. The presence of this cytotoxin in bacterial cultures or in stool water can readily be demonstrated utilizing a tissue culture technique. When added to almost any type of tissue culture preparation the cytotoxin will produce characteristic cytopathic changes in the cells.

While there is no specific antitoxin available to this cytotoxin, this cytopathic activity can be readily neutralized by adding antitoxin to C. sordellii (which cross reacts with the cytotoxin of C. difficile) or by use of polyvalent antitoxin. This reaction forms the basis for a highly specific clinical test for detecting the presence of the cytotoxin in the stool water of patients with diarrhea specifically caused by C. difficile overgrowth.

More recently, an entirely different toxin has been isolated from this organism. Unfortunately, this particular toxin has been termed an "enterotoxin". This term should not be confused with the various enterotoxins described above that alter small bowel absorptive capacity. The "enterotoxin" derived from C. difficile is a tissue invasive factor and elicits a marked inflammatory reaction in the colon. The fact that this "enterotoxin" is far more lethal in experimental animals and induces a marked inflammatory reaction in the intestine strongly suggests that this toxin, rather than the cytotoxin, is the tissue invasive factor from C. difficile that is primarily responsible for the production of clinical colitis. Nevertheless, demonstration of the cytotoxin utilizing the tissue culture technique remains an extremely useful way to detect overgrowth of this organism in patients.

The clinical course of enterocolitis produced by clostridial overgrowth is extremely variable. The patient may experience only very mild diarrhea of a short duration or, at the other end of the spectrum, may manifest a rapidly progressive, fulminating colitis with perforation and death. Classically, the patient gives a history of antibiotic intake prior to the onset of the GI symptoms. As noted above, the disease can probably follow the administration of virtually any antibiotic although ampicillin and clindamycin are commonly implicated. The disease may follow either the oral or parenteral administration of the antibiotic. The syndrome has been described after only one or two doses of an antibiotic but most typically comes on during a 1-2 week course of antibiotics. The symptoms may begin as long as 4 weeks after the administration of the drug has been stopped.

The onset of the colitis is usually heralded by the development of diarrhea. At first, the diarrhea may be watery and of large volume. However, the disease may then progress to a typical dysentery-like syndrome with frequent small-volume bowel movements that may contain pus, mucus or blood. Most patients also experience considerable abdominal cramping, abdominal tenderness, fever and elevation of the WBC. There is considerable variability, however: some patients may experience only a very mild diarrheal disease with no systemic manifestations while other patients may experience severe toxicity with temperature elevations to 104-105°F and WBC of 30,000 to 50,000.

On proctoscopic examination most patients will exhibit inflammation and friability of the colonic mucosa. Biopsy, if done, will show ulceration and crypt absesses, and a smear of the mucosa will reveal many WBC's. In this type of colitis, pseudomembranes are particularly prominent and may be grossly visible through the sigmoidoscope. These gross findings, however, can be segmental. In some cases that have minimal changes in the rectum, colonoscopy will reveal typical inflammatory changes and pseudo-

TABLE IV

ISOLATION RATES OF C. DIFFICILE FROM STOOLS

Patient category (reference)	Culture technique	No. tested	No. with C. difficile (%)
Antibiotic-associated PMC	Blood agar with kanamycin	20	20 (100)
	Heat-shock subculture	5	4 (80)
	Selective agar with cefoxitin plus cycloserine	10	8(80)
Bartlett unpublished experience*	Selective agar with cefoxitin plus cycloserine	32	31 (97)
Antibiotic-associated diarrhea	Brucella base blood (BMB), Clostrisel and selective agar	30	5 (16)
	Selective agar with cefoxitin plus cycloserine	11	2 (18)
Postoperative diarrhea	Blood agar with kanamycin	28	5 (18)
Gastrointestinal disease unrelated to antimicrobials	Heat-shock subculture	20	0 (0)
Healthy adults+	Multiple media	137	4 (3)

*Stools contained the cytopathic toxin.
+No specific attempt was made to detect C. difficile.

membranes in more proximal segments of the colon. In severe cases the ulcerative inflammatory process may extend into the terminal small bowel. If barium enema studies are undertaken, there is commonly a diffuse ulcerative process involving the colon that is essentially indistinguishable from ulcerative colitis or the segmental colitis of Crohn's disease.

One of the most important findings in this disease is the presence of grossly visible pseudomembranes. Certainly the presence of such lesions should strongly suggest this diagnosis. However, it should be recognized that clostridial colitis may occur without the presence of grossly visible pseudomembranes and, contrarywise, pseudomembranes may be seen in other forms

of colitis including amoebiasis, infection with Shigella, ischemic colitis, etc.

Typically the disease lasts several weeks but many reported cases have protracted diarrhea for as long as eight weeks. Some severely ill patients have developed toxic megacolon and colonic perforation.

There are 3 features of this syndrome that are important in making a specific diagnosis. First, most patients will give a history of recent antibiotic intake. Occasionally patients are encountered who are not aware that they have been on antibiotics so that it is necessary to vigorously pursue this possibility even to the point of having a relative search the patients home and medicine cabinets for antibiotics. Second, as discussed above these patients will commonly have pseudomembranes present on proctosigmoidoscopic examination. Third, and most importantly, greater than 90% of patients with this syndrome will have detectable cytotoxin present in stool water. Samples of stool water will produce marked cytopathic changes in tissue culture cells and these cytopathic changes will be specifically prevented by either polyvalent clostridial antitoxin or antitoxin to C. sordellii. The results of this test in different groups of patients with established pseudomembraneous colitis or with other forms of the disease is summarized in Table V.

