

DEVELOPMENT OF A MODEL
FOR EXPERIMENTAL COLITIS
IN THE RAT AND CAT USING
ACETIC ACID

CENTRE FOR NEWFOUNDLAND STUDIES

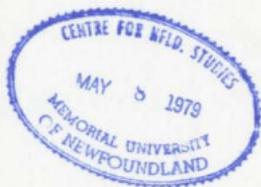
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Development of a Model for Experimental Colitis in the Rat and Cat
Using Acetic Acid.

by

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ABSTRACT

Diffuse, topical application of dilute acetic acid to the serosal surface of the rat colon, or standardized intraluminal (per rectum) instillation of the agent into rat or cat colon, induced a reproducible, diffuse colitis in a dose-response manner. The lesions were reproduced with 100% reliability and were evaluated up to 60 days in the rat, and 21 days in the cat, when healing generally occurred. Histopathological features of this chemically-induced colitis were diffuse ulceration of the distal colon down to the muscularis mucosae or superficial aspect of the submucosa, occurrence of pseudopolyp-like structures, alterations in crypt-depth and mucus secretion, and a transmural, non-specific inflammatory response. Histochemical monitoring revealed an increase in alkaline phosphatase activity as the lesion developed, while mucus staining was markedly decreased. Electron microscopy revealed the mechanism of this agent on the colonic mucosa and indicated initial epithelial sloughing and mucosal edema followed by rapid infiltration of inflammatory cells from submucosal vessels and of erythrocytes from damaged mucosal capillaries. The histopathological pattern observed here was similar to that of an edematous burn response of the colonic mucosa or that induced by other caustic agents, indicating that the colonic mucosa of various species reacts in a similar manner to mechanical or chemical insult. Motility alterations recorded in the colons of cats afflicted with acetic acid-induced lesions, demonstrated a pattern similar to that observed in individuals with inflammatory bowel disease or other diarrheal states. Initial suppression of unstimulated motor

activity was followed by a subsequent hyperactive response, while that of urecholine-stimulated activity also demonstrated the initial decrease but returned to near normal values by the end of the experimental period. Suppression of urecholine-stimulated activity during the period of severest diarrhea (greatest ulceration), was not characteristic of that reported in patients with colitis and may indicate some temporary impairment of nervous organization. The opposite was observed during the period of healing and regeneration of the mucosa in animals where the colonic motility was observed to be greatly increased, perhaps reflecting an increased sensitivity. Preliminary trials with corticosteroid and sulfanilamide compounds demonstrated a therapeutic value for the sulfanilamides (Salazopyrin) in significantly reducing the severity of the lesions following pre- and post-induction of colitis treatment in rats.

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INTRODUCTION

Inflammatory Bowel Disease

The human colon is basically a storage organ which also exhibits important absorptive functions, particularly in the conservation of water. However, this organ is not essential to the continuance of life. Its primary role of storage exposes the colonic mucosa for a longer period to the diverse array of exogenous chemical, bacterial, viral, fungal, protozoal, phytic and helmenthic agents which may be present in the gastrointestinal tract. For this reason, inflammatory disorders of the colon, many of which are the direct result of these exogenous agents, are common. They may present occasionally as extensions of disease of the small intestine, but more often as primary illness involving a considerable length of the colon.

Bercovitz (1973) classified the primary inflammatory diseases of the colon as:

1. Ulcerative colitis; acute or chronic, primarily involving the mucosa and submucosa.
2. Granulomatous colitis (also often referred to as regional enteritis, Crohn's disease of the colon, segmental or transmural colitis); involving all regions of the colonic wall.
3. Pseudomembranous colitis.
4. Ischemic colitis.
5. Bacillary (Shigella) dysentery.

6. Amebic (*Entamoeba histolytica*) dysentery and other protozoan dysenteries.
7. Other inflammatory disorders such as tuberculosis, Staphylococcal, typhoid and necrotizing colitis, sarcoidosis and radiation-induced colitis, as well as other "unclassified" inflammations.

Of the many types of colonic inflammations, all tend to produce anatomic lesions closely resembling one another and it is often impossible on gross and even microscopic examination to identify the precise etiologic agent (Robbins, 1974). In accordance with Bercovitz's list of inflammatory disorders, the two primary colonic inflammations are ulcerative and granulomatous colitis. While other inflammatory disorders may be attributable to other systemic diseases, the etiology of both ulcerative and granulomatous colitis remains obscure.

The history of inflammatory bowel disease, more specifically ulcerative colitis, extends back over a century, its origin lost amid diagnostic confusion with infectious and non-infectious diarrheal states. In 1875, Wilks and Moxon first delineated ulcerative colitis as a separate disease entity from other forms of colitis caused by bacilli or parasites. For many decades it was considered a disease of industrialization. Prominent in Europe and North America, its incidence is notably higher among caucasians than non-whites, and with an ethnic variation best demonstrated in the western Jewish population (Acheson, 1965). Since then ulcerative colitis has been diagnosed with increasing frequency over the past two decades on every continent of the world, including the black population of North America. Kirsner (1974b) attributed this spread to both an actual increasing

disease incidence, as well as to improved health services to the indigent and within impoverished countries.

Granulomatous colitis has been a distinct entity for a much shorter time. Crohn and colleagues presented a classic description of regional ileitis in 1932, while the first case of granulomatous colitis was not documented until 1934 by Colp. Following this discovery, Rappaport and coworkers (1951) reviewed 100 cases of regional enteritis and found colonic involvement in approximately half, although it was usually less severe. Over the past four decades a marked overlap between the two diseases developed, not only pathologically but in anatomic distribution. It became convenient to consider these two colitis forms as a single entity grouped under the heading of chronic ulcerative colitis. Only since the fundamental description of segmental (ulcerative) colitis by Yarnis and Crohn in 1960, has appreciable literature become available on the differentiation of these two colonic conditions. While at present they are considered to be two distinct disease entities (Schleisenger & Fordtran, 1973), overlap or co-existence of these two diseases within a single individual has been reported (Voitk et al., 1976).

Despite its long history, many salient features about inflammatory bowel disease remain obscure. The predominant age of onset being between 10 and 19 years of age, coupled with the highly increased risk of colonic carcinoma in longstanding cases of chronic ulcerative colitis (Robbins, 1974), makes it a most mysterious and menacing disease. Detailed histopathology, description of course and complications encountered in these diseases have been well documented (Schleisenger & Fordtran, 1973) and

only emphasizes the relative non-specific nature of the inflammatory reaction, the uncertain and unpredictable course, and the variable response to medical and surgical treatment. Ulcerative colitis is perhaps the most prominent colonic inflammation and few diseases, including connective tissue disorders, are more debilitating, depressing and threatening to the individual (Kirsner, 1971). In light of the continuing increase in incidence of inflammatory bowel disease, the development of an experimental animal model would be most useful in understanding the various types of pathogenic responses of the colon, in testing of possible therapeutic agents, in the study of healing phenomenon, and in its role as a possible precursor lesion of colonic cancer.

Experimental Colitis

The search for an animal model for inflammatory bowel disease, such as ulcerative and granulomatous colitis, is difficult as the human form of the disease may well be produced by the altered reactivity of an individual, an agent or agents to which the individual is exposed, or both. Therefore, there are diverse combinations of conditions capable of producing inflammation of the colon. Furthermore, one agent may initiate the process while another may promote its progression. The possibility for these interactions are dynamic within the gastrointestinal tract, as it has a great diversity of exposure to exogenous agents (Pfeiffer, 1977).

The production of an experimental animal model for inflammatory bowel disease may be approached from three angles:

1. the search for a procedure involving an immunologic, bacterial, or viral imposition, which induces a lesion similar to that observed in humans and which acts by the same mechanism from which the human disorder is speculated to arise
2. the search for a naturally occurring disease process in animals that resembles the human disease
3. the development of a practical working model which reliably induces, in a convenient laboratory species, a disorder which can be easily produced and controlled, and which histopathologically resembles the human disorder, but which may be induced by an artificial method unlike that which is responsible for the human disease.

Since the pathogenesis of inflammatory bowel disease probably involves more than one etiologic factor, investigation of each possible mechanism, clinical and experimental, should help to clarify the nature of the disease. The development of an experimental technique for producing colitis in laboratory animals would be of great value, but relatively few successful attempts to reproduce colitis have been recorded in the literature. A review of those reported reveals limited laboratory investigations of each suggested etiologic agent recorded in the literature (MacPherson & Pfeiffer, 1976). Many and varied techniques have been used in attempts to produce experimental colitis in various animal species, but their significance in relation to the clinical condition in humans is dubious.

At present there is no animal disease or lesion which spontaneously develops and possesses a high frequency of occurrence with close anatomic

and histologic similarity to human inflammatory bowel disease to serve as a convenient and reliable animal model. Therefore, a great need exists for the development of such a model which can be used to investigate pathogenic mechanisms, delayed tissue responses, and novel therapeutic measures.

Spontaneous Colonic Lesions Observed in Animals

Neither ulcerative nor granulomatous colitis has been successfully reproduced experimentally. A search for spontaneous lesions resembling these disease processes has been carried out over a wide range of laboratory and domestic animals. Although many types of inflammatory lesions have been described in these animals, none has had the total characteristic histopathology of ulcerative or granulomatous colitis.

Rodent Colitis:

Stewart and Jones (1941) first reported a spontaneous inflammatory disease in the cecum of the rat. This inflammation was predominantly perivascular. Prominent lymphangitis and lymphostasis were noted and after healing there was extensive scarification at the ulcer sites, in some cases producing a stricture.

Ediger et al., (1974) documented a spontaneous outbreak of colitis in a conventional production colony of Fdc:(SW) mice. This was characterized by increased mortality and a high incidence of rectal prolapse. The most common features were a variably enlarged colon and rectal prolapse. Prior

to this, Brennan et al., (1965) described colitis and accompanying diarrhea in mice experimentally induced with Citrobacter freundii. Ediger isolated this same microorganism from the colon of mice affected with the spontaneous colitis. Brynjolfsson and Lombard (1969) studied the epithelial changes in these prolapsed recti and found that the lesions closely resembled those seen in human colitis cystica.

Acute and chronic ileitis have been reported in the golden Syrian hamster by Boothe and Chevillie (1967). While this process appeared to be limited to the terminal ileum, it caused marked enlargement and thickening of this region. Severe coagulative necrosis of the villi and extension of purulent material into the submucosa and muscularis resulted in a process which closely resembled human regional enteritis, without the appearance of granuloma.

Primate Colitis:

Stout and Synder (1969) reported a fatal, ulcerative colitis-like lesion in four Siamang gibbons. The animals developed the disease under clinical circumstances involving emotional stress introduced by a socio-environmental upheaval. Clinically, the symptoms were comparable to acute Shigella dysentery; however, none of the animal's cage-mates developed diarrhea or colitis.

Porcine Colitis:

The great bulk of gastrointestinal diseases of domestic animals are

inflammatory, many with close parallels to human disease. However, these inflammatory intestinal processes are only described in the various veterinary texts, without particular emphasis on etiology. Terminal ileal lesions resembling human regional enteritis, grossly and microscopically, were described by Emsbo (1951). The incidence of this disease was approximately one percent of all animals slaughtered and has been observed as two main types, muscular and mucous. Haeltermann and Hooper (1967) noted a similar disease in swine of northern Europe. However, this finding did not resemble the human disease and was presumably a result of infection with Salmonella choleraesuis. A number of other investigators (Cross & Kohler, 1969; Glika, 1968; and Moon, 1965) have also reported a naturally occurring colitis in young swine. However, its pathogenesis has not been elucidated.

Equine Colitis:

Rooney et al., (1963) reported a sporadic, acute, fatal disease in horses of unknown etiology, characterized primarily by severe non-bloody diarrhea. Investigation suggested endotoxemia as the etiologic factor. Gross pathology was most severe in the cecum and ventral colon. It occurred in horses of all ages and was usually preceded by a stressful situation one to two weeks prior to its onset. The relationship of this entity to human colitis is not clear, but it demonstrated that "stressful" situations could induce a colitis in horses which may have catastrophic results.

Bovine Enteritis:

Johne's disease is a chronic enteritis observed in cattle, sheep, and goats, and which is caused by Mycobacterium paratuberculosis. However, it does not resemble Crohn's disease or ulcerative colitis. Although it often starts in the ileum, it may involve both the small and large intestines. Mitchell et al., (1970) studied this process and indicated a possible relationship between regional enteritis and sarcoidosis, or the etiologic role of a mycobacterium, but the present evidence remains inconclusive.

Feline Colitis:

Only one report of feline inflammatory bowel disease could be found in the literature. Ewing (1972) encountered a two year old female cat exhibiting tenesmus following defecation, and dripping of fresh blood from the anus. A healthy appetite without weight gain was evident. Following proctoscopic examination, an ulcerative colitis-like condition was noted. After treatment with Salicylazosulfapyridine the animal's colonic mucosal appearance returned to normal.

Canine Colitis:

Perhaps the most promising non-human species with respect to spontaneous colitis is the dog. As early as 1954, Strande et al. reported two cases of inflammatory bowel disease in cocker spaniel dogs, which closely resembled regional enteritis of humans. This disease

process was also documented by VanKruiningen (1972), who stated that this canine form of regional enteritis was comparable, if not identical, to that of man.

Of particular interest in this area is a relapsing type of colitis occurring predominantly, or possibly exclusively, in boxer dogs. (Kennedy & Cello, 1966). The disease usually begins in puppyhood or shortly thereafter and has a 2:1 preference for female dogs. The affected animals have a long history of loose or poorly formed stools prior to the presentation of bloody, mucus-covered stools. The disease is characterized by remissions and exacerbations, many of the latter triggered by pregnancy, change of environment or feed. Anorexia, fever, malaise, and weight loss are late manifestations of the disease.

Lawson et al., (1975) studied a number of cases of this chronic histiocytic canine colitis in such dogs. The earliest discernable histologic changes were focal epithelial cell degeneration and acute inflammation occurring along the luminal surface of the large intestine. In advanced stages the colon was denuded and the submucosa was thickened. Upon autopsy the characteristic gross lesions were confined to the colon and rectum, varying from irregular ulceration to a diffuse or patchy mucosal thickening with only minor ulceration. The unique microscopic feature of the chronic lesion was an infiltration of the lamina propria and submucosa by large, pale macrophages whose cytoplasm contained much PAS-positive material. Crypt abscesses and pseudopolyps were not, however, features of the disease stages examined (Kennedy & Cello, 1966).

This documentation is interesting, the disease sharing features of both granulomatous and ulcerative colitis. It is probably not a suitable model for either, because its occurrence is not reliable or common. Kennedy and Cello (1966) stated that its histology was similar to that of Whipple's disease and even closer to an infection of the intestine involving regional lymph nodes, as observed in cattle and caused by an acid-fast bacillus, Mycobacterium johney. Mottet (1971) suggested that canine colitis is not simply a specific disease, but that dogs suffer from a number of entities which present as colitis. Canine cases reported to date document a range of manifestations; regional enteritis, ileocoloitis, coloproctitis, and segmental granulomatous colitis as well as extraintestinal lesions such as perianal fissures, hepatic granulomas and ulcerative dermatitis as seen in the human form of the disease. Other cases have presented manifestations of mucosal colitis corresponding to a spectrum of mucosal forms in man, including ulcerative colitis, cystica profunda, and self-limited proctitis (VanKruiningen, 1972). Smith and Jones (1957) confirmed the existence of a regional cicatrizing enterocolitis in dogs as well. All these cases were documented with the hope of stimulating interest in them as research models for the human disease.

Features common to inflammatory diseases of both canine and human colonic epithelium include; cryptitis, regenerative hyperplasia, loss of PAS staining, cystic downgrowth into the submucosa, mucosal limitation, cellular response in the lamina propria, and mucosal hyperemia, congestion, and hemorrhage. It is important to recognize that the dog was the first species to be shown to experience a substantial range of spontaneous colonic diseases similar to that of man. Furthermore, it is the purebreds

that have resulted from generations of inbreeding and linebreeding that possess the disease susceptibilities (VanKruiningen, 1972).

Experimental Colitis Induced By Vascular Impairment

Since Virchow's (1853) postulation that impaired vasculature was involved in the development of gastric ulcer, subsequent support for this theory has surfaced (Guth et al., 1975; Pfeiffer & Sethbathki, 1971) and vascular phenomena have been suggested by many workers seeking an organic etiologic explanation for ulcerative colitis (Fairburn, 1973; Shorter & Shephard, 1975). Kirsner (1961) reported that injection of cholinergic compounds, acetylcholine, carbachol, neostigmine and methacholine in dogs have produced bloody diarrhea and a rectal mucosa resembling that seen in the early stages of ulcerative colitis in man. However, the symptoms disappeared rapidly after cessation of administration of these agents. The histological appearance of the colon in dogs given methacholine demonstrated vascular hyperemia, congestion, dilation and hemorrhage, presumably secondary to the severe muscular contractions of the bowel wall. The findings however, were not similar to those of human colitis. Further, lesions did not occur in dogs given large quantities of laxatives over long periods of time (1-2 years), indicating that mere increase in propulsive motility of the bowel wall was not sufficient to produce colitis. The colitis observed after prolonged administration of histamine or the histamine releaser, compound 48/80, in dogs is interesting in relation to the concept emphasizing the role of histamine or some other factor in increasing capillary permeability in the pathogenesis of ulcerative colitis (Kirsner, 1961).

Mechanical obstruction of blood flow to the colon resulted in varying degrees of mucosal ulceration in dogs (Marston et al., 1969). Earliest changes following interruption to the arterial supply to the colon included the radiological "thumbprinting" of the affected segment, due to mucosal edema and hemorrhage, which reverted to normal within a few weeks. Increased numbers of peripheral leucocytes were noted and were directly related to the extent of devascularization and mucosal necrosis.

Pathological appearances, dependent upon the degree of ischemia inflicted, varied from slight mucosal congestion to extensive necrotic ulceration. The lesions were generally similar to those seen in human ischemic colitis but differed by the smaller number of hemosiderin-laden macrophages and the lesser degree of fibrosis. Furthermore, the very early changes created and studied in this experiment are not often seen by the clinical pathologist who is usually presented with mature, florid lesions. Occasional strictures were noted which corresponded to the focal strictures in human cases resulting from previous ischemic episodes. Extensive necrotic ulcers with full-thickness loss of mucosa and replacement of the submucosa with granulation tissue were similar features observed in both the experimental and clinical material (Marston et al., 1969).

Immunological Aspects of Experimental Colitis

Bacterial Infection:

Histologic studies of colonic tissue from humans with inflammatory bowel disease show changes analogous to infection caused by established

pathogens; for example, ulcerative colitis resembles bacillary dysentery (Mottet, 1971). Broberger and Perlmann (1959) found autoantibodies to human colon in the sera of patients with ulcerative colitis and suggested that their occurrence was a result of stimulation by bowel bacteria. Perlmann et al., (1965) showed that these sera also reacted with polysaccharide antigen obtained from sterile rat colon and that the reaction could be inhibited by polysaccharide antigen from certain strains of Escherichia coli. They suggested that stimulation, perhaps by the heterogenic antigen of Kunin, occurring in many bowel organisms, might be responsible for the formation of anticolon antibodies in patients with ulcerative colitis.

Asherton and Holborrow (1966) injected rabbits with dead bacteria in Freund's complete adjuvant and one month later found autoantibodies present against colon. The antigen with which the autoantibodies reacted behaved like mucus and was detected in the colon and sometimes in the ileum and stomach. Polysaccharide preparations of the colon inhibited the reaction of the autoantibodies with the colon in the sera tested.

Injection of some strains of E. coli into the footpads of rats produced an inflammatory disease characterized by diarrhea, bleeding, and severe lesions restricted to the colonic mucosa, including constant presence of ulcerations (Halpern et al., 1967). Symptoms and lesions were chronic and the disease was produced with both live and dead bacteria. This experimental colitis seemed to be the result of an immunological process. No cross-reaction could be observed between the E. coli which produced the disease and any colonic antigen, excluding the

possibility of an autoimmune process. The hypothesis that the experimental colitis was the result of an immunological breakdown of colonic bacterial balance was supported by the fact that the disease could be prevented in these animals by daily oral administration of live E. coli.

Zweibaum et al., (1968) immunized rats with certain strains of E. coli and produced an experimental colitis which by way of symptoms, chronic course, site of lesions, and histological characteristics, was similar to human ulcerative colitis. This experimental disease could also be prevented by oral administration of living E. coli of an appropriate type. Immunization of rats with homologous tissue of the colonic mucosa membrane induced chronic ulcerative cecitis (Mitschke & Kracht, 1968). This disease occasionally occurs spontaneously with the presence of Salmonella enteritidis. Immunization of rabbits with homologous colonic mucosa from rats that had been exposed to this cecitis for long periods of time led to the formation of precipitating antibodies against rabbit colon. However, colonic lesions were never induced.

Cooke et al., (1968) found that colonic autoantibodies were induced in over fifty percent of rabbits immunized with E. coli derived from patients with ulcerative colitis. Although there were no morphological changes in the mucosal epithelium, histochemical enzyme studies demonstrated an increase of glucose-6-phosphate dehydrogenase and alkaline phosphatase, which may be related to early mucosal damage in ulcerative colitis.

Germfree rats monocontaminated with anerobic microorganisms, Clostridium difficile, or another Clostridium species (strain G.62) produced

autoantibodies to colon antigen (Hammarstrom et al., 1969). These antibodies were not found in the sera of germfree rats, germfree rats monocontaminated with various other bacteria, conventional rats of germ-free origin, or conventional Sprague-Dawley rats. The anticolon antibodies of ulcerative colitis patients or rabbit colon that had been immunized with rat colon antibodies, but their specificity was different with no cross-reaction between C. difficile antigen and colon antigen. The other possible mechanisms for autoantibody production in this model were immunogenic alteration of gastrointestinal mucins by bacterial degradation, adjuvant effects of bacterial products, or both of these phenomena (Hammarstrom et al., 1969).

Colitis in young pigs was reported (Cross & Kohler, 1969; Glika, 1968; Moon, 1965), but its pathogenesis not elucidated. Neonatal pigs monocontaminated with E. coli produced a mild enteritis and colitis as well (Staley et al., 1970a). Cellular changes were minimal until edema of the lamina propria developed 144 hours after monocontamination. Intracellular lipid increased after bacterial penetration but cellular degeneration did not appear to result from subsequent edema of the subepithelial lamina propria or impaired circulation which was thought to be the result of intravascular endotoxins. However, once E. coli were present in the circulatory system for a minimum of 60 hours, colitis developed in the neonatal pigs. (Staley et al., 1970b). The morphologic response sequence involved accumulation of macrophages in the lamina propria, swollen terminal capillary endothelia, adherence of fibrin and platelets to the endothelial cell membranes, extravascular fibrin and platelets and erythrocytes, and edema of the lamina propria and submucosa concurrent with

lymphangiectasis and perilymphangitis. The attachment and penetration of E. coli onto ileal and colonic epithelium of neonatal pigs was similar in both areas and occurred with equal frequency.

Colonic epithelial antibodies were implicated in the pathogenesis of ulcerative colitis due to the presence of antibodies to fetal colonic epithelium in the sera of children with the disease (Broberger & Perlmann, 1962). However, these epithelial changes may be secondary and the result of microvascular insufficiency (Donnellan, 1966). Hawk et al., (1967) considered the etiologic factors(s) to act directly on the structures of the submucosa in Crohn's disease of the colon, the earliest detectable lesion coinciding with submucosal edema (Meadows & Batsakis, 1963). E. coli-infected pigs generally displayed edema of the submucosa of the colon (Staley et al., 1970a), and mesocolon (Sojka, 1965), suggesting that ultrastructural alterations in the vascular compartments occur after the penetration of the microorganism. E. coli endotoxins (McGrath & Stewart, 1969; McKay et al., 1966), bacillus anthracis toxins (Dalldorf et al., 1969), or staphylococcal enterotoxins (Finegold, 1967) administered intravenously all produced structural changes in the blood vascular system.

Experimentally induced colitis in mice was also accomplished by using a bacterium, C. freundii, which produced diarrhea as well (Brennan et al., 1965; Brynjolfsson & Haley, 1967). Further attempts to produce chronic ulcerative colitis bacterially with infiltrates of feces and rectal mucosa obtained from affected patients were also unsuccessful (Victor et al., 1950).

Allergy and Hypersensitivity

In the research laboratory the colon has been shown to participate in many types of immunologic reactions. Direct hypersensitivity reactions are characterized by the presence of circulating, measurable, serologic antibody titers. Colonic lesions were produced by the combination of these antibodies with the fixed tissue antigen (Bicks, 1965).

In 1920, Auer produced a severe inflammation at the site of a previously induced, non-specific irritation, by injection of a large dose of a specific antigen to a sensitized animal. Kirsner et al., (1959) induced an 'Auer reaction' in the distal colon of rabbits sensitized by crystalline egg albumen, producing a form of 'colitis' after prior mild irritation of the bowel with dilute formalin, followed by intravenous or intraperitoneal administration of the specific antigen. This 'Auer colitis' was characterized histologically by superficial ulceration and hemorrhage, with infiltration of polymorphonuclear leucocytes, plasma cells, and lymphocytes. Direct evidence of the antigen-antibody nature of the Auer reaction in the colon, found localized only at the site of the lesions, was soon provided (Kraft et al., 1963). Intrarectal instillation of a specific antigen (crystalline egg albumen) in sensitized rabbits was shown to induce a colitis in the distal colon previously exposed to a mild formalin solution which locally increased the capillary permeability (Ford & Kirsner, 1964). The 'Auer colitis' presumably developed on the basis of an antigen-antibody reaction.

The colon of the rabbit and dog has been shown to participate in

the Arthus, Auer, and Schwartzman reactions (Bicks *et al.*, 1965), and in mucosal cell reactions (Bicks & Walker, 1962). None of these has produced chronic, progressive lesions. There is no information available on whether any treatment will modify or prevent them.

The colon also participates in a delayed hypersensitivity reaction. This is similar to rejection of homografts (Bicks & Rosenberg, 1964), tuberculin sensitivity, and contact dermatitis, in that immunologically competent cells, not serologic antibody, are necessary to produce the histologic lesions (Bicks, 1965).

Rosenberg and Fischer (1964) demonstrated the participation of the guinea pig colon in a chronic, delayed hypersensitivity reaction of the contact dermatitis type. Sensitization and daily rectal instillation of 2,4-dinitrochlorobenzene (DNCB) resulted in microscopic, necrotic, mucosal edema and perivascular cuffing. Contact dermatitis fulfills the criteria of a specific hypersensitivity reaction and this type of immunologic state can be localized in a specific area of the gastrointestinal tract. The guinea pig, monkey, pig and man react identically in terms of delayed hypersensitivity (Rostenberg & Hoerberlin, 1950). DNCB, a well-known investigative tool in dermatology, to which man, guinea pigs, monkey and swine also react identically, was studied as a contactant. It was then demonstrated that lesions in the gastrointestinal tract could be produced locally by a contactant in animals sensitized to the substance via the skin (Bicks & Rosenberg, 1969). The severity of the chronic delayed hypersensitivity reaction produced in the guinea pig colon by DNCB was dependent upon the frequency of application of the

contact allergen. The intensity of the reaction could be modified in a non-predictable manner by Immuran, an immunosuppressive agent (Bicks et al., 1965).

Rabin and Rogers (1977) utilized DNCB in rabbits to elicit a non-specific cellular immune reaction in the colon, producing histologic changes compatible with ulcerative colitis and also leading to the production of lymphocytes sensitized to colonic antigen. This corresponds to reports of patients with ulcerative colitis having lymphocytes which are cytotoxic to colonic cells in vitro.

In the light of early studies with DNCB, the effect exerted on chronicity by neostigmine, Freund's adjuvant, and endotoxin, were tested in addition to the contactant on the guinea pig colonic lesions (Bicks et al., 1969a). Neostigmine, a motor stimulant, produced hyperemia, hemorrhage, and colonic ulceration in dogs when administered daily; the latter was thought to be due to smooth muscular spasm, and vascular stasis (Moeller and Kirsner, 1954). Bicks et al. (1969b) reported microscopic ulceration and partial glandular destruction in pigs treated with DNCB. The lamina propria showed a chronic inflammatory infiltrate and the vessels exhibited perivascular congestion (fig. 3). The Freund adjuvant group had a more grossly severe lesion. The neostigmine-treated animals exhibited crypt abscesses in all animals, the first production of such lesions in an experimental model (Bicks et al., 1969a).

Ceredig et al. (1977) utilized a subrenal capsular implantation method for organ growth as a model for delayed hypersensitivity. Rat fetal colon implants induced specific adult, anti-fetal tissue immune-reactions resulting in ulceration of the implanted mucosa. Serum samples contained antibodies reactive with fetal colonic mucosa but not with adult mucosa - indicating an immune reaction in adult rats to fetal antigens deficient in the adult animal. This model may be useful for investigating immunologically determined colonic inflammatory disease.

Other Immunological Phenomenon:

An autoimmune pathogenesis for ulcerative colitis was supported by the evidence, in the sera of patients with the disease, of anticolon autoantibodies, but such antibodies could not be found in all patients with the disease and no experimental reproduction of the disease had ever been achieved by direct immunological methods (Broberger and Perlmann, 1959). The only experimental indication of the possibility of producing anticolon autoantibodies in animals was by immunization with heterologous colonic mucosa (Bernier et al., 1960; Holborrow et al., 1963; Koffler et al., 1962; and Zweibaum et al., 1967).

In 1936, Reichert and Mathes described the production of a colitis-like disease in dogs after cannulation of intestinal lymphatics and injection of sclerosing solutions. Leveen et al. (1961) prepared an antigen from dog colon rendered sterile and injected into ducks and rabbits. Shortly after intravenous injection of the anticolon anti-

bodies produced in ducks or rabbits into dogs, a bloody diarrhea developed (within 1 - 2 days), resulting from induced ulcerative lesions not unlike those seen in the acute phase of ulcerative colitis. The induced disease was subject to remissions and exacerbations, also similar to that seen in the human disease, but chronic lesions could not be induced. In long-term experiments, the animals ceased the continued production of antibodies because continuous destruction of the mucosa did not occur, denying access to the circulating blood in which antibody production was stimulated. In this study the pathogenesis appeared to be initiated with infection, ischemia, or trauma which caused destruction of colonic tissue (Leveen et al., 1961). Colonic protein apparently gained access to the circulation and antibodies were formed against colonic tissue. The antibodies caused further destruction of the remaining colonic tissue, establishing a vicious circle. However, the histological changes observed were difficult to evaluate due to the use of Freund's adjuvant for the development of adequate titers of the rabbit antidog serum (Kraft et al., 1962).

Organ-specific, antigen-antibody reactions in animals have been extensively studied (Seegal and Bevans, 1957; Smadel and Farr, 1937). Previous attempts to produce colitis in animals have utilized histamine, foreign proteins (Kirsner, 1961) long-acting parasympathomimetic drugs (Moeller and Kirsner, 1954) or local injections of endotoxin (Goldgraber and Kirsner, 1958). However, colonic participation was passive and dependent upon generalized sensitization procedures followed by local antigen injections. Bicks and Walker (1962) attempted to show that colonic mucosa was the antigen or that a systemic immuno-

logic reaction could be localized in the colon without prior sensitization. They succeeded in producing an immunologic colitis in dogs by immunization with an anticolon rabbit serum administered intravenously. An acute immunological reaction developed, primarily limited to the colon but with evidence of systemic involvement. This was characterized by mucosal infiltration which was most marked at the base of the crypts, submucosal edema, venous dilation, and perivascular cuffing. The exact mechanism, however, was not elucidated.

Rogers and Rabin (1977) reported colonic changes following transfer of colon-sensitized lymphocytes into normal guinea pigs. After eight weeks, the colon of these animals showed only minimal changes including mucin depletion, cellular immaturity, increased mitotic rate, and occasional crypt spanning.

Oriol Palou et al. (1967) produced experimental ulcerative colitis with autoantibodies in the rat by immunization with the colonic mucus of dogs. The lesions were confined strictly to the colon, the clinical symptoms were similar to that of the human disease, and an elevated titer of antibodies specifically against the glandular cells of the colon, was found. The symptomology, gross nature of the lesions, and particularly the histology, resembled that of human rectocolitis.

Injection of aqueous extracts of adult guinea pig colon and fetal colon in complete Freund's adjuvant into rabbits induced the formation of antibodies against substances which are a constituent of

mucus-producing cells and their products, and which are present both in the colon of adult and fetal guinea pigs (Hausamen et al., 1969a). Further study revealed that injection of rabbit antisera against antigens from fetal and adult guinea pig gastric mucosa resulted in an acute inflammation confined to the stomach in guinea pigs. However, antisera against antigens from adult and fetal guinea pig colonic mucosa induced only a mild capillary dilation in the colon of the animal. No histological changes of the mucosa were seen in rabbits with circulating autoantibodies against constituents of colonic mucosa (Hausamen et al., 1969b).

Carrageenan-Induced Colitis:

Watt and Marcus (1973) opened a new route of experimentation of inflammatory disease of the colon by use of extracts of various red seaweeds, commonly Euchema spinosum, fed in drinking water to guinea pigs, rabbits, rats, or mice. The active macro-molecule of these extracts was carrageenan, a sulfated polysaccharide of high (100,000-800,000) molecular weight. Mild acid hydrolysis resulted in a degraded product with a molecular weight of less than 30,000, but which retained its original sulfate content and polyanionic properties. The degraded product was found in guinea pigs and rabbits to be more ulcerogenic than the native or undegraded form.

The structure and biological activity of carrageenan was investigated by Anderson (1967), who pointed out that the substance existed in two forms, kappa and lambda, their biological activity being vastly different and due to only a very small structural change.

The effect of degraded and undegraded kappa and lambda carrageenan on the gastrointestinal tract was characterized by their ability to prevent or diminish histamine-induced, experimental peptic ulcer.

From 1969 to 1973, Watt and Marcus (1969, 1970a, 1970b, 1970c, 1971, 1972, 1973) and Marcus and Watt (1969, 1971a, 1971b) investigated the effects of degraded and undegraded carrageenan on the colon of various laboratory species. A 1% aqueous solution of the red seaweed produced ulcerations in the guinea pig within 5 months, the same extract causing ulceration in rabbits within 3 months. The animals gained weight but when sacrificed, multiple ulcerations of the cecum were evident. Degraded carrageenan produced these lesions as well as lesions in rats and mice, although in mice they were only histologically evident. In rats the lesions were macroscopic and often accompanied by strictures (Watt & Marcus, 1973).

In rabbits 5% degraded carrageenan resulted in the onset of fulminating disease with animals dying of intestinal bleeding in 10 to 14 days. A 1% solution resulted in a lesser tendency for diarrhea but bleeding occurred within 3 weeks, while 0.1% produced ulcers only after 3 months in only 50% of the animals. Inflammatory changes and ulcerations occasionally extended deep into the submucosa (Watt & Marcus, 1973).

The pathology of the lesions was investigated extensively in the guinea pig and rabbit as the lesions could be produced in these animals

within 3 weeks with the degraded product, and were accompanied by diarrhea and occult blood and mucus. Feeding the rabbits 0.8 mg/kg body weight of carrageenan daily for 3 months resulted in ulceration of the colon as well as marked hyperplastic mucosal changes, including pseudopolyps and polypoidal lesions of a glandular type. In animals fed 0.07 mg/kg, gross ulcers of the colon and adverse microscopic changes were observed (Watt and Marcus, 1970a; 1970c).

The ulcers first appeared in the cecum, involving the colon and rectum at a later stage. They were confined to the mucosa and associated with acute or subacute inflammatory cellular infiltrate at the base or margins. Crypt abscesses and cystic dilation were present. After 5 - 6 weeks, the ulcers were in various stages of healing (Watt and Marcus, 1973).

Food grade carrageenan was observed to have no effect on the gastrointestinal tract of guinea pigs, rats, monkeys, or pigs (Abraham et al., 1972; Benitz et al., 1973), nor did 2% degraded carrageenan in milk, or lower concentrations (0.02 and 0.20%) in water fed to guinea pigs (Abraham et al., 1974). Rats exposed to a degraded extract of E. spinosum, such as that used in the therapy of peptic ulcer disease, developed neither colonic nor cecal ulcerations. although squamous metaplasia of the rectal mucosa was observed (Fabian et al., (1973).

In-depth studies (Abraham et al., 1972; 1974) have shown that carrageenan is either absorbed by, or transported to the cecum of these animals and that the ability of the macrophages in the lamina propria to endocytose and store the substance is closely related to cecal ulceration. Initiation of the ulcerogenic process occurred by the uptake of the carrageenan by the macrophages, bringing about stimulation and release of lysosomal enzymes with damage to the surrounding tissue. Lysosomal hydrolases have the ability to degrade carrageenan; the presence of autophagic vacuoles and myeloid bodies derived from lysosomes in the epithelial cells regarded as good indicators of cell damage and were probably a contributing factor. When the macrophages did not endocytose the macromolecular material, ulceration did not ensue (Abraham et al., 1974).

Dietary neomycin has been shown to reduce the number of polymorphs present but the macrophage and lymphocyte responses, as well as the degree of ulceration, were not affected (Sharrat et al., 1971). Antibiotics, such as neomycin, also had no effect on the capacity of macrophages to take up carrageenan nor did they alter the increase of lysosomal hydrolases (Fabian, 1972). Thus, it appeared that the amount of carrageenan absorbed, and its storage at a specific site in macrophagal lysosomes, may have been responsible for the production of cecal ulceration in the guinea pig.

The absence of ulceration in guinea pigs fed carrageenan in milk is especially interesting since most carrageenans are used as food additives in products where milk is a major constituent. The binding

of carrageenan to milk has been reported (Anderson, 1967), and milk proteins are known to be taken up by endocytosis in macrophages (Helminen and Ericsson, 1968). The binding of the carrageenan to milk may have made it unavailable for endocytosis by the macrophages of the lamina propria (Abraham et al., 1974).

More recent work indicated that other high molecular weight, sulfated products such as sulfated amylopectin (Marcus and Watt, 1971b; Watt and Marcus, 1972) and sodium lignosulfonate caused similar lesions in the colon of these animals. Sulfated amylopectin, a synthetic polysaccharide derived from potato starch, caused lesions in the guinea pig even at 0.1%, with only minor differences from those fed degraded carrageenan. Sodium lignosulfonate, a molecule of sulfonated phenylpropane units, is a by-product from the manufacture of pulp and paper from spruce trees and has a molecular weight of 20,000. This substance also produces lesions in the guinea pig at an incidence of 80 - 100%, depending upon the duration and concentration used (Watt and Marcus, 1973).

The selection of carrageenan as an agent in the production of experimental colonic inflammatory disease was based on its use in the treatment of peptic ulcer disease and as a common food additive in recent years. However, there are difficulties with this approach. One must consider the possibility that different species may react differently to exogenous agents, such as carrageenan, and that any one, or a combination of exogenous agents, may have a primary role in the genesis of human ulcerative colitis and there is no evidence that carrageenan

is one of these. Bonfils (1970) stated that in 200 patients receiving the sulfated products as treatment, there was no evidence of ulcerative colitis in any patient. Also, in countries such as Ireland, Japan, and in the South Pacific Islands, where those seaweeds constitute part of the diet, the incidence of ulcerative colitis is lower than in most industrialized countries.

Although these lesions grossly, and to a large extent histologically, resembled those of the human disease, there were some distinct differences. Remissions and exacerbations were nonexistent, except by intermittent administration of the agent, and necrosis of the crypts occurred at all levels rather than being confined to the base. The lesions resulting from 5% carrageenan in the drinking water of guinea pigs, resulted in areas of epithelial stripping, submucosal edema, and little or no macrophage infiltration, which occurred within a few days of administration. However, the acute lesion was probably due to an osmotic factor rather than due to the carrageenan per se, since it can be produced by feeding the animals other osmotic cathartics such as 1% sodium sulfate or 2% sodium cyclamate in their drinking water (Sharrat et al., 1971).

The most important criticism that Sharrat et al. (1971) noted with regard to the carrageenan technique related to the anatomical site of the lesions in carrageenan-induced ulceration, although Mottet (1970) felt the histological features were probably due to secondary infection of the ulcerated granuloma. It appeared that the cecum of nonruminant herbivores, guinea pigs and rabbits, was capable of absorbing carrageenan

in sufficient quantities to produce a granulomatous type of response with subsequent ulceration, a reaction not observed in the histological spectrum of human ulcerative colitis. For this reason, they believed that the guinea pig lesion would not adequately serve as a model for the study of ulcerative colitis (Sharrat et al., 1971).

Other Novel Techniques Attempted For Experimental Induction of Colitis:

Inflammatory and ulcerative changes have been induced in the colon of dogs, rabbits, monkeys, guinea pigs and rats by various techniques. In all cases the lesions tend to be acute, superficial and transient, and disappear with discontinuance of the inciting agent.

A lesion strikingly similar to that of ulcerative colitis was produced by means of irradiation of laboratory rats and rabbits (Friedman & Warren, 1942; Sommers & Warren, 1955). In 346 parabiotic rat pairs irradiated, crypt abscesses were observed in 9% of the animals and were very similar to the lesions of the human disease. However, while the colonic lesions were developing, additional changes characteristic of radiation disease were developing in other organs but the appearance of crypt abscesses preceded the point of maximal radiation damage.

Long et al., (1935) autopsied rats fed inert materials such as kaolin, and noted that in animals fed large amounts, pathologic abnormalities developed in the intestinal mucosa. However, pathologic lesions were not produced with sufficient regularity to warrant the assumption that

these substances produced intestinal disease. Rats fed diets including Karaya gum, bran, or a poor grade of psyllium materials were found to be more susceptible to the development of lesions in the cecum (Hoelzel et al., 1941). Severe lesions were found in three of five rats fed large amounts of Karaya gum for periods of 396 to 711 days. Rinehart and Greenberg (1948) induced a colitis in monkeys deficient in folic acid. These lesions bore some similarity to those of human ulcerative colitis.

A regional enteritis was produced in the colon of dogs whose ileocecal lymphatics were mechanically obstructed (Poppe, 1941). A similar lesion was induced in the terminal ileal region of swine by Kalima et al., (1976) by formalin fixation of the regional lymph nodes.

High intramuscular doses of cholinergic drugs also produced a bloody diarrhea in dogs, suggesting that colonic contraction induced by repeated injections of acetylcholine fortified by neostigmine, resulted in mucosal hemorrhage and colonic ulceration (Lium, 1939). This also involved excessive secretion of mucus and severe goblet cell depletion, a prominent feature of mucosal changes in active human colitis. The colonic mucosa can also be rendered anoxic by the chronic contraction of the muscularis. Since the colon contracts after sympathectomy, postganglionic sympathectomy was tried with dogs in attempts to induce ulcerative colitis-like lesions in the colon. Histological alterations comparable to a single phase of the human disease were produced but did not mimic the full-blown condition (Berger & Lium, 1960).

Other attempts recorded by Kirsner (1974b) and others included:

- (1) induction with enzymes, including lysozyme (Prudden et al., 1950);
- (2) broad spectrum antibiotics (Katz et al., 1977; Humphrey et al., 1977). intraperitoneal epinephrine, histamine (Brasher et al., 1955), and histamine-releasers; (3) experimental obstruction (Glotzer & Phil, 1966), in some cases augmented with parasympathomimetic drugs; (4) local injection of collagenase; (5) intraperitoneal injection of phenylbutazone in rats; (6) experimental "self-stimulation" by intracerebral electrodes promoting indecision and frustration in monkeys; (7) repeated injection of cholinergic drugs (methacholine, neostigmine, acetylcholine); (8) intravenous injection of staphylococcus toxin in dogs and rabbits (Rigdon & Leff, 1936); (9) intraperitoneal injection of adrenalin in cats and rabbits; (10) oral administration of virulent dysentery bacilli to Rhesus monkeys; and (11) application of Shigella toxin to canine colonic explants. All produced colonic mucosal lesions. However, the relationship of these experimental conditions to the clinical phenomenon as observed in man is dubious.

For every successful attempt inducing a non-specific lesion, there have been as many, or more, unsuccessful attempts. Professor Kirsner (1974b) listed those carried out in his laboratory alone: (1) intrarectal instillation of concentrated solutions of gram-negative bacterial endotoxin; (2) introduction of bile, intestinal or pancreatic juice, fecal infiltrates from colitis patients, lysozyme or hyaluronidase infusion into surgically established ileocolonic pouches; (3) injection of dog, rabbit or guinea pig colon normal or altered, injection of methacholine into dogs and

and rabbits, and the injection of saline extracts of rectal biopsy tissue from patients with active ulcerative colitis into the rectum of monkeys.

Electron Microscopy in Inflammatory Bowel Disease:

Although inflammatory colonic diseases, such as ulcerative and granulomatous colitis, are becoming increasingly more common, their etiology remains obscure and the amount of literature concerning ultrastructural aspects of these diseases is scanty. In many cases, e.g., for selected liver diseases, ultrastructural studies on diseased tissue have helped elucidate the pathogenesis of the disease process and often uncovered the etiologic agent(s). Although there have been many gross and histopathologic studies on tissue from patients with ulcerative or granulomatous colitis in many stages of the disease, few investigators have turned to the electron microscope for assistance. As late as 1965 the structure of the normal colonic mucosa was just being elucidated. (Donnellan, 1965; Deane, 1964). Those studies reported in the literature are concerned with advanced epithelial dysplasia (Otto & Gebbers, 1976) and observations on polymorphonuclear leucocytes in the mucosa (Dobbins, 1975). This has resulted in the description of clinical cases presenting as acute or chronic disease. Little, if any, information as to pathogenesis, monitoring of clinical course, or etiology has been revealed.

Although many experimental models and naturally occurring animal lesions have been brought forth, little ultrastructural investigation has been conducted. Cockrell and Krehbiel (1972) studied the ultrastructural

changes in histiocytic ulcerative colitis in a boxer dog, this being a singular report involving this highly valuable lead in a non-human model. Of the many experimental models presented, many have not been even characterized histologically, their pathogenetic mechanisms remaining undisclosed. While it is important to develop a suitable animal model, the elucidation of the pathogenesis and etiology of these process is not less important

Motility in Inflammatory Bowel Disease:

Motor-related changes in the colon have been suggested as being etiologically important in many diseases, such as, irritable bowel syndrome (Harvey & Read, 1972; Snape et al., 1977), megacolon (Connell, 1961), diverticular disease (Findlay et al., 1974; Parks & Connell, 1969), constipative or diarrheal conditions (Connell, 1974; Waller & Misiewicz, 1972), and perhaps even large bowel carcinoma (Walker & Walker, 1969). Abnormal colonic motor activity has also been considered as either being etiologically related to ulcerative colitis (Fairburn, 1973), or to be an altered physiological process associated with this disease (Almy, 1961; Code et al., 1952; Kern et al., 1951; Spriggs et al., 1951). To date it has not been possible to determine whether these altered motility patterns are a cause or consequence of the disease inasmuch as colonic motor function in pre-colitis states has not yet been delineated.

Of the many experimental, colonic inflammatory lesions induced over the wide range of species used, few, if any, have included any type

of physiological testing. Since colonic motor activity appears to be one such parameter which is affected by inflammatory bowel disease, monitoring during various stages of the induction and duration of an experimental colonic lesion would be informative.

The Acetic Acid Ulcer Technique:

Peptic ulcer is a high incidence gastrointestinal disorder with a chronic course and the possibility of acute hemorrhage or perforation which can result in some instances in mortality. Like inflammatory bowel disease, numerous attempts utilizing many experimental approaches have been tried in an effort to elucidate the etiology of peptic ulcer. Again, this important information remains undetermined.

Takagi et al., (1970) originally developed the acetic acid model for chronic gastric ulcers. Okabe and Pfeiffer (1971, 1972) and Okabe et al., (1971a) modified this technique for the induction of duodenal as well as gastric ulcers. This convenient technique proved useful for the development of discrete, chronic experimental ulcers in both locations. These ulcers were reproducible in 100% of the animals treated and bore a high degree of anatomic and histologic similarity to human chronic ulcer. Considerable information has accumulated on the action of drugs and other agents on this experimental lesion, elucidating many aspects of chronic ulcer formation, exacerbation and healing in both normal and drug-altered states.

Although the lesions characteristic of human inflammatory bowel disease exhibit a diffuse pattern, pilot experiments by Professor Pfeiffer (personal communication) suggested that further modifications of this technique might produce a diffuse colonic lesion with histopathological similarities to those of inflammatory bowel disease. The aim of the research project described in the following pages was to develop a colonic mucosal lesion which was readily reproducible, of diffuse character, located in the distal colon and would hopefully exhibit histopathological features similar to the human disease. The nature of the research required to perform this task constitutes an endeavor in experimental gastrointestinal histopathology. Accordingly the specific objectives of this study were:

- 1) Development of a colonic lesion using acetic acid in a convenient laboratory animal which would hopefully exhibit a diffuse nature, 100% reproducibility, rapid development, chronicity, low incidence of complications and mortality, and histopathological characteristics common to human forms of inflammatory bowel disease.
- 2) Utilize various histochemical characterizations and/or morphological parameters to observe lesion development and healing as well as quantitate the severity of the lesion induced.
- 3) Ultrastructural elucidation of the pathogenetic process.
- 4) Measurement of motor activity during early and late experimental colitis development, compared to appropriate control

preparations.

- 5) Conduct pilot tests on agents which are used to treat the human form of the disease and which might alter lesion development or healing.

The purpose of this thesis is to describe in detail the experimental procedures and findings with respect to the above objectives. The manner in which these experiments have both succeeded in some ways, and failed in others, to create a model of inflammatory bowel disease in these animals will be delineated.

MATERIALS AND METHODS

Induction of Colitis in Rats:

Two modes of administration of the dilute acetic acid were used: serosal and intraluminal. Each method utilized approximately 450 male Sprague-Dawley rats with an average body weight of 275 grams.

Serosal Application:

The animals were anesthetized with ether, a midline incision was made and the descending colon exposed. A cotton-tipped applicator was saturated with one of the various concentrations of the acetic acid used (10, 25, 35, or 50%). The volume of acid contained within the cotton was approximately 0.3 ml. The exposed serosal surface of the

descending colon was "painted" with the acidic solution and after 30 seconds the excess fluid was removed with a dry applicator. The abdomen was closed with Clay-Adams 9 mm. stainless steel wound clips and the rat allowed to recover. In control animals, physiological saline was substituted for acetic acid.

Intraluminal Application:

A 12 mm. length of Intramedic PE 240 polyethylene tubing was fitted to a 15 gauge Luer stub adapter and the open distal end sealed off with a drop of fiberglass resin. This rounded tip facilitated smooth passage of the tubing through the colonic lumen. The first 8 mm. of the tubing was perforated with 4 lines of holes 90° apart, each one 0.5 mm. from the next in line, but spaced alternatively every 0.25 mm. around the circumference (Figure 1). A 1 cc. tuberculin syringe was fitted into the adapter.

All animals to be treated intraluminally had their food removed 2 days prior to administration of the agent. Water was allowed ad libitum. This helped clear the colon of any fecal pellets which might impede spread of the acid. The animals were lightly anesthetized with ether and the tubing passed full-length through the rectum so that the Luer stub rested against the animal's anus. Half a milliliter of one of the various concentrations of diluted acetic acid (10, 25, or 50%) were slowly injected into the lumen of the large bowel. After 10 seconds in situ, the remaining acidic solution was withdrawn. The lumen was then flushed with 3 successive 0.5 ml. washes of physiological saline. Control

animals received 4 aliquots of the saline solution instead of acetic acid.

All animals were housed in groups of 4 in identical cages, maintained on Purina Laboratory Chow and water ad libitum, and body weight recorded weekly. The animals were sacrificed at screening intervals of 1, 2, 3, 5, 7, 9, 21, and 60 days.

A mucosal ulcer index was developed for the colon and the criteria utilized to classify the gross severity of lesions of the mucosal surface in rats are outlined in Table 1 (page 48). These criteria were applicable to the mucosal features of animals treated either serosally or intraluminally.

The histopathology of these colonic lesions was rated semi-quantitatively with an index, the criteria of which are outlined in Table 2 (page 49). This index was also applicable to both modes of treatment with respect to mucosal and submucosal changes. Crypt depths were measured with a micrometer ocular and averaged from at least 50 counts per histological section.

Induction of Colitis in Cats:

Pilot trials with cats utilizing intraluminal administration of 25% acetic acid failed to induce inflammatory colonic lesions within 3 days following administration. A 50% solution of the agent was then administered intraluminally through a piece of polyethylene tubing constructed in a similar fashion to that used in the rat (Figure 1). In this instance, the tubing length was 18 cm. and the diameter increased to PE 320 with

the first 12 cm. perforated. The proximal end of the tubing was tapered to fit a 15 gauge Luer stub adapter which was fitted to a 3 cc. syringe. The cats had their food removed for 2 days prior to administration of the agent, but water was allowed ad libitum, and cats were tranquilized with Ketaset (Rogar/STB) prior to intraluminal colonic instillation, per rectum, of 3 ml. of 50% acetic acid. Control animals received 3 ml. of physiological saline. The acid was allowed to remain in situ for 30 seconds after which it was withdrawn and the lumen washed with 3 rinses of saline solution.

A mucosal ulcer index was developed to classify the gross lesions of the mucosal surface of the cat colon. The criteria utilized to delineate these stages are outlined in Table 3 (page 51).

Rat Time-Lapse Studies:

To investigate the pathogenesis of these lesions, time-lapse samples were taken from 3 animals treated in a similar fashion for the same length of time. Tissue samples were taken from animals treated with the optimal concentration of acid for both techniques. These were 10% intraluminally and 35% serosally. The time periods used were: normal colon, colon from animals from which food was withheld for 2 days, saline control, immediately following administration of the acid, 10 seconds, 5, 15, 30, 60, and 120 minutes and 4, 8, 12, 18, 24 and 72 hours following administration of the acid.

Processing of Excised Tissue:

a) Routine Paraffin-Embedded Samples:

The animals were anesthetized with ether, a midline incision made and the descending colon exposed. In serosal treatments where connective tissue adhesions and fistulous tract formation was often observed, these tissues were removed to expose the colon. Once exposed, the entire descending colon, from splenic flexure to the distal point at which it enters the pelvic girdle, was excised. The colon was opened longitudinally with scissors and washed quickly in physiological saline at 37°C to remove fecal material and other debris. The colon was then sliced into pieces no more than 15 mm. square with rat and 8-9 mm. with cat tissue, and immersed in alcoholic Bouin's fluid. The animal was then sacrificed.

The tissues were fixed for 2 days, rinsed in 70% alcohol, dehydrated through an increasing alcohol series, cleared in xylene, and embedded in paraffin (Tissueprep, Fisher) at 56.5°C. Paraffin sections were cut at 8 to 10 microns and mounted on albumen-coated slides. Routine hematoxylin and eosin staining was supplemented by staining corresponding sections with Masson's Trichrome (Gurr, 1956), and Periodic Acid-Schiff (McManus & Mowry, 1960). A control slide was processed with each group of PAS slides to ensure validity.

b) Electron Microscopy Tissue:

Immediately following washing of the excised tissue in saline, a small piece of the desired area was dissected from the colon with a scalpel blade. This piece of tissue chunk was placed in a pool of Karnovsky's (1965) gluteraldehyde-paraformaldehyde fixative buffered to pH 7.4 with

sodium cacodylate and diced into pieces no larger than 4 mm. square. Following this the tissue was rinsed in buffer, post-fixed in 2% osmium in s-collidine, stained en bloc with saturated uranyl acetate, dehydrated through a graded alcohol series, cleared in acetone and embedded in Spurr's resin.

Thin plastic sections (1 micron thickness) were cut from trimmed blocks on a Huxley-LKB microtome, flame mounted on slides and stained for 10 seconds with hot (60°C) 1% toluidine blue in 1% sodium borate. Ultrathin sections were cut on glass knives on a Reichert UmO2 Ultramicrotome, mounted on 200 mesh copper grids coated with Formvar (Ernest Fullam) supporting film, and stained for 1 minutes in lead citrate before observation with a Phillips 300 Electron Microscope operating at 60 Kv.

c) Scanning Electron Microscopy:

Tissue for scanning electron microscopy was obtained at the same time as that for the transmission electron microscopy work. It was fixed and buffer-rinsed in a similar fashion, sonicated at 28 Hz for 60 seconds to remove mucus adhering to the mucosal surface, osmicated and dehydrated through a progressive alcohol series. The tissue was then placed into a Freon 113 (Dupont of Canada) intermediary solvent for critical point freezing in Freon 13 (Dupont of Canada). The prepared tissue pieces were then mounted on aluminum stubs with silver paint and the edges built up with a silver paste to reduce charging.

Special Stains:

Monitoring of alkaline phosphatase activity in the mucosa of the rat during the time-lapse and routine screening interval experiments was performed using a technique modified by Clark (1973). Tissue was chopped into small chunks, 3 mm. square, and fixed in cold acetone, dehydrated in acetone, cleared in chloroform, and embedded in paraffin at 56°C under vacuum.

Silver staining for myenteric ganglia in excised cat colon at the various screening intervals following acid administration was also attempted utilizing a method described by Schofield (1960). However, no results could be obtained with this procedure after several attempts.

Motor Activity Recording:

A total of 32 adult male and female cats with an average body weight of 3 kg., each acting as its own control, were utilized in this aspect of the study. The animals were housed individually in identical cages, maintained on Purina Cat Chow and water ad libitum, and had their weight monitored weekly throughout the course of the experiments.

a) Pre-Colitis Measurements:

The animals were anesthetized with pentobarbital (Somnotol, MTC Pharmaceuticals) intravenously, and the descending colon of each animal exposed through a midline abdominal incision. A strain gauge-type transducer (Jacoby et al., 1963 and shown in Figure 2) was linked to a transducer force amplifier designed by the Technical Services Branch of

the Faculty of Medicine (figure 3), and to a Beckman 10 inch strip recorder, was sutured to the colon with 4-0 silk approximately 3 cm. from the distal point where it enters the animal's pelvic girdle. Motility was recorded at an amplification of 10mV and at a chart speed of 127 mm./min. Unstimulated colonic motor activity was recorded for 10 minutes for each act in vivo, after which 0.05 ml. of Urecholine (bethanechol chloride - Merck, Sharp & Dohme) was injected under the serosa with a 27 gauge needle proximal and distal to the transducer. Equal volumes of physiological saline were injected in control animals. Stimulated colonic motor activity was recorded for 20 minutes. The transducer was then removed, the incision closed with 2-0 chromic interrupted stitches, and the skin closed with 1-0 silk horizontal mattress stitches. A week later the animal was ready for the induction of the experimental lesions.

b) Post-Colitis Measurements:

At intervals of 3, 7 and 21 days following administration of the acetic acid, the animals were again subjected to unstimulated and Urecholine-stimulated motility recording in vivo. At the end of this recording period the colon was excised for histological examination and the animal sacrificed.

A motility index was adapted from Misiewicz et al., (1971) in which $M.I. = [\Sigma(\text{contraction height} \times \text{duration}) / \text{total time}]$. Contraction height and duration were measured in millimeters directly from the strip recording while total time was expressed in minutes. An index, as delineated in Table 3 (page 51), was used for gross pathological evaluation

of the colonic lesions. Significance of differences in motility indices were tested by Student's t-test.

The Effect of Drug Administration on the Lesion:

Two pilot experiments were conducted to test the effect of 3 agents on the development and course of the experimental lesions in rat colon.

A trial was conducted with 26 male Sprague-Dawley rats: one group of 10 received 2 ml. of Betnesol (betamethasone disodium phosphate - Glaxo-Allenburys) at a dosage of 0.5 mg. per rat daily, a second group of 10 received 2 ml. of Meticortelon (Prednisilone as sodium hemisuccinate - Schering Corp.) at a dosage of 5 mg. per rat daily, and a third group of 6 rats acted as a control and received 2 ml. of physiological saline at similar intervals. Each animal received the agent by rectal enema daily for 3 days prior and 3 days following intraluminal administration of 10% acetic acid.

Another trial utilized 22 male Sprague-Dawley rats; one group of 8 received 250 mg. of Salazopyrin (Salicylazosulfapyridine - Pharmacia) daily perstomach tube as well as a 0.5 Betnesol enema daily for 7 days prior to induction of the lesion and for 7 days post treatment. The second group of 8 animals received only the Salazopyrin at the 250 mg./day dosage over this period while the third, control group, received only physiological saline.

Attempts With Bile Acids:

Intraluminal instillation, per rectum, of 20 mM deoxycholic acid and 40 mM taurocholic acid (Sigma), hourly for a period of 6 hours and daily for 7 days, yielded no gross or microscopic lesions or mucosal abnormalities in the rats treated. Cannulation of the bile duct, routing the continuous flow of bile acid of the rat directly into its colon via a stab wound through the colonic wall tied off with a purse-string suture of 3-0 silk, also yielded no results - even over an extended period of 90 days.

Lymphatic Obstruction:

Two approaches to lymphatic obstruction were attempted in order to induce colonic ulceration according to a procedure described by Kalima et al., (1976) with pigs. Formalin-Evan's Blue destruction of regional colonic lymph nodes as well as their surgical removal from this area failed to induce inflammatory lesions of the colonic mucosa in 8 cats.

Photography:

All photomicrographs were taken on a Zeiss Photomicroscope II on Kodak Panatomic X (ASA 32, Din 16) film, with the VG 9 green contrast filter. Electron micrographs were taken on Kodak Electron Microscope film 4489 (ESTAR thick base) at 50 mV for a 2 second exposure on a Phillips 300 Electron Microscope. Photomicrographs were grouped and plated, rephotographed and printed on 8½ by 11 Kodak Glossy Single Weight Ektamatic SC F paper.

RESULTS

Experimental Colonic Lesions in the Rat:

(i) Serosal Application of Acetic Acid:

Lesions were induced within three days of serosal administration of 25, 35 and 50% acetic acid. Animals treated with the lower concentration, i.e., 10% acetic acid, and saline treated control animals did not develop lesions. The pH of the various acidic solutions used can be found in Table 4 (page 52). Figure 4 illustrates the dose-response relationship exhibited by the rat colon treated serosally with the various concentrations of acetic acid. The criteria used to classify these lesions on a gross pathological scale are outlined in Table 1 (page 48), their numerical index values can be examined in Table 5 (page 53). The rapidity of lesion development and severity was correlated with the concentration of acid used. The greatest overlap of standard error of the mean was found to be in the early stages of lesion development, the latter stages being highly separable.

An unfavourable complication of this mode of application of the acid was the formation of a serosal inflammatory reaction involving the formation of excessive amounts of connective tissue. The degree of the reaction was dependent upon the concentration of acid administered, and this phenomenon resulted in the adhesion of adjacent viscera, in particular loops of the small intestine, pancreas, spleen and seminal vesicles

Table 1: Rat Colitis Ulcer Index - Mucosal Surface Observations.

Value	Description
1	Mucosa has normal appearance but less pink in color.
2	Mucosal inflammation, reddened, petechial bleeding, increased visible mucus
3	Ulcerated areas up to 7 mm. diameter, some slit-like and extending along basal region of the longitudinal folds.
4	Diffuse ulcerations from 15 to 30 mm. diameter. Mucosal surfaces between lesions are inflamed and present occasional pseudopolypoidal-like structures. Ulcer base is clean with fresh bleeding.
5	Diffuse, deep ulcerations, up to 45 mm in diameter, interspersed with areas denuded of surface mucosa appearing as shallow lesions. Adjacent mucosa is inflamed. Ulcer base appears covered with a fibroid, granular material with no evidence of fresh bleeding.
6	The most severely ulcerated areas are deep excavations resulting in an extremely thin, friable bowel wall. These areas have yellowish, pyogenic color and may be site of perforation or fistulous tracts. Adjacent areas are inflamed but may exhibit a reddish-black hemorrhagic appearance.

Table 2: Histopathological Index for Experimental Colitis in Rats
Observed in Routine Histological Screening Intervals.

STAGE 1:

- Mucosa: Scalloped profile of surface epithelium due to edema of lamina propria.
Bridges of separated surface epithelium.
Crypts are elongated with dilated bases (36 μ m) and exhibit cellular degeneration.
Decreased goblet cell mucus and increased surface mucus.
Lamina propria swollen with slight polymorphonuclear leucocyte infiltration and dilated lacteals.
Disorganized muscularis mucosae with fibers separated by edema.
- Submucosa: Edema, dilation of lymphatic vessels and veins, margination of white blood cells in veins.

STAGE 2:

- Mucosa: Thinner with shorted crypts dilated up to 125 μ m and composed of injured, degenerating cells.
Mucus content reduced in goblet cells.
Epithelial bridges ruptured, polymorphs and erythrocytes invade the lamina propria and escape into the lumen.
Thick mucus coat covers luminal aspect of the mucosa.
- Submucosa: Increased prominence of collagenous fibers and connective tissue matrix. Cellular population slightly increased.

STAGE 3:

- Mucosa: Heavy infiltration of polymorphs and erythrocytes.
Isolated remnants of colonic crypts remain.
Surface epithelium appears cuboidal.
- Submucosa: Polymorph infiltration, erythrocyte diapedesis, venous stasis, thickening of collagenous fibers.

STAGE 4:

- Mucosa: Loss of surface epithelium with polymorph accumulation at the luminal surface and around the muscularis mucosae.
Accumulation of a homogeneous hyaline-like substance in the lamina propria.
Extravasated erythrocyte accumulation around the muscularis mucosae.

STAGE 5:

- Mucosa: Excavations up to 675 μm , some extending below muscularis mucosae.
- Adjacent areas have heterogeneous mixture of hyaline-like material and necrotic debris which extends unbounded into the lumen.
- Muscularis mucosae absent or degenerating.
- Islands of inflamed mucosa, resembling pseudopolyps, scattered with their epithelial boundary extending to the muscularis mucosae. Crypts are elongated (450 μm) or shortened and dilated (60 μm).
- Adjacent mucosa may slope into lesion with cuboidal-like epithelial boundary extending a short distance over lesion. Crypts may be shortened, deranged, and dilated with polymorph and erythrocyte infiltration. In nearby areas mucosa is swollen with enlarged lacteals and elongated crypts (up to 300 μm).
- Submucosa: Heavily infiltrated with polymorphs, large amounts of collagenous connective tissue forming well-organized networks in areas where the polymorph population is reduced.

STAGE 6:

- Mucosa: Similar to Stage 5 with some areas thinner, resulting from sloughing or covered by a thick hyaline matrix.
- Submucosa: Granulation tissue in some areas with well-organized collagenous fiber network with large numbers of fibroblasts. Fibers are oriented in same plane as circular muscle fibers of normal muscularis mucosae.

STAGE 7:

- Mucosa: Fibrotic infiltration of granulation tissue from submucosa extends into region of mucosa but numerous polymorphs still evident.
- No epithelium present and polymorph-infiltrated necrotic debris sloughs off into the lumen.
- Submucosa: Highly organized fibrosis with extensive network of capillaries extend perpendicular to the serosa upward into the mucosa.
-

Table 3: Cat Colonic Ulcer Index - Gross Luminal Surface Morphology.

Value	Description
1	Mucosa appears normal.
2	Mucosal folds still well-defined but slight inflammation present.
3	Mucosa inflamed, haustral folds edematous with petechial bleeding. Punched out ulcers enlarge to form slit-like lesions in the trough of folds (up to 10 mm. in length). Colonic wall retains shape and tonicity.
4	Some areas diffusely ulcerated (up to 15 mm. diameter) in ring-like fashion. Lesions extend up and down haustral folds of adjacent inflamed mucosa and have clean, reddish base. Colonic wall lies flat and flaccid.
5	Colonic wall edematous and rigid except in areas of ulceration (up to 25 mm. diameter). Sharp delineation between inflamed and non-inflamed mucosa. Pseudopolyp-like structures scattered throughout ulcerated areas. Ulcer bases are deep and exhibit reddish-black color.
6	Colonic wall is rigid in areas of inflammation and edematous in areas of lesion. Lesions still extensive but bases less hemorrhagic and more fibroid in appearance. Adjacent mucosa is inflamed.

Table 4: The pH of Various Concentrations
of Acetic Acid.

Acetic Acid Concentration (%)	pH
0.5	2.80
1.0	2.65
5.0	2.35
10.0	2.20
25.0	2.00
35.0	1.75
50.0	1.45

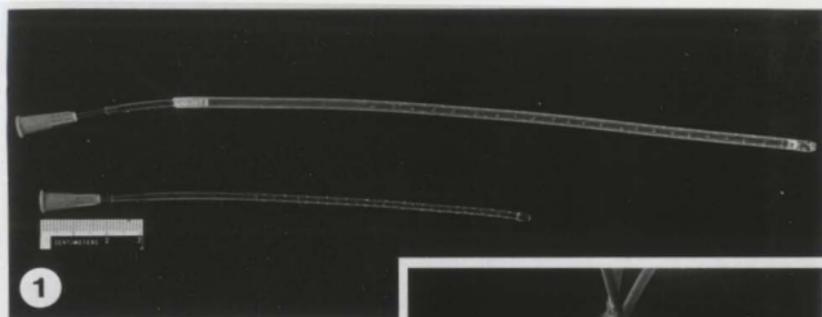
Table 5: Mean Colonic Ulcer Indices (Gross Observation) for the Rat Treated Serosally With Various Concentrations of Acetic Acid at Several Intervals. Each Value Represents the Mean of 10 to 20 Rats \pm S.E.M.

Interval (days)	Concentration of Acetic Acid			
	50%	35%	25%	10%
1	2.5 \pm 0.77	1.9 \pm 0.75	1.7 \pm 0.50	1.5 \pm 0.40
2	3.3 \pm 0.70	2.2 \pm 0.80	2.0 \pm 0.62	1.8 \pm 0.43
3	3.8 \pm 0.55	3.2 \pm 0.77	2.5 \pm 0.60	1.9 \pm 0.61
5	5.6 \pm 0.46	4.1 \pm 0.71	2.9 \pm 0.54	1.6 \pm 0.41
7	6.0 \pm 0.60	4.8 \pm 0.69	3.7 \pm 0.70	1.5 \pm 0.30
9	-	5.6 \pm 0.65	4.5 \pm 0.51	1.3 \pm 0.25
21	-	4.3 \pm 0.90	3.6 \pm 0.82	1.2 \pm 0.10
60	-	3.7 \pm 0.98	3.1 \pm 0.97	1.1 \pm 0.05

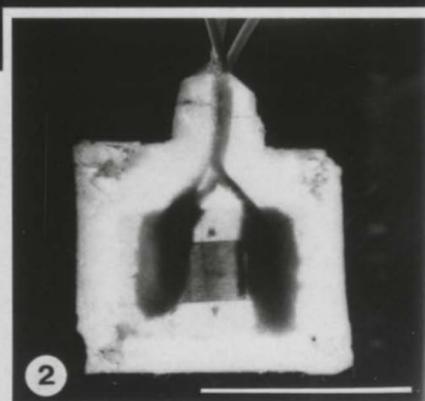
Figure 1: Intraluminal instillation tubes for administration of the acetic acid solutions, constructed of polyethylene tubing fitted to a Luer stub adapter and perforated for various lengths from their proximal end. The upper tube was that used with cats, while the lower one was used with rats.

Figure 2: Extraluminal contractile force strain gauge transducer used in recording motor activity in the cat colon. The light area surrounding the internal circuitry plate is composed of Silastic (Dow Corning) which was sutured by its four corners to the colonic serosa. The white line near the bottom of the photograph is the scale which represents 1 cm.

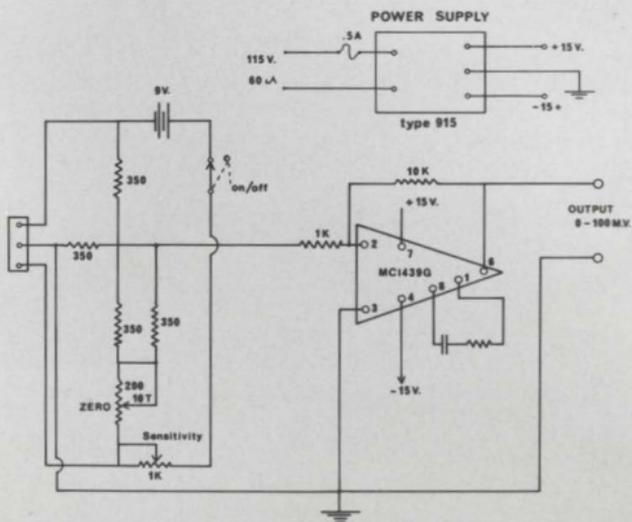
Figure 3: Circuit diagram of the force transducer amplifier constructed to amplify signals monitored by the contractile force strain transducer conducted to the strip recorder.



1



2



3

FORCE TRANSDUCER AMPLIFIER

(Figure 5). This resulted in the formation of a large mass of abdominal viscera tightly bound in a connective tissue sheath. Often, after stripping this tissue away, the colon was found to be extremely enlarged, sometimes up to 30 mm. in diameter. In this megacolon-like condition the colonic wall was thin, friable and pyogenic (Figure 6). In other instances this condition was the direct result of mechanical expansion due to the formation of a fistulous tract with a loop of the small intestine. The colonic lumen was filled with large volumes of intestinal chyme. Perforation and fistulous tract formation had a 90% occurrence after seven days in animals treated with 50% acetic acid, but was observed less often, in 20% of all animals, nine days after treatment with 35% acid.

The general appearance of the serosally-treated rats was unhealthy, and the animals presented with loose, paste-like stools within two to three days following administration of the acid. The stools often appeared tarry or were accompanied by fresh bleeding. The animals lost their normal vitality and sat hunched in the cages with a ruffled coat, often with blood around their snout and forepaws and fecal material adhering to their anal region and tail. Animals treated with the higher concentrations had swollen abdominal regions as a result of the formation of the adhesious mass of tissue and viscera forming on the colonic serosa. Weight monitoring for 21 days indicated that the average weight change in these animals was dependent upon the severity of the ulceration which was in turn dependent upon the concentration of acid used (Table 6, page 57).

The optimal concentration of acetic acid for this mode of application

Figure 4: Dose-response relationship of colitis ulcer index following serosal application of acetic acid in various concentrations to the descending colon of the rat. The concentrations are given in percent and each point represents the Mean \pm S.E.M. of 12 to 20 rats.

Figure 5: (rat, 50% acetic acid, serosal, 7 days) The pancreas of the rat engulfed in the inflammatory connective tissue exudate forming on the visceral aspect of the serosa following treatment with 50% acetic acid (paraffin, H&E, 100X, scale = 25 μ m).

Figure 6: (rat, 50% acetic acid, serosal, 5 days) Transmural view of the colonic wall of the rat following administration of 50% acetic acid serosally. There is extensive necrosis of all layers and the serosal inflammatory exudate has been stripped off prior to fixation of the tissue (paraffin, H&E, 100X, scale = 25 μ m).

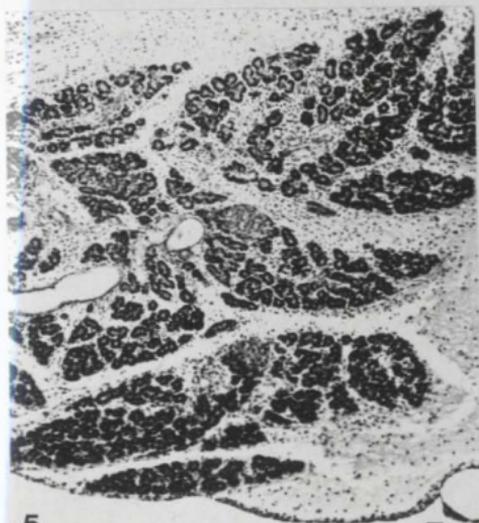
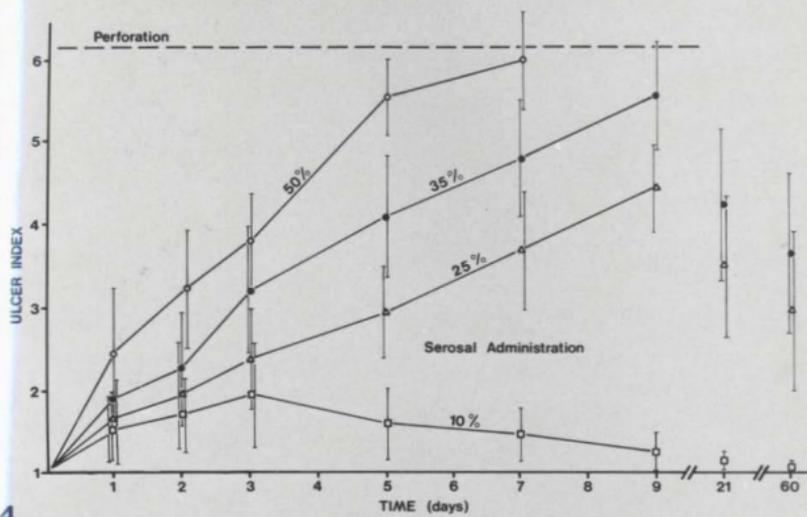


Table 6: Average Weight Change in Grams Observed Over a Period of 21 Days in Rats Treated Either Serosally or Intraluminally With Various Concentrations of Acetic Acid.

Treatment Mode	Concentration of Acetic Acid			
	10%	25%	35%	50%
Serosal	+ 29.53	- 33.33	- 41.25	- 50.15
Intraluminal	+ 7.50	- 17.25	-	-

was determined to be 35%. This selection was based on the rapidity of lesion development, severity, chronicity and prevalence of complications. Figure 7 shows the gross appearance of rat colon treated serosally with 35% acetic acid in toto contrasted to that of the saline controls. The characteristic mucosal morphology of these colons is shown in Figure 8. The histopathology of these lesions is described in the following paragraphs.

The histology of the normal, saline treated, control rat colon is illustrated in Figure 9. The initial histopathological features of the serosally-induced acetic acid lesion were structural disorganization or separation of the cellular elements composing the serosa and muscularis externa, sometimes accompanied by hemorrhage from the intramuscular blood vessels (Figure 10). In some areas there was an early emigration of polymorphonuclear leucocytes (polymorphs) into the connective tissue stroma of the submucosa which migrated toward the muscularis mucosae (Figure 11), while other areas exhibited an initial, edematous swelling with a relatively small increase in cellular components (Figure 12). In focal areas of the mucosa the lacteals became packed with white blood cells that emigrated into the lamina propria (Figure 13), while at the same time the submucosa underwent leucocytic infiltration. The muscularis mucosae soon became indistinct in these focal areas, was often densely packed with polymorphs and appeared only as a fibrous connective tissue network stretching across these areas (Figure 14).

The mucosa meanwhile, underwent an edematous reaction, the lamina propria appeared less cellular and the crypts assumed a tortuous config-

Figure 7: Gross perspective of the rat colon, in toto, showing the typical response to acetic acid administered serosally (b and c) as compared to the saline control (a). b illustrates a colon 3 days following administration of the agent and c is a colon 7 days after similar treatment. The distal end is to the left.

Figure 8: The same tissues as in Figure 7 with their mucosal aspect exposed. a is from a saline control animal; b from an animal treated serosally with 35% acetic acid 3 days earlier; and c is from an animal treated as in b but sacrificed after 7 days. The distal end is at the left. Note the diffuse character of the lesions and their anatomical localization in the descending colon.

Figure 9: (rat, physiological saline, serosal, 3 days) The histology of the saline control rat colon. The mucosa, muscularis mucosae and superficial submucosal regions are shown (paraffin, H&E, 160X, scale = 25 μ m).

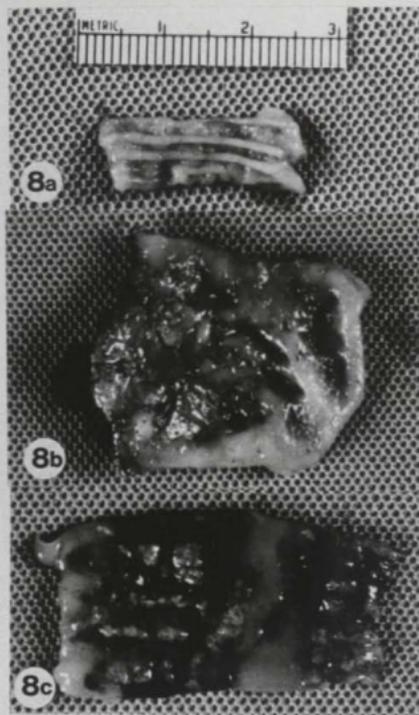
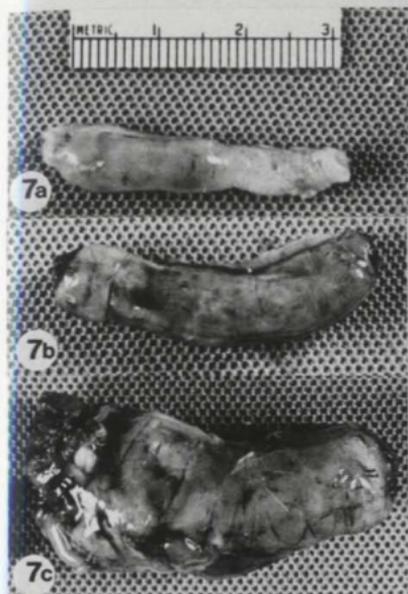


Figure 10: (rat, 35% acetic acid, serosal, 12 hours) Early response of the colon to serosal administration of the agent showing edematous separation of the muscle fibers in the muscularis externa, as well as intramuscular hemorrhage. The submucosa demonstrates an edematous response with an increase in prominence of collagenous fibers (paraffin, H&E, 160X, scale = 25 μ m).