Since the disease can be relatively prolonged and, in some cases, very severe, specific antibiotic treatment is recommended. Table VI shows the antibiotic sensitivities, in vitro, of C. difficile. As is apparent, the organism is quite sensitive to a number of antibiotics. However, it is apparent that not only must the organism be sensitive to the antibiotic but, equally important, the antibiotic must be non-absorbable and must attain a high concentration in the colonic contents. In this regard, vancomycin is the drug choice. At least one controlled trial has established the usefulness of this antibiotic for the treatment of clostridial colitis. The usual minimal inhibitory concentration of vancomycin for C. difficile is 0.2 to 1.6 μg/ml. When 500 mg of vancomycin are given orally four times daily one anticipates a concentration of greater than 1000 μg/ml in the stool water. This type of therapy will cost the patient approximately $25.00 to $40.00/day. The clinical response is excellent in nearly all cases. The diarrhea, fever and abdominal pain improve rapidly over a 24-48 hour period. The organism disappears from the stool as does the cytotoxin.

Because of the great expense associated with the use of vancomycin, an alternative antibiotic is bacitracin. As seen in Table VI, C. difficile is much less sensitive to bacitracin. On the other hand, the drug is not absorbable and high concentrations presumably can be achieved in stool water. It should be emphasized that a number of cases have now been reported in which there are one or more recurrences of the disease following apparent successful treatment with one of these antibiotics. The recurrences are associated with a return of symptoms and with demonstrable cytotoxin in the stool water. It should be emphasized, therefore, that patients should be carefully followed for several months after apparent successful therapy and should be told to return to the physician should symptoms recur.

There is one final clinical point that should be made with respect to the development of this clinical syndrome. Patients who have a well established diagnosis of chronic inflammatory bowel disease (ulcerative colitis or Crohn's disease) typically have episodes of increased severity of

TABLE V

TISSUE CULTURE ASSAYS OF STOOLS FOR A CYTOTOXIN WHICH IS NEUTRALIZED BY C. SORDELLII ANTITOXIN

Patient category	No. tested	No. positive (%)
Antibiotic-associated diarrhea Confirmed PMC	71	68 (96)
"Colitis" without pseudomembranes*	27	13 (46)
Normal sigmoidoscopy	20	3 (15)
No sigmoidoscopy performed	90	21 (23)
Gastrointestinal disease unrelated to antibiotic usage	158	1 (1)
Healthy adults	29	0 (0)
Healthy neonates	82	2 (2)

*Criteria for "colitis" was a colonic mucosa which was granular, friable, or hemorrhagic, or showed erythema and edema.

symptoms with increased diarrhea, fever, elevation of the WBC and other systemic manifestations. In the past, these recurrent episodes of increased clinical activity have simply been considered to be part of the natural history of these inflammatory bowel syndromes. However, one recent provocative publication has reported that approximately 40% of patients who have an established diagnosis of inflammatory bowel disease will become positive for the stool cytotoxin of C. difficile during exacerbation of the symptoms. Furthermore, the clinical symptoms associated with these exacerbations may respond to vancomycin therapy. If these reports are true then in addition to patients developing the syndrome following antibiotic administration, the physician should also be alert to the possibility that patients with either Crohn's disease or ulcerative colitis will experience worsening symptoms due to the superimposition of a colitis caused by infection with C. difficile.

TABLE VI

IN VITRO SUSCEPTIBILITY OF C. DIFFICILE

Antimicrobial agent	No. of strains	Cumulative % with MIC (μg/ml) of:																
		≤1	2	4	8	16	32	64	128	256	>256	512	>512	1,024	≥1,024	2,048	4,096	≥4,096
Clindamycin	38	0	3	18	74	82	82	84	87	87	—[a]	87	—	87	100			
N-demethyl clindamycin	39	3	5	41	79	79	82	82	85	87	—	87	100					
N-demethyl clindamycin sulfoxide	39	0	0	0	0	0	44	82	82	82	—	82	100					
Clindamycin sulfoxide	39	0	0	0	0	0	56	82	82	82	—	82	100					
Lincomycin	39	0	0	0	0	33	79	82	85	85	—	85	—	100				
Penicillin G[b]	39	3	51	82	100													
Ampicillin	39	74	90	100														
Carbenicillin	39	0	0	0	5	54	74	100										
Cephalothin	39	0	0	0	0	5	67	100										
Cefoxitin	39	0	0	0	0	0	3	21	95	100								
Cycloserine	38	0	0	0	0	0	0	0	0	0	—	8	—	42	100			
Nalidixic acid	39	0	0	0	0	0	0	0	8	87	—	100						
Tetracycline	39	51	51	54	54	56	56	100										
Chloramphenicol	39	0	0	46	85	87	97	100										
Metronidazole	39	87	100															
Co-trimoxazole[c]	38	0	0	5	18	34	53	58	61	66	100							
Erythromycin	39	62	74	74	74	74	74	74	74	74	—	74	—	—	100			
Rosamicin	38	53	71	76	76	76	76	76	76	76	—	76	—	92	100			
Neomycin	38	0	0	0	0	0	0	0	13	45	—	76	—	87	—	92	92	100
Gentamicin	39	0	0	0	0	0	3	18	74	92	—	95	—	95	100			
Vancomycin	39	72	92	100														
Rifampin	39	100																
Miconazole	23	0	0	0	0	17	100											
Bacitracin[b]	39	0	3	5	8	49	90	100										

[a] —, Not applicable; susceptibility testing performed for higher concentrations of drug.
[b] MIC expressed as units per milliliter.
[c] MIC = concentration of sulfamethoxazole and trimethoprim (combined in a ratio of 20:1 by weight) required for growth inhibition.

4) Colitis Produced By Infection With E. histolytica:

Patients who present to the physician with an acute colitis, particularly in the southern portions of the United States including Dallas, may have colitis associated with an infection by pathogenic amoeba rather than pathogenic bacteria. Since the differential diagnosis of inflammatory colitis routinely involves ruling out this particular type of infection, the next section of this protocol gives a very brief review of the clinical and pathological characteristics of amoebiasis so that these characteristics can be contrasted with those produced by bacterial colitis.

The major phases in the life cycle of the parasitic amoebae are illustrated in Fig. 26. Just as the pathogenicity of bacteria is determined by the plasmids that the organisms carry, the pathogenicity of E. histolytica also is determined by the presence of genetically encoded structural components. Several recent studies that deal with the ultrastructure of pathogenic Entamoeba histolytica have provided a possible explanation for the mechanism of tissue invasion. Under the electron microscope trophozoites isolated from patients with active amoebic colitis show a definite "fuzzy" layer approximately 300A wide surrounding the outer limiting membrane. The cytosol of the pseudopodia is usually less electron-dense than the central portion of the cell and contains few or no vacuoles. In contrast, numerous vacuoles are present in the central portion of the cytoplasm and each of these structures is surrounded by a limiting unit membrane resembling the plasmalemma suggesting that they are derived by phagocytic activity. It should also be pointed out that a fuzzy layer is seen lining the interior surface of the limiting membrane of these vacuoles. The endoplasmic reticulum

LIFE CYCLE OF E. histolytica

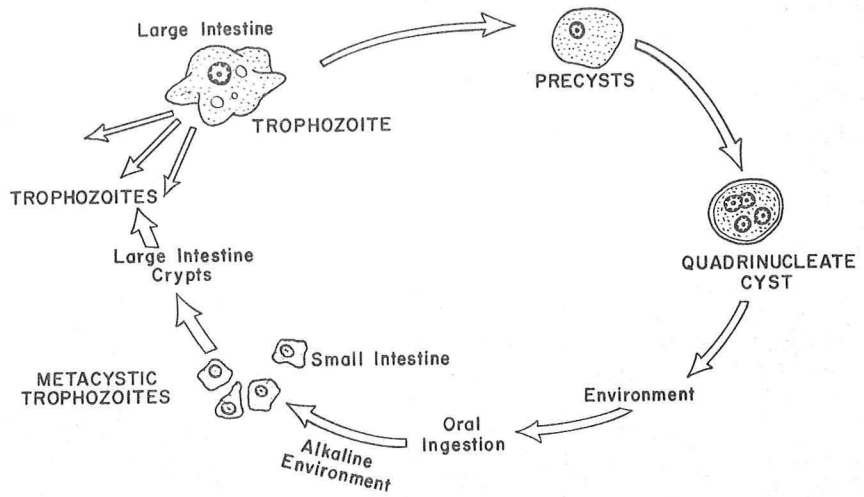


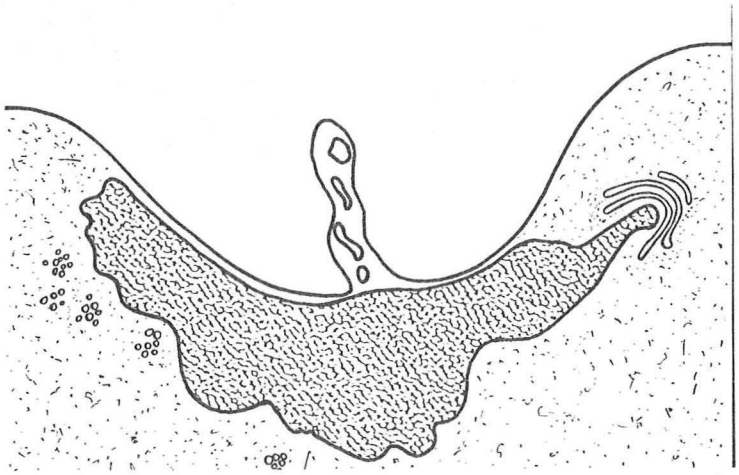
FIG. 26

consists of a number of small tubules and vesicles within the cytosol but no well-defined Golgi complex or mitochondria are seen in Entamoeba histolytica. There are, however, glycogen particles scattered throughout the cytosol. Of particular importance is the observation of peculiar structures, illustrated diagrammatically in Fig. 27, lying just beneath the limiting membrane on the periphery of the amoeba. These structures, surface lysosomes, consist of an amorphous background of material surrounded

by a limiting membrane. The limiting membrane of the lysosome comes into immediate juxtaposition with the plasmalemma so that the contents of the lysosome are separated from the exterior of the amoeba by only the thickness of the double plasma unit membrane. On the interior margins of the lysosome

Surface lysosome in Entamoeba histolytica

FIG. 27



one commonly sees tubular structures in close relationship to the lysosomes that resemble primitive Golgi tubular complexes. In addition, there commonly are collections of polyribosomes in the cytosol immediately adjacent to the lysosome. These structures suggest active protein synthesis is taking place and histochemical studies have shown the presence of various lytic enzymes in these surface lysosomes. Of particular interest, as also shown in Fig. 27, is the finding of an apparent "trigger" mechanism extending outward from the surface lysosome to the exterior of the amoebic cell. This "trigger" is a complex tubular structure and presumably in some manner results in rupture of the surface lysosome and discharge of the hydrolytic enzymes contained therein when the plasmalemma encounters a target cell. Scanning electron microscopy of Entamoeba histolytica reveals that the surface of the amoeba contains a number of these lysosomes that occupy small depressions in the limiting plasma membrane. One can visualize a single "trigger" projecting outward from the middle of each of these depressions.