Figure 11: (rat, 35% acetic acid, serosal, 12 hours) Margination of leucocytes in submucosal vessels and subsequent emigration into the surrounding connective tissue. There is a slight infiltration of these cells into the lamina propria of the mucosa as well (paraffin, H&E, 160X, scale = 25 μ m).

Figure 12: (rat, 35% acetic acid, serosal, 12 hours) Another form of early response of the colon to serosal application of the agent. Superficial submucosal veins and lymphatic vessels appear dilated (paraffin, H&E, 160X, scale = 25 μ m).

Figure 13: (rat, 35% acetic acid, serosal, 1 day) Early mucosal response to serosally applied acetic acid, showing dense infiltration of the lamina propria by lymphocytes and plasma cells (paraffin, H&E, 160X, scale = 25 μ m).

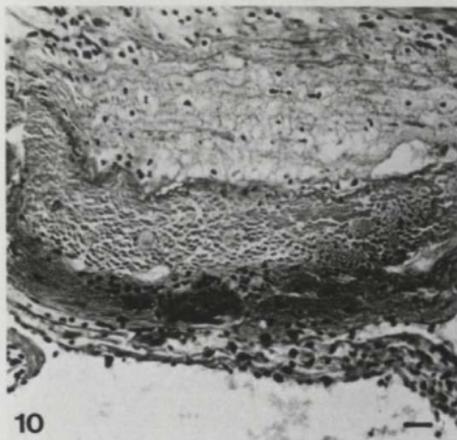
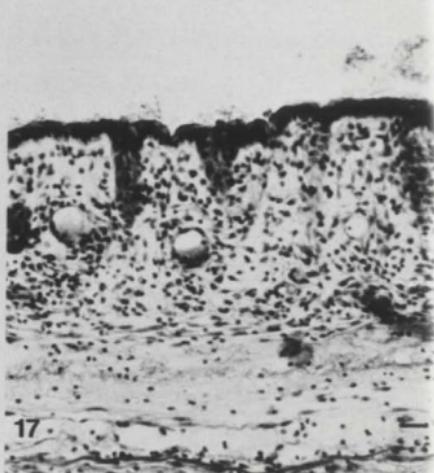
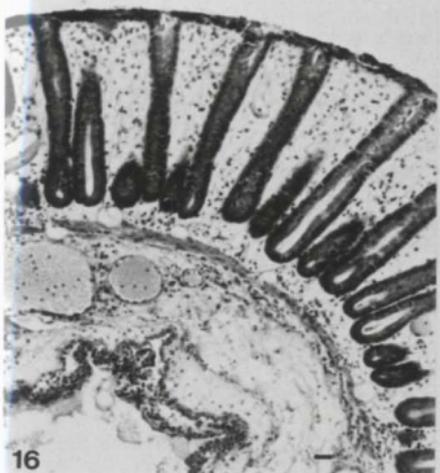
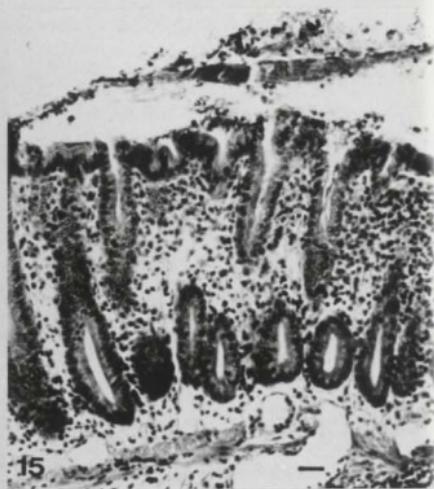
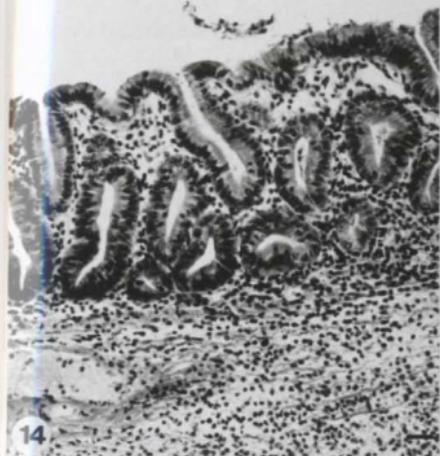


Figure 14: (rat, 35% acetic acid, serosal, 1 day) Infiltration of the mucosal lamina propria, muscularis mucosae, and submucosa with inflammatory cells. The muscularis mucosae becomes indistinct with an increase in collagenous fibers in the superficial submucosa. Mucosal crypts appear to be distorted and dilated slightly (paraffin, H&E, 160X, scale = 25 μ m).

Figure 15: (rat, 35% acetic acid, serosal, 1 day) Rupture of the surface epithelium with escape of inflammatory cells and other debris into the colonic lumen (paraffin, H&E, 100X, scale = 25 μ m).

Figure 16: (rat, 35% acetic acid, serosal, 1 day) Edematous swelling of the mucosa so that it appears to have fewer crypts. The submucosa experiences inflammatory cell emigration which spreads upward into the mucosa (paraffin, H&E, 160X, scale = 25 μ m).

Figure 17: (rat, 35% acetic acid, serosal, 1 day) Advanced stages of mucosal degeneration 1 day post-acid. Crypts have become degenerated in their basal region, the mucosal thickness has been reduced, the muscularis mucosae is indistinct and collagenous fiber deposition in the superficial submucosa is heavier (paraffin, H&E, 160X, scale = 25 μ m).



uration. The crypts appeared less numerous as a result of the swelling, often appearing shortened and dilated. The thickness of the mucosa also became reduced (Figures 15, 16, 17). Focal hemorrhage and polymorph infiltration occurred, filling the lamina propria and rupture of the surface epithelium appeared in localized areas allowing streaming of this infiltrate into the colonic lumen (Figure 16). Sloughing of the surface epithelium followed, and these focal areas developed into enlarging ulcerations bounded by regions of degenerating epithelium (Figure 18). The bowel wall thickness appeared densely packed with polymorphs from the muscularis externa to the superficial, eroded luminal surface. The muscularis mucosae became indistinct in these ulcerated areas, with focal hemorrhage emanating from the submucosal vessels, and the muscularis externa was altered with polymorph infiltration and a connective tissue exudate present on the serosal surface (Figure 19). The epithelium of adjacent mucosa tapered into the edges of the lesions while the lamina propria of the closest segments appeared edematous with slight infiltration, and often exhibited a distorted crypt organization (Figure 20, 21).

A progressive connective tissue organization in the submucosa pushes the polymorph infiltration upward and out into the lumen in a dense band. A layer of necrotic debris was observed lining the luminal aspect of the bowel wall (Figure 22). The organization of the submucosa progressed steadily (Figure 23), and once it had spread to the most luminal position in the bowel wall, epithelium from adjacent mucosa stretched out over the surface of this connective tissue mass and effectively separated it from the overlying layer of inflammatory cells and other debris (Figure 24).

Figure 18: (rat, 35% acetic acid, serosal, 3 days) A transmural view of rat colon noting extensive inflammatory cell infiltration of the submucosa and muscularis externa. The mucosa exhibits distorted crypts, edema of the lamina propria and inflammatory cell infiltration. The mucosa tapers into the open lesion which has eroded down to the level of the muscularis mucosae which is no longer present in this region (paraffin, H&E, 32X, scale = 50 μ m).

Figure 19: (rat, 35% acetic acid, serosal, 3 days) General degeneration of the colonic mucosa, appearing totally denuded of surface or crypt epithelium and is heavily infiltrated with inflammatory cells, but not reduced in height, leaving tall pillars of lamina propria heavily infiltrated with cellular components (paraffin, H&E, 32X, scale = 50 μ m).

Figure 20: (rat, 35% acetic acid, serosal, 3 days) The abrupt edge of a lesion showing the condition of the adjacent mucosa which exhibits distorted cryptic arrangement. There is a heavy polymorph infiltration and the muscularis mucosae has disappeared. Note the blood vessel packed with erythrocytes (paraffin, H&E, 160X, scale = 25 μ m).

Figure 21: (rat, 35% acetic acid, intraluminal, 3 days) Tapering of the mucosa into an open lesion with the epithelium assuming a cuboidal configuration at its distal end which begins to migrate out over the lesion surface. There is a heavy polymorph infiltration of the lamina propria (paraffin, H&E, 160X, scale = 25 μ m).

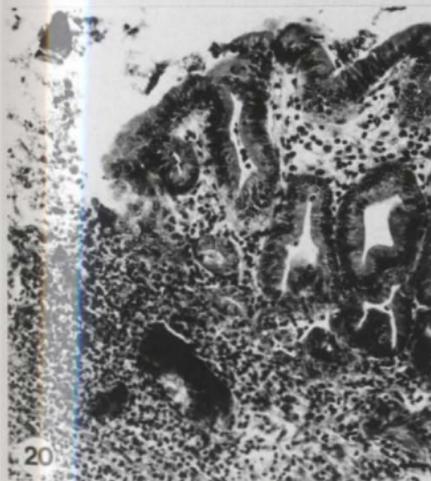
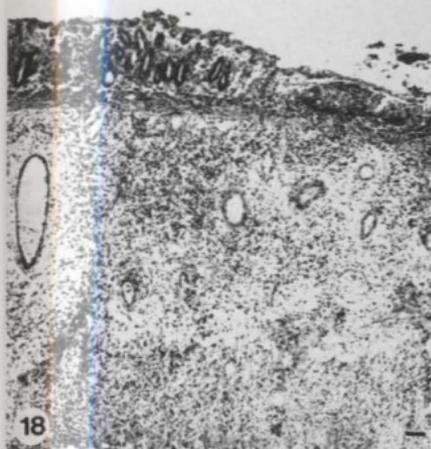
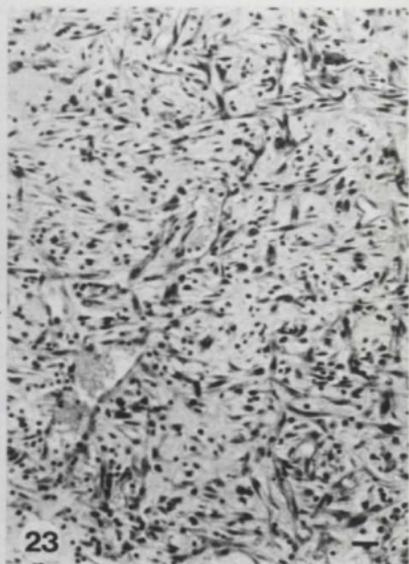
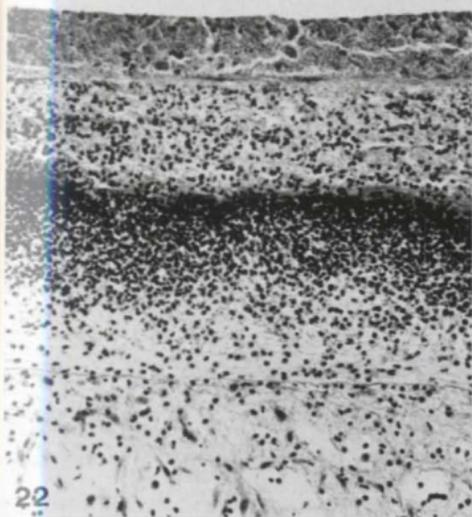


Figure 22: (rat, 35% acetic acid, serosal, 5 days) Layer of eosinophilic necrotic debris overlying the degenerated mucosa of rat colon. The heavy polymorph infiltration is localized around the level of the muscularis mucosae which is barely discernable. There is a collagenous fiber increase in the submucosa (paraffin, H&E, 100X, scale = 25 μ m).

Figure 23: (rat, 35% acetic acid, serosal, 5 days) Organization of the granulation tissue which occupied the area of a lesion 5 to 7 days following administration of the agent. This mass is localized in the submucosa and capillaries can be seen forging their way through the tissue (paraffin, H&E, 100X, scale = 25 μ m).

Figure 24: (rat, 35% acetic acid, serosal, 7 days) Edge of a lesion exhibiting mucosal infolding and outgrowth of the surface epithelium over the lesion separating the inflammatory exudate from the underlying tissue. Note the columnar nature of the epithelial cells on the adjacent mucosa and its cuboidal configuration on the surface of the lesion (paraffin, H&E, 160X, scale = 25 μ m).

Figure 25: (rat, 5% acetic acid, intraluminal, 3 days) Rat colonic mucosal response to intraluminal instillation of 5% acetic acid. There is distortion of the crypts resulting in uneven mucosal thickness but the submucosa elicits little response (paraffin, H&E, 100X, scale = 25 μ m).



The mucosa bordering these segments bounding the lesions was markedly increased in height and exhibited elongated crypts and an edematous swelling of the lamina propria. The muscularis mucosae retained its normal architecture while the submucosa in these areas also exhibited edematous swelling with a slight polymorph infiltration.

(ii) Intraluminal Application of Acetic Acid:

Colonic intraluminal instillation, per rectum, resulted in the development of a lesion within three days using 10, 25 and 50% acetic acid in all animals treated. Lower concentrations, 0.5, 1.0 and 5%, did not induce significant ulceration, with 5% eliciting only a weak inflammatory reaction. Lesions were observed in only 10% of the animals treated with 5% acid and were of a superficial, fast-healing nature. (Figures 25, 26). The pH of the acidic solutions utilized in the intraluminal technique can also be found in Table 4 (page 52).

Figure 27 illustrates the dose-response relationship of the rat colon to intraluminal administration of 10, 25 and 50% acetic acid and mean, colonic ulcer indices are delineated in Table 7 (page 67). The criteria used in the ulcer index were the same as those used for the surface morphology in serosally treated rats, and can be found in Table 1 (page 48). Again, the severity of the lesion with respect to morphology and time, can be correlated with the concentration of acid used.

The selection of an optimal concentration of the acetic acid for intraluminal instillation was made after the evaluation of the

Figure 26: (rat, 5% acetic acid, intraluminal, 3 days) A focal erosion in rat colon exhibiting mucosal infolding on both peripheries with streaming of an inflammatory exudate into the colonic lumen. Mucosal edema and lifting of the surface epithelium from the underlying lamina propria is shown in adjacent regions (paraffin, H&E, 100X, scale = 25 μ m).

Figure 27: Dose-response relationship of colitis ulcer index following intraluminal application of acetic acid into the descending colon of the rat. Concentration is given in percent. Each point represents the Mean \pm S.E.M. of 12 to 20 rats.

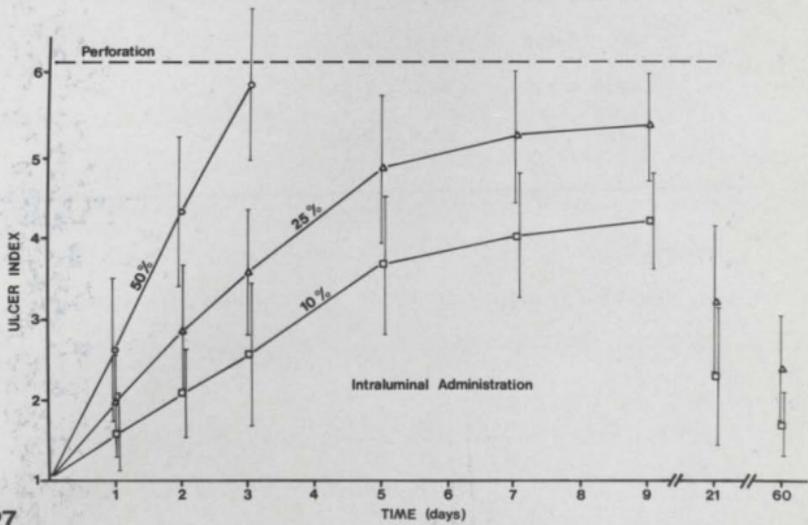


Table 7: Mean Colonic Ulcer Indices (Gross Observation) for the Rat Treated Intraluminally With Various Concentrations of Acetic Acid at Several Intervals. Each Value Represents the Mean of 10 to 20 Rats \pm S.E.M.

Interval (days)	Concentration of Acetic Acid		
	50%	25%	10%
1	2.6 \pm 0.85	2.1 \pm 0.68	1.6 \pm 0.48
2	4.3 \pm 0.90	2.9 \pm 0.79	2.1 \pm 0.53
3	5.8 \pm 0.98	3.6 \pm 0.79	2.6 \pm 0.85
5	-	4.9 \pm 0.89	3.7 \pm 0.83
7	-	5.2 \pm 0.80	4.1 \pm 0.75
9	-	5.4 \pm 0.65	4.2 \pm 0.61
21	-	3.3 \pm 0.96	2.4 \pm 0.83
60	-	2.4 \pm 0.63	1.7 \pm 0.42

morphologic and histopathologic processes observed with the various concentrations. The occurrence of complications and their incidence was also taken into consideration. An acidic solution of 10% was found to give the most favourable results. It produced lesions within 3 days which maintained their integrity until day nine. The lesions were not as chronic as those induced with 25% intraluminally or 35% serosally, but they did not exhibit as severe a tissue reaction with as many complications. Fifty percent acetic acid, intraluminally, consistently produced perforation and death of the animal within three to four days following administration.

Figure 28 illustrates the characteristic appearance of the response of the rat colon to 10% acetic acid administered intraluminally. The mucosal appearance of these respective colons can be seen in Figure 29. This figure illustrates the diffuse character of these experimentally induced lesions.

The general appearance of these animals was again unhealthy and resembled that of the serosally treated animals except that the abdominal region rarely appeared swollen as a result of connective tissue reaction. Weight monitoring for 21 days indicated that the average weight change was again dependent upon the severity of the ulceration, which was in turn influenced by the concentration of the acid used (Table 6, page 57).

Screening of colonic tissue from intraluminally treated rats revealed a mucosal histopathology slightly different from that observed in the serosally treated animals. Histological examination of tissues

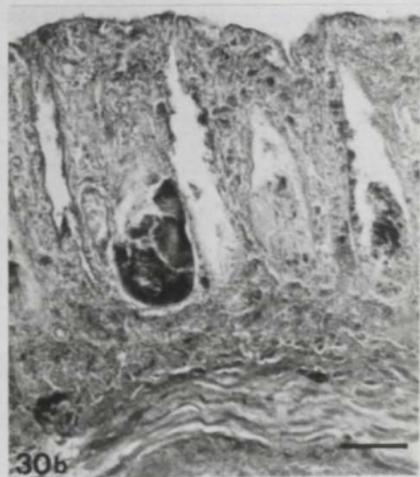
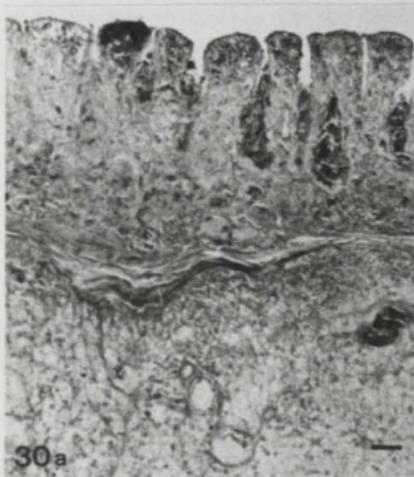
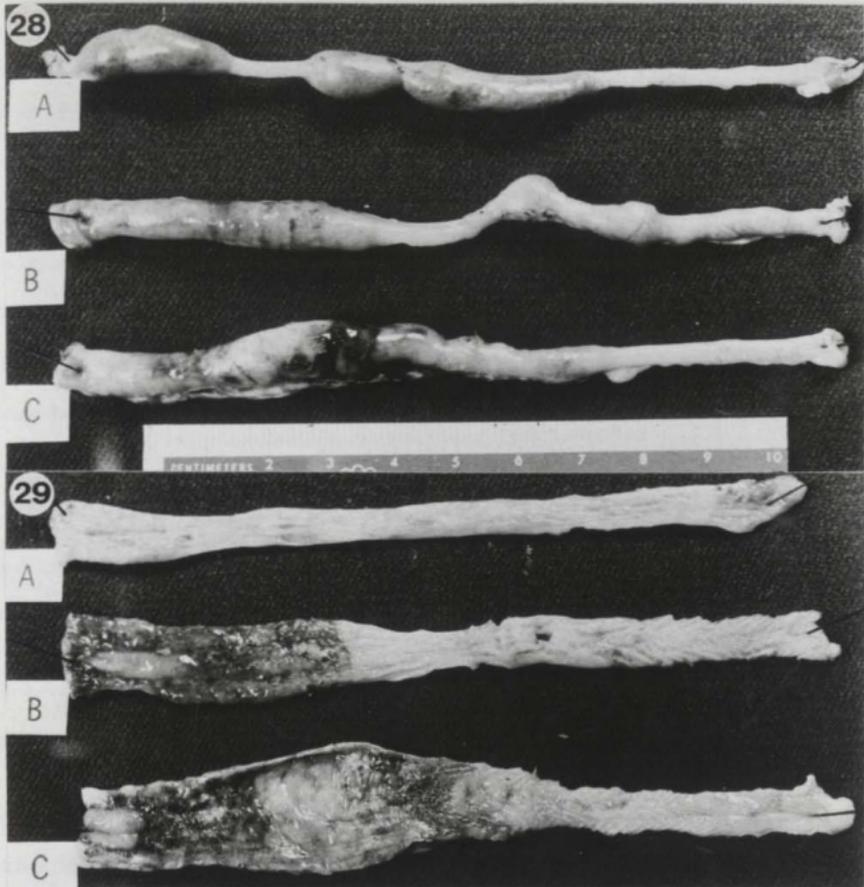
Figure 28: Gross perspective of the rat colon (external surface) showing the typical response to acetic acid (B and C) as compared to the saline control (A). B illustrates a colon 3 days following intraluminal application of 10% acetic acid, while C is a colon 7 days after similar treatment. The distal end is at the left.

Figure 29: The same 3 tissues as in Figure 28 with their mucosal aspect exposed. A is from a saline control; B, from an animal treated intraluminally with 10% acetic acid 3 days earlier, and C is from an animal treated as in B but sacrificed after 7 days. The distal end is again to the left and note the diffuse character of these lesions.

Figure 30: (rat, 10% acetic acid, intraluminal, 1 day)

(a) Degenerating epithelium 1 day following intraluminal administration of the agent. Sloughing of the surface and superficial neck region crypt epithelium is complete. The lamina propria is filled with a coagulative matrix (paraffin, H&E, 160X, scale = 25 μ m).

(b) Higher magnification of these pillars of lamina propria showing the coagulative matrix and the large dark areas which are degenerated blood vessels and extravasated erythrocytes. The muscularis mucosae is edematously separated (paraffin, H&E, 380X, scale = 25 μ m).



resected at the first screening interval, 1 day following administration of the acid, revealed a series of features dependent upon distance from the area in direct contact with the acetic acid. Mucosal regions which came into direct contact with the full-strength solution revealed a pillar-like arrangement of lamina propria representing the original crypt arrangement except that the epithelial cell lining had been sloughed off except for a few isolated cells deep in the base of the crypts. The submucosa became thicker and exhibited thick collagenous fibers embedded in a dense connective tissue matrix (Figures 30a, 30b). There was minimal polymorph emigration from the superficial submucosal vessels, which became enhanced in later stages, leading to massive migration of cells from these submucosal vessels (Figures 31a, 31b) and into the lamina propria of the mucosa.

The pillars of lamina propria became filled with an eosinophilic hyaline-like substance resembling protein coagulation. An enlarging population of erythrocytes mixed with this dense matrix as time progressed. Polymorphs began to appear in the basal region of the lamina propria around the level of the muscularis mucosae with a few of these cells scattered in the more superficial levels of the mucosa (Figure 32). As this infiltration continued, the dense band of cells moved up through this matrix and as well infiltrated the muscularis mucosae (Figures 33,34).

In adjacent regions of the mucosa, i.e., those areas exposed to saline-diluted volumes of the acid, the epithelial loss was not as severe, although there was a degeneration of the cellular elements of the crypts as well as on the luminal surface. The lamina propria became filled with

Figure 31: (rat, 10% acetic acid, intraluminal, 1 day) The early response of the submucosal vascular channels. Figure 31b shows the artery under higher magnification where active emigration of polymorphs is underway. These migrating inflammatory cells pass through the muscularis mucosae and infiltrate the lamina propria (paraffin, H&E, 100X, and 380X, scale = 25 μ m).

Figure 32: (rat, 10% acetic acid, intraluminal, 1 day) The early response of the mucosa. The mucosa appears stripped of epithelial cells, the lamina propria filled with a homogeneous matrix which has a great affinity for eosin. The polymorph infiltration from the submucosal vessels is beginning in the lowest part of the lamina propria and the darker areas higher up in the matrix are pools of erythrocytes which have escaped from damaged capillaries (paraffin, H&E, 160X, scale = 25 μ m).

Figure 33: (rat, 10% acetic acid, intraluminal, 2 days) The polymorph infiltration has spread upward through the matrix of the lamina propria and in some instances is escaping into the colonic lumen through ruptures in the naked limiting membrane (paraffin, H&E, 160X, scale = 25 μ m).

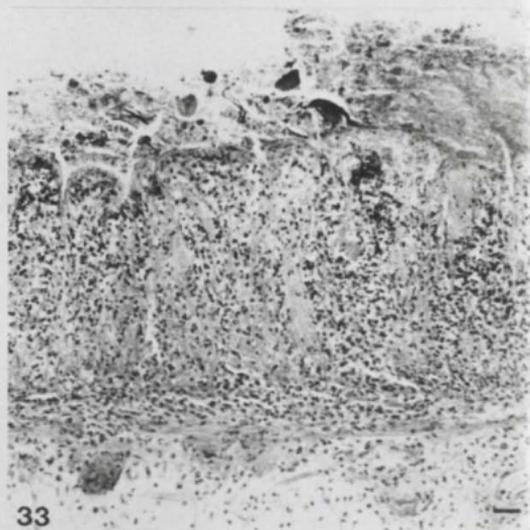
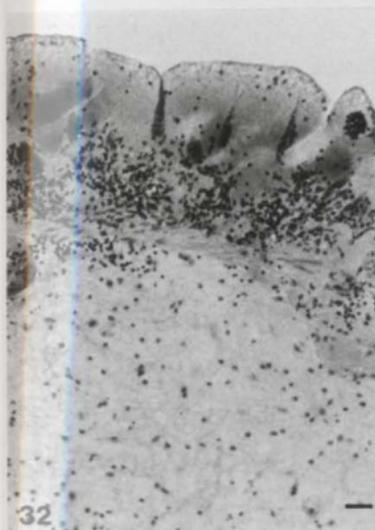
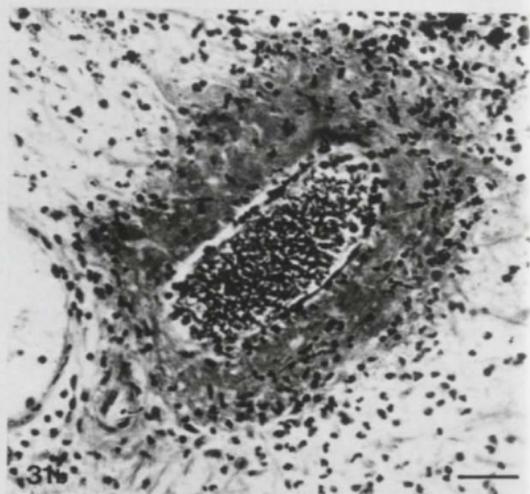
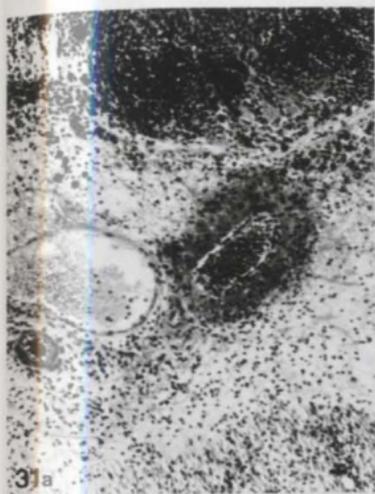
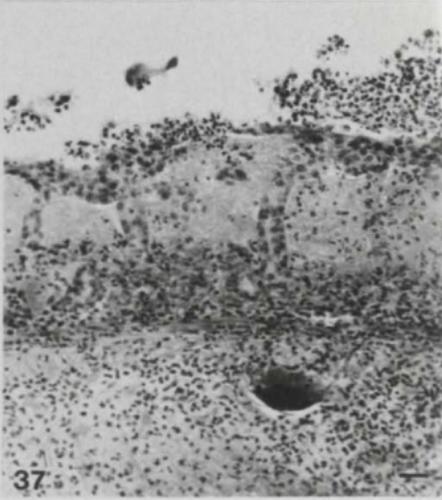
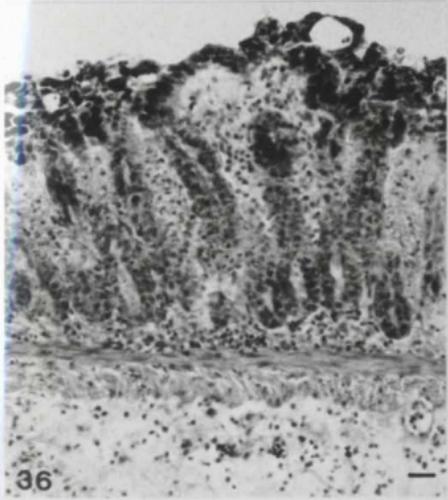
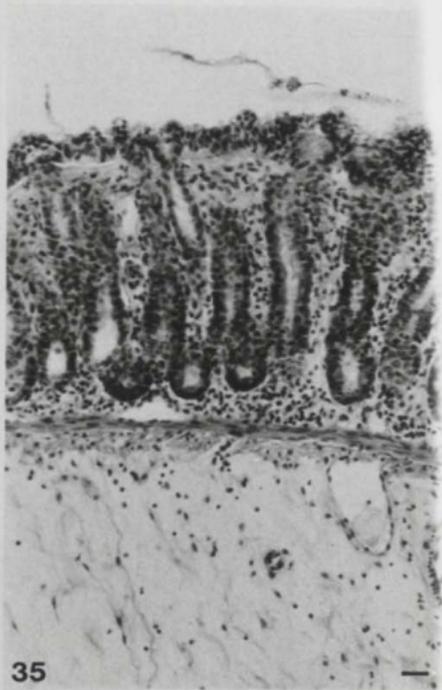
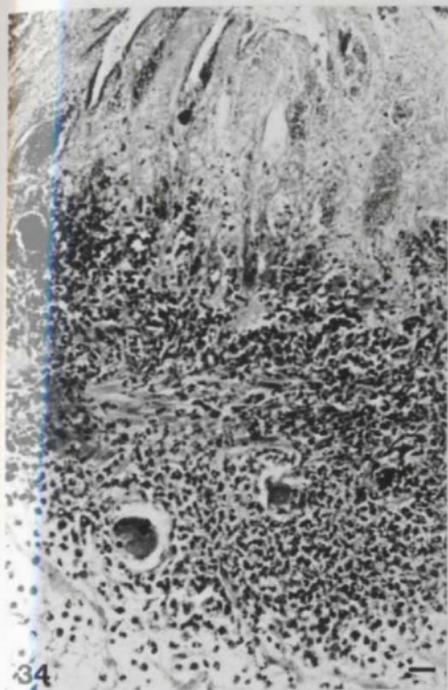


Figure 34: (rat, 10% acetic acid, intraluminal, 1 day) Dense infiltration of polymorphs appears in the superficial submucosa within the muscularis mucosae and up into the lamina propria of the mucosa. The lamina propria pillars above this infiltration appear to be completely degenerated (paraffin, H&E, 160X, scale = 25 μ m).

Figure 35: (rat, 10% acetic acid, intraluminal, 1 day) Areas not directly affected with full strength acid exhibit increased cellular components in the lamina propria and a slight edematous swelling in this area. The submucosa is extremely edematous (paraffin, H&E, 160X, scale = 25 μ m).

Figure 36: (rat, 10% acetic acid, intraluminal, 2 days) Continued degeneration of the area shown in Figure 35 with the crypts appearing indistinct in their lower regions and an irregular mucosal outline on the luminal aspect (paraffin, H&E, 160X, scale = 25 μ m).

Figure 37: (rat, 10% acetic acid, intraluminal, 3 days) Mucosal degeneration continues from Figures 35 and 36 with filling of the lamina propria with the homogeneous eosinophilic matrix. The mucosa is now reduced in height with the crypts indistinct and inflammatory cells extending upward from the submucosa through the lamina propria and out into the colonic lumen (paraffin, H&E, 160X, scale = 25 μ m).



the same hyaline-like matrix as observed in the adjacent areas and there was a small, initial polymorph infiltration. Mucosal crypts were less evident within the matrix and the surface epithelium lost its columnar architecture. Individual muscle fibers of the muscularis externa appeared to have become separated from one another and polymorphs had taken up position in these gaps. The polymorph infiltration around the muscularis mucosae migrated upward in a fashion similar to that observed in the adjacent areas with some streamers having escaped into the colonic lumen through focal epithelial rupture, forming an inflammatory exudate in the lumen (Figures 35, 36, 37). The relationship between these two areas can be seen in the photomicrograph (Figure 38).

From this point the process proceeded in a similar fashion, but at a slightly altered rate in these two areas. There was very dense infiltration of polymorphs around the region of the muscularis mucosae which itself became indistinguishable. This cellular invasion extended upward into the matrix-filled mucosal region as well as into the superficial aspects of the submucosa (Figure 39). The mucosal areas experiencing this infiltration soon underwent complete degeneration to form open lesions where the cellular infiltrate passes freely into the colonic lumen to form dense streams of inflammatory exudate which extended from the lesion out over the adjacent mucosa (Figures 40, 41).

Mucosa immediately adjacent to these lesions exhibited grossly dilated and tortuous crypts, a lamina propria with slight polymorph infiltration but a distinct muscularis mucosae. The submucosa in these regions was still heavily infiltrated with polymorphs and the superficial

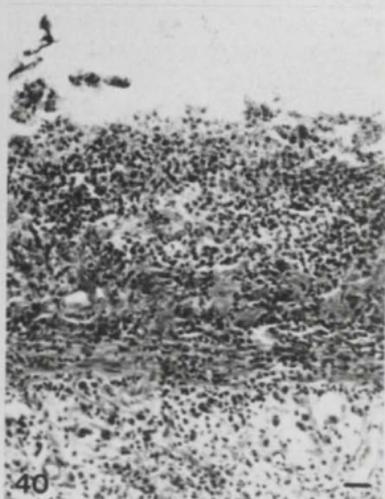
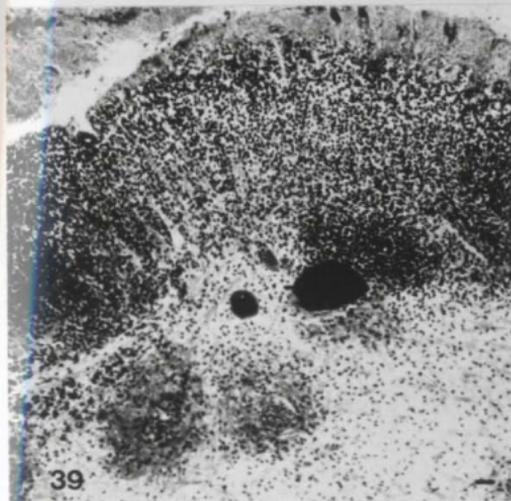
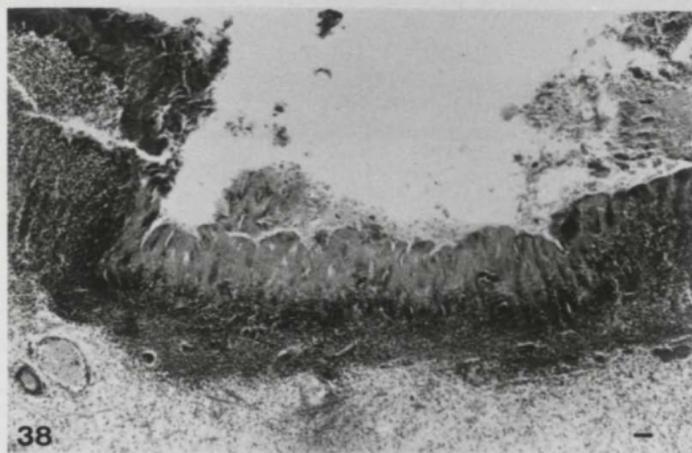
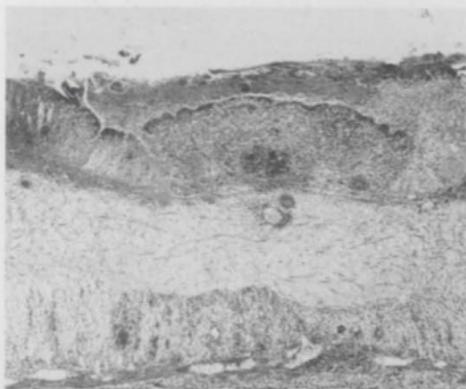


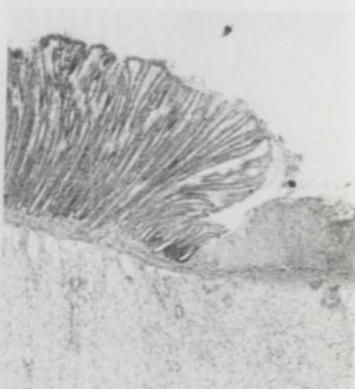
Figure 41: (rat, 10% acetic acid, intraluminal, 3 days) The submucosa is edematous with collagenous fibers appearing more numerous and thicker, the muscularis externa has become degenerated into a connective tissue network extending perpendicular to the lumen, and the mucosa is heavily infiltrated with inflammatory cells and areas not eroded are covered by an inflammatory exudate streaming from lesions eroded down to the level of the muscularis mucosae or superficial submucosa (paraffin, H&E, 32X, scale = 50 μ m).

Figure 42: (rat, 10% acetic acid, intraluminal, 3 days) Edematous mucosa adjacent to an open lesion. The crypts are extremely elongated and the lamina propria swollen. The submucosa is also edematous (paraffin, H&E, 32X, scale = 50 μ m).

Figure 43: (rat, 10% acetic acid, intraluminal, 3 days) A photomicrograph showing the variation in crypt depth during stages of lesion formation. The mucosa immediately adjacent to the lesion is reduced in height, has degenerating crypts and a thinner underlying muscularis mucosae. That further from the lesion is markedly increased in height with a corresponding increase in crypt depth and obvious edematous swelling. The submucosa also exhibits a high incidence of inflammatory cells (paraffin, H&E, 100X, scale = 25 μ m).



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blood and lymphatic vessels appeared to have undergone stasis. Cellular inflammatory elements continued to escape from these vessels into the surrounding connective tissue matrix. Mucosal regions which adjoined those immediately adjacent to the lesions exhibited a marked increase in height with elongated crypts, edematous lamina propria with slight polymorph infiltrate, a distinct muscularis mucosae and an inflammatory reaction in the underlying submucosa (Figures 42,43).

The reaction of the muscularis externa was similar to that observed following the serosal application of the agent except that it developed later in the process. The initial alternation was a slight separation of the muscle fibers with occasional polymorph evident in these spaces (Figure 44). By the third day following application of the agent the muscularis became vacuolated, the spaces filled with a connective tissue matrix extending from the submucosa, and with a fibrous network of collagen that extended in a perpendicular fashion and which was penetrated by a large number of capillaries. There was a slight polymorph infiltration into this region as well as the formation of a small inflammatory reaction on the visceral aspect of the serosa which remained more or less intact (Figures 45a, 45b).