The earliest lesion identifiable in the large intestine is usually a tiny, pinhead-size ulcer with hyperemic margins and edema of the surrounding mucosa. This ulcer is very superficial and reaches only down to the muscularis mucosa. Commonly there is a small amount of whitish exudate within the ulcer region. In a smaller number of cases the earliest lesion appears as a more extensive superficial erosion with clearcut margins and irregular shape. Bleeding is uncommon in these early lesions. Later the ulcers enlarge and form more typical "buttonhole" lesions up to 1 cm in diameter with excavation of the ulcer base and irregular, over-hanging margins. A "wet preparation" made from the discharge of these ulcers is commonly rich in living trophozoites. Histological sections taken of the margins of such ulcers also commonly show organisms invading the submucosal tissue. The polymorphonuclear leukocytic response to tissue invasion by the amoeba is relatively less intense than to bacterial invasion so that pus cells are relatively few in number in tissue sections and in the

mucoid secretions from the ulcer region. In a number of cases the intact mucosa between ulcers is edematous but otherwise relatively normal. However, it should be emphasized that in many cases the mucosa may be diffusely involved and appear as an erythematous, granular, fragile mucosa which, for practical purposes, is indistinguishable from other forms of colitis such as acute Shigellosis or idiopathic ulcerative colitis.

Uncommonly, amoebic infection of the colon takes the histological form of an "amoeboma". This is usually present as a single lesion in which simultaneously there are destructive changes produced by the amoeba, inflammation and granulation tissue overgrowth but little, if any, fibrosis. Grossly the mucosal surface is ulcerated and covered with a greyish, nodular exudate. The wall of the intestine is very much thickened and is firm, inelastic and irregular. While most amoeboma occur as a solitary lesion found predominantly in the cecum or rectum, occassionally these lesions may be multiple.

The clinical presentation of patients with amoebic dysentery or colitis varies enormously from patients with very mild or essentially no symptoms to patients who present with fulminating disease and overwhelming toxicity. Acute amoebic colitis usually begins abruptly and symptoms may be present for only a few days or few weeks at most. In patients with very mild symptoms there is usually involvement of the rectal or rectosigomoid areas but cecal involvement is not clinically apparent. Complications are rare although an occasional amoeboma is identified. This form of amoebic colitis is probably the most commonly encountered clinical syndrome. In more severe disease patients often present with diffuse colonic involvement. There may be marked fluid loss and ECF depletion, grossly bloody diarrhea with rare episodes of massive bleeding and occasional local complications such as colonic perforation or formation of amoeboma. In its most fulminating form diffuse amoebic colitis may present with the clinical picture of "toxic megacolon" which is indistinguishable from the syndrome seen in idiopathic ulcerative colitis.

Amoebic colitis also may present with a more chronic course in which there is a low grade, continuous or intermittent diarrhea, commonly without blood, that occurs over many months or years. This type of chronic amoebic colitis may be localized or diffuse in its distribution in the colon and may lead to weight loss, general debility, protein-losing enteropathy and persistent iron deficiency anemia. If an amoeboma is present, the mass lesion in the cecum or rectosigmoid may be mistakenly diagnosed as carcinoma.

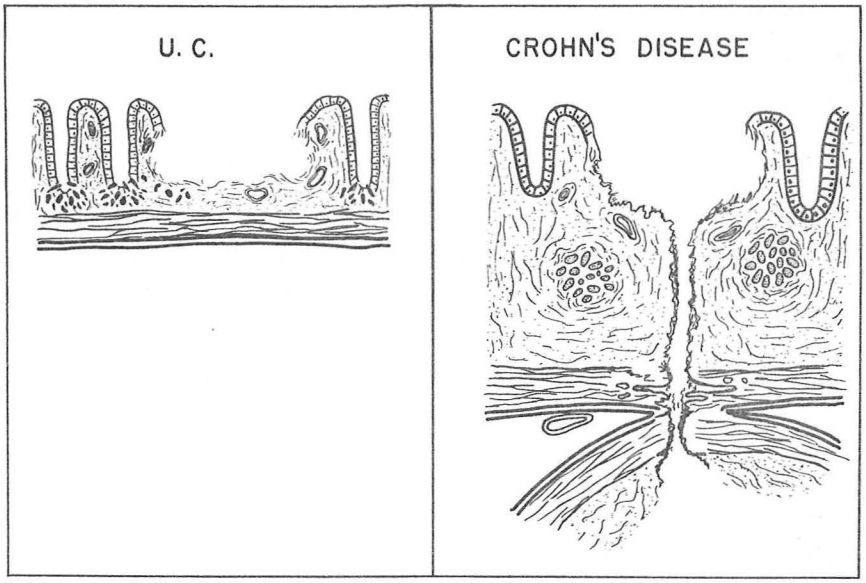
5) The Characteristics of Colitis Produced by Idiopathic Crohn's Disease And Ulcerative Colitis:

In any patient presenting with acute or chronic colitis, the physician must also seriously entertain the diagnosis of either Crohn's disease or ulcerative colitis. There are no specific tests available for the definitive diagnosis of either of these diseases. The diagnosis, therefore, involves ruling out all other known causes of enteritis and colitis and the establishment of a chronic clinical course that is compatible with these two diseases.

As illustrated in Fig. 28, the typical pathology seen in ulcerative colitis and in Crohn's disease is very different. Ulcerative colitis

usually involves a very superficial inflammatory process in which there are collections of pus cells beneath the colonic crypts (crypt absesses).

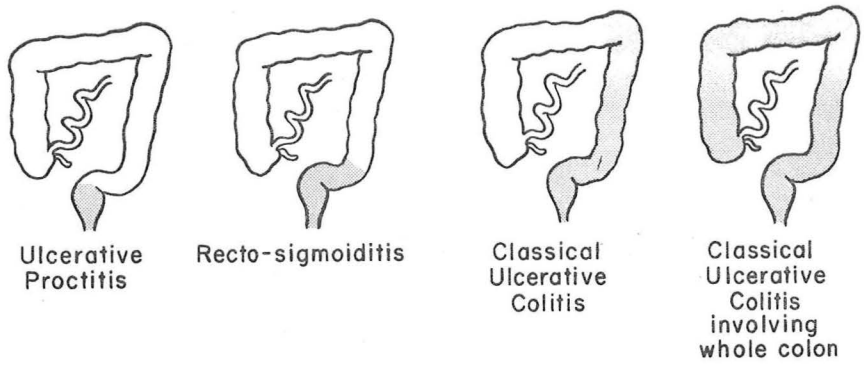
FIG. 28



These absesses may coalesce and form grossly visible ulcers. Thus, when viewed through the sigmoidoscope the colon appears erythematous and ulcerated, bleeds easily and exudes pus. Except in the most severe cases, the inflammatory reaction does not penetrate through the wall of the intestine to involve the serosal surface. Thus, the muscular layers and serosal surface remain thin, translucent and free of an inflammatory reaction. In contrast, in Crohn's disease there is an extensive infiltration of the bowel wall with inflammatory cells that does involve the muscular layers and the serosal surface. As a consequence, the involved area of intestine usually becomes tightly adherent to any structure that is adjacent to it. There are two other histological findings that are very characteristic of Crohn's disease and that are important in the differential diagnoses. First, in approximately one-third of the patients the mucosal ulcerations burrow deeply through the intestinal wall and form either fistulae or sinus tracts to adjacent structures. Secondly, in some patients (the incidence varies from about 10% to 45% depending upon age and location) typical granulomas are found in the submucosal tissues. Thus, the finding of granulomas in intestinal biopsies or the demonstration of fistula tracts is very suggestive of the diagnosis of Crohn's disease.

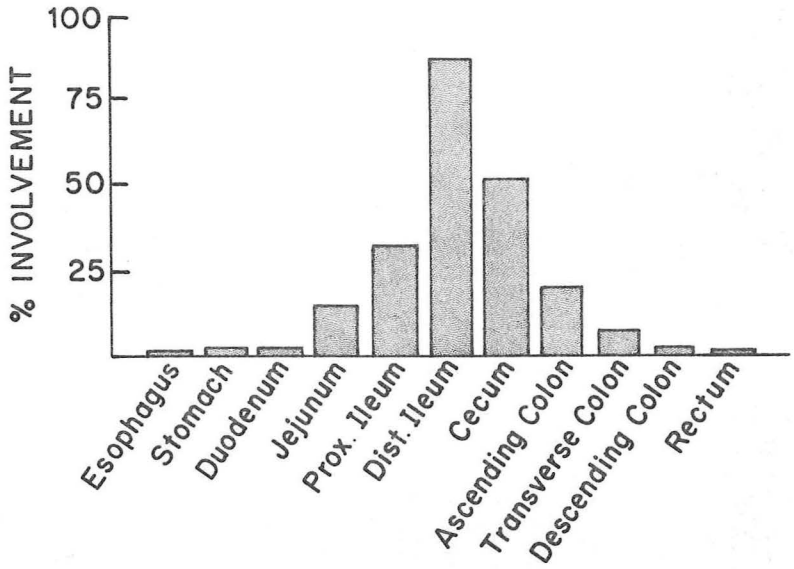
Another feature of these illnesses that is important in the differential diagnosis has to do with the distribution of the areas of involvement in these two syndromes. As shown in diagrammatic fashion in Fig. 29, ulcerative colitis is found exclusively in the colon. Furthermore, it almost always involves at least the distal rectum but may involve more proximal portions of the colon as a continuous, ulcerative process. Thus, as illustrated in Fig. 29, a patient may have ulcerative proctitis or may have involvement of the sigmoid colon, the left colon or the entire colon. Again, it should be emphasized that > 95% of the cases of ulcerative colitis will have involvement of the rectum and the disease will extend more proximally as a continuous process all the way to the ileocecal valve. Ulcerative colitis probably never involves the small intestine (the entity of "back-wash" ileitis should not be considered as pathological involvement of the ileum by ulcerative colitis).