Focal areas of adjacent mucosa exhibited a pseudopolyp-like inflammatory swelling, often with epithelial bridges forming on their luminal aspect. These bridges stretched from crypt to crypt and formed a large gap between the basement membrane of the epithelium and the underlying lamina propria. The inflammatory reaction in these structures was mainly edematous swelling with a slight cellular infiltration exhibited (Figures 46,47).

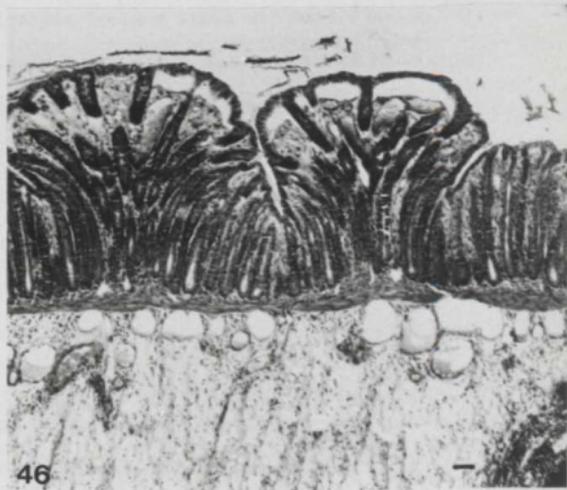
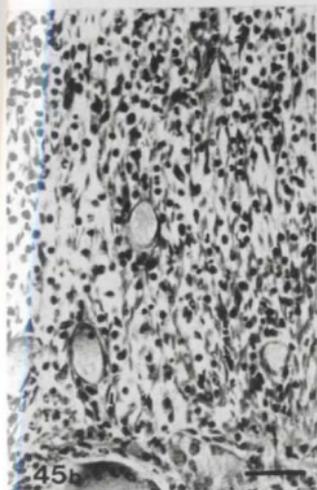
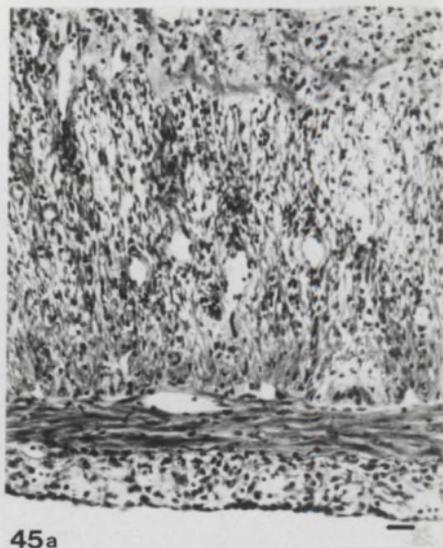
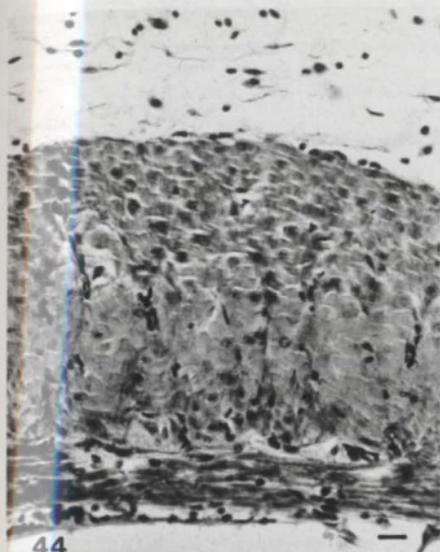
Figure 44: (rat, 10% acetic acid, intraluminal, 1 day) The initial stages of edematous separation of the muscle fibers in the muscularis externa of the rat colon (paraffin, H&E, 160X, scale = 25 μ m).

Figure 45: (rat, 10% acetic acid, intraluminal, 3 days)

(a) 3 days following administration of the agent the majority of the muscularis externa has become degenerated and replaced with a connective tissue network extending in a perpendicular fashion to the lumen. In the spaces between these fibers, polymorphs are often observed (paraffin, H&E, 160X, scale = 25 μ m).

(b) Higher magnification of this collagenous fiber arrangement where once the muscle fibers of the muscularis externa were located. Capillaries are prominent as are polymorphs (paraffin, H&E, 380X, scale = 25 μ m).

Figure 46: (rat, 10% acetic acid, intraluminal, 3 days) Edematous swelling of adjacent areas of mucosa exhibiting epithelial bridging and lifting from the lamina propria, a scalloped luminal appearance for the mucosa as a result of the edematous swelling prevalent mainly in the upper regions of the mucosal lamina propria. Note the dilated appearance of the superficial submucosal veins and lymphatic vessels (paraffin, H&E, 32X, scale = 50 μ m).



Interpersed within the ulcerations were many islands of residual mucosa exhibiting various degrees of inflammation. They usually had tapering edges which extended down to the remnants of intact muscularis mucosae which stretched out from under these structures (Figure 48).

Areas exhibiting only focal necrosis, with intact muscularis mucosae, began to repair themselves between the fifth and seventh day following administration of the agent. The mucosal inflammatory infiltrate began the initial organization and an epithelial plate grew outward from regions of adjacent mucosa over the surface of the connective tissue inflammatory cell mass. Adjacent mucosa however, often displayed markedly distorted crypts, cryptitis and an inflamed lamina propria (Figure 49).

By the ninth day post-acid, lesions still actively exuding inflammatory elements into the colonic lumen often became covered by a layer of necrotic debris which had a strong affinity for eosin (Figure 50). The submucosa and muscularis externa, which had been heavily infiltrated with polymorphs and collagenous fibers in a dense connective tissue matrix, exhibited a highly organized arrangement. In the submucosa the fibers extended in a parallel fashion to the colonic surface while those in the muscularis externa extended perpendicular to the surface.

Colons of rats treated intraluminally with 50% acetic acid exhibited an extremely necrotic transmural degeneration (Figure 51). This stage was often reached within three days following administration of the agent and led to perforation of the colonic wall and death of the animal.

Figure 47: (rat, 10% acetic acid, intraluminal, 3 days) Edematous swelling of mucosa slightly removed from the acid-induced lesion. More peripheral mucosa has a thinner degenerating appearance (paraffin, H&E, 60X, scale = 25 μ m).

Figure 48: (rat, 10% acetic acid, intraluminal, 5 days) Poly-poidal-like island of residual inflamed mucosa in the midst of an area ulcerated down to the level of the muscularis mucosae which is no longer visible except in the area under this structure. It is surrounded by a cellular inflammatory exudate and it also exhibits dilated crypts, incomplete epithelial boundaries, edematous swelling and slight infiltration of inflammatory cells into the lamina propria (paraffin, H&E, 60X, scale = 25 μ m).

Figure 49: (rat, 10% acetic acid, intraluminal, 5 days) Mucosa adjacent to ulcerated regions exhibiting thickened sub-mucosa, edematous swelling in the muscularis externa, and prominent cryptic dilation of the mucosa (paraffin, H&E, 100X, scale = 25 μ m).

Figure 50: (rat, 10% acetic acid, intraluminal, 5 days) Hyaline mass of necrotic material forming a thick layer over the luminal aspect of the ulcerated colonic wall which is packed with polymorphs (paraffin, H&E, 380X, scale = 25 μ m).

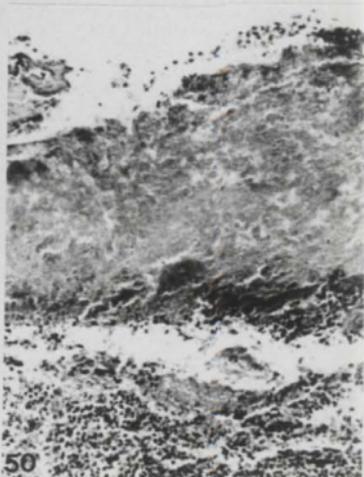
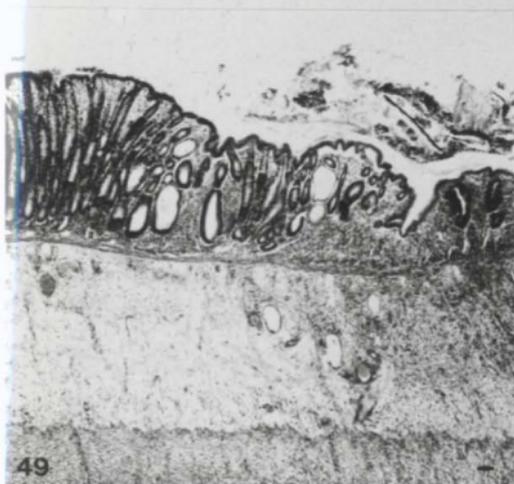
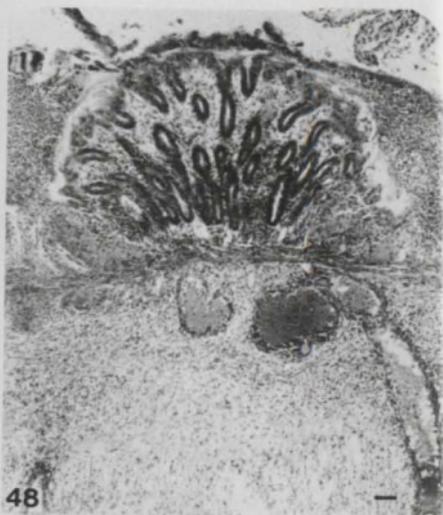
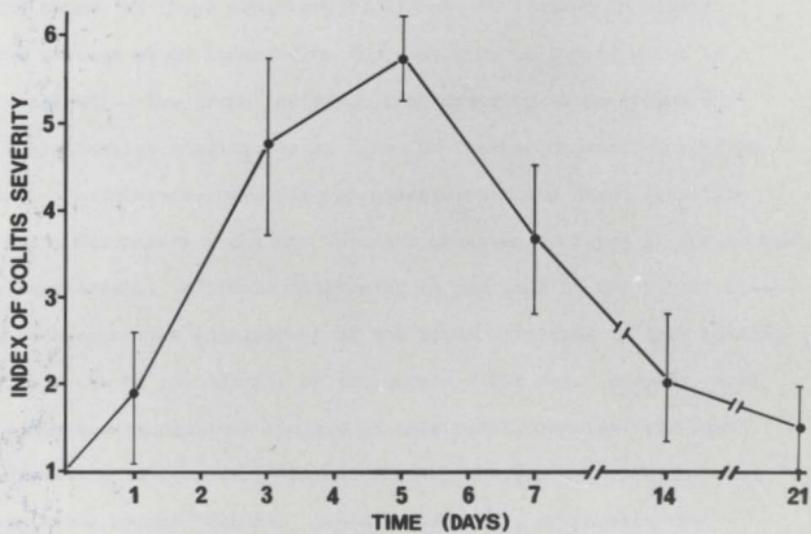
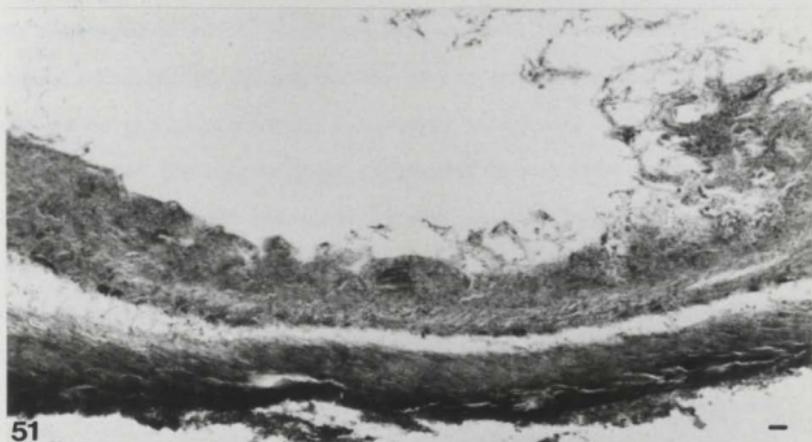


Figure 51: (rat, 50% acetic acid, intraluminal, 7 days) The marked necrotic appearance of all layers of the colonic bowel wall following administration of high concentrations of the agent intraluminally (paraffin, H&E, 32X, scale = 50 μ m).

Figure 52: Graphic illustration of the severity and development of colonic lesions in cats treated with 50% acetic acid intraluminally as evaluated by the colitis index based on gross observation. Each point represents the Mean \pm S.E.M. of 7 to 10 cats.



Experimental Colonic Lesions in Cats

The incidence of perforation and unfavourable tissue reactions at the higher intraluminal concentrations of the acidic solution in rats was thought to be the result of the relatively thin bowel wall of this species. The search for a convenient laboratory animal with a thicker colonic wall resulted in the selection of the cat. Intraluminal administration of 25% acetic acid resulted in a slight mucosal inflammation by three days but failed to induce lesions. Utilization of 50% acetic acid resulted in the development of diffuse, colonic lesions in 100% of animals treated within three days of administration. Figure 51 shows the typical luminal appearance of the colon after this period of time.

The course of these experimental lesions is plotted in Figure 52 and the average ulcer index value for each time period is shown in Table 8 (page 82). The graph indicates peak severity to be around day 5, with a progressive healing, or at least no further degeneration, from that point. Perforation, pericolic abscesses, fistulous tract formation and serosal inflammatory reactions were not observed over the 21 day course of these experiments. Fibrotic thickening in the wall of the distal colon caused a megacolon-like enlargement of the region proximal to this partial stenosis in a few of the animals by the twenty-first day. However, most animals exhibited generalized healing at this point, ranging from less severe ulceration to completely normal mucosal morphology. Fibrous scar tissue was not a common feature. Table 3 (page 51), delineates the morphologic criteria used to evaluate the severity of the lesion on a gross basis. The gross intraluminal mucosal appearance of the cat

Table 8: Mean Colonic Ulcer Indices for the Cat at Selected Intervals. Each Value Represents the Mean of 7 to 10 Cats \pm S.E.M.

Interval (days)	Mean Ulcer Index \pm SEM
1	1.9 \pm 0.76
3	4.8 \pm 1.10
5	5.7 \pm 0.54
7	3.7 \pm 0.87
14	2.1 \pm 0.73
21	1.6 \pm 0.45

colon three days following exposure to 50% acetic acid compared with that of the saline control animals is shown in Figure 53.

Over the duration of the twenty-one day experiments, the cats exhibited minimal discomfort associated with loose stools which sometimes appeared tarry or were accompanied by fresh bleeding. Table 9 (page 85), demonstrates a slight weight loss in treated animals during the experimental periods while the saline control animals gained an average of 0.22 kg. No deaths occurred during the experimental periods.

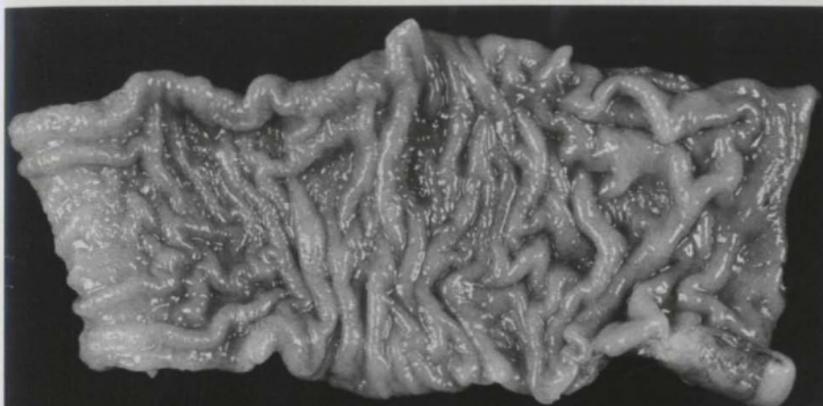
Histopathologically, the cat colon exhibited a similar tissue reaction to the intraluminal instillation of acetic acid to that demonstrated by the rat. Mucosal edema, polymorph infiltration of the lamina propria and superficial regions of the submucosa, dilated crypts, breakdown of the muscularis mucosae, and erosion of the epithelium and underlying mucosal elements with release of inflammatory cells into the colonic lumen, were all features of the lesion in the cat as well. Generally, the submucosa exhibited a less marked reaction to the agent while mucosal ulceration appeared to resemble that of the rat. The muscularis externa and serosa were rarely, if ever, affected (figures 54 to 58).

Comparison of the Histopathology of Lesions in Rat Colon with
the Two Modes of Application:

Figure 59 illustrates the histopathological course of both intraluminal and serosal application of acetic acid to the rat colon. The observations are limited to those occurring in the mucosa and submucosa of the colon. In both cases, treated animals developed diffuse colonic lesions of the

Figure 53: (a) The mucosal appearance of the normal, saline control, descending colon of the cat. It is flaccid and maintains haustral folds when stretched out on the material.

(b) The mucosal appearance of the cat colon 3 days after intraluminal instillation of 50% acetic acid. The colon is thickened and shrinks when excised, the haustral folds are less distinct due to mucosal edema and areas between these folds are openly ulcerated. There is a thick layer of mucus giving the colon a glossy appearance.



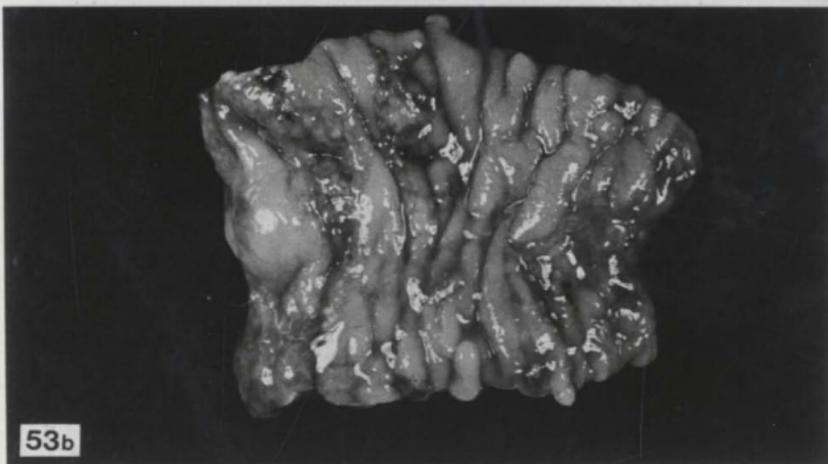
53a



CENTIMETERS

2

3



53b

Table 9: Average Weight Change in Kilograms Observed
Over a Period of 21 Days in Cats Treated
Intraluminally With 50% Acetic Acid.

Group	Weight in Kilograms		
	0 Days	21 Days	Change
Treated	2.66	2.46	- 0.20
Saline Controls	2.74	2.96	+ 0.22

Figure 54: (cat, 50% acetic acid, intraluminal, 1 day) Polymorphonuclear leucocyte infiltration of the lamina propria in the cat treated intraluminally with 50% acetic acid (paraffin, H&E, 380X, scale = 25 μ m).

Figure 55: (cat, 50% acetic acid, intraluminal, 3 days) Cryptic distortion and branching near the periphery of a lesion. Note the active margination of polymorphs in the large submucosal vein (paraffin, H&E, 100X, scale = 25 μ m).

Figure 56: (cat, 50% acetic acid, intraluminal, 5 days) A large mushroom-shaped mass of inflammatory cells and connective tissue which is in the initial stages of organization in those regions within the bowel wall. Adjacent mucosa exhibits dilated crypts and no underlying muscularis mucosae (paraffin, H&E, 32X, scale = 50 μ m).

Figure 57: (cat, 50% acetic acid, intraluminal, 7 days) The muscularis externa of a cat treated 7 days prior. Although the submucosa has undergone organization of the granulation tissue, the muscularis externa has remained intact (paraffin, H&E, 380X, scale = 25 μ m).

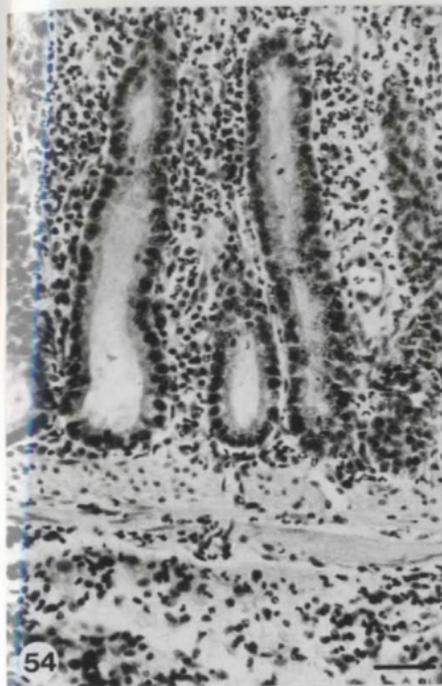
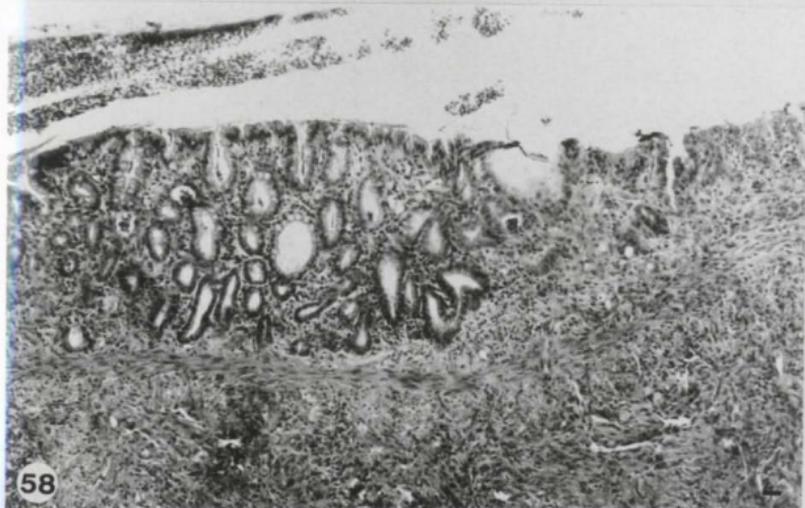
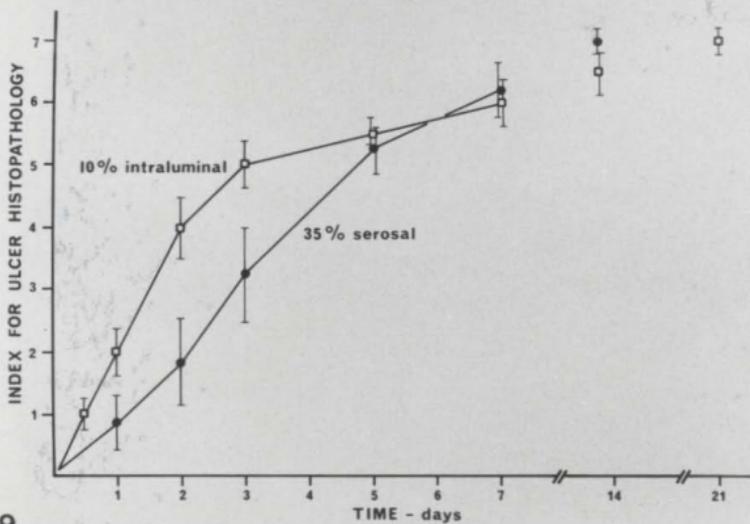


Figure 58: The characteristic appearance of the cat colonic mucosa 14 to 21 days following intraluminal instillation of 50% acetic acid. The mucosa adjacent to the crypt possesses contorted crypts, some dilated, and an intact muscularis mucosae. In the area of the lesion, organization of the granulation tissue has begun as has surface epithelial outgrowth and crypt formation from this. A cellular inflammatory exudate can be seen just above the mucosa (paraffin, H&E, 100X, scale = 25 μ m).

Figure 59: Graphic illustration of the histopathologic response of the rat descending colon to the application of optimal concentrations of acetic acid applied by the two modes. Each point represents the Mean \pm S.E.M. for 10 to 20 rats.



58



59

severity indicated by the ulcer index (Table 2, page 49). The brief histopathological descriptions of the step-wise gradations of severity indexed in this table were taken from the histological screening of excised tissue from colons treated by both techniques at the various screening intervals. This table outlines the development of the mucosal lesions and the extent of morphological alteration exhibited by the submucosa. The serosa and muscularis externa were generally affected more commonly in the serosal technique, although they did undergo similar changes but with lesser severity and later in the disease process than animals treated intraluminally. The common alterations in these deeper bowel layers were edematous separation of muscle fibers followed by polymorph infiltration into these spaces and the establishment of a fibrous connective tissue network (Figures 44, 45). In these instances the serosa exhibited a similar reaction. A connective tissue inflammatory exudate accumulated on the visceral aspect of the serosa in both techniques, although again the development occurred earlier and far more commonly and extensive in the serosally treated animals.

Evaluation of the above information collected on both modes of application for the agent resulted in the selection of 10% acetic acid as the most desirable technique for induction of these lesions. This concentration and mode produced a semi-chronic lesion in 100% of all rats treated, involved mainly the mucosa and superficial region of the submucosa, and exhibited the minimal incidence of complications.

Mechanism of Development of the Lesion Induced by Intraluminal
Acetic Acid:

Timed interval sampling of tissue excised from rats in the earliest stages of lesion development was undertaken. For additional clarity of histological features, the tissue was embedded in plastic resin for the study of cytologic detail by light microscopy. Electron microscopy of certain aspects of these stages also helped to elucidate the mechanism behind development of these lesions.

The normal histology of the rat colon embedded in plastic can be observed in Figures 60 to 64. These photomicrographs illustrate the various typical features of the mucosa and submucosa of the rat colon. Figures 65 to 73 are transmission and scanning electron micrographs of these same features which help to clearly document the histology and ultrastructure of the normal, control, rat colon.

Figures 74 through 111 are a pictorial documentation of the development of the colonic lesions induced by 10% acetic acid administered intraluminally and are based on timed interval sampling. Immediately following completion of administration of the acid and washing the colonic lumen with saline, the mucosa exhibited cellular disruption of the epithelium. The goblet cells of the surface and crypt epithelium responded to the acid by releasing their stored quantities of mucus granules. At the same time the surface epithelium assumed a ragged appearance as many of the cells, both absorptive and goblet, were shed from the underlying reticular basement membrane. The colonic lumen contained bits of these cells as well as many intact cells (Figure 74). The cellular response deeper in the crypts was limited and the cellular components of the muscularis mucosae and submucosa appeared normal.

Figure 60: (rat, normal colon) The histological appearance of the normal, saline control, rat colon embedded in plastic resin and sectioned at 2 microns (plastic, toluidine blue, 160X, scale = 25 μ m).

Figure 61: (rat, normal colon) Histology of the surface and crypt epithelial cells as well as the cellular components of the lamina propria (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 62: (rat, normal colon) Histology of the basal regions of the mucosal crypts and of the muscularis mucosae of the rat colon (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 63: (rat, normal colon) Cross section of the mucosa showing the arrangement of the crypts and the cellular components of the lamina propria (plastic, toluidine blue, 630X, scale = 25 μ m).

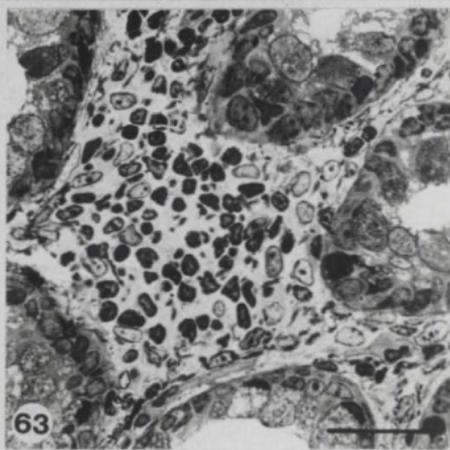
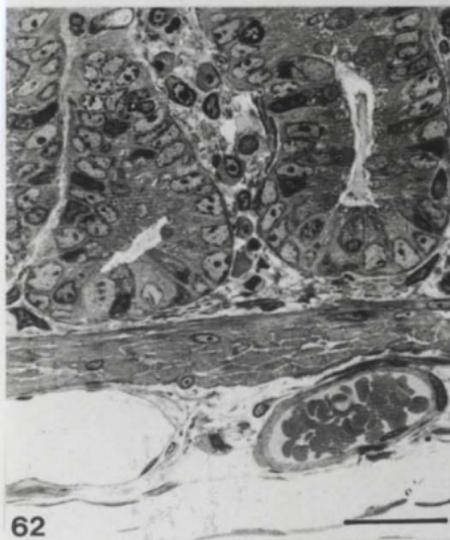
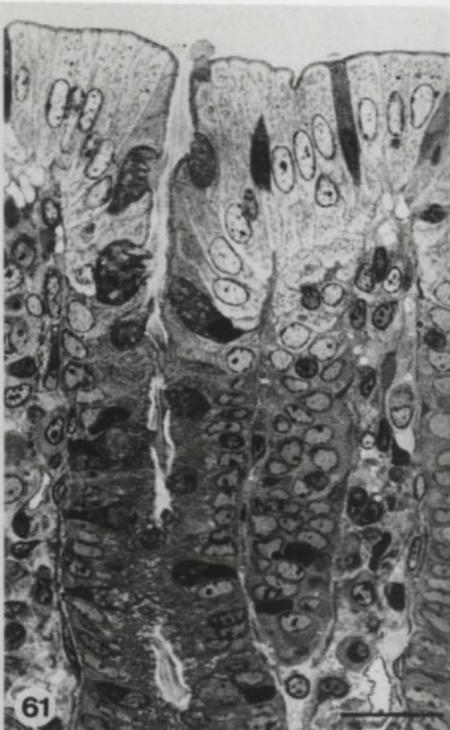
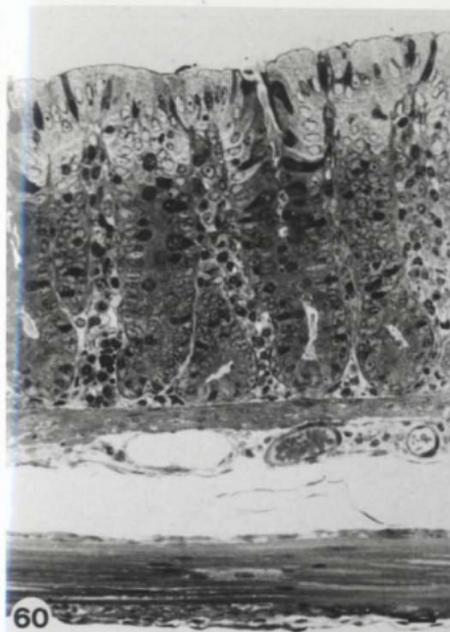


Figure 64: (rat, normal colon) The submucosa of the rat colon showing a blood vessel and connective tissue stroma (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 65: (rat, normal colon)

(a) Surface epithelial cells of normal rat colon with their microvilli and cellular organelles (4,175X, scale = 1 μ m).

(b) Higher magnification of the microvilli showing tiny vacuoles just under the luminal aspect of the cell membrane (8,650X, scale = 1 μ m).

(c) Higher magnification of the microvilli showing the glycocalyx covering and lying between these structures as from well as the terminal web formed by the filaments emanating from them. A typical desmosomal connection can also be seen (25,300X, scale = 1 μ m).

(d) Cross section of a group of surface epithelial cells showing their interdigitations which helps them adhere to one another in the lower cell regions. Mitochondria are also abundant (8,650X, scale = 1 μ m).

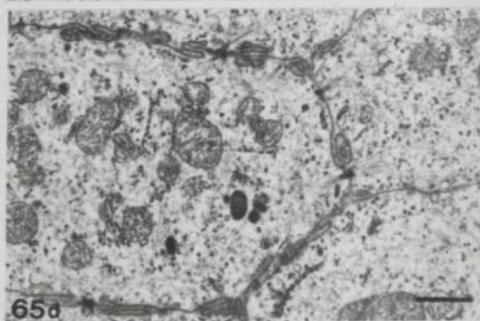
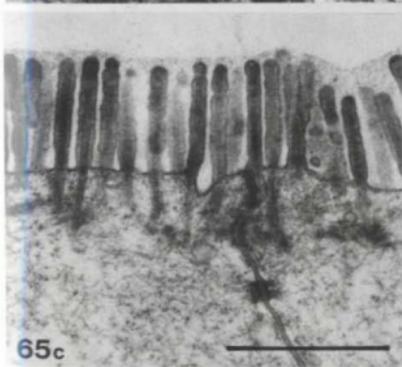
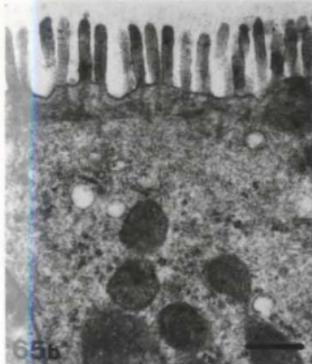
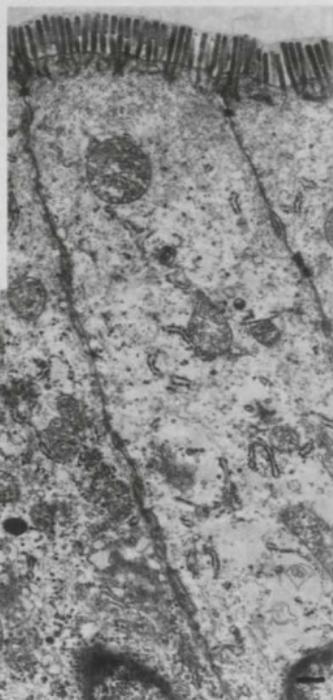
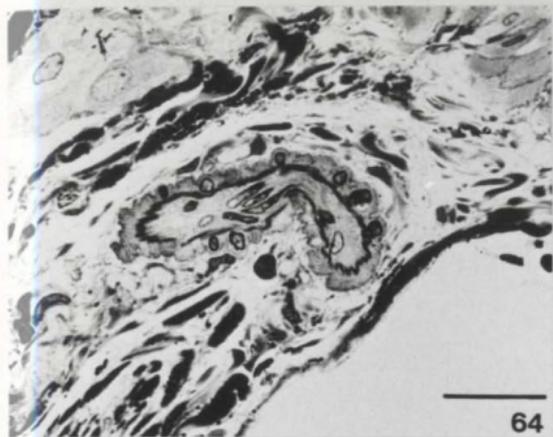


Figure 66: (rat, normal colon) The interdigitation of cellular processes at the base of the epithelial cells of rat colonic mucosa. The basement membrane is situated under this (25,300X, scale = $1\mu\text{m}$).

Figure 67: (rat, normal colon) Crypt epithelial absorptive cells wedged among each other and possessing extremely long microvilli (4,175X, scale = $1\mu\text{m}$).

Figure 68: (rat, normal colon) Mucosal epithelium at the crypt base showing a goblet cell filled with mucus droplets and a number of absorptive cells with their characteristic vacuoles situated near the luminal cell membrane. Active Golgi complexes are located superior to the nuclei of these cells (4,175X, scale = $1\mu\text{m}$).



Figure 69: (rat, normal colon) The luminal aspect of epithelial cells deep in the crypts showing their typical vacuolated appearance, cellular junctions and tall microvilli (25,300X, scale = $1\mu\text{m}$).

Figure 70: (rat, normal colon) The typical consistency of the lamina propria in normal rat mucosa. The basal epithelium of a crypt is seen to the right while the lamina propria is composed of bundles of collagenous fibers and other cellular components (7,085X, scale = $1\mu\text{m}$).

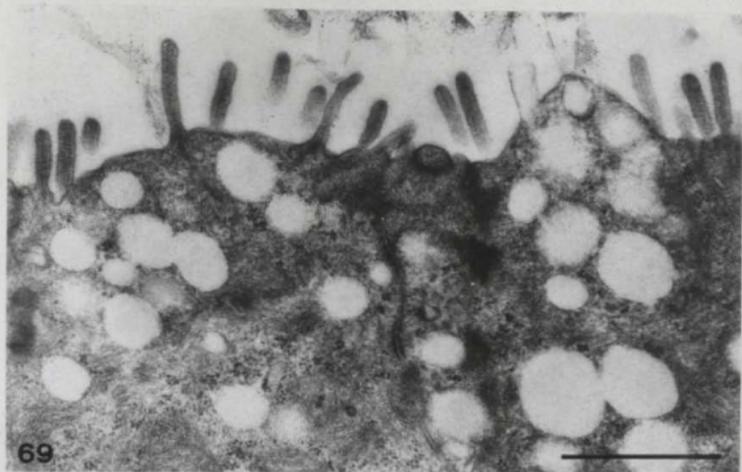


Figure 71: (rat, normal colon) Ultrastructure of the normal muscularis mucosae of the rat. The basal region of the lamina propria can be seen near the top while the bottom right is the lumen of a capillary (10,375X, scale = 1 μ m).

Figure 72: (rat, normal colon) Lymphocytes in the lumen of a mucosal lacteal (8,605X, scale = 1 μ m).

Figure 73: (rat, normal colon) Scanning electron micrograph of the mucosal surface of the normal rat colon (95X, scale = 25 μ m).

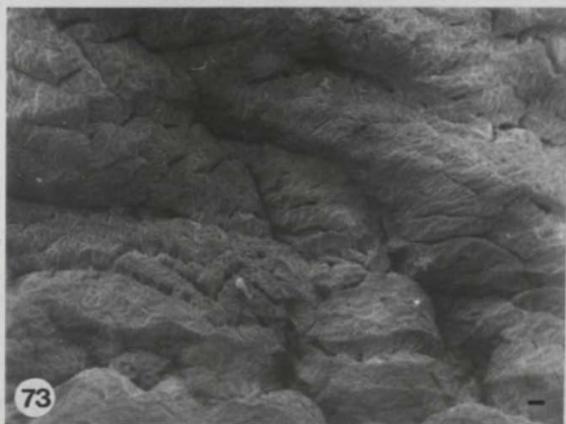
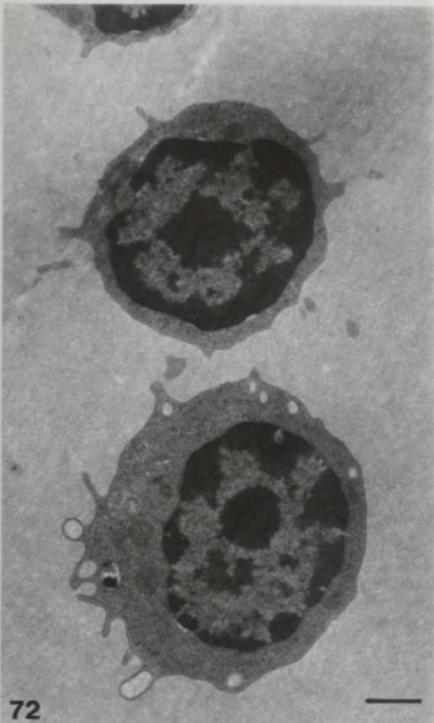
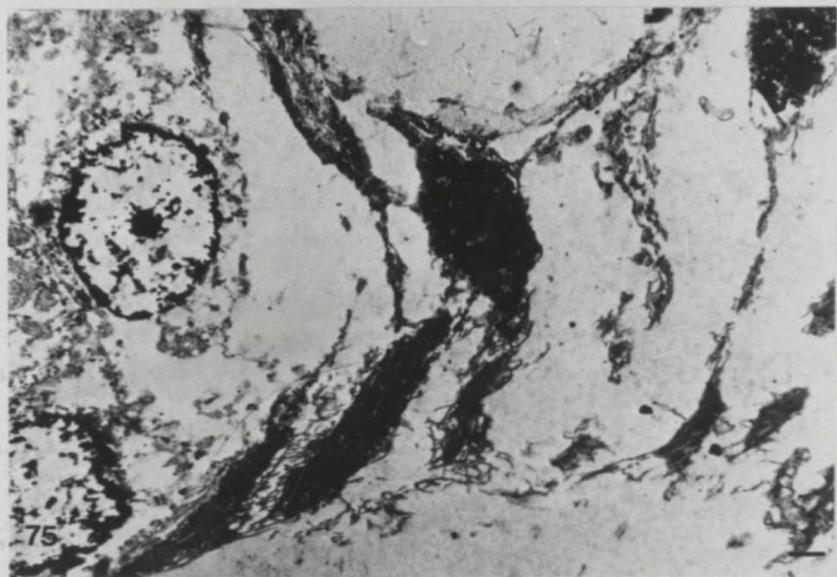
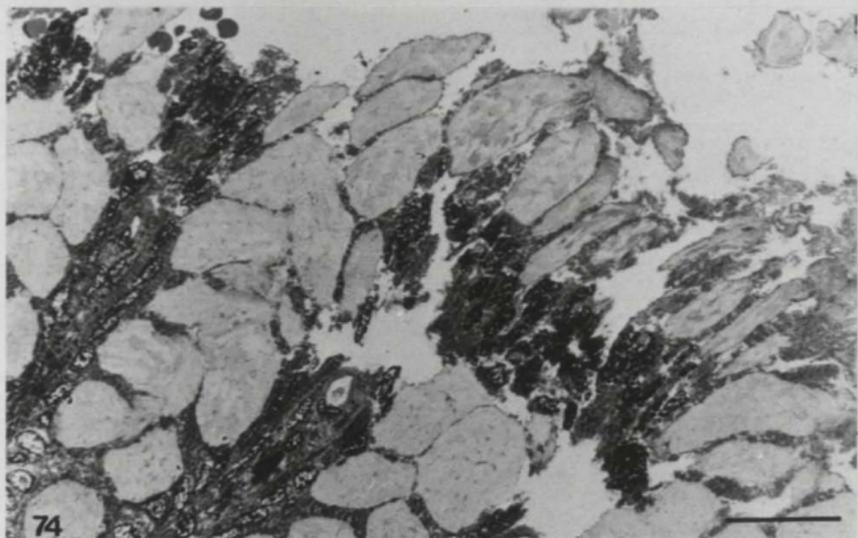


Figure 74: Response elicited by the colonic mucosa of the rat immediately following intraluminal administration of 10% acetic acid. There is sloughing of the surface epithelial cells as well as a minimal number from the superficial regions of the neck of the crypts. Pieces or entire cells may be sloughed in this initial response. Note that the goblet cells are empty of stored quantities of mucus (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 75: An electron micrograph of the same time period illustrated in Figure 74 showing the severity of cellular degeneration inflicted in these superficial cells by the acetic acid and their lifting from the basement membrane, which in the majority of cases remains intact (4,935X, scale = 1 μ m).