FIG. 29



In contrast, Crohn's disease typically involves very different portions of the gastrointestinal tract. In autopsy cases, for example, the most commonly involved area of the gastrointestinal tract is the distal illeum, as shown in Fig. 30. The second commonest area of involvement is the cecum and right colon. More distal areas of the colon and more proximal areas of

FIG. 30



the small intestine may be involved, but the incidence of involvement is much less. When the patient presents to the physician and is evaluated by X ray and endoscopic procedures, three separate clinical syndromes are defined. The most common type of patient (about 45% of all cases) present with X ray evidence of involvement of only the terminal small bowel. A second group of patients (approximately 35-40% of all cases) present clinical evidence that the disease process involves the terminal small bowel and a portion of the cecum or right colon. Finally, approximately 10% of cases of Crohn's disease present with evidence of involvement of only

the colon. This entity is referred to as Crohn's colitis or granulomous colitis to distinguish it from ulcerative colitis. When Crohn's disease involves the colon it often manifests a different type of distribution. As shown in Fig. 31, for example, these differences in distribution of the disease process is an important differential point in distinguishing Crohn's disease of the colon from ulcerative colitis. For example, Crohn's disease may involve the colon and the small bowel. The disease process may be predominantly on the right side of the colon or may involve skip leisons in the colon. Finally, any portion of the colon may be

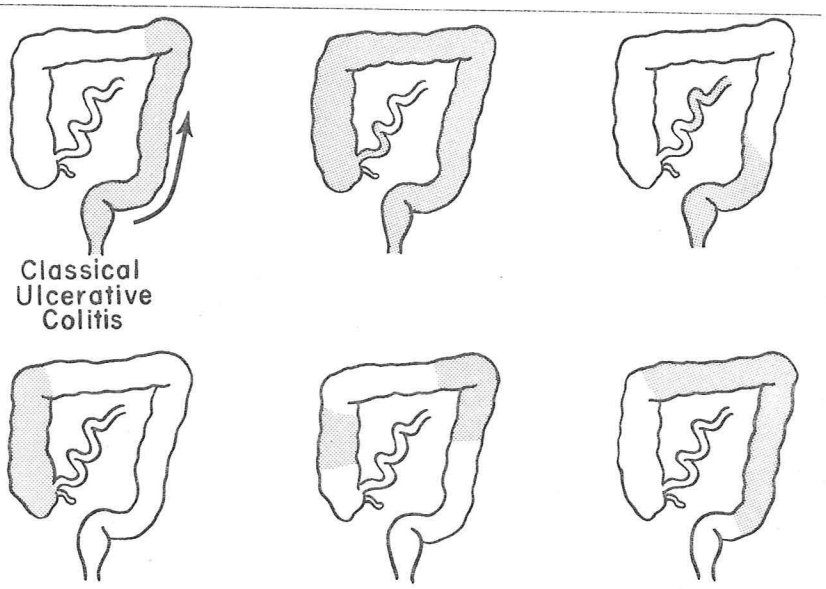


FIG.31

involved but the rectum may be spared. It should be emphasized that any of these findings should strongly suggest that one is dealing with Crohn's disease and not ulcerative colitis. Again, it should also be emphasized that classical ulcerative colitis nearly always involves the rectum and extends proximally as a continuous ulcerative process. In addition, the finding of granulomous or the formation of fistulae also strongly suggests that the colitis is due to Crohn's disease and not ulcerative colitis. In summary, then, ulcerative colitis is usually differentiated from Crohn's disease primarily on the basis of the distribution of the disease processes.

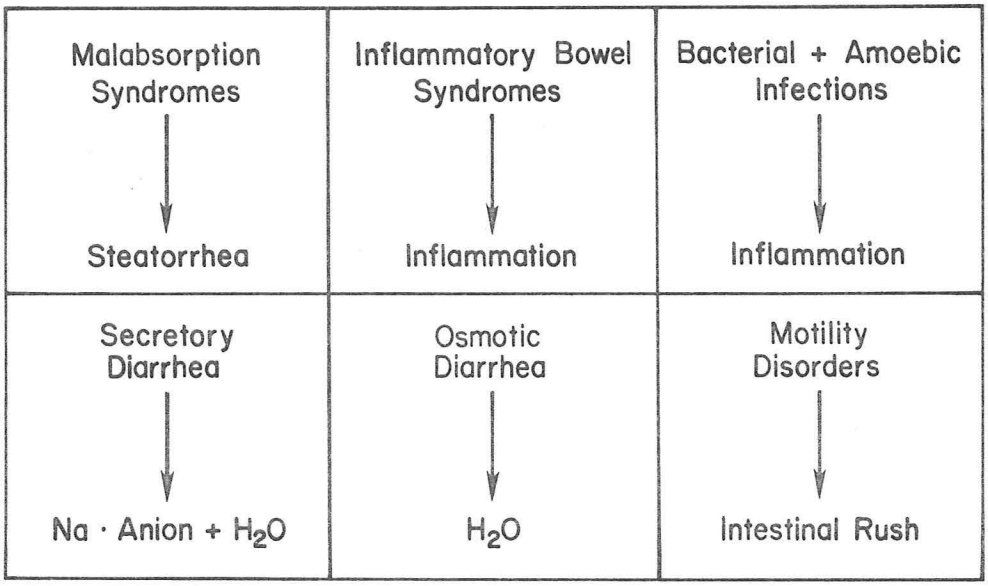
III. APPROACH TO THE PATIENT WITH COLITIS

The most common presenting symptom in patients with acute or chronic inflammatory colon disease is diarrhea. This symptom, however, is not specific for the inflammatory colon diseases but is also seen in a number of other gastrointestinal disorders. Thus, the initial diagnostic event must be directed at the identification of that group of patients who do have inflammatory colon disease as opposed to the other clinical syndromes also presenting with the symptom of diarrhea.