At the ultrastructural level the separation of the surface epithelial cells from the basement membrane was clearly defined, with the latter retaining its normal appearance. The cytoplasm of these cells appeared vacuolated and scattered, losing its homogeneous appearance. The nuclear chromatin and nucleoplasm exhibited a similar precipitated appearance. Following the epithelium into the crypt revealed cells in a similar condition peeling away from the basement membrane in a sheet (Figure 75).

Within 15 seconds following completion of acid administration, the cellular response had spread deeper into the crypts. On the luminal surface the mucosa appeared ragged with sloughing of epithelial cells while those of the superficial neck region of the crypts also appeared to be degenerating. Deeper in the mid-crypt region, the cells begin to appear disrupted. Goblet cells were empty of mucus granules and the cytoplasm of these cells, as well as others, appeared vacuolated. Some of the cells at the base of the crypt, as well as some in the lamina propria also exhibited cytoplasmic vacuolation. The muscularis mucosae and submucosa still retained their normal architecture (Figures 76 to 80).

Electron microscopy revealed that the crypt epithelial cells were degenerated with precipitation of the cellcytoplasm and nuclear material. Cellular boundaries were not apparent and the microvilli at their luminal aspect were distorted (Figures 81, 82). Cells deep in the crypt had cytoplasm that appeared normally homogeneous except for large spherical vacuoles. At higher magnification, these vacuoles proved to be the remnants of the mitochondria. These organelles exhibited a limiting membrane not noticeably different from that of their normal configuration

Figure 76: (rat, 10% acetic acid, intraluminal, 5 minutes)

Appearance of the surface epithelium approximately 5 minutes following administration of the agent. The surface epithelial cells are being sloughed into the lumen of the colon (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 77: (rat, 10% acetic acid, intraluminal, 5 minutes)

Vacuolation of epithelial cell cytoplasm accompanied by a coagulated appearance, release of stored mucus and relative lack of nuclear chromatin in the epithelial cells further down the crypts shortly after intraluminal administration of the agent (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 78: (rat, 10% acetic acid, intraluminal, 5 minutes)

Epithelial cells near the base of the crypts at this same stage, exhibiting slightly increased and larger vacuolation than normal in rat colon (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 79: (rat, 10% acetic acid, intraluminal, 5 minutes)

The muscularis mucosae remains apparently unaffected by the acetic acid at this early stage of lesion development (plastic, toluidine blue, 630X, scale = 25 μ m).

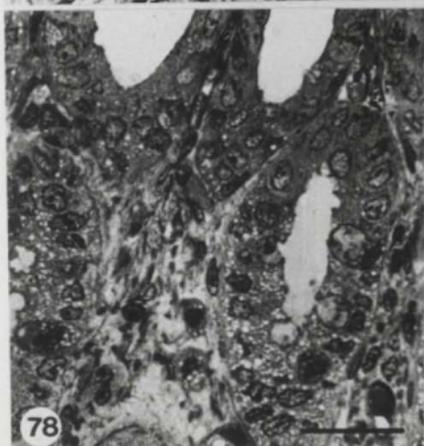
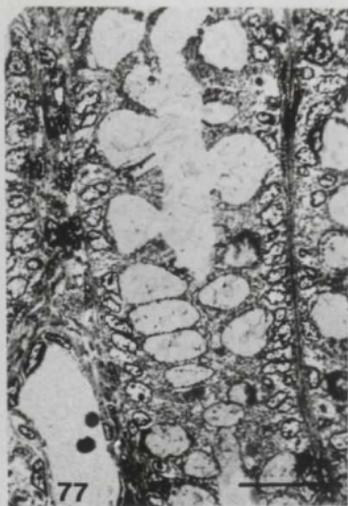
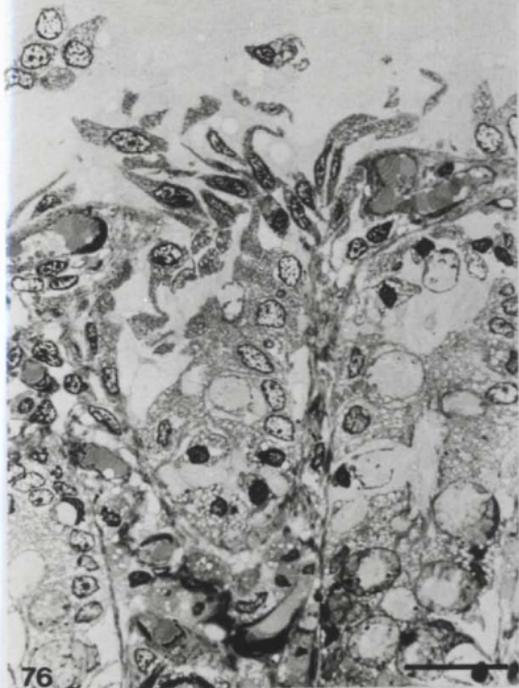


Figure 80: (rat, 10% acetic acid, intraluminal, 15 minutes)

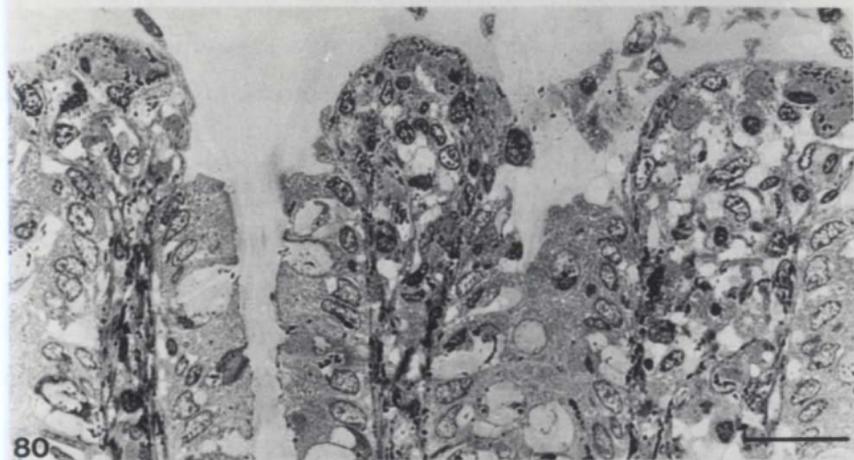
The appearance of the colonic mucosa approximately 15 minutes following intraluminal administration of the agent. The surface epithelium and that of the superficial crypt neck region has been sloughed leaving a bald pinnacle of lamina propria projecting out into the lumen. The epithelium further down the crypt can be seen to have a vacuolated cytoplasm (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 81: (rat, 10% acetic acid, intraluminal, 15 minutes)

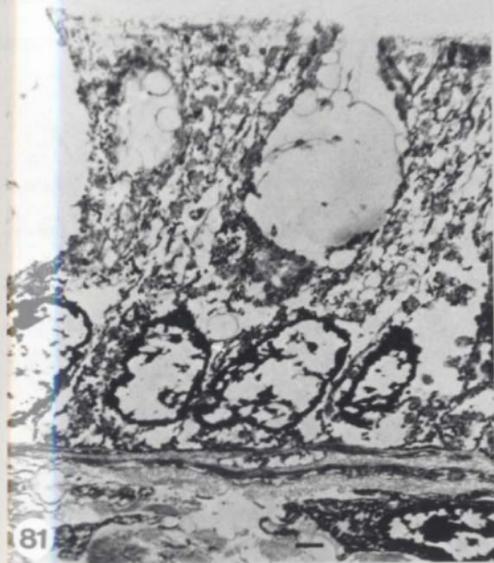
Fine structure of the epithelial crypt cells with vacuolated cytoplasm as seen under the light microscope. The mucus has been released from the goblet cells and their cytoplasm and nucleoplasm has a coagulated appearance. Cellular organelles are not evident but the cells remain adherent to the basement membrane and their microvilli appear long and scraggly (4,175X, scale = 1 μ m).

Figure 82: (rat, 10% acetic acid, intraluminal, 15 minutes)

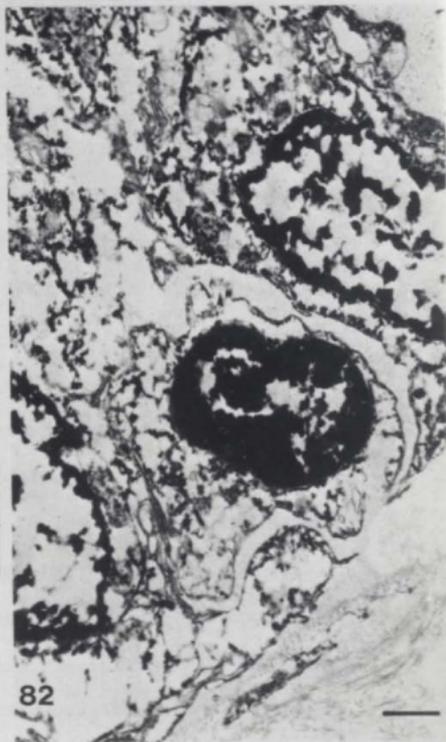
A higher magnification of the basal region of these degenerated cells. In this case, the cells are beginning to experience an edematous swelling of their intercellular spaces near the point of attachment to the basement membrane (8,650X, scale = 1 μ m).



80



81



82

but the internal organization of the organelle was completely disrupted. The interdigitations between adjacent cells were not as tight as usual and had become blunter, with increased intercellular spaces (Figure 83).

Within five minutes the surface epithelium and that of the superficial regions of the crypts, mostly goblet cells, had been sloughed off. This epithelial stripping continued down the crypt and by thirty to sixty minutes following acid administration, over one half the crypt had been denuded of epithelial cells, leaving only a pillar of lamina propria projecting into the colonic lumen (Figure 84). The effect of the acid was now exhibited by the muscularis externa, where the most superficial muscle fibers appeared vacuolated (Figure 85).

Ultrastructurally, epithelial cells deep in the crypts exhibited a sheet-like separation from their basement membrane. Higher magnification revealed distorted microvilli and degenerating cellular boundaries whose junctional complexes had maintained their integrity holding the cells together in the sheet (Figure 86).

By the eighth hour post-acid, areas which had direct contact with the acetic acid exhibited a mucosal architecture consisting only of tall columns of lamina propria. These pillars of connective tissue appeared thicker than normal as a result of an edematous reaction in the matrix. The underlying muscularis mucosae also assumed a shrunken, vacuolated appearance, while the submucosa was beginning to undergo an edematous response (Figures 87, 88). Scanning electron microscopy shows this absence of cellular components on the surface and within the crypts (Figure 89).

Figure 83: (rat, 10% acetic acid, intraluminal, 1 hour)

(a) Epithelial absorptive cells at the base of the crypts exhibiting a vacuolated appearance. The cytoplasm and other cellular organelles appear normal (12,650X, scale = 1 μ m).

(b) Higher magnification of these vacuoles in Figure 83a reveals that they are destroyed mitochondria which have become edematous, losing all internal structure but retaining an intact limiting membrane (25,300X, scale = 1 μ m).

Figure 84: (rat, 10% acetic acid, intraluminal, 1 hour) The sloughing of the superficial crypt epithelium as well as that of the surface while that further into the crypts has a vacuolated appearance. This is a typical feature in the early stages of lesion development (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 85: (rat, 10% acetic acid, intraluminal, 1 hour)

Vacuolation of the superficial aspects of the muscularis externa (upside down in this instance). The submucosa exhibits thickened collagenous fibers (plastic, toluidine blue, 630X, scale = 25 μ m).

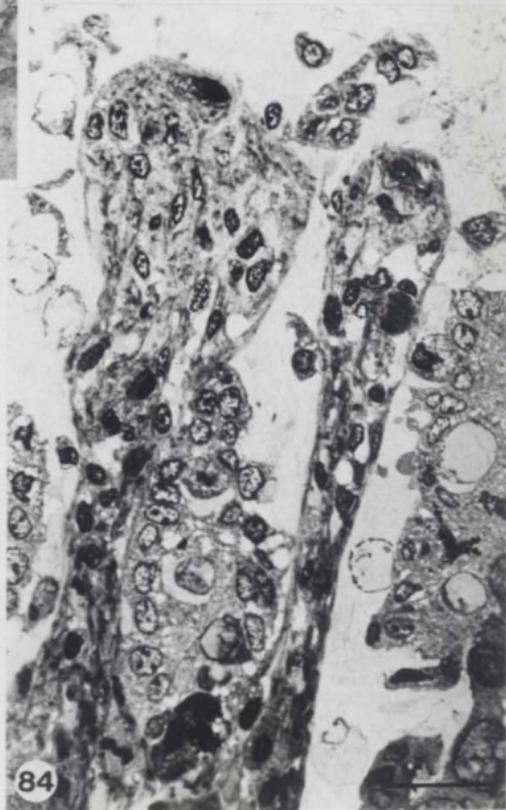
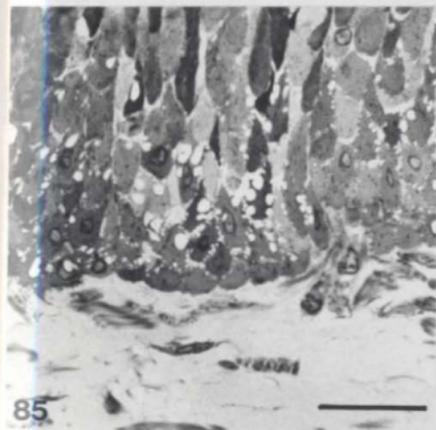
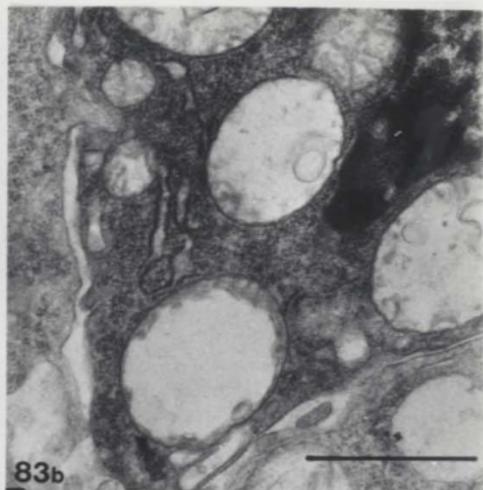
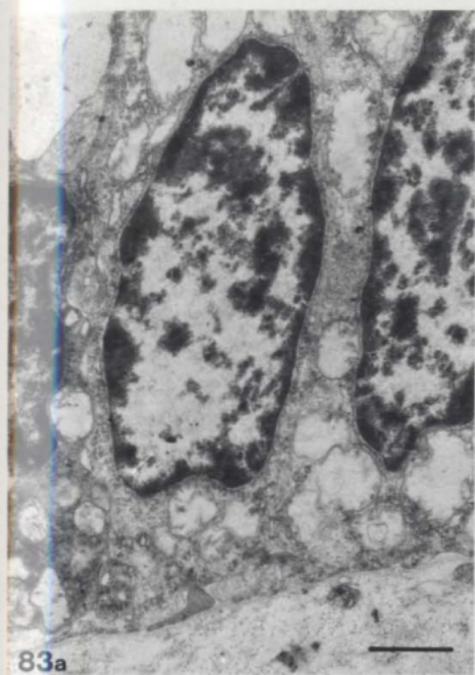


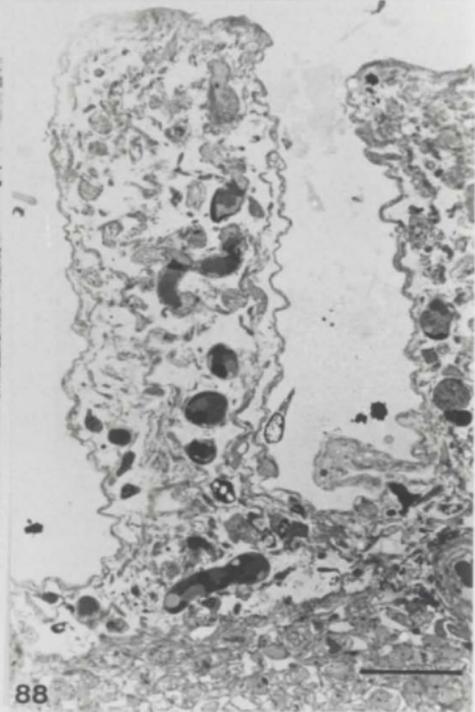
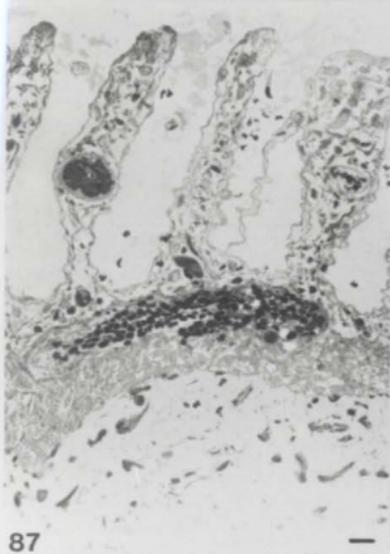
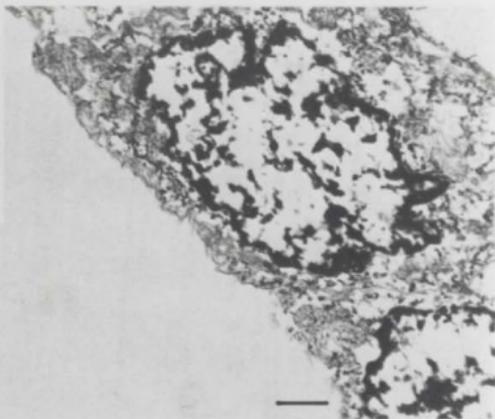
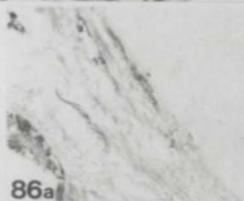
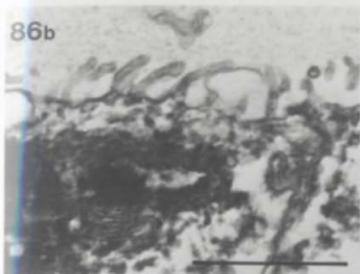
Figure 86: (rat, 10% acetic acid, intraluminal, 4 hours)

(a) Crypt epithelium in the superficial neck region of the crypt, peeling away from its basement membrane (left) in a sheet. The cytoplasm and nucleoplasm of these cells exhibits severe coagulation with destruction of cellular organelles but they manage to adhere to one another (8,650X, scale = $1\mu\text{m}$).

(b) Higher magnification of the luminal aspect of these cells showing the disturbed cellular cytoplasm and altered microvilli. Cell junctional complexes are not distinct but the cells are tightly apposed to one another (25,300x, scale = $1\mu\text{m}$).

Figure 87: (rat, 10% acetic acid, intraluminal, 8 hours) Rat colonic mucosa showing the entire surface and crypt stripped of epithelium leaving only a tall pillar of lamina propria whose capillaries are packed with erythrocytes. Lacteals in the basal regions are also packed with plasma cells and lymphocytes. The muscularis mucosae has an edematous appearance (plastic, toluidine blue, 380X, scale = $25\mu\text{m}$).

Figure 88: (rat, 10% acetic acid, intraluminal, 8 hours) Higher magnification of one of these pillars of lamina propria showing the edematous reaction as well as the packing of all capillaries with erythrocytes. Edematous separation of the muscularis mucosae is clearly evident here (plastic, toluidine blue, 630X, scale = $25\mu\text{m}$).



Both the cellular and collagenous fiber composition of the submucosa increased and additional connective tissue matrix appeared to be laid down as the material assumed a denser appearance. The walls of the superficial blood and lymphatic vessels began to elicit diapedesis of erythrocytes and polymorphs into the surrounding connective tissue matrix (Figure 90). The vacuolation of the muscle fibers of the muscularis externa progressed at a steady rate (Figure 91).

Meanwhile, in mucosal regions which had come into contact with the saline-diluted acid following administration of the acetic acid, in many cases adjacent to these regions previously described, the cellular response was not as severe or as rapid. Figure 92 illustrates the typical response where there was formation of epithelial bridges on the colonic surface. These represented the sloughing of entire sections of surface epithelium spanning the lamina propria between two crypts. The cells were still held together by their interdigitations which prevented separation. Although there was a release of the entire volume of stored mucus from the goblet cells, cellular degeneration was not as prevalent. This pattern progressed, the time involved varied and depended upon the concentration of acid encountered at the particular site, until the entire surface epithelium, as well as superficial crypt epithelium, had become unattached from the underlying basement membrane. The cells in these superficial regions appeared to undergo vacuolative degeneration while those deeper in the crypts were not as severely affected (Figures 93, 94).

The mucosa soon appeared to have dilated crypts in which the superficial aspects appeared frayed as the cells sloughed off into the

Figure 89: (rat, 10% acetic acid, intraluminal, 8 hours) A scanning electron micrograph of the colonic surface and down into the crypts 8 hours following administration of the ulcerogenic agent. The lack of cells both on the surface and in the crypts is clearly evident (795X, scale = 25 μ m).

Figure 90: (rat, 10% acetic acid, intraluminal, 8 hours) A series of photomicrographs illustrating the increase in both inflammatory cells and collagenous fibers within the submucosa around the 8th hour post-acid (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 91: (rat, 10% acetic acid, intraluminal, 8 hours) The initial response of the muscularis externa to 10% acetic acid administered intraluminally occurring later in the process. It is evidenced by vacuolation throughout the muscle layer (plastic, toluidine blue, 630X, scale = 25 μ m).

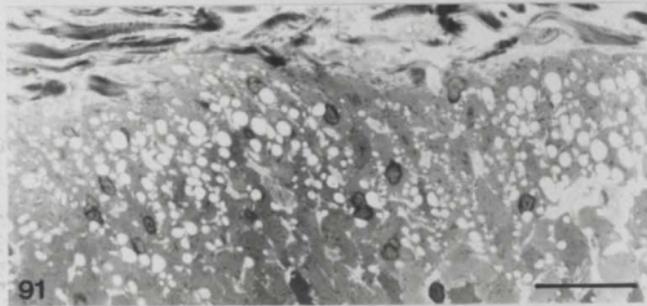
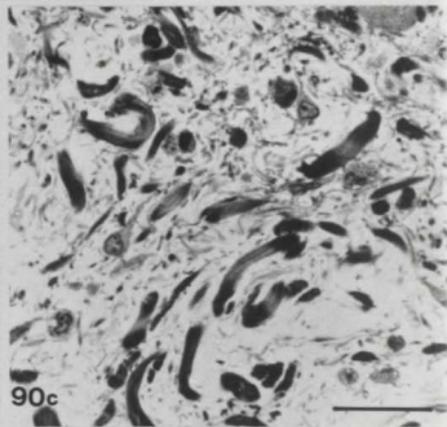
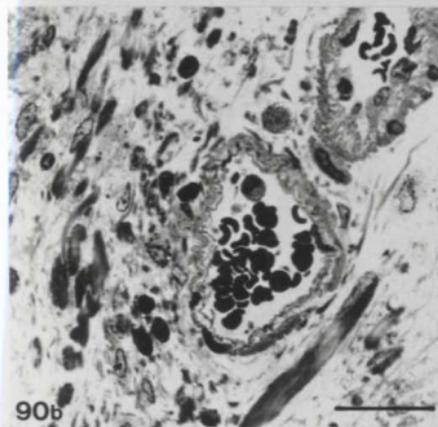
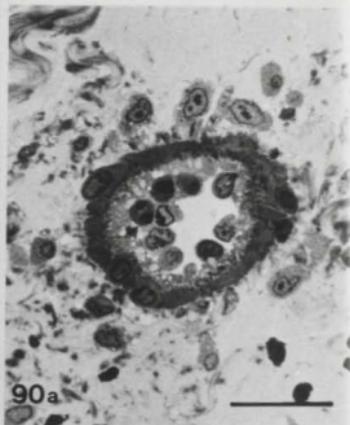
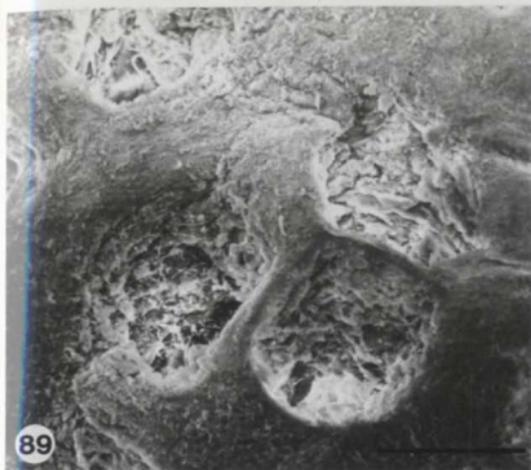


Figure 92: (rat, 10% acetic acid, intraluminal, 5 minutes)

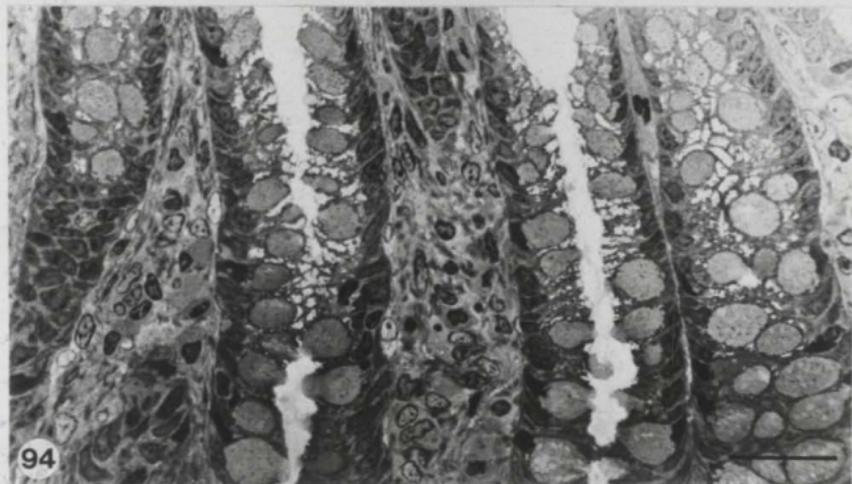
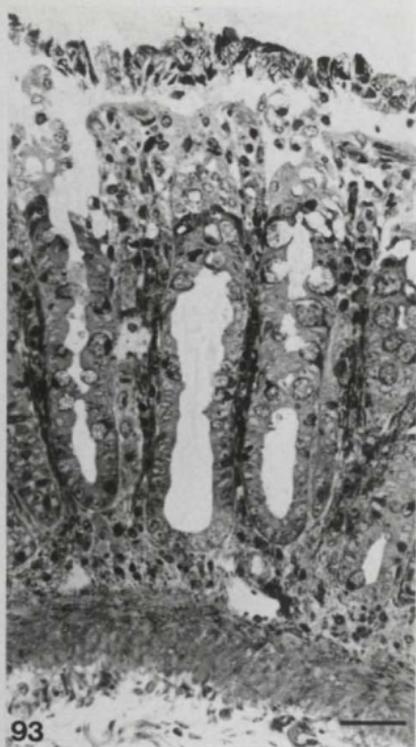
The mucosal response to acetic acid in areas removed from sites directly affected by the agent. The initial reaction was a lifting of the surface epithelium from the lamina propria forming an epithelial bridge (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 93: (rat, 10% acetic acid, intraluminal, 5 minutes)

Continuation of this sloughing and degeneration a little into the crypt, with the crypt epithelium further down becoming flattened and giving the crypt the appearance of being dilated (plastic, toluidine blue, 380X, scale = 25 μ m).

Figure 94: (rat, 10% acetic acid, intraluminal, 5 minutes)

Degeneration of the crypt epithelium continuing downward toward the base of the crypt with the cells exhibiting increasing cytoplasmic vacuolation in their apical regions (plastic, toluidine blue, 630X, scale = 25 μ m).



colonic lumen. The cells behaved in a similar fashion to those in the more severely affected regions but their crypt epithelium absorptive cells became flattened rather than vacuolated. These cells lost their columnar configuration while the adjacent goblet cells retained their bulbous shape and projected out above the others into the lumen of the crypt. The basal crypt epithelium, lamina propria and muscularis mucosae appeared more or less normal (Figure 95).

Electron microscopy revealed that these cells had shortened, stubby microvilli and a restricted amount of cytoplasmic vacuolation. The majority of the cytoplasmic vacuolation occurred just under the luminal surface of these cells throwing the cell membrane, without microvilli in some instances, into a humped configuration. In areas where these cells appeared to be lifting from the basement membrane, the cellular interdigitations were short and blunt. On other boundaries they appeared to be just able to maintain attachment to the adjacent cells, being connected with a reduced number of junctional complexes and the intracellular spaces appeared swollen, allowing the interdigitations to become free of one another and dangle in the enlarged space (Figures 96, 97, 99, 100, 101). These cells also had an increasing number of opaque or dense bodies within their cytoplasm, possibly lysosomes or cytosegrosomes.

The nuclei also had an irregular, shrunken appearance in these cells. Epithelial cells deeper in the crypts exhibited relatively normal nuclei although their cytoplasm contained a number of these large spherical or ovoid, opaque bodies. In some cases, erythrocytes could be observed

Figure 95: (rat, 10% acetic acid, intraluminal, 30 minutes) The response of adjacent mucosa to diluted acetic acid. Although the superficial epithelium is sloughed almost as rapidly, the crypt epithelium undergoes a different sort of degeneration resulting in a flattening of the typically columnar cells into configurations resembling that of squamous epithelium. In these cases, the shells of the goblet cells which are now empty of granules, project out into the lumen like small nodules (plastic, toluidine blue, 630X, scale = 25 μ m).

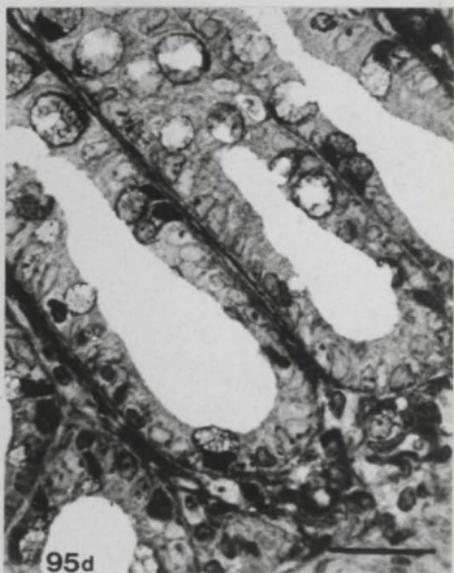
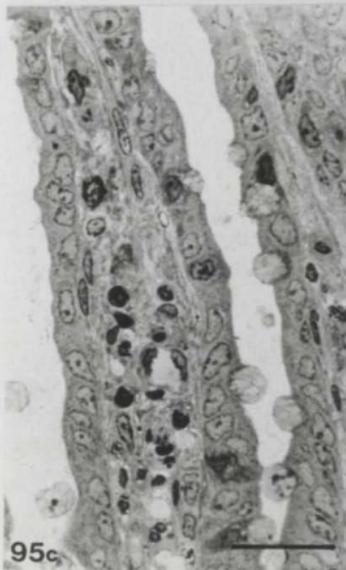
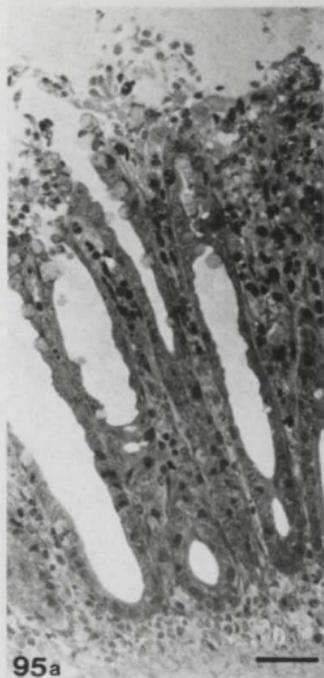
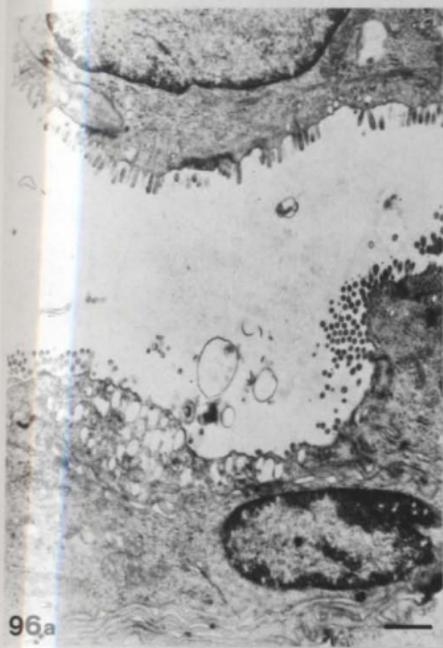


Figure 96: (rat, 10% acetic acid, intraluminal, 8 hours)

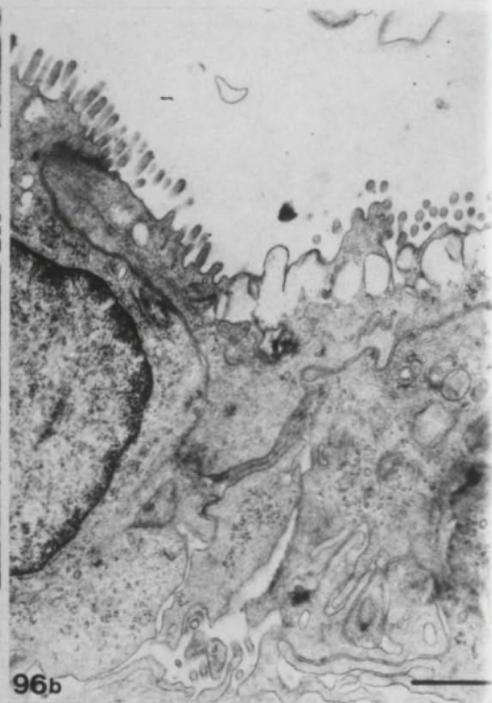
(a) Crypt epithelial cells undergoing degeneration following treatment with 10% acetic acid. The cells appear flattened, squamous-like, with altered microvilli and extreme vacuolation just under the luminal cell membrane (4,175X, scale = 1 μ m).

(b) Higher magnification of this cytoplasmic vacuolation. Cell junctional complexes appear to be maintained in the luminal regions, whereas in the basal regions there is swelling of the intercellular spaces and disengagement of the intercellular interdigitations (7,085X, scale = 1 μ m).

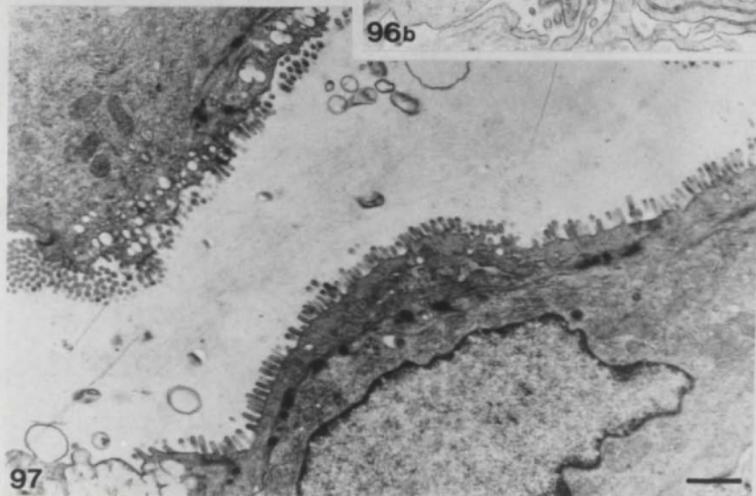
Figure 97: (rat, 10% acetic acid, intraluminal, 8 hours) Crypt epithelial cells undergoing degeneration. Adjacent cell in some cases (bottom) appear to overgrow their neighbour and form dense junctional complexes with it. The cellular organelles of these cells do not exhibit any sign of damage (8,650X, scale = 1 μ m).



96a



96b



97

Figure 98: (rat, 10% acetic acid, intraluminal, 1 day)

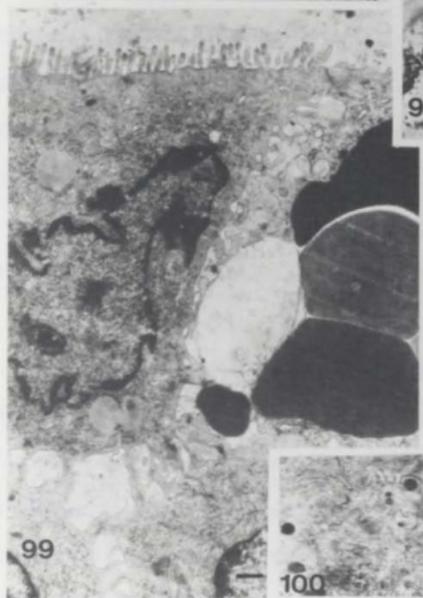
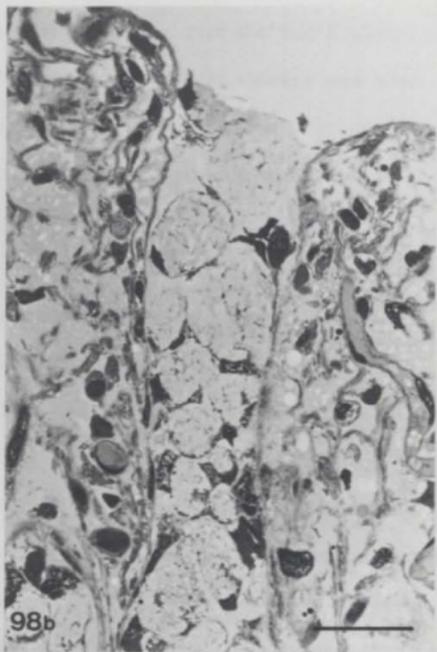
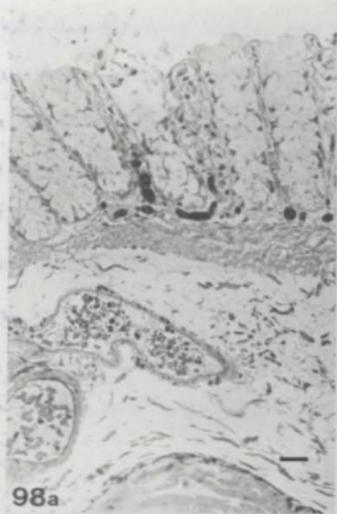
(a) The bowel wall of rat colon adjacent to areas severely affected by the acetic acid. The surface epithelium is sloughed, crypt cells appear vacuolated, the muscularis mucosae has an edematous separation, and the submucosa exhibits the beginning of a cellular infiltration into its already edematous matrix (plastic, toluidine blue, 160X, scale = 25 μ m).

(b) Higher magnification of 98a, showing the coagulative-type matrix filling the non-cellular areas of the lamina propria and vacuolated appearance of the goblet cells in the crypts (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 99: (rat, 10% acetic acid, intraluminal, 1 day) Crypt epithelium becoming separated from its surrounding neighbours through swelling of the intercellular spaces. Erythrocytes have invaded the epithelium immediately adjacent to this cell. There are a number of large opaque bodies in various locations throughout the cell cytoplasm and the nucleus has a peculiar morphology (7,085X, scale = 1 μ m).

Figure 100: (rat, 10% acetic acid, intraluminal, 1 day) A crypt epithelial cell resting on the mass of cellular debris which was once the lamina propria. Erythrocytes, polymorphs and other pieces of cells, as well as occasional bacterial invasion occupy this region (7,085X, scale = 1 μ m).

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outside blood vessels in the lamina propria bordering these cells (Figure 99).

Within twenty-four hours, the mucosa of these regions had a characteristic morphology as illustrated in Figure 98. This arrangement was also similar to that of the adjacent mucosal regions in that edematous swelling of the lamina propria was the prominent feature except that the crypts were still lined with degenerating villular remnants of the epithelium. In most cases these cells were large, swollen, vacuolated, membrane-bound structures, some with only a barely distinguishable nucleus. In other areas the crypts were lined with degenerated epithelial cells whose cytoplasm appeared to have undergone coagulative necrosis.

The next step in lesion development for the mucosal regions was a continued increase in edematous swelling of the lamina propria and submucosa, accompanied by a polymorph infiltration, and in the submucosa establishment of large amounts of connective tissue fibers and matrix. In some cases the spaces which once were crypts become filled with colonic bacteria and other debris which on occasion penetrated into the region of the lamina propria (Figure 102).

By eighteen to twenty-four hours post-acid, there usually was a massive emigration of erythrocytes and polymorphs from mucosal and superficial submucosal vessels into these two bowel regions. These cells became lodged in the fibrillar connective tissue network representing the remnants of the muscularis mucosae, and soon packed this area and moved into the mucosal region (Figure 103). The mucosa became filled with these blood vascular elements, retained for the most part by the

Figure 101: (rat, 10% acetic acid, intraluminal, 1 day) Epithelial cells sloughing off the basement membrane. These cells become unattached from one another's interdigitations and float free into the lumen of the crypt. Opaque bodies are evident in the cytoplasm of both cells (7,085X, scale = $1\mu\text{m}$).

Figure 102: (rat, 10% acetic acid, intraluminal, 1 day)

(a) The epithelial-stripped columns of lamina propria have the spaces which were once cell-lined crypts filled with necrotic debris from sloughed cells and great number of colonic bacteria which also appear to have penetrated the bounding membrane of the lamina propria and form nodules and other small aggregations within the necrotic material filling the lamina propria (plastic, toluidine blue, 380X, scale = $1\mu\text{m}$).

(b) Fine structure of the material filling the crypts showing the bacterial and other cellular necrotic debris that make up this material (8,650X, scale = $1\mu\text{m}$).

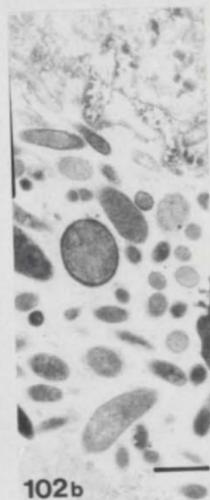
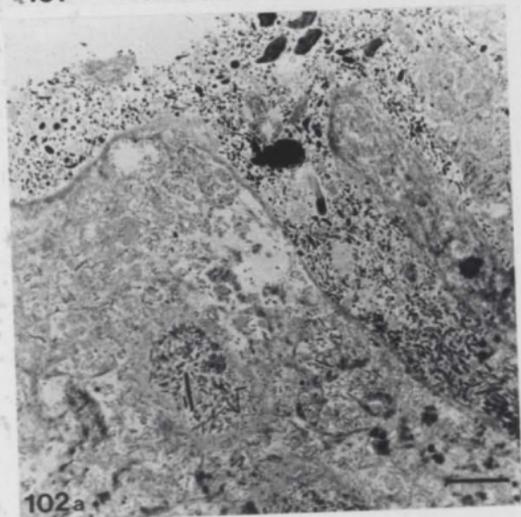
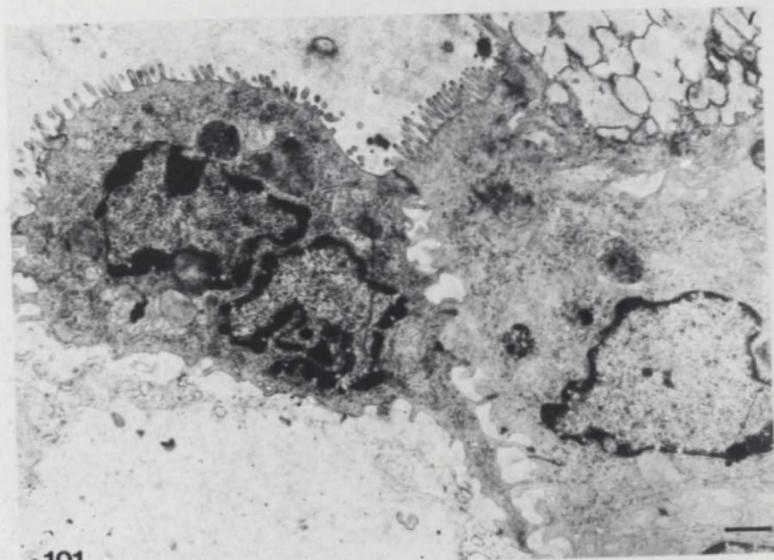
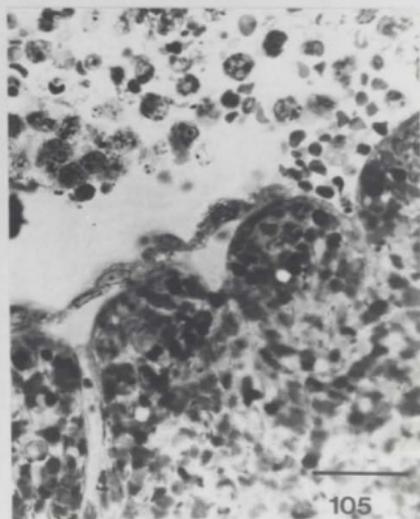
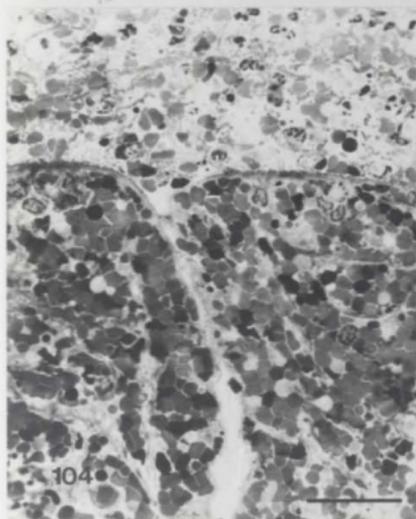
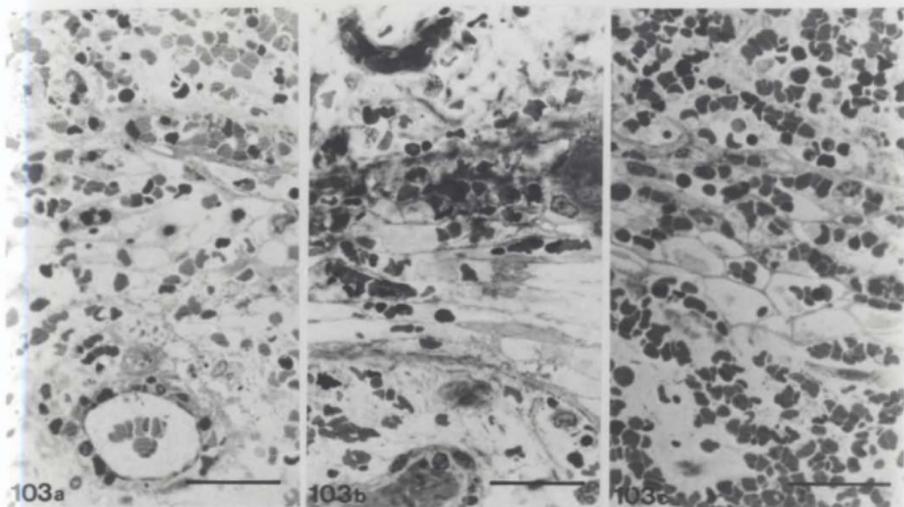


Figure 103: (rat, 10% acetic acid, intraluminal, 2 days) This series of photomicrographs (a,b &c) illustrate the initiation of polymorph emigration from the submucosal vessels, their migration into and through the muscularis mucosae, and their eventual packing of the lamina propria and lodging in the connective tissue network which now occupies what used to be the muscularis mucosae (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 104: (rat, 10% acetic acid, intraluminal, 2 days) The lamina propria, limited only by a layer of connective tissue and packed with erythrocytes and polymorphs. Exuded cellular components can be seen in the colonic lumen (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 105: (rat, 10% acetic acid, intraluminal, 2 days) Epithelial migration out over this fragile mucosa from adjacent inflamed mucosal regions. This squamous-like tissue sheet attempts to completely cover the degenerated area (plastic, toluidine blue, 630X, scale = 25 μ m).



reticular basement membrane of the epithelium and a thin connective tissue sheath enclosing the lamina propria (Figure 104, 111). An epithelial cell layer began to grow out over the edges of these regions affecting a repair of the defect and formed a squamous-like layer. However, the cellular elements packing the friable mucosa ruptured the limiting material and passed out into the colonic lumen (Figure 105). The submucosa contained increasing amounts of connective tissue as well as an increasing number of polymorphs, both of which begin to infiltrate the superficial aspects of the muscularis externa (Figure 106).

From this condition it was only a small step to total ulceration and free movement of these blood vascular elements into the colonic lumen. The erythrocyte population decreased while the major cell type predominating in the region was that of polymorphonuclear leucocytes. These cells passed into the mucosal region from the submucosa in great numbers and formed a dense layer on the surface of the submucosa. On the superficial aspect of this cellular layer another thinner layer of necrotic debris accumulated, infiltrated heavily with colonic bacteria (Figures 107, 110, 112). Scanning electron microscopy of this eroded surface revealed a highly cellular appearance (Figure 108). The muscle fibers of the muscularis externa became widely separated and the space between them filled with connective tissue fibers and matrix, erythrocytes and polymorphs (Figure 109).

As the lesions grew older, seven days post-acid onward, granulation tissue organization began in the submucosa and extended gradually up into the polymorph-infiltrated mucosal region. Along with this a continuing

Figure 106: (rat, 10% acetic acid, intraluminal, 3 days) Open lesion formation with polymorphs and other degenerated mucosal debris being sloughed into the lumen. Active polymorph emigration can still be observed from the submucosal artery (plastic, toluidine blue, 380X, scale = 25 μ m).

Figure 107: (rat, 10% acetic acid, intraluminal, 3 days) Higher magnification of the luminal aspect of this cellular accumulation in the mucosa. The polymorph-necrotic debris layer is capped by a sheet of colonic bacteria (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 108: (rat, 10% acetic acid, intraluminal, 3 days) A scanning electron micrograph of the colonic surface at the third day post-acid showing the highly cellular nature of the exudate exposed to the colonic lumen (475X, scale = 25 μ m).

Figure 109: (rat, 10% acetic acid, intraluminal, 3 days) The muscle fibers of the muscularis externa exhibiting edematous separation by the third day. The spaces formed are quickly filled with emigrating polymorphs and fibroblasts which continue to lay down more collagenous material between the fibers (plastic, toluidine blue, 630X, scale = 25 μ m).

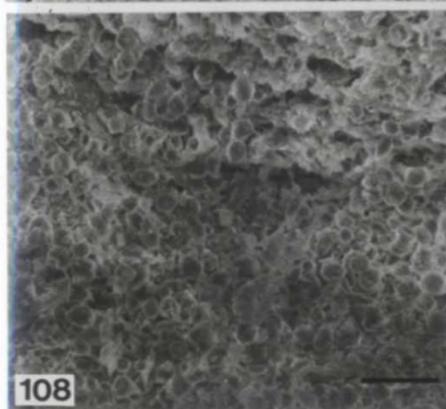
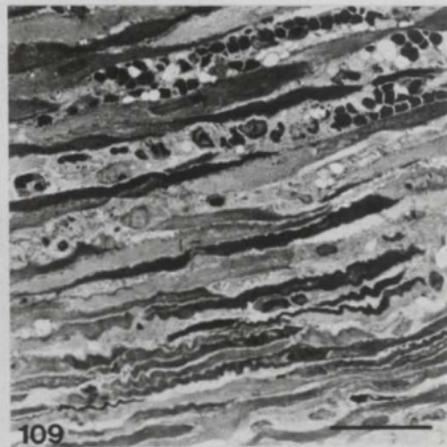
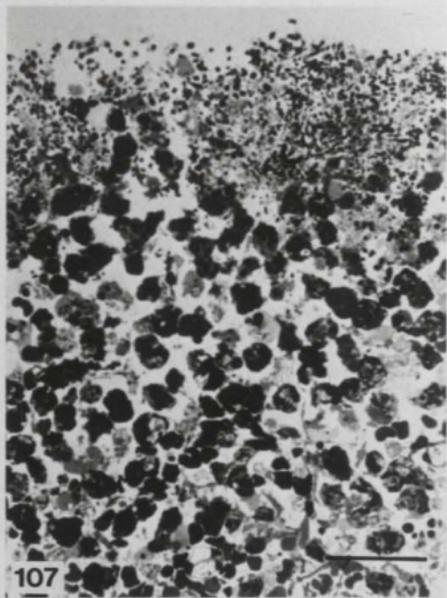
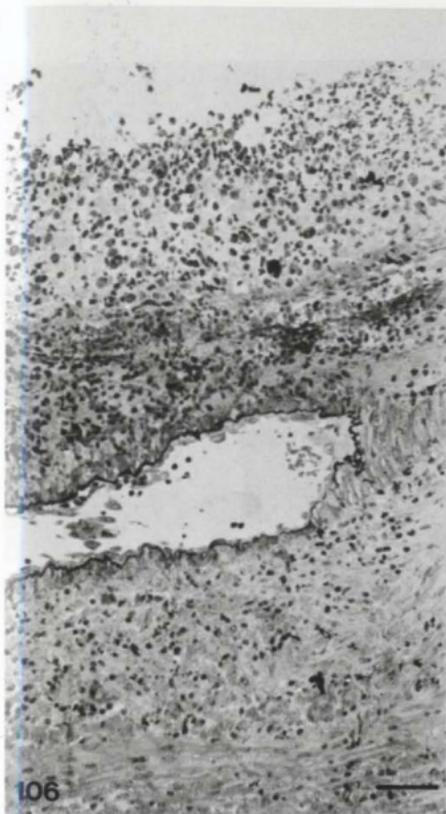
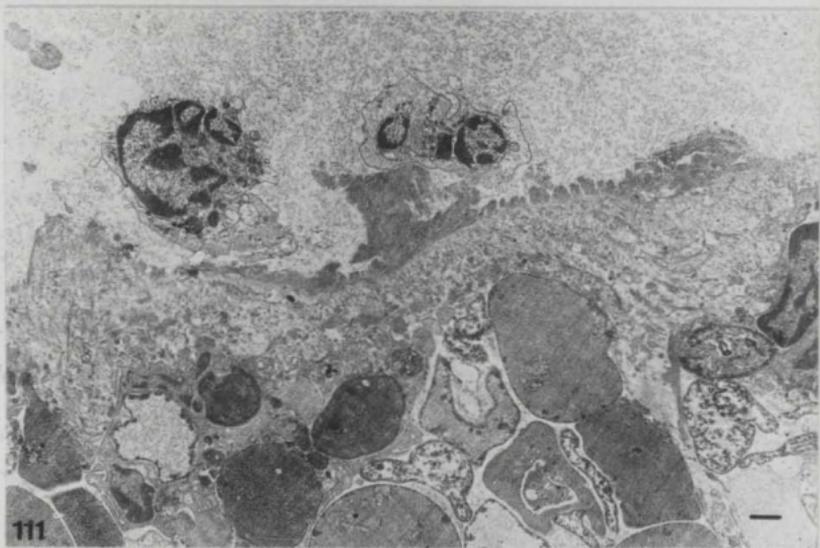
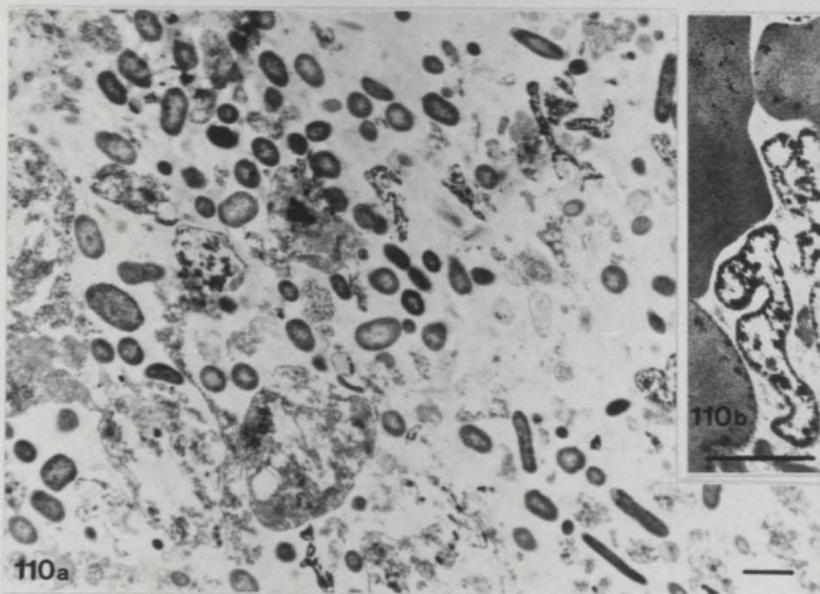


Figure 110; (rat, 10% acetic acid, intraluminal, 3 days) Fine structure of the necrotic layer which overlies the open lesions in the rat colon. There is a large number of bacteria present and deeper in the layer, almost adjacent to the base of the lesion, erythrocytes (b) and membrane-bound sacs can also be seen in this debris as they are exuded from the bowel wall (15,000X, scale = 1 μ m).

Figure 111: (rat, 10% acetic acid, intraluminal, 3 days) The fragile limiting membrane of the rat colonic mucosa 3 days after administration of 10% acetic acid. The lamina propria is packed with polymorphs, other inflammatory cells, erythrocytes and other necrotic debris (4,175X, scale = 1 μ m).



migration of epithelial cells spread out over the granulation tissue-inflammatory cell mass from the adjacent mucosa (Figure 113). This generalized trend of lesion development is well illustrated in the group of photographs portraying the main stages of this process.

Healing of the Lesion:

Healing of the acetic acid lesion followed two main patterns: 1) a return to normal architecture as seen in the majority of animals treated intraluminally with 10% acetic acid, and 2) the formation of a fibrous scar tissue, most common after higher intraluminal concentrations of the agent and in serosal applications. The experimental colonic lesions in the rat have not been observed to become re-activated within the sixty day period over which they were studied.

Excised colon from rats treated intraluminally with 10% acetic acid observed in the latter stages of the experiment, twenty-one and sixty days, usually appeared to have regained normal mucosal morphology upon gross observation. However, upon histologic examination, focal areas of grossly distorted mucosal histology were often encountered. The anomalies ranged from dilated, branched and distorted crypt configuration, to cryptic downgrowth through the muscularis mucosae into the submucosa to mucosal cryptitis forming large sealed pockets, usually containing varying amounts of mucus (Figures 114, 115). These areas generally represented regions which had subsequently regenerated from less severe mucosal ulceration.

The colons of other animals, in particular those treated with

Figure 112: (rat, 10% acetic acid, intraluminal, 21 days) Note that although the submucosa has undergone organization, there is still a fair degree of inflammatory exudate sitting on the luminal surface (plastic, toluidine blue, 380X, scale = 25 μ m).

Figure 113: (rat, 10% acetic acid, intraluminal, 21 days) Growth of the surface epithelium of the rat mucosa out over a region of organizing granulation tissue (plastic, toluidine blue, 630X, scale = 25 μ m).

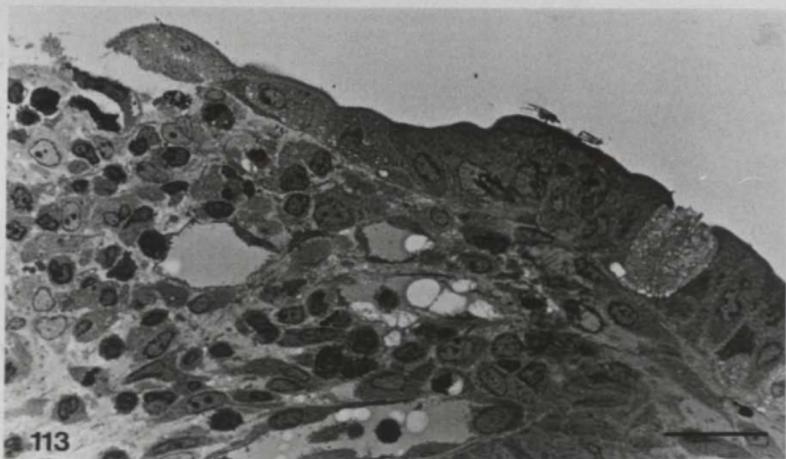
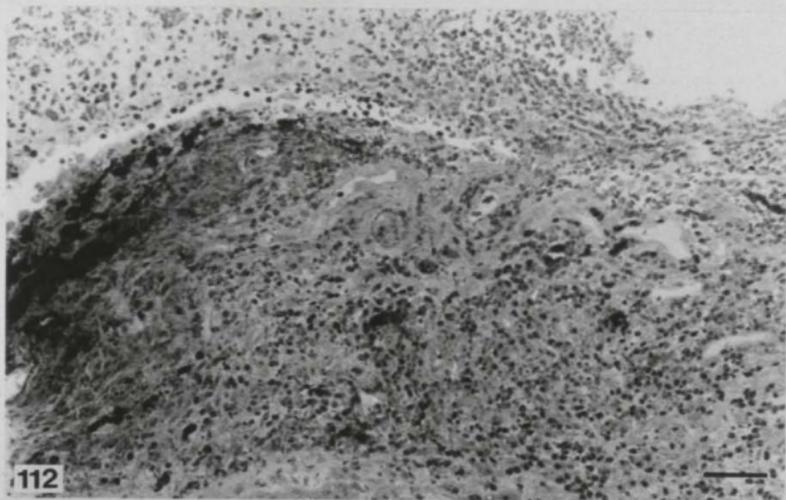
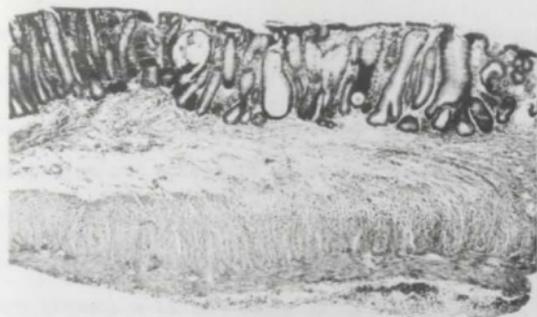


Figure 114: (rat, 10% acetic acid, intraluminal, 21 days) Distorted crypt configuration in the mucosa, cryptic dilation, and organization of the submucosa and muscularis externa in areas which had exhibited more severe degeneration (paraffin, H&E, 32X, scale = 50 μ m).

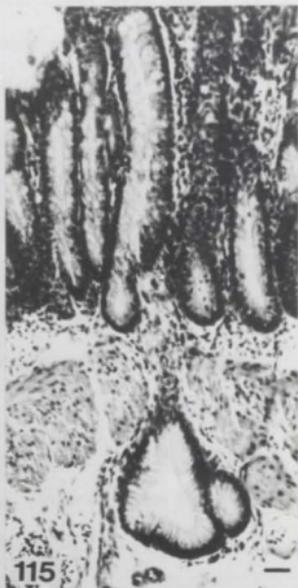
Figure 115: (rat, 10% acetic acid, intraluminal, 60 days) Down-growth of a crypt through the muscularis mucosae into the submucosa of the rat colon (paraffin, H&E, 160X, scale = 25 μ m).

Figure 116: (rat, 10% acetic acid, intraluminal, 21 days) Luminal aspect of the rat colonic wall showing organization of the granulation tissue and the exudation of the polymorph infiltration into the lumen (paraffin, H&E, 160X, scale = 25 μ m).

(b) The region of the muscularis externa from the same tissue section which has become occupied by granulation tissue following muscle fiber degeneration. Capillary growth in a perpendicular fashion to the luminal surface can be seen near the base of this tissue mass (paraffin, H&E, 160X, scale = 25 μ m).



114



115



116a



116b

stronger concentrations of acid, exhibited scar tissue upon gross observation at these later screening intervals. Histologic examination revealed that these areas, which had been focal erosions at one time, were now occupied by a granulation tissue mass in various stages of organization. These areas of scarring were highly cellular and exhibited bands of collagenous fibers extending in a parallel orientation to the colonic lumen. The surface of this granulation tissue plug was usually devoid of epithelium and consisted only of a loose array of this tissue network capped by a band of polymorphs, bacteria and necrotic debris (Figure 116a). Extending upward from the muscularis externa through this mass of granulation tissue, a large number of developing blood vessels forged through the cicatrization (Figure 116b). On the periphery of these scars the mucosa was beginning to infiltrate the granulation tissue. Epithelium grew out over the edges, separating inflammatory cell exudate from the granulation tissue mass, and the crypts extended downward. Cryptic regeneration on the periphery of these scars occurred and often appeared abnormal, exhibiting branching, dilation and a tortuous configuration (Figures 117, 118).

Rats treated intraluminally with 25% acetic acid often still exhibited large areas of scarification at sixty days post-acid. These extensive scars had not yet begun the reparatory process, while other areas showed evidence of having been involved in this process.

Assessment of Lesion Severity:

Alkaline Phosphatase Activity:

Figure 117: (rat, 10% acetic acid, intraluminal, 21 days)

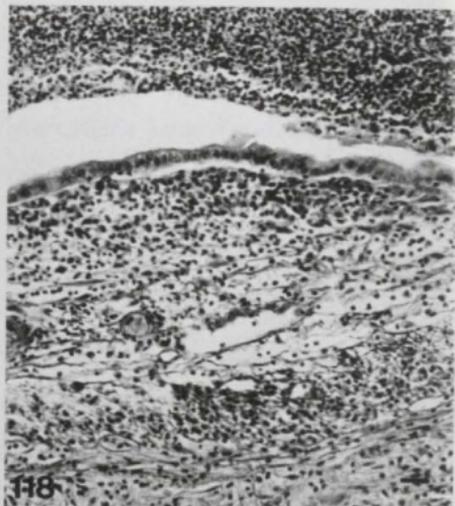
Regenerating mucosa of rat colon, the surface epithelium growing outward and down, beginning to separate the inflammatory cell exudate from the organizing granulation tissue. Adjacent crypt configuration is also altered (paraffin, H&E, 160X, scale = 25 μ m).

Figure 118: (rat, 10% acetic acid, intraluminal, 21 days)

Cuboidal-type epithelium covering the granulation tissue of a focal mucosal regeneration, again effectively separating the inflammatory cellular exudate from the underlying tissue (paraffin, H&E, 160X, scale = 25 μ m).

Figure 119: (rat, normal colon) The alkaline phosphatase activity of normal rat colon (paraffin, cobalt sulfide, 100X, scale = 25 μ m).

Figure 120: (rat, 10% acetic acid, intraluminal, 18 hours) Initial alkaline phosphatase activity localized around the submucosal channels. The blackening in this area hallmarks active polymorphonuclear emigration into the surrounding tissue (paraffin, cobalt sulfide, 160X, scale = 25 μ m).



Monitoring of alkaline phosphatase content of the polymorphonuclear leucocytes, by histochemical assay, was useful in evaluating the relative activity and degree of the inflammatory response in the above experiments. The alkaline phosphatase activity of the normal, control colon, is illustrated in Figure 119. Monitoring for this enzyme activity in the early stages of lesion development, less than twenty-four hours, yielded little information as these stages were mainly characterized by edematous swelling accompanied by plasma cells and lymphocyte emigration.

By twenty-four hours post-acid, enzyme activity could be localized around the muscularis mucosae and superficial aspects of the submucosa, especially surrounding the blood vessels in this level. This dense localization around these vessels indicated the start of the polymorph emigration onto the edematous surroundings (Figure 120). These migrating cells radiated away from the vessels out into the submucosa and up through the muscularis mucosae into the mucosa (Figures 121 a, b, c).

Areas of focal degeneration of the mucosa three days following acid administration became progressively blackened by histochemical staining, around the muscularis mucosae, with the reaction spreading into both the mucosa and superficial aspects of the submucosa. When open lesions developed, the superficial layer of the bowel wall appeared as a solid black mass (Figure 121d). Subsequent formation and invasion of granulation tissue in the form of scarification, restricted the localization of the alkaline phosphatase activity to the very superficial aspect of the bowel wall, the remainder having been exuded. There was little, if any, alkaline phosphatase activity present in the mass of

Figure 121: A series of photomicrographs showing alkaline phosphatase localization in the rat colon exposed to 10% acetic acid intraluminally. All sections have been paraffin embedded and stained with cobalt sulfide.

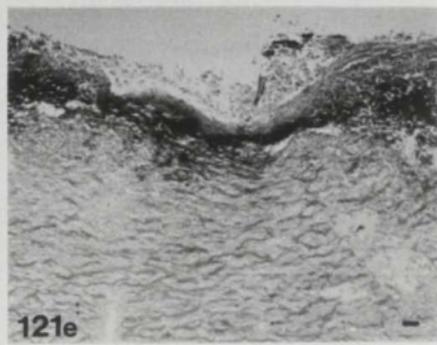
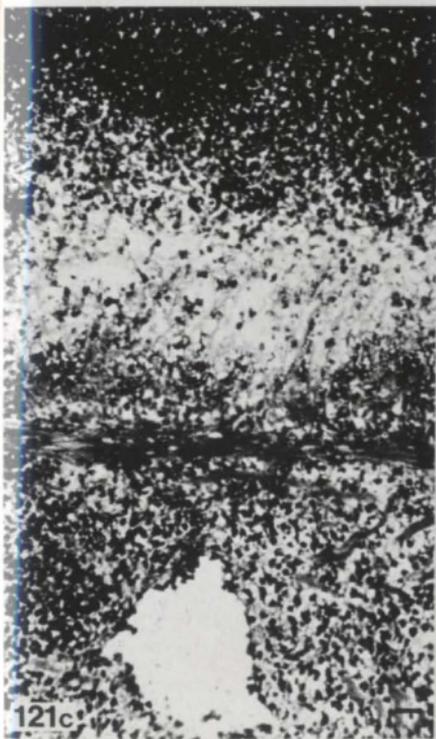
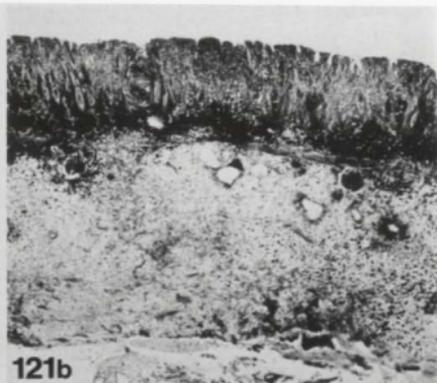
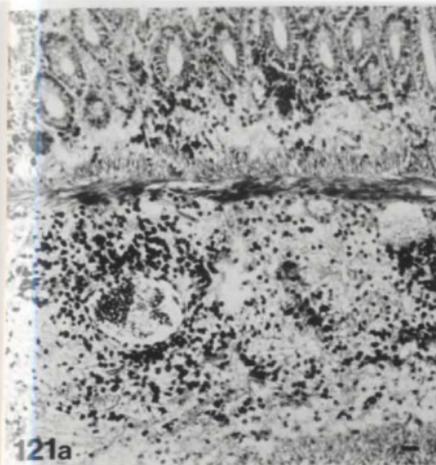
(a) 18 hours following acid administration. Emigration of polymorphs from submucosal vessels into connective tissue, up through the muscularis mucosae and into the lamina propria of the mucosa (100X, scale = 25 μ m).

(b) Further migration into the mucosa but exhibiting a noticeable localization around the muscularis mucosae and lower regions of the mucosal lamina propria by 1 day following acid administration (32X, scale = 50 μ m).

(c) Higher magnification of (b) showing polymorph infiltration beginning to move upward (160X, scale = 25 μ m).

(d) Localization of the alkaline phosphatase activity on the peripheral aspect of the open lesion by the third day following lesion induction. There is still some activity in the lower regions, especially around the muscularis mucosae which is now reduced to a connective tissue network (100X, scale = 25 μ m).

(e) 21 days following acid administration exhibiting a mass of granulation tissue with the alkaline phosphatase activity restricted to the very superficial aspect of this mass, the rest having been extruded into the lumen as it was sloughed off. Note absence of activity in the granulation or scar tissue (32X, scale = 50 μ m).



connective tissue which formed the scar (Figure 121e).

Areas exhibiting longstanding inflammation of fourteen days, demonstrated an intense alkaline phosphatase reaction within the lamina propria of the inflamed mucosa (Figure 122). Areas adjacent to ulceration also demonstrated this localization in those regions of the mucosa immediately adjacent to the ulceration.

Mucus Content of the Mucosa:

Timed interval sampling allowed close observation of the behaviour of mucus production in the mucosal goblet cells. Figure 123a illustrates the routine PAS staining pattern of normal, control, rat colon. The staining was uniformly dense in the surface epithelium and superficial regions of the crypts, but the staining intensity faded in the deeper crypt regions.

Figures 123b to 123g shows mucin distribution during development of these lesions. There was an initial release of the accumulated stores of mucus in the surface and crypt epithelial goblet cells in the form of a cloud-like swirl of PAS-positive material in the lumen and crypts. Although the superficial epithelium degenerated, the goblet cells deeper in the crypt epithelium continued to display mucin. As the cellular degeneration progressed down into the crypt, the PAS staining activity was lost until only mucocoele-like deposits of mucus were evident in the degenerated mucosa. Open lesions manifested no mucus unless there were small mucocoeles trapped within the inflammatory exudate and debris, or if there was some laying on the surface of the exudate, released from

Figure 122: (rat, 10% acetic acid, intraluminal, 14 days)

Alkaline phosphatase activity indicating an inflammatory response within the lamina propria 14 days post-acid in a region of the mucosa exhibiting elongated crypts. Additional activity is localized around the surface of the muscularis externa which has been affected in this area (paraffin, cobalt sulfide, 100X, scale = 25 μ m).

Figure 123: Monitoring of the mucus production within the goblet cells of the rat colonic mucosa before and after administration of 10% acetic acid intraluminally.

(a) The normal PAS staining pattern of the rat colonic mucosa (paraffin, PAS, 160X, scale = 25 μ m).

(b) Response of the mucosa immediately following the intraluminal administration of 10% acetic acid. There is a release of most of the stored mucus in a cloud-like fashion (paraffin, PAS counterstained with hematoxylin, 160X, scale = 25 μ m).

(c) Five minutes following administration of the agent. Surface epithelium has been sloughed as well as that of the superficial region of the crypts. PAS activity is restricted to cells further down the crypts where the acidic solution has not affected the cells to such a severe degree. There is a layer of exuded mucus covering a portion of the luminal surface (paraffin, PAS, 160X, scale = 25 μ m).

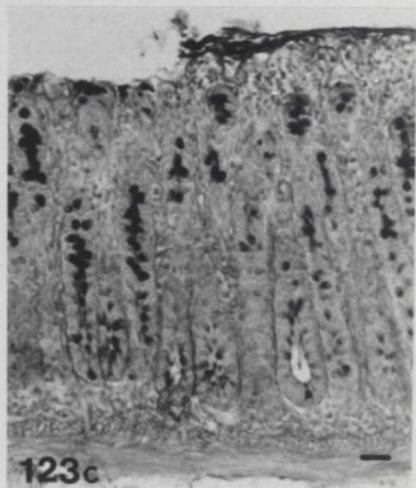
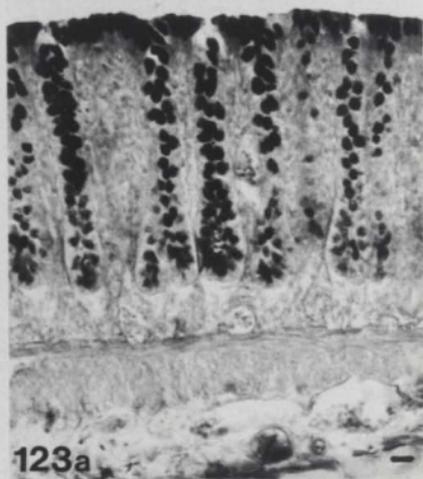
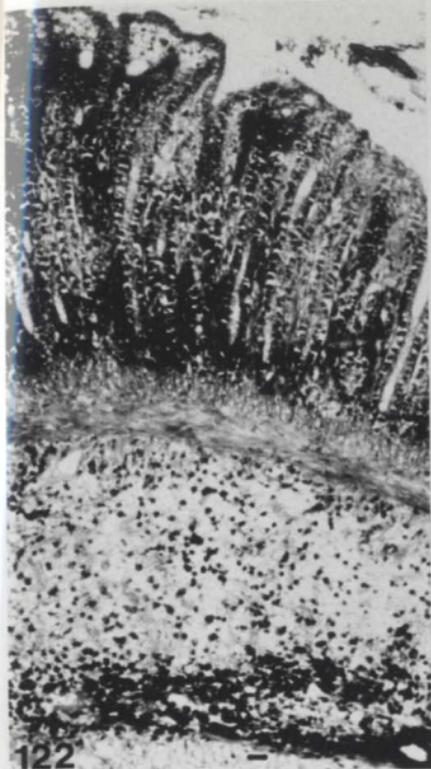
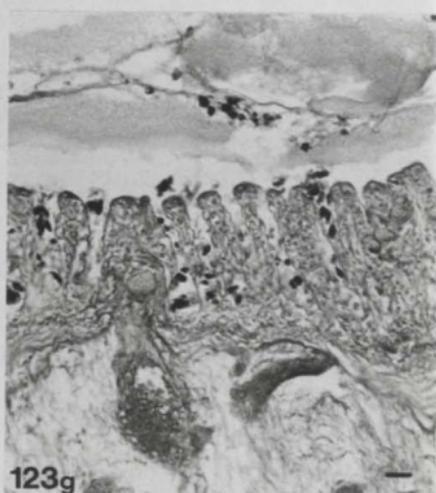
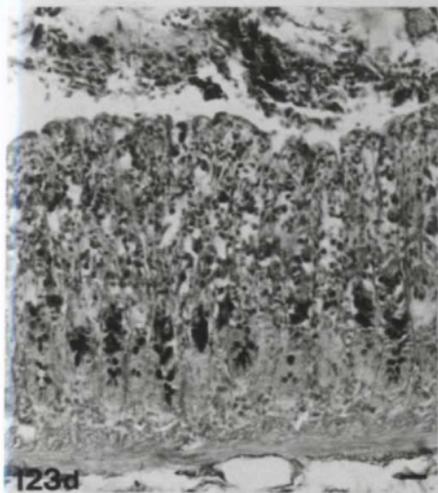


Figure 123: (d) Further degeneration of the crypt epithelium, moving down the crypt 18 hours following administration of 10% acetic acid intraluminally. Mucus production is now limited to the very basal aspect of the crypts (paraffin, PAS, 160X, scale = 25 μ m).

(e) Complete crypt degeneration and mucus staining here has been released into the crypt as a mucocoele-like structure by 24 hours following administration of the acid (paraffin, PAS, 160X, scale = 25 μ m).

(f) The surface of the mucosa by 24 hours following 10% acetic acid intraluminally has now eroded and mucus production remains practically nil (paraffin, PAS, 160X, scale = 25 μ m).

(g) Mucosa now composed only of pillars of lamina propria with the occasional goblet cell sticking to the base of the crypt or has been extruded and is lying in the crypt. (paraffin, PAS, 160X, scale = 25 μ m).



goblet cells in the adjacent epithelium.