Fig. 32 summarizes the major clinical syndromes that commonly present with more serious or prolonged diarrhea that must be considered in any patient who presents to the physician or to the emergency room with the

chief complaint of an increased frequency or liquidity of his/her bowel movements. The first major group of diseases are classified under the general category of malabsorption syndromes. This category includes a large number of individual illnesses all of which are characterized by

FIG. 32



failure to digest or failure to absorb at least dietary fat (in some cases dietary carbohydrate and protein also will be malabsorbed). This group of diseases include such diverse entities as pancreatic insufficiency, blind-loop syndromes, sprue and obstruction of the intestinal lymphatics. This group of diseases is usually identified by performing either a qualitative or quantitative stool fat determination. The finding of excessive amounts of fat in the stool (steatorrhea) essentially identifies a patient as belonging to this category of disease. A second group of patients will present with a moderate to very large-volume, watery diarrhea. Usually, they will manifest no steatorrhea and no symptoms of systemic illness (no fever, elevation of the WBC). Such patients are generally separated into two groups depending upon whether the diarrhea is due to a secretory process or due to the presence of an osmotically active material in the gut lumen. The secretory diarrhea may be due to many specific causes such as the production of substances either in the gut lumen or in the vascular space (from tumors) that induce the secretion of an isosmotic fluid. The diarrhea is often of a very large volume and persists even during fasting. The osmotic pressure of the stool water is usually fully accounted for by the content of electrolytes. Osmotic diarrhea is, on the other hand, usually produced by the presence of osmotically active molecules in the intestinal lumen that pull water from the vascular space and cause diarrhea. Such syndromes generally produce diarrhea of only moderate volumes and the diarrhea ceases with fasting. Often, there is an "osmotic gap" in that the osmotic pressure of the stool water cannot be accounted for by the concentration of sodium, potassium and appropriate anions. Finally, there are also a group of illnesses in which there appears to be a primary motility disorder. The diarrhea in these cases is presumably caused by rapid intestinal transit

through the gastrointestinal tract.

In contrast to these major syndromes presenting with steatorrhea, intestinal rush or a large volume, watery diarrhea, there are two other groups of illnesses that present primarily with evidence of colonic (and small bowel) inflammation: such patients generally fall into two categories including those who have idiopathic inflammatory bowel disease (ulcerative colitis and Crohn's disease) and individuals who have bacterial or amoebic infections of the colon. Clearly, the initial diagnostic event is to determine that one is dealing with one of these diseases that is capable of producing acute inflammation of the colon and to separate this group of patients from those patients who have acute or chronic diarrhea due to the presence of steatorrhea, secretory or osmotic diarrhea or motility disorders.

Most patients falling into the category of inflammatory colitis will give a history of frequent, small-volume diarrhea. Not uncommonly a patient will describe the passage of fresh or old blood as well as mucus or pus. The symptoms may vary in duration from only a few days to many months or years depending upon the underlying disease. Commonly there is evidence of tissue invasion/destruction with systemic symptoms, fever and an elevation in the WBC. The illness may vary, however, from a very mild syndrome to one of overwhelming toxicity and death.

The major diagnostic procedures that should be carried out in such patients are summarized in Fig. 33. The initial diagnostic procedure that should be undertaken is to subject the patient to rectosigmoidoscopic examination without preliminary preparation of the colon. The purpose of this

FIG. 33

DIAGNOSTIC PROCEDURES

1) Rectosigmoidoscopy (Colonoscopy)
2) Mucosal Smear For WBC
3) Specimens For Bacterial Cultures
4) Scrapings For E. histolytica
5) Barium Enema/Colonoscopy

procedure is to establish that the patient has inflammation of the colon as manifested by erythema, friability, ulceration and exudation. Occasionally some of the diseases that fall into this category (e.g. Crohn's disease or

antibiotic associated colitis) will spare the rectum and it will be necessary to perform colonoscopy in order to identify the diseased portion of the colon. During this examination several other diagnostic procedures should be carried out including preparation of a mucosal smear for pus cells, the obtaining of fecal material for bacterial cultures and the obtaining of mucosal scrapings in order to look for E. histolytica in a "warm-stage" preparation. Under circumstances where the disease is more prolonged, it may also be necessary to obtain a barium enema or to perform colonoscopy in order to obtain specific information on the distribution of the inflammatory process in the colon and terminal small bowel. The specimens for bacteriological culture should be taken immediately to the laboratory. The bacteriology laboratory has the capability of culturing pathogenic Shigella, Salmonella, Campylobacter and Yersinia. Most of these organisms can be identified within 24-48 hours: however, Yersinia may require many days or several weeks to grow out. E. coli can also be cultured: however, in order to establish that a particular E. coli is tissue-invasive, and, therefore, the probable cause of acute colitis, would require the performance of a test of tissue invasiveness such as the Sereny test. Such tests are not routinely available in the hospital lab but, in special circumstances, might be performed in one of the research laboratories. C. difficile cannot be cultured routinely in the hospital laboratory: however, the cytotoxin present in stool water of patients infected with this organism can be detected using the tissue culture test discussed earlier (Dr. James Luby, Infectious Disease section).

By following these diagnostic procedures the patient will be identified as having an inflammatory process of the colon. The physician is then faced with the differential diagnosis of a relatively large number of diseases that can produce such "colitis". It should be emphasized that these various illnesses cannot necessarily be distinguished on the basis of clinical behavior, the appearance of the colon or the finding of pus cells in the colonic exudate. Nearly all of the specific diseases that can cause an inflammatory bowel syndrome are capable of producing ulceration, friability and bleeding from the colon and biopsies in nearly all of these illnesses will show inflammatory changes and crypt absesses. The findings that are important in differentiating the various causes of colitis are reviewed in the following three paragraphs.