Mucosa immediately adjacent to and bordering the lesion, as well as inflamed residual pseudopolyp-like remnants of mucosa, exhibited a marked reduction in mucin content (Figures 124, 125). However, the more distant but thickened and edematous mucosa exhibited elongated crypts and demonstrated only a slight reduction in mucin content, often with a thick luminal surface coat (Figure 126). Areas exhibiting regenerating mucosa demonstrated an abnormal pattern of mucus distribution, the PAS staining intensity usually appeared reduced or in some cases absent (Figures 127 to 131).

Crypt Depths:

During histological screening the varying height of the colonic mucosa during the conditions leading to, or associated with, ulceration was recorded. Table 10 (page 129), displays the average dimensions of various mucosal and submucosal structural features during stages of degeneration and ulceration. The features measured included: crypt depth from luminal surface to the base of the cells in the bottom of the crypts; the width of the lamina propria from the base of the crypt epithelial cells to the superficial aspect of the muscularis mucosae; the thickness of the muscularis mucosae; the submucosa from the base of the muscularis mucosae to the superficial aspect of the muscularis externa; the thickness of the muscularis externa, and the thickness of the serosa. These values were averaged from at least fifty counts from each histological section from the excised colon for each of ten to twelve rats treated intraluminally

Figure 124: (rat, 10% acetic acid, intraluminal, 3 days) Mucus production indicated by PAS staining in mucosal regions bordering on the periphery of lesions. Note that the mucus production in these regions (a and b) is much reduced, in some cases (b) limited to exuded quantities lying on the luminal surface (paraffin, PAS, 100X, scale = 25 μ m).

Figure 125: (rat, 10% acetic acid, intraluminal, 5 days) PAS staining activity in a pseudopolyp-like structure in the midst of a colonic lesion in the rat treated 5 days earlier with 10% acetic acid intraluminally. Again, PAS activity is reduced in this inflamed region (paraffin, PAS, 100X, scale = 25 μ m).

Figure 126a and 126b: (rat, 10% acetic acid, intraluminal, 5 days) The PAS activity in two regions of rat colonic mucosa treated intraluminally with 10% acetic acid and exhibiting mucosal inflammation and edema of various degrees (paraffin, PAS, 100X, scale = 25 μ m),

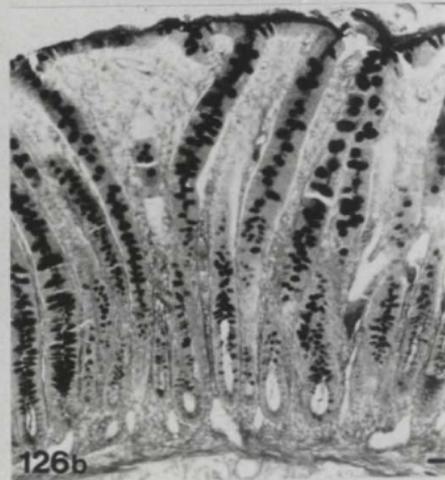
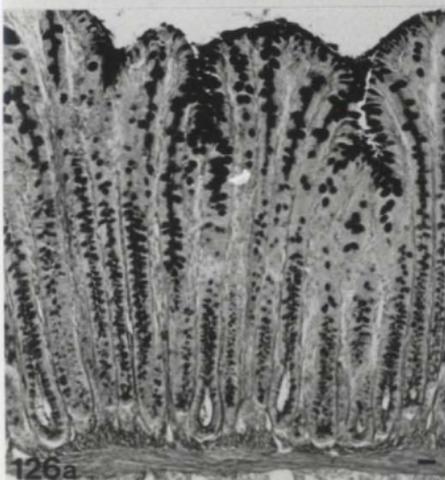
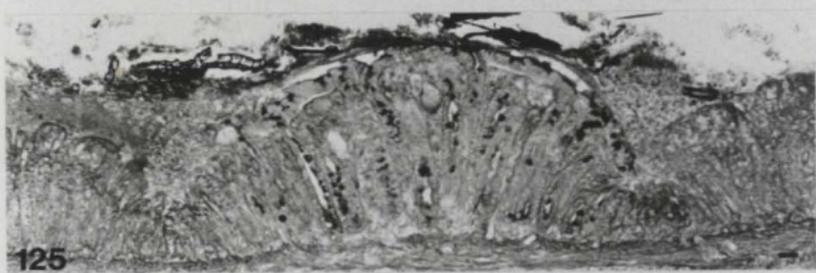
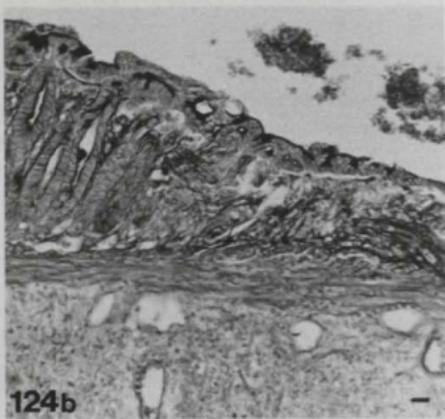


Figure 127: (rat, 10% acetic acid, intraluminal, 21 days) Mucus production in mucosa adjacent to areas of granulation tissue in rat colon treated intraluminally 21 days prior with 10% acetic acid. Crypts in this area exhibit distorted configuration and reduced PAS staining intensity (paraffin, PAS, 100X, scale = 25 μ m).

Figure 128: (rat, 10% acetic acid, intraluminal, 21 days) Mucosal regeneration of a focal lesion showing growth of surface epithelium over the plug of granulation tissue as well as crypt invasion into this material. Mucus production on the periphery is slightly reduced while being almost absent from the new surface epithelium covering this defect (paraffin, PAS, 100X, scale = 25 μ m).

Figure 129: (rat, 10% acetic acid, intraluminal, 21 days) Rat colonic mucosa 21 days following intraluminal administration of 10% acetic acid showing cryptitis and distorted cryptic arrangement. Areas exhibiting this abnormal morphology demonstrate reduced or no mucus production (paraffin, PAS, 100X, scale = 25 μ m).

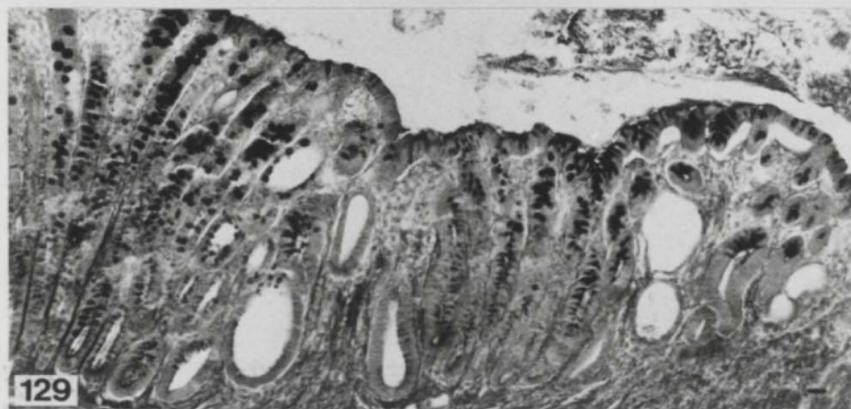
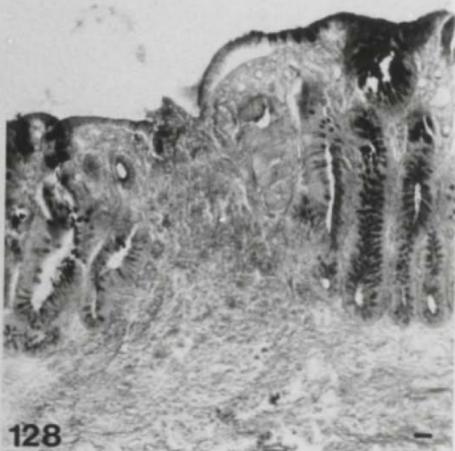
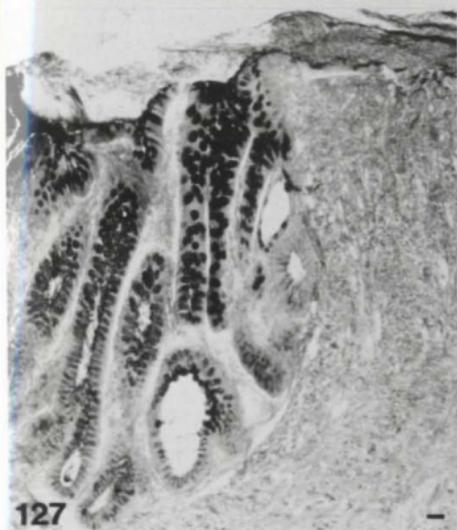


Figure 130: (rat, 10% acetic acid, intraluminal, 60 days) Mucosa of healed rat colon showing cryptic downgrowth into the submucosa and intense mucus production indicated in the ectopic gland (paraffin, PAS, 160X, scale = 25 μ m).

Figure 131: (rat, 10% acetic acid, intraluminal, 60 days) Large vacuole in the healed rat mucosa, the lower portion of this cyst lined with columnar epithelium exhibiting a reduced mucus production compared to that of adjacent cells (paraffin, PAS, scale = 25 μ m).

Figure 132: Graphic illustration of the variation in crypt depth and submucosal thickness in the various stages leading to ulceration of the rat colonic mucosa. Both increase in value during the edematous inflammatory reaction but subsequently decline once degeneration of the mucosa results in ulceration within 3 days.

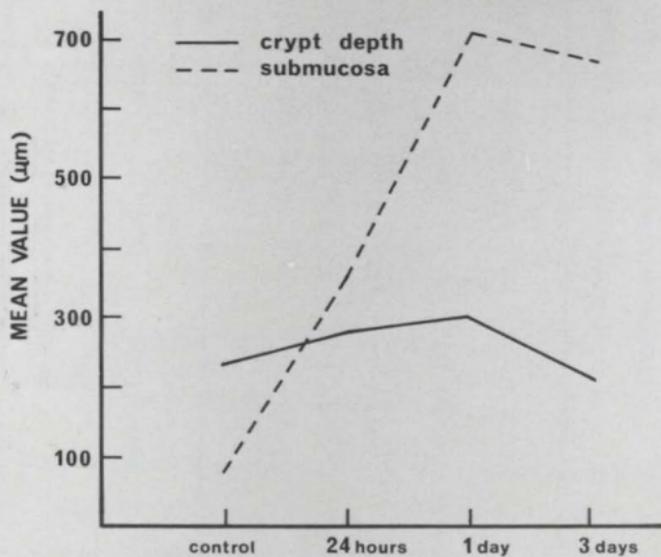


Table 10: Quantitative Measure of Inflammatory Responses in the Colonic Bowel Wall of Rats in Experimental Colitis.*

Condition	Mucosal Crypt Depth	Lamina Propria	Muscularis Mucosae	Submucosa	Muscularis Externa	Serosa
Control	228.6 ± 1.19	36.6 ± 1.28	39.5 ± 1.89	79.6 ± 1.58	206.5 ± 1.33	38.9 ± 1.11
12 Hours	271.8 ± 1.85	20.4 ± 1.29	25.3 ± 1.63	365.8 ± 2.70	203.2 ± 1.55	39.2 ± 1.13
1 Day	293.1 ± 1.67	13.0 ± 1.42	13.1 ± 1.11	695.2 ± 2.81	247.3 ± 1.51	83.3 ± 1.40
3 Days Degenerating	189.6 ± 1.70	13.6 ± 1.63	13.8 ± 1.14	663.6 ± 2.08	-	61.8 ± 1.82
3 Days Adjacent	474.0 ± 1.58	21.8 ± 1.60	24.7 ± 1.10	556.0 ± 2.00	145.5 ± 1.11	48.51 ± 1.11

* Induced by standardized, intraluminal application of 10% acetic acid

Each value indicates Mean ± S.E.M. of 10 to 12 rats.

All measurement are expressed in μm

with 10% acetic acid.

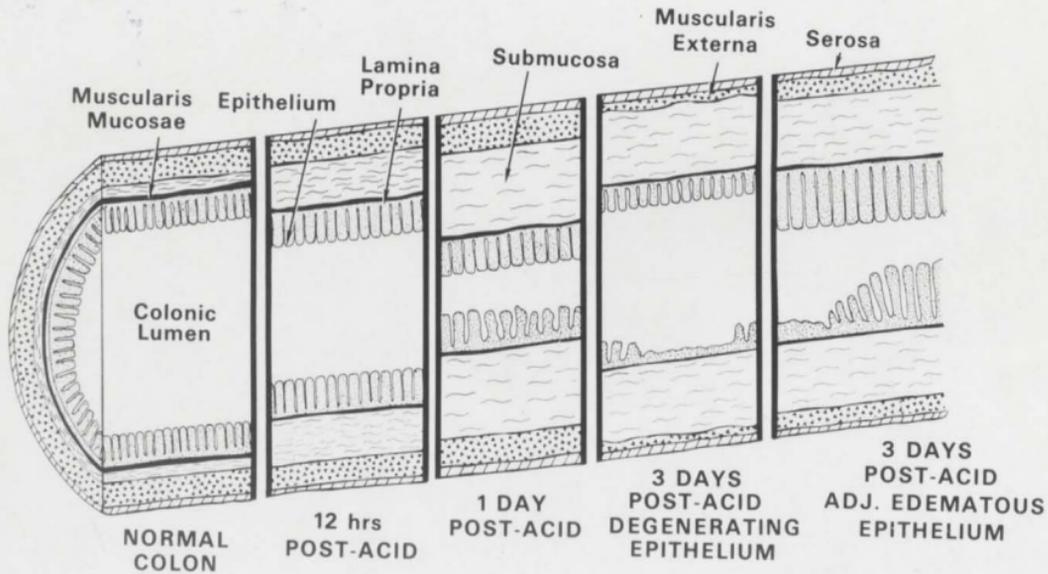
The crypt depth values were of principal interest. While degenerating epithelium had a mean crypt depth of $189.6 \pm 1.70\mu\text{m}$, as opposed to a normal control value of $228.6 \pm 1.19\mu\text{m}$, the inflamed mucosa adjacent to the ulceration exhibited markedly elongated crypts averaging $474 \pm 1.58\mu\text{m}$. Figure 132 is a graphic illustration of the variation in crypt depths over the various stages of lesion development, as well as the values for submucosal thickness at these same screening intervals. The graph illustrates the steady increase in both values up to degeneration of the mucosa and open lesion formation. Figure 43 (page 75), is a photomicrograph showing this variation in mucosal dimensions over the various stages of degeneration and anatomical proximity to the lesions.

A diagrammatic illustration of the mean values for all bowel wall structures measured is drawn to scale and can be examined in Figure 133. The numerical values for this diagram are outlined in Table 10 (page 129). This figure facilitates comparison of the bowel wall thickness, of the particular components, over the various stages of lesion development.

Colonic Motor Activity in Experimental Lesions Induced With Acetic Acid.

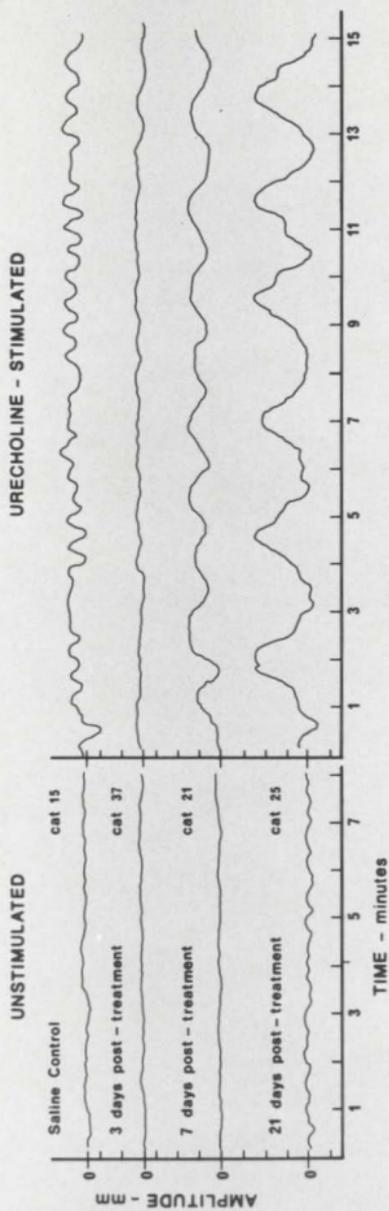
Figure 134 is a reproduction of selected tracings of phasic contractions taken from typical motility recordings of four cats at the various time intervals. All recordings were conducted at the same amplification (10 mV) and chart speed (127 mm/min.), making direct visual comparison possible.

Figure 133: Diagrammatic illustration of the response of the colonic bowel wall to the intraluminal instillation of 10% acetic acid in the rat at various screening intervals leading to ulceration. The dimensions of the various layers at each time interval are drawn to scale.



600 μ m

Figure 134: Unstimulated and urecholine-stimulated colonic motor activity from a cat representative of the time interval sampled. These motility tracings are taken directly from the strip recording. In cases other than saline control, post-treatment refers to the number of days following intraluminal administration of 50% acetic acid.



A decrease in both unstimulated and urecholine-stimulated motor activity was evident on the third day following administration of the acid. By the seventh day post-acid, the spontaneous activity remained less than that of the saline-treated control animals without colitis. The amplitude of the stimulated activity approached that of controls but the number of contractions per unit time were reduced while their duration was increased. Cats recorded twenty-one days following administration of the acid revealed an increased spontaneous motor activity compared to control animals. The stimulated activity was also increased over the control state in both wave amplitude and duration, thus reducing the frequency but increasing propulsive action. Tables 11 to 14 provide the numerical values for the various motility indices recorded for each animal before and after induction of colitis in the unstimulated and urecholine-stimulated conditions.

The cat motor responses are summarized by motility indices shown in the histogram, Figure 135. These averaged figures again demonstrate an initial significant decrease followed by a subsequent increase in colonic motor activity observed following induction of the experimental lesions. Figure 136 illustrates the trend of the mean motility indices in both the unstimulated and urecholine-stimulated conditions. As in the histogram, both lines show a decrease in activity, more pronounced in the stimulated condition. The recovery of unstimulated activity followed a linear progression leading to hyperactivity, whereas that of the stimulated activity had a more rapid initial activity increase followed by a smaller but steady increase during the latter stages of the experiment, leading to activity not significantly different from that observed before the induction of the lesions.

Table 11: Comparative Motility Indices for Each of 7 Cats
 Before and After Treatment With Normal Saline
 (Saline Control Animals) in Unstimulated and
 Urecholine-Stimulated Conditions.

Cat #	Unstimulated		Stimulated	
	Before	After	Before	After
12	1.45	0.78	3.99	4.20
5	0.01	0.24	2.23	2.79
31	0.66	0.32	6.39	5.84
10	0.01	0.35	14.58	12.97
32	0.01	0.01	3.73	3.95
15	0.01	0.03	7.96	7.67
11	0.01	1.03	11.11	12.46
Mean Value	0.31	0.39	7.14	7.13
S.E.M.	0.21	0.14	1.68	1.56

Table 12: Comparative Motility Indices for Each of 10 Cats Before and 3 Days After Intraluminal Administration of 50% Acetic Acid in Unstimulated and Urecholine-Stimulated Conditions.

Cat #	Unstimulated		Stimulated	
	Before	After	Before	After
16	0.81	0.01	7.77	0.01
14	0.07	0.01	2.70	0.71
30	0.05	0.01	5.18	(11.09)*
37	0.01	0.01	4.29	1.46
36	0.30	0.01	11.12	2.34
19	0.16	0.08	2.82	0.52
1	0.47	0.01	3.05	1.03
2	0.01	0.01	5.60	0.01
3	0.10	0.01	3.28	2.18
18	0.01	0.01	3.86	0.01
Mean Value	0.20	0.02	4.97	0.92
S.E.M.	0.08	0.01	0.84	0.30

* This figure omitted from calculation of mean stimulated motor activity 3 days post-acid due to its evident incompatibility with an overwhelming majority of other.

Table 13: Comparative Motility Indices for Each of 8 Cats
 Before and 7 Days After Intraluminal Administration
 of 50% Acetic Acid in Unstimulated and Urecholine-
 Stimulated Conditions.

Cat #	Unstimulated		Stimulated	
	Before	After	Before	After
17	0.01	0.77	1.11	7.98
34	1.50	0.01	5.96	11.68
4	0.01	0.01	1.13	4.47
20	1.02	0.44	1.60	2.40
21	0.56	0.01	2.22	5.51
7	0.01	0.55	2.31	6.67
8	0.40	0.01	1.76	4.89
9	0.22	0.38	2.10	5.45
Mean Value	0.47	0.27	2.27	6.13
S.E.M.	0.19	0.11	0.55	0.98

Table 14: Comparative Motility Indices for Each of 7 Cats
 Before and 21 Days After Intraluminal Administration
 of 50% Acetic Acid in Unstimulated and Urecholine-
 Stimulated Conditions.

Cat #	Unstimulated		Stimulated	
	Before	After	Before	After
23	0.01	1.19	1.49	10.29
22	0.01	1.59	1.71	8.14
25	0.40	1.59	3.67	9.09
26	0.11	0.75	2.42	6.42
27	0.10	1.06	1.88	8.88
28	0.20	0.99	1.59	6.73
29	0.24	1.18	2.87	9.15
Mean Value	0.15	1.06	2.23	8.38
S.E.M.	0.05	0.12	0.03	0.53

Figure 135: Comparison of motility indices of unstimulated and urecholine-stimulated cat colon from various time intervals. Motility Index = $[\xi(\text{contraction height} \times \text{duration})/\text{total time}]$. The number of animals in each sample is given over each bar. Significance is indicated by the asterisk over this number ($p > 0.05$). All bars represent the Mean \pm S.E.M.

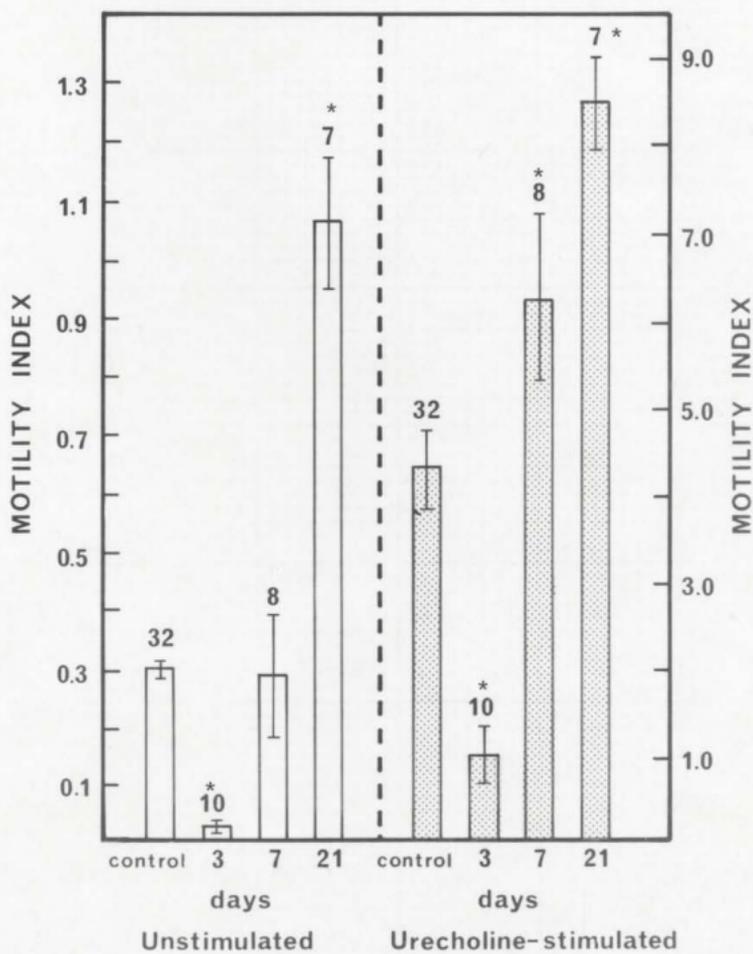
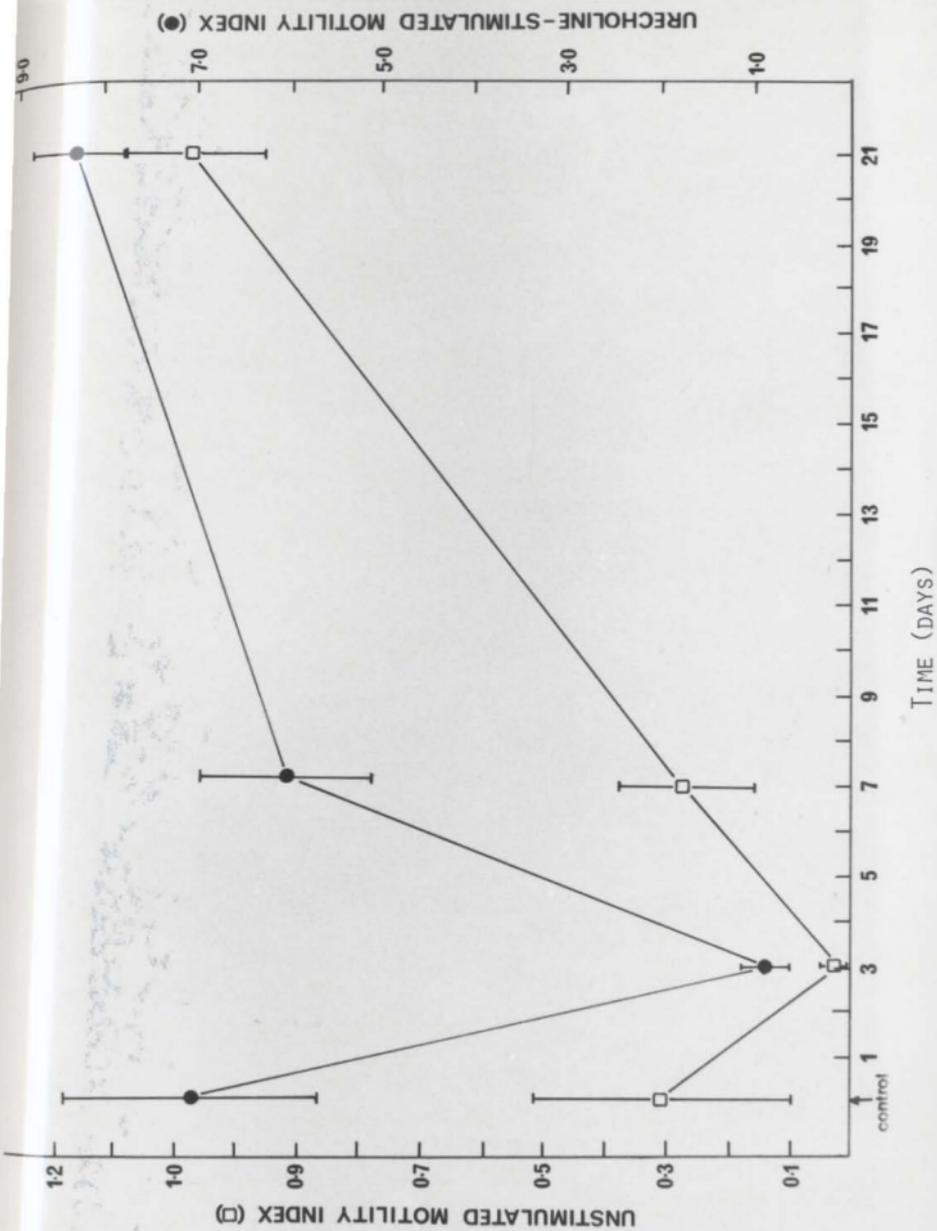


Figure 136: Graphic illustration of the mean motor activity indices of the groups of cats sampled at the various time intervals in both unstimulated and urecholine-stimulated conditions. Each point represents the Mean \pm S.E.M. for a group of 7 to 10 animals.



Preliminary Findings on Effects of Selected Pharmacologic Agents
on Lesion Development and Healing:

The first trial utilized corticosteroids administered daily via rectal enema. Betnesol (betamethasone disodium phosphate) at a dosage of 0.5 mg./day and Meticortelone (sodium hemisuccinate) at 5 mg./day were administered on each of three days prior to acid administration, on the day the acid was administered, and for each of three days after this time, did not result in any significant decrease in ulcer severity. Figure 137 is a composite showing the typical luminal appearance of rat colon treated with 10% acetic acid in association with one of the corticosteroids. Coupled with the numerical indices for each group of rats shown in Table 15 (page 142), the values plotted on the histogram, Figure 139, demonstrate the failure of these agents to modify the colonic reaction to the agent in this limited trial.

The second preliminary drug test in rats involved per os administration of Salazopyrin (salicylazosulfapyridine) at a dosage of 250 mg./day alone, or coupled with a Betnesol enema (0.5 mg./day). Daily administration of these therapeutic agents for seven days prior to intraluminal instillation of 10% acetic acid, on the day of lesion induction, and for seven days post-acid revealed that Salazopyrin alone resulted in a decreased ulcer index value as compared to that of the control animals not receiving any drug. The significant decrease exhibited in this group was not reflected in animals receiving Salazopyrin plus the Betnesol enema. This latter group did not exhibit a significant decrease in lesion severity. Figure 138 shows the luminal appearance of colons treated with these agents

Figure 137: The effect of corticosteroid compounds on the development of the acetic acid lesions after a 3 day pre- and post-treatment schedule with these agents in rat colon.

- (a) Saline control colon, received saline for the 6 days and 10% acetic acid intraluminally.
- (b) Colon treated with 0.5 mg./day Betnesol (Pred-(betamethasone disodium phosphate) via rectal enema and also received 10% acetic acid.
- (c) Colon treated with 5 mg./day Meticortelone (Prednisolone as sodium hemisuccinate) via rectal enema and also received 10% acetic acid.

Figure 138: The effect of a sulfanilamide compound on the development and healing of colonic lesions induced with 10% acetic acid intraluminally following 7 days of pre-treatment with the drug, per os, and followed by 7 days of post-treatment.

- (a) Saline control animal.
- (b) Colon from animals receiving 250 mg. of Salazopyrin (Salicylazosulfapyridine) daily.
- (c) Colon from animals receiving 250 mg. of Salazopyrin (Salicylazosulfapyridine) daily coupled with a 0.5 mg. daily Betnesol (betamethasone disodium phosphate) rectal enema.

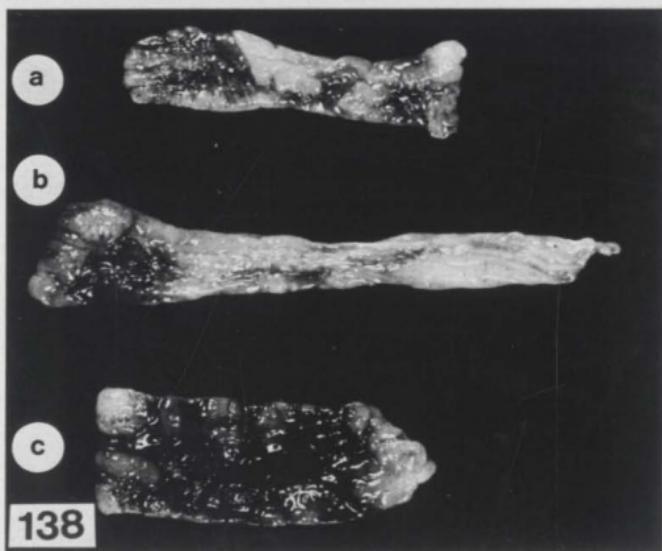
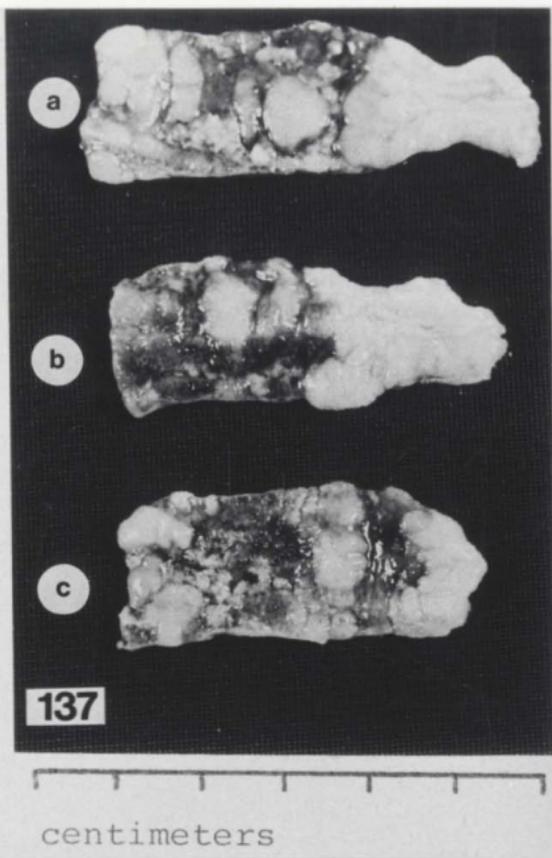


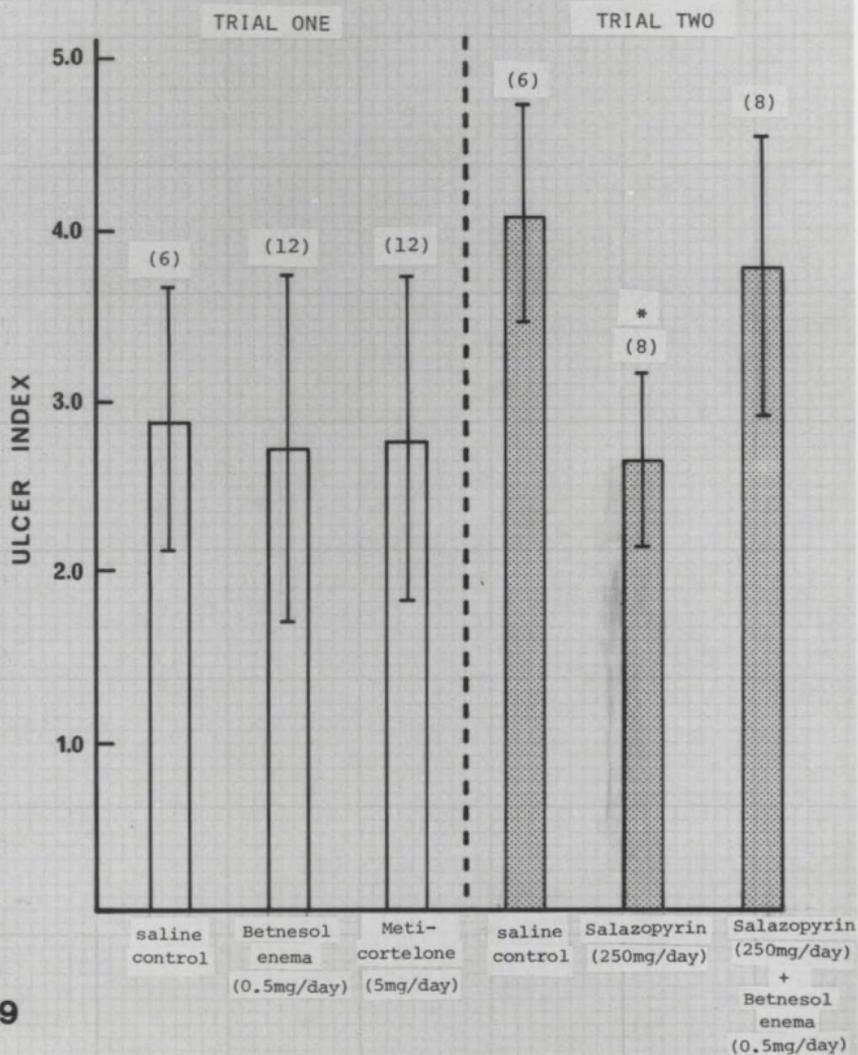
Table 15: Mean Mucosal Ulcer Index Values (Gross Observations) for Rat Colon Treated With Therapeutic Agents 3 Days Before and After Intraluminal Administration of 10% Acetic Acid. Each Value Represents the Mean \pm S.E.M. of 6 to 12 Rats.

Saline Control	2.80 \pm 0.76
Betnesol (0.5 mg/day) ¹	2.67 \pm 0.98
Meticortelone (5 mg/day) ²	2.74 \pm 0.94

¹ Betamethasone disodium phosphate, a corticosteroid by Glaxo-Allenburys Ltd.

² Prednisolone as sodium hemisuccinate by Schering Corp. Ltd.

Figure 139: Graphic illustration of the colonic response to various therapeutic agents following pre- and post-treatment during induction of the lesion. Trial One had a 3 day pre- and post-treatment administration of the agents while Trial Two, was extended to 7 days either side of administration of 10% acetic acid. The number of animals in each sample is given above the bar in parentheses, the S.E.M. is indicated by the vertical bar, and the asterisk (*) indicates a significant value by the Student's t-test ($p > 0.05$).



compared with that of the control animal. The numerical values for the mean ulcer indices of these three groups can be examined in Table 16 (page 145) and are plotted in the histogram, Figure 139.

Comparative Histopathology of Human Ulcerative Colitis Biopsies:

A limited number of colonic mucosal biopsies were obtained from patients in various stages of clinical ulcerative colitis. Examination of these tissues in paraffin and plastic resin by light and electron microscopy was performed for comparison of this disease with the experimental acetic acid lesion. The normal mucosal morphology of the human colon is shown in Figures 140 and 141, taken from a Turttox prepared slide.

Histopathologic features of biopsies from patients with active hemorrhagic or granulocytic colitis revealed an ulcerated mucosa with the occurrence of pseudopolyps. Unlike those structures observed in the rat lesions, the muscularis mucosae under the human structures was also degenerated (Figure 142). Although crypt dilation was a feature common to both lesions, the crypts of the human pseudopolyps exhibited lesions through the epithelium into the lamina propria (Figure 143). The lamina propria of the pseudopolyps in the human was packed with inflammatory cells, a feature not as markedly demonstrated in corresponding rat structures. Both species however, did exhibit reduction in PAS staining in the goblet cells of these crypts. The epithelium of the human pseudopolyps was a uniform cuboidal type, in contrast to the normal columnar epithelium observed in the rat, where only at the base of these structures did the epithelium become cuboidal as it began migrating out

Table 16: Mean Mucosal Ulcer Index Values (Gross Observations)
 for Rat Colon Treated With Therapeutic Agents 7
 Days Before and After Intraluminal Administration
 of 10% Acetic Acid. Each Value Represents the
 Mean \pm S.E.M. of 6 to 8 Rats.

Saline Control	4.10 \pm 0.75
Salazopyrin (250 mg/day) ¹	2.70 \pm 0.50 ²
Salazopyrin (250 mg/day) + Betnesol (0.5 mg/day) ³	3.65 \pm 0.83

¹ Salicylazosulfapyridine by Pharmacia.

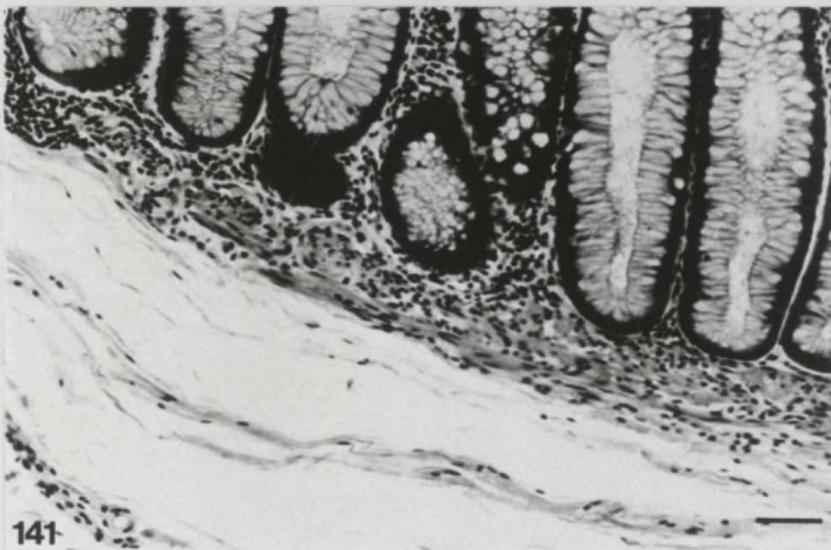
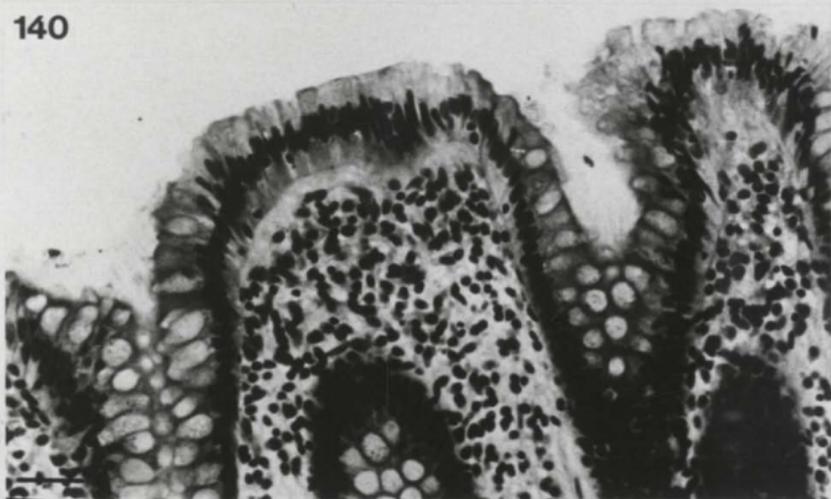
² This value significant by the t-test to .01

³ Betamethasone disodium phosphate by Glaxo-Allenburys Ltd.