In this part of the country, amoebiasis is always an important possible cause of colitis. As reviewed in Fig. 34, there are a number of findings that would suggest that this is a possible etiology in a given

DIAGNOSIS	SUGGESTIVE FINDINGS	DEFINITIVE DIAGNOSIS
AMOEBIASIS	1. History of Contaminated Water/Food to Endemic Area 2. Duration May be Short or Long 3. Isolated Ulcers in "Normal" Mucosa 4. Few WBC in Proportion to Degree of Inflammation 5. Disease Localized to Cecum or Rectosigmoid Colon 6. Prompt Response to Flagyl	1. Demonstrate E. histolytica 2. Elevated CF Test a) Seramoeba Test b) Indirect HA Test

FIG. 34

patient. The patient may have had a history of intake of contaminated water or food or travel to a endemic region such as Mexico, Central America or South America. However, it should be emphasized that amoebiasis can be acquired within the city of Dallas. If sigmoidoscopic examination reveals isolated ulcers in an apparently normal mucosa, amoebiasis should be suspected. However, most cases of amoebiasis have diffuse erythema, ulceration and friability that is indistinguishable from other forms of inflammatory bowel disease. Another observation of importance would be the finding of relatively few pus cells on the mucosal smear when there is clearly marked inflammatory changes in the colon. Nearly all of the other causes of inflammatory colitis will exude large numbers of WBC's. Finally, the presence of localized disease in the cecum or rectosigmoid colon or a very prompt symptomatic response to Flagyl also suggests amoebiasis. The definitive diagnosis of this disease, however, depends upon either 1) the demonstration of E. histolytica in the stools or mucosal scrapings or 2) a diagnostic elevation of one of the serological tests for amoebiasis. Two such tests are now available at Parkland Hospital: the Seramoeba test and the indirect hemeagglutination test. A negative Seramoeba test is reliable: however, a positive Seramoeba test may represent a false positive and should be confirmed with the indirect hemeagglutination test.

As outlined in Fig. 35, there are a number of tissue invasive bacteria that should be considered as potential causes for an inflammatory colitis. Certainly all patients should be cultured for Shigella, Salmonella and Campylobacter. In certain circumstances one may also have to consider the possibility of a tissue invasive E. coli, Yersinia or infection with C. difficile. Patients who have acquired a Shigella or Salmonella commonly give a history of intake of contaminated water or food or exposure to another

DIAGNOSIS	SUGGESTIVE FINDINGS	DEFINITIVE DIAGNOSIS
SHIGELLA SALMONELLA	1. History of Contaminated Water/Food Exposure to Sick Individuals 2. Self Limited (< 2 weeks)	Isolate Organism
CAMPYLOBACTER	1. History of Exposure to Animals or Animal Products 2. Maybe Prolonged to 8-10 weeks	Isolate Organism
ENTEROINVASIVE E. coli	1. History of Contaminated Water/Food Travel to Endemic Area	Isolate Organism and ⊕ Sereny Test
C. DIFFICILE	1. History Antibiotic Intake 2. Exascerbation of U.C./C.D. 3. May be Prolonged or Recurrent 4. Pseudomembranes	Demonstrate Cytotoxin

FIG. 35

sick family member or child. Infection with these two organisms is almost always self-limited and virtually never lasts longer than two weeks.

Infection with Campylobacter should be suspected when the history indicates exposure to animals, animal products or unpasturized milk. The disease may be prolonged. Certainly all patients with inflammatory colitis should be carefully questioned with respect to the use of antibiotics. A history of antibiotic intake in the recent past or the finding of pseudomembranes on examination of the colon should immediately raise the possibility that one is dealing with colitis due to overgrowth of C. difficile. The definitive diagnosis of these illnesses will depend upon isolation of a pathogenic Shigella, Salmonella, Campylobacter or Yersinia. Definitive diagnosis of clostridial overgrowth depends upon the demonstration of the cytotoxin in the stool water of such patients.

The third group of illnesses that must be considered in the differential diagnosis of acute and chronic colitis include the two idiopathic diseases ulcerative colitis and Crohn's disease. There is no definitive way to make either of these diagnoses. Rather, the diagnosis is based upon the presence of a chronic inflammatory reaction with a certain characteristic distribution within the gastrointestinal tract. As discussed in detail

FIG. 36

DIAGNOSIS	SUGGESTIVE FINDINGS	DEFINITIVE DIAGNOSIS
ULCERATIVE COLITIS	1. History of Prolonged Disease 2. Typical Location (rectum with variable involvement of more proximal colon)	
CROHN'S DISEASE	1. History of Prolonged Disease 2. Typical Location (ileocolitis, ileitis or segmental colitis) 3. Granuloma 4. Fistulae	

above, chronic ulcerative colitis almost always involves the rectum and may involve more proximal portions of the colon as a continuous process. The disease, however, probably never crosses the ileocecal valve to involve the small bowel. Crohn's disease, on the other hand, most commonly involves the terminal ileum and right colon (ileocolitis) or the terminal ileum alone (ileitis). It may involve the colon alone, but when it does so typically involves the right colon, spares the rectum or produces a segmental colitis. In about one-third of the patients one may find granuloma in the submucosal tissue or fistula tracts in various portions of the gastrointestinal tract. Again, it should be emphasized, that these two diagnoses depend upon a demonstrated chronic course and exclusion of all other known forms of colitis.

Apart from these major causes of colitis, other diseases may occasionally have to be considered. In elderly patients ischemic colitis is occasionally a problem. Rarely infiltrative tumors present as "colitis". The various venereal diseases, particularly in homosexuals, can produce a distal proctitis.

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