Figure 140: (human, normal colon) The histology of the normal human colonic mucosa. The surface epithelium and superficial aspect of the lamina propria are illustrated here (Turtox prepared slide, H&E, 320X, scale = 25 μ m).

Figure 141: (human, normal colon) The histology of the lower regions of the normal human colonic mucosa showing the basal region of the crypts, lamina propria and the muscularis mucosae (Turtox prepared slide, H&E, 380X, scale = 25 μ m).

140



141

Figure 142: (human, active colitis) Pseudopolyp in ulcerated mucosa in a mucosal biopsy during active disease. Note the lymphoid follicle in the mucosa, vast infiltration of polymorphs, absence of muscularis mucosae through the entire region, distortion and dilation of crypts in the pseudopolyp (paraffin, H&E, 100X, scale = 25 μ m).

Figure 143: (human, active colitis) Higher magnification of the pseudopolyp showing rupture of the crypt epithelium and emigration of inflammatory cells from the capillaries into the surrounding lamina propria (paraffin, H&E, 380X, scale = 25 μ m).

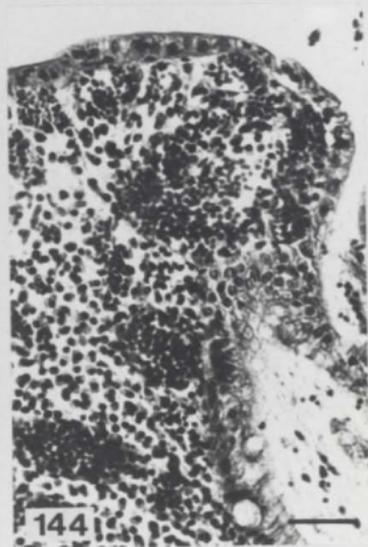
Figure 144: (human, active colitis) Surface region of the pseudopolyp showing the cuboidal nature of the surface epithelium, packed capillaries, and dense infiltration of the lamina propria with inflammatory cells (paraffin, H&E, 380X, scale = 25 μ m).



142



143



144

over the lesion. The mucosal capillaries of the human pseudopolyps were packed with erythrocytes (Figure 144).

The base of the human lesion was filled with a granulocytic, inflammatory infiltration of polymorphs, while adjacent areas less eroded had the surface epithelium stripped off, leaving the lamina propria filled with an inflammatory cellular infiltrate. An inflammatory cell exudate was often seen lying on the surface of this lamina propria exposed to the colon lumen, probably escaping from a lesion through a crypt or directly through the bare lamina propria and into the lumen (Figure 145). This was a feature shared with the early stages of the rat lesion, where the epithelial cells stripped off their basement membrane and left the lamina propria exposed.

Patients in remission from symptoms of colitis demonstrated a mucosa which appeared normal upon sigmoidoscopy but which, upon histological examination, revealed a mucosa heavily infiltrated with adipose tissue. These adipose cells were of various sizes and were accumulated in the lamina propria between the crypts, usually pressed against the basement membrane of the epithelium, and in some cases, notably the surface regions, extended right into the epithelium and between developing cells (Figure 146). In these sections, the basement membrane appeared thickened and stained deeply basophilic with toluidine blue (Figure 146c). One of the most notable ultrastructural similarities shared with the rat colon was the alteration in microvilli on the surface of these cells; in both cases appearing blunt and shortened.

Figure 145: (human, active colitis) Mucosal biopsy from a patient with active colitis. Sloughing of the surface epithelium, increased inflammatory cell prominence in the lamina propria and presence of an inflammatory cell exudate are illustrated (plastic, toluidine blue, 380X, scale = 25 μ m).

Figure 146: (human, colitis in remission) A series of photomicrographs from a patient in remission illustrating the fatty infiltration of all regions of the mucosa.

- (a) Adipose cell infiltration of the lamina propria and between epithelial cells of the surface epithelium (plastic, toluidine blue, 380X, scale = 25 μ m).
- (b) Higher magnification showing fat cells wedged between basement membrane of the crypt epithelium and lamina propria (plastic, toluidine blue, 630X, scale = 25 μ m).
- (c) Adipose cells lodged in the surface epithelium and just under the basement membrane. Note increased basophilia of the basement membrane in patchy areas (plastic, toluidine blue, 630X, scale = 25 μ m).

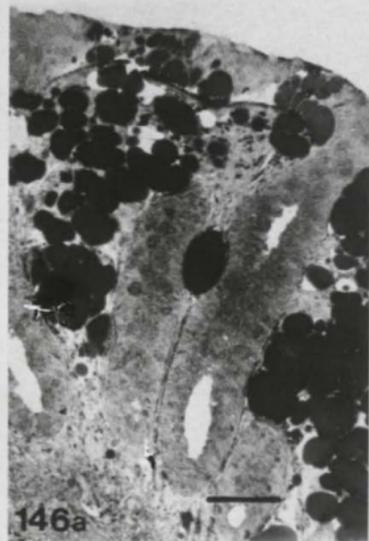
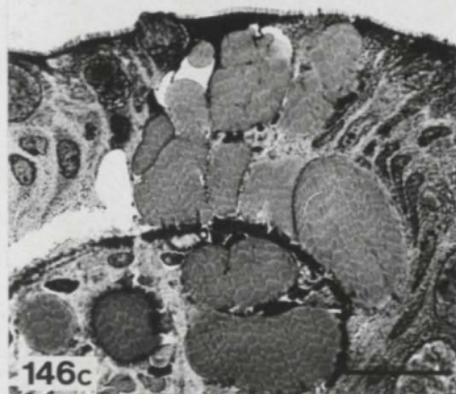
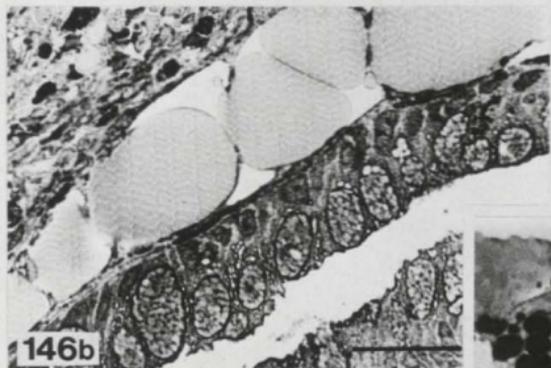
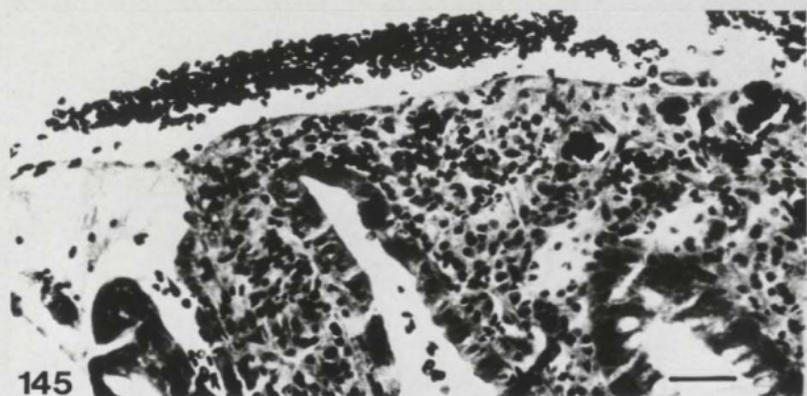
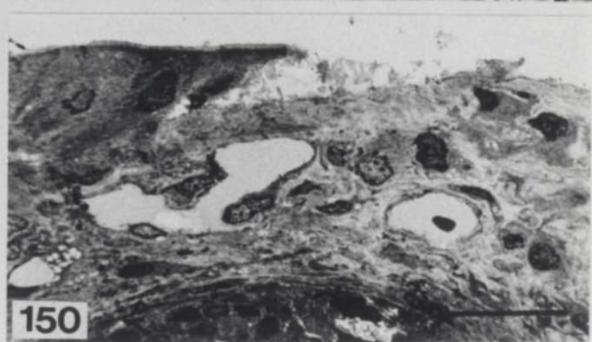
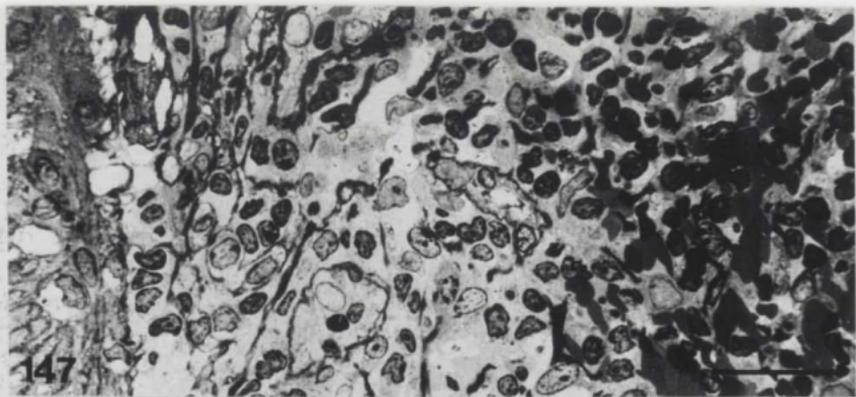


Figure 147: (human, chronic colitis) Submucosal infiltration of collagenous fibers, polymorphs, and erythrocytes in the chronic stages of human disease (plastic, toluidine blue, 630X, scale - 25 μ m).

Figure 148: (human, chronic colitis) Mucosal biopsy from a patient with chronic disease showing abnormal configuration of the mucosa. This one is a large epithelial lined cavity, the microvilli of these cells visible in the lumen. Epithelium rich in goblet cells has started growth over the top of this epithelial invagination (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 149: (human, chronic colitis) Growth of an abnormal surface epithelium over a granulation tissue region in the mucosa (plastic, toluidine blue, 630X, scale = 25 μ m).

Figure 150: (human, chronic colitis) The growing edge of this plate of surface epithelium extending out over the granulation tissue in a patient with chronic ulcerative colitis (plastic, toluidine blue, 630X, scale = 25 μ m).



In other cases, e.g., in patients whose attacks of colitis had been more severe or of a more chronic nature, the biopsies revealed a thick collagenous fiber, polymorph, and erythrocyte cellular infiltration in the submucosa. This extended up to the superficial luminal regions where a surface epithelium was not always present to cover this cellular connective tissue mass. In some areas the epithelium had begun growing out over this irregular stroma and often exhibited altered morphology of the epithelial cells and crypts (Figures 147 to 150). This feature was also shared with the rat lesion except that the new epithelium in these animals exhibited a much more uniform configuration in migration over these area, The crypts however, often appeared distorted in fashions similar to those seen in the human disease.

DISCUSSION

Histopathology of the Lesion:

As illustrated by the introduction, a large array of techniques have been utilized by various investigators in efforts to produce a model for colonic inflammatory bowel disease. Problems common to these models are that lesions are not reproducible in high percentages of animals treated, are not located in those regions of the colon where human inflammatory bowel disease is usually manifest, do not afflict the colonic mucosa in a diffuse manner, do not possess similar histopathologic features, or do not display chronicity or recurrent exacerbations. The present model, which is a modification of the acetic acid method used by Okabe and Pfeiffer

and others (1971, 1972; Okabe et al., 1971a; Takagi et al., 1970) for the production of discrete gastric or duodenal ulcers, offers several unique features. However, it still falls short of constituting an ideal model - a condition which may never exist because animals do not reliably present spontaneously with these colonic ulcerative diseases.

The gastroduodenal acetic acid ulcer model has been proven to induce a chronic erosion of the glandular portion of the rat stomach lasting up to 100 days via topical application of 30% acetic acid (Okabe & Pfeiffer, 1971). Acetic acid was originally chosen by Takagi et al. (1970) as an ulcerogenic agent administered via submucosal injection because of its known ability to induce damage to the gastric mucosa (Davenport, 1966; Johnson & Overholt, 1967). Topical application, however facilitated localization of the defect in any desired anatomical location. Since this time much literature has accumulated on modifications of this model, its use in the study of normal ulcer healing, and the therapeutic effects of many anti-inflammatory or other agents in the treatment of this disease (Takagi & Abe, 1974; Okabe et al., 1971b,c,d; Okabe & Pfeiffer, 1973).

Lesions observed with the present method of acetic acid administration were produced in a regular dose-response fashion with either serosal or intraluminal administration of the optimal concentrations (35% for serosal and 10% for intraluminal) of acetic acid. They were diffuse, could be induced in the distal colon, or more proximally if desired and were reproducible in 100% of treated animals in both the cat and rat species. Serosal application of higher concentrations of the acid (50%) caused the formation of an inflammatory exudate on the serosa

resulting in adhesion of adjacent viscera which further induced formation of pericolic abscesses, perforation and fistulous tract formation. One of the prominent features of the experimental acetic acid-induced gastric ulcer also was adhesion of the ulcer base to adjacent organs, in this case the liver, pancreas or omental fat (Okabe & Pfeiffer, 1972). This phenomenon was theorized to possibly influence the healing process of these defects as the pyloric sphincter was in some cases adhered, thereby possibly causing gastric retention by pyloric obstruction. While the lesions were usually of more severe nature in the colon when this adhesionous tendency was exhibited, chronicity could not be attributed to this phenomenon. Once the colon became involved in this type of process, the wall was usually too badly necrotized to allow healing and perforation resulting in death of the animal usually followed. However, when administered at their respective optimal dosages in the cat and rat, the colonic acetic acid lesions were not associated with any serious side effects. A marked transmural inflammatory response was a feature of both acetic acid techniques used in this study in the rat colon, but it extended only into the submucosa in the cat. With respect to the rat colon, the response appeared earlier and was more marked with the serosal application of the agent. Intraluminal application of the agent generally localized the inflammatory response within the mucosa and submucosa, extending to the deeper bowel regions only in the later stages of the inflammatory process and with increased concentrations. Re-activation of these lesions over the 60 day course of the experiments was not observed. The fact that the gastric acetic acid erosions began initial healing at 40 to 60 days but were still ulcerated by day 100, appears to be a feature observed only in the stomach of the rat but not in the cat (Okabe & Pfeiffer,

1971, 1972). The very nature of the gastric environment, with its prevalent low pH due to hydrochloric acid production may be a factor behind the prolongation of these gastric defects. More prolonged trials and studies with psychic, drug or physical stressing factors might reveal a similar pattern of lesion duration in the colon.

Histopathologically, the acetic acid colitis model did exhibit a number of general features commonly observed in human forms of non-specific colonic inflammation. Initial observations of these lesions were mucosal edema with packing of the blood and lymphatic vessels, the latter with lymphocytes and plasma cells, resulting in swelling of mucosal height. This was followed by extravasation of erythrocytes into the lamina propria and petechial mucosal hemorrhages which subsequently enlarged and suppurated. Submucosal edema was a common feature in both techniques, resulting in a complete early transmural inflammatory response in serosally-treated colon but which occurred later or with increased acid concentration after the intraluminal application of the agent. Mucosal margins around these lesions become infolded but often formed epithelial bridges over the edematous lamina propria which resulted in additional epithelial sloughing, producing lesions of varying diameter. These defects erode past the muscularis mucosae only in the latter stages of the disease process at which stage the submucosa experiences a granulation tissue reaction. The early lymphocytic response in the mucosa turns to a polymorphonuclear leucocyte infiltrate and the lamina propria, muscularis mucosae and the superficial submucosa. Pseudopolyp-like structures were observed mainly in the intraluminal technique. As in chronic stages of inflamed colon, there was formation of fistulous tracts associated with megacolon and

edematous thickening and rigidity were also seen in the latter stages of the rat model. Fibrosis was occasionally evident in the cat colon only after 21 days post-acid and in no more than 25% of the animals treated.

While pathologists present varying opinions on the classification of ulcerative colitis and Crohn's or granulomatous colitis, Morson and Dawson's (1972) extensive treatment of gastrointestinal pathology and Mottet's (1971) text devoted to these two forms of inflammatory bowel disease, do present a number of histopathological points which they commonly considered to be distinguishing features of these two colonic disorders.

The predominant feature of granulomatous colitis is of course the appearance of the sarcoid or tuberculoid reaction in the affected tissue, which is found in 50-70% of all cases. Granuloma, epithelioid and Langhan's giant cells were not observed in any of the excised colonic tissue examined from rats or cats treated with either technique in the present experiments.

Fissuring, knife-like clefts deep into the bowel wall, are considered a feature just as important as granuloma in Crohn's colitis. This type of lesion was occasionally observed in the experimental lesions but were probably nothing more than artifacts as there was no granulation tissue, epithelioid or giant cells of the Langhan's or foreign-body type lining these passageways, as seen in the human disease.

Even in the absence of granuloma and fissures, transmural in-

flammation with widening of the submucosa by edema is a distinguishing feature of human granulomatous or Crohn's colitis. Focal collections of lymphocytes are hence found scattered in all layers of the bowel wall as well as in the serosa and peri-intestinal fat. This phenomenon is rarely seen in human ulcerative colitis where inflammation is generally restricted to the mucosa and superficial aspect of the submucosa, extending below this only in acute fulminating acses. Where epithelial destruction occurs, the inflammatory infiltrate spreads into the submucosa and this was a common feature of the rat and cat experimental lesion. Transmural inflammation was a prominent feature in the serosal application of the acetic acid, occurring early in lesion development. Intraluminally-treated animals often exhibited this but usually later in the disease process and with higher concentrations of the acid. In the human form of Crohn's colitis, there is a marked increase in the amount of collagen in the bowel wall, especially in the submucosa. This generally spares the muscularis externa from degenerative change. Again, collagen fiber accumulation, leading to rigidity in latter stages, was a feature common to both modes of acetic acid application. Serosally, the collagen appeared early and infiltrated the submucosa as well as spreading extensively into the muscularis externa. The intraluminal technique however, usually resulted in increased amounts of collagen in the submucosa and muscularis mucosae with a superficial invasion of the muscularis externa. In these latter cases, the muscularis externa usually regenerated quickly and the majority of collagenous deposits was localized in the submucosa which subsequently began organization of the granulation tissue.

Histopathological features that both ulcerative and granulomatous

colitis share include crypt abscesses. Although sometimes thought to be a distinctive feature of ulcerative colitis, they are quite commonly observed in Crohn's colitis but are not present in as large numbers as in ulcerative colitis. Though not specific for ulcerative colitis, crypt abscesses are particularly conspicuous in this disease, possibly as a result of the diffuse nature of the mucosal process. They are thought to be the result of a damaged mucous membrane allowing an accumulation of polymorph and luminal microorganisms which, due to a blocked crypt, either burst into the lumen contributing pus to the feces or into the loose connective tissues of the submucosa. These defects were commonly observed in the colonic biopsies obtained from patients with ulcerative colitis for this study. Structures resembling these defects were also occasionally seen in the rat tissue but were attributed to artifacts even though an inflammatory cell infiltrate was sometimes present in the lumen. If not artifactual these breaks in the epithelium were eliciting inflammatory exudate out into the crypt rather than the other pattern as observed in the human tissue. In the human tissue, it is these defects which result in epithelial undermining and subsequent sloughing. Mucosal undermining was occasionally observed in the acetic acid lesions but mucosal infolding at the edges of the lesions was more prominent. Gentle tapering of the mucosa into these erosions was also a common feature in the rat and cat experimental lesion.

The undermining of the mucosal margin in ulcerative colitis is attributed to the development of pseudopolyps. These structures may be short or extremely long. These polypoidal-like structures observed in the intraluminally-induced lesion of the cat and rat were always short,

plump, semi-circular remnants of inflamed tissue rather than stalked in appearance. The epithelium usually tapered downward infolding to seal off the tissue island by contacting the underlying muscularis mucosa, which was in all cases preserved under these structures. In both human and animal instances though, the tissue remnant exhibited edema, tortuous crypts with dilation, as well as mucus depletion.

In active human colitis, the most striking feature is congestion and dilation of the blood vessels, particularly in the mucosa and submucosa. This is accompanied by edema and an inflammatory infiltrate confined to the lamina propria of the mucosa which is at first composed mainly of lymphocytes, plasma cells and eosinophils. This again was a common feature of the intraluminal acetic acid lesion, although it was also concurrently associated with submucosal infiltration and edema, the mucosal reaction however slightly preceding that observed in the submucosa after the intraluminal application of the agent and vice versa after the serosal application. An important difference in the intraluminal cases however, was the absence of epithelial cells at the time this response was being elicited in the lamina propria.

A significant feature of human ulcerative colitis is the relative lack of fibrosis, the inflammatory exudate being of the type which does not provoke formation of granulation tissue with fibrosis. In severe disease there may be an increase in the amount of collagen in the superficial mucosa. The rat, and to a lesser degree, the cat, both experience large amounts of collagen fiber establishment in the intraluminal and serosal techniques which later give rise to the formation of granulation

tissue.

In fulminating forms of ulcerative colitis, where transmural inflammation occurs, there is extensive loss of the mucosa with surviving patches displaying intense vascular congestion and edema with a mild inflammatory infiltrate. This pattern was often exhibited in areas adjacent to ulceration in the acetic acid lesion, although vascular congestion was not a constant feature. In the human form where mucosal ulceration occurs, the submucosa largely disappears exposing the deeper muscle coats which are usually covered only by a thin layer of granulation tissue. The fibers of the muscularis externa became separated by edematous exudate and may appear stretched and thin. In the rat and cat lesions this occurred only after severe ulceration of the mucosa and submucosa, where the muscle fibers were intermingled with a dense inflammatory cell infiltrate. The common pattern observed here was a thick submucosa filled with collagenous fibers and polymorphs, which formed granulation tissue and quickly began the initial stages of organization. The muscle fibers of the muscularis externa often appeared as those in the human condition after the serosal technique in animals but were less common after the optimal intraluminal dosage.

At the highest concentrations of acetic acid in both techniques (50%), or in the instances where the lesions had continued to degenerate rather than heal, the rat colon appeared extremely necrotic. Its general appearance was not unlike that described by Morson and Dawson (1972) for necrotizing colitis. They stated that this form of colitis was the result of acute ischemia with tissue necrosis followed by secondary

invasion of the bowel wall by Clostridium welchii. Although these organisms are normal gastrointestinal tract commensals, they will invade the bowel wall if it becomes necrotic as a result of impaired blood supply or infarction. Microorganisms penetrated the bowel wall in these stages of acetic acid lesion development, as was clearly evident in ultrastructural examination of the tissue samples.

In summary, when compared to human forms of inflammatory bowel disease such as granulomatous and ulcerative colitis, the acetic acid lesion in rats and cats was not an ideal model for either. This experimental lesion shared features that were either common to both, as non-specific inflammatory phenomenon, or one feature distinctive to one form of human disease and another which was characteristic of another form.

Experimentally, the acetic acid lesion bears histopathological features common to those documented by Melynk et al. (1966) in the dog colon exposed to burn treatment. In the dog lesions the early phase appeared erythematous with superficial epithelial necrosis and tissue separation, with some areas denuded of epithelium and covered with a fibrin exudate containing inflammatory cells. Pronounced edema and extravasated erythrocytes were also common in the lamina propria and submucosa with the muscularis mucosae appearing necrotic over the central region of focal degeneration but also identifiable around the edges. The base of this lesion was also composed of a dense population of mixed inflammatory cells, prominent vascular changes and severe edema. Although this was most common in this region it also extended into the muscularis mucosa and varying distances into the submucosa. On occasion when focal

necrosis of the submucosa and muscularis externa occurred, a mild serosal inflammatory reaction was observed. These lesions, like those of the acetic acid techniques were sharply demarcated with some mucosal infolding of the surrounding edges.

Kirsner and Elchlepp (1957) also reported a similar type of reaction in the rabbit colon exposed to formalin. Those sensitized with egg albumen prior to formalin (1-2%) exposure exhibited features resembling the more severe stages of the acetic acid lesion. These two experimental lesions illustrate that the colon reacts in a similar fashion to various forms of injury - mechanical, physical or chemical - over a wide range of animal species.

Ultrastructural Observations:

Time-interval studies of thin and ultrathin plastic-embedded sections helped elucidate the mechanism of acetic acid lesions induced via intraluminal application of the agent. Harding and Morris (1977) studied the effect of 1N acetic acid, applied to the gastric serosa, on the mucosal epithelium and noted a similar process to that observed in this study. Six to seven hours after application of the agent, ultrastructural examination revealed a large region of surface epithelial cell desquamation over the site of acid application. As the lesion expanded, the cells on the edge of the lesion degenerated leaving a denuded area composed of only basal lamina and the underlying lamina propria. This was similar to the initial stages observed after the intraluminal administration of acetic acid. Harding and Morris did

not observe hemorrhagic lesions as the time period studied was too short to allow their development.

A review of the literature pertaining to ultrastructural studies on tissue affected with inflammatory bowel diseases such as ulcerative or granulomatous colitis revealed only a small amount of information was available on this cellular perspective of the diseases, reports consisting mainly of preliminary observations made on selected cases of these diseases, and often based upon no more than one or two biopsies.

Lumb (1960) described the structure of the upper portion of the colonic mucosa as complex; a single layer of columnar surface epithelium resting on a thick reticular fiber layer which in turn overlies the surface capillaries. The earliest electron microscopic change observed in ulcerative colitis was a progressive degeneration of this reticular or basal layer. This process originated in tiny focal areas and spread to involve more and more of the fiber layer (Donnellan, 1966). A similar reticulin layer degeneration was observed in shigellosis and post-radiation proctitis, suggesting that it was not a specific feature for ulcerative colitis but rather the result of intestinal edema (Gonzalez-Licea & Yardley, 1966a). There was a progressive decrease in the degree of PAS staining of this reticular layer, visible by light microscopy, which later extended into the lamina propria. These reticulin fibrils were composed of collagen. Thin plastic-embedded sections of human colonic tissue in stages of remission and healing exhibited unusually intense basophilic staining of the basal lamina of the surface and crypt epithelium. A visible increase in thickness accompanied the increased staining intensity

of this layer. The colonic response to acetic acid in the rat indicated that the basal lamina was left stripped of epithelial cells, a similar process occurring in the gastric mucosa of the rat treated serosally with this agent (Harding & Morris, 1977). The lamina propria here remained intact until the lamina propria filled with extravasated erythrocytes and polymorphs, the layer only then rupturing and releasing this cellular inflammatory exudate into the colonic lumen and regions which were once crypts of Leiberkuhn.

Another prominent feature in the early phases of ulcerative colitis was an increase in size of the surface capillaries in the mucosa (Donnellan, 1966). Although dilated, they contained no erythrocytes and the endothelial lining appeared normal. The invasion of plasma cells and lymphocytes gradually extended from the deeper portions of the mucosa up towards the surface, filling the lamina propria. In the apical regions these cells were scattered irregularly but in deeper regions they were often seen in small clusters around the blood vessels. These vessels in the rat mucosa also appeared filled with lymphocytes and plasma cells. However, the capillaries of the mucosa were also packed with erythrocytes with diapedesis beginning to occur and involving the blood vascular elements from both vessel types. At later stages of this experimental process there was a great extravasation of erythrocytes from the remaining blood vessels of the mucosa as well as those in the superficial regions of the submucosa.

Many workers (Dick & Grayson, 1961; Dobbins, 1975; Flick *et al.*, 1962; Gear & Dobbins, 1968; Gonzalez-Licea, 1966; Gonzalez-Licea &

Yardley, 1966a) considered the hallmark of active disease to be the presence of polymorph infiltration of the lamina propria and crypts, which may occasionally extend into the submucosa. Plasma cells and lymphocytes also infiltrated the lamina propria in varying degrees (Gear & Dobbins, 1968). Eosinophils (Bercovitz & Sommers, 1966; Matts, 1961; Sommers & Bercovitz, 1966; Wright & Truelove, 1966) were also noted to be increased in some cases and this was also manifest in paraffin and plastic sections of the early stages of the experimental rat lesion. Donnellan (1966) reported however, that PAS-positive macrophages were virtually absent.

Intracellular polymorphs have been reported to appear as if they were degranulating and ingesting organelle components from the epithelial cells, while their intercellular counterparts remained intact and phagocytically inactive (Dobbins, 1975). Although the polymorphs observed in ulcerative colitis have been reported by a number of workers to remain extracellular and non-invasive to individual epithelial cells (Donnellan, 1965; Gonzalez-Licea, 1966; Mottet, 1971; Nagle & Kurtz, 1967; O'Connor, 1972; Shnitka, 1964; Hirsch, 1965; Dobbins, 1975) stated that he found many instances of probable intracellular invasion in biopsies in 10 of 14 patients. Flick *et al.* (1962) and Gonzalez-Licea and Yardley (1966a) claimed that these polymorphs appeared to migrate through both glandular and surface epithelia but Dobbins (1975) reported that this intracellular invasion occurred in the epithelial cells on the luminal surface, and into the the absorptive cells rather than the goblet cells which generally appeared empty of mucous granules.

Dobbins (1975) also reported that polymorphs frequently packed the lumen of capillaries and, although normal in appearance, they were occasionally seen to degranulate and release lysosomes into the lumen of the capillary. Cells appearing to move across the capillary wall were always noted to be extracellular, the endothelial cells maintaining their normal junctional complexes. Occasionally the capillary endothelium exhibited prominent dilation of the endoplasmic reticulum and decreased cytoplasmic density when adjacent to a degranulating polymorph. In the rat tissue, the acetic acid moved quickly through the mucosa and very early in the process (5 to 15 minutes) the lymphocytes and other cellular elements were observed to have extremely vacuolated cytoplasm, the nucleus however remained normal in appearance. The endothelial cells also appeared to have cytoplasmic vacuolation following acetic acid administration and the early hemorrhage of these vessels, although not common, was not rare, filling the lamina propria with erythrocytes and other vascular elements.

While the rat lesion did not exhibit true crypt abscesses, there are conflicting reports as to whether there is a polymorph infiltration in these structures. Donnellan claimed that this was observed only occasionally while Gear and Dobbins (1968) stated that crypt abscesses as defined by Warren & Sommers (1949), exhibited collections of polymorphs at their base in the tissue they examined.

Lumb and Protheroe (1955) claimed that occasionally this polymorph infiltration extended into the muscularis mucosae and submucosa in ulcerative colitis. The lamina propria appeared edematous and the sub-epithelial reticular layer may appear diminished or absent (Donnellan,

1966). In the rat tissue, the polymorph invasion of the muscularis mucosae was a standard feature, the bulk of this cellular invasion emanating from the submucosal vessels up through it into the lamina propria where a minimal emigration from the packed vessels was occurring. The lamina propria was consistently edematous in the rat, experimental lesion but the reticular layer of the epithelial cells basement membrane resisted degeneration long after it was stripped of its epithelial coat.

Gear and Dobbins (1968) stated that the human surface and crypt epithelium retained normal shape and configuration appearing flattened and basophilic with a tendency to nuclear hyperchromatism only later in the pathogenesis. In the rat colon the surface epithelial cells were shed immediately but in areas adjacent to regions which had come into direct contact with the 10% acid the crypt cells did demonstrate this flattening and nuclear abnormality. Ultrastructural studies showed that these cells exhibited fewer microvilli, which were shorter and blunt, and cytoplasmic vacuolation under the cell membrane. These three features were also observed in the human tissues examined by Donnellan (1966) and Gear and Dobbins (1968). In addition, they noted a shrinking of the cell and an increase in the intercellular spaces. This was also observed in crypt cells in the rat colon; the intercellular spaces along the sides and bases of the cells became swollen and the cellular fingers which once interdigitated between adjacent cells were free and dangled in the spaces or appeared short and blunt. Usually though the junctional complexes near the surface of the cell, those which were normally the more sturdy, remained intact until the last moment before the cell was shed.

Donnellan (1966) reported that in all cases the mitochondria and endoplasmic reticula of these cells remained intact. This was disputed by Cockrell and Krehbiel (1972) who studied a case of canine histiocytic ulcerative colitis and reported that the endoplasmic reticulum had become dilated, and mitochondria scarce and accompanied by vacuole formation. Likewise, in our experimental rat tissue there was dilation of the endoplasmic reticula with internal destruction and swelling of the mitochondria in crypt epithelial cells. Gonzalez-Licea (1966) also stated that epithelial cell damage in ulcerative colitis was marked by the formation of cytosegrosomes, dilation of rough endoplasmic reticula, mitochondrial alterations, and a decrease in size and number of the microvilli. He stated that the epithelial and capillary basement membranes were visible even after the reticulin had disappeared. This again was the case in the rat tissue.

Otto and associates (1975) studied the ultrastructural pathology of 37 ulcerative colitis biopsies, in one of the most extensive investigations reported, and found that among epithelial changes exhibited, alterations in the microvilli and glycocalx of the surface epithelia were prominent. This extraneous coat was decreased in intensity and the microspheres were also decreased in number (Cockrell & Krehbiel, 1972; Otto et al., 1975). Extreme cytoplasmic vacuolation was also evident. These alterations were interpreted as the morphological basis for partially impaired mucosal block, as seen in IgA secretory piece deficiency and these authors considered this to be an essential pathogenic process in ulcerative colitis. The decrease in glycocalx density was also noted in rat tissue crypt cells and the extreme vacuolation noted made assessment of the

microspheres difficult.

In these cases, epithelial necrosis was not noted until the blood supply to the surface mucosa had become markedly reduced with the sub-epithelial capillaries undergoing a progressive diminution and thickening with occlusion of the vessels by platelet thrombosis. This could not be observed in the surface epithelium of the rat as it was so rapidly shed, but the crypt epithelial necrosis began after the lamina propria underlying it had become filled with erythrocytes, polymorphs and colonic bacteria - a feature noted by Aluiwhare (1971a) in Crohn's disease of the colon.

Aluiwhare (1971b) also noted a high percentage of transformation of lymphocytes into cells exhibiting a cartwheel structure in the nuclei which was thought to be transforming nucleoli. These structures were not observed in the rat tissue but an alteration of nuclear chromatin distribution in the nuclei of crypt epithelial cells, changing from an even distribution to peripheral dispersion, was noted.

Bacteria were also observed in the deeper bowel layers in Crohn's disease of the colon (lamina propria and submucosa) as well as some distance from the ulcerated areas of the bowel (Aluiwhare, 1971c). Viruses, bacteria, and bacterial residues were not however, identified in the phagolysosomes (vacuoles containing phagocytosed material and lysosomal enzymes), some of which were cytosegrosomes (membrane-bound bodies containing cytoplasmic organelles undergoing digestion) observed in the epithelial cells themselves. In over 20 mouse colons, 6 normal and 7

with ulcerative colitis, bacteria were not seen in comparable cases (Aluiwhare, 1971c), nor have they been observed in the colonic wall by many workers studying the fine structure of the normal or colitic colon (Donnellan, 1966; Gonzalez-Licea, 1966). Bacteria have also been observed in shigellosis (Takeuchi *et al.*, 1965) where they are thought to be etiologic agents. Their presence in Crohn's disease may be of some etiologic significance (Aluiwhare, 1971a) and it is of interest that Mitchell and Rees (1970) have suggested the possibility of a transmissible agent in Crohn's disease. Bacteria were very evident in treated rat colon after ulceration had occurred and extended deep into the mucosa and submucosa.

Epithelial cell abnormality was not a prominent feature of Crohn's disease of the colon, whereas in ulcerative colitis it was more obvious and probably occurred earlier (Aluiwhare 1971b). It appears then that the site of primary abnormality is not the mucosal epithelium in Crohn's colon, and the earliest detectable change observed with the electron microscope was the increased proportion of lymphocytes and plasma cells in the lamina propria, as compared with the normal or inflamed colon. The prominent, unusual nucleoli of these lymphocytes were probably an indication of excessive activity.

In summary, it is evident that these two colonic inflammatory disorders, whose distinctiveness has often been the subject of controversy, exhibit very definite ultrastructural differences. Again, ultrastructural features of the experimental rat colitis fell between these two diseases, showing some common features to each human disease, but were entirely

similar to either.

Assessment of Lesion Severity:

Indices for human inflammatory bowel disease are currently topics of much discussion and controversy. Many parameters, including cellular, enzymatic, and clinical, have been suggested, such as: granulocyte activity (Kane et al., 1974), serum proteins (Marnier et al., 1975), small intestinal disaccharide activity (Gudmand-Hoyer et al., 1975), serum orosomucoid (Jensen et al., 1976), nitroblue tetrazolium testing (Ward & Eastwood, 1976), serum tryptophan (Beeken, 1976), carcinoembryonic antigen (Isaacson, 1976), and lysozyme activity (Nugent et al., 1977; Hylander et al., 1976; Falchuck et al., 1975; Dobbins et al., 1976; Dronfield & Langman, 1975). The validity of many of these biochemical assessments remains disputed. Also, morphological parameters have been utilized by various workers, such as were utilized here, including measures of crypt depth, alkaline phosphatase activity and mucus content of the mucosa.

Both Mottet (1971) and Morson and Dawson (1972) suggested that in active ulcerative colitis, as well as in mucosal regions affected by granulomatous colitis, depletion of goblet cells was exhibited. Periodic acid-Schiff staining of rat and cat colonic mucosa in the various stages of ulcer development and healing illustrated that this lesion exhibited this depletion. Mucus depletion in the initial states was perhaps a protective action taken by the epithelial cells of the mucosa against the chemical insult. As epithelial degeneration progressed the goblet cells

were no longer able to continue mucus production except for those cells located in the base of the crypts where the acid damage was not as severe. These lower cells however, underwent a degenerative process at a later stage and there was no mucus synthesis evident in the degenerating areas. Mucosal segments immediately adjacent to the lesions also exhibited a decreased mucus production as well as the pseudopolyp-like islands of inflamed residual mucosa. More distant regions exhibited extremely elongated crypts, and an almost normal amount of mucus-containing cells, although the intensity of the staining was decreased. Bustos-Fernandez et al. (1976) also observed a reduction in the mucus content of colonic goblet cells following intraluminal administration of acetic acid in their attempts to induce experimental diarrhea in rats. Acetic acid at a low pH (2.9) was absorbed by the colonic mucosa and caused a reduction in water and electrolyte absorption which was specific for this organic anion, and was not caused by hydrochloric acid at a similar pH.

Decreased depth of the intestinal crypts of Lieberkuhn was noted by several investigators studying the similarity between implant rejection and features observed in human celiac disease (Ferguson & Parrott, 1973; Holmes et al., 1971). Holden and Ferguson (1976) also observed a similar depth reduction in colonic allografts in mice undergoing rejection. This general reduction in crypt depth observed in these segments of degenerating mucosa was also a phenomenon observed in the acetic acid colitis model. In our tissues, regions of degenerating mucosa, as well as areas adjacent to the lesions, exhibited a reduction in crypt depth, while areas of inflamed edematous mucosa and the pseudopolyp-like remnants demonstrated crypts with a marked increase in depth.

The alterations of alkaline phosphatase activity were directly correlated with the number of polymorphs in the colonic mucosa at any stage. Jervis (1965) stated that alkaline phosphatase was present in significant but variable amounts in the striated border of the epithelial cells lining the small intestine. In the rat there was a decreasing gradient from duodenum to ileum, while the striated border of the colon did not normally demonstrate any enzymatic activity. Histological sections however were said to exhibit some activity in the colonic lamina propria. This, however, was not exhibited in any of the control rat colon processed by the technique outlined by Clark (1973). Since many workers consider the emigration of polymorph to be the hallmark of active human disease (Dick & Grayson, 1961, Dobbins, 1973, Flick et al., 1962; Gear & Dobbins, 1968; Gonzalez-Licea & Yardley, 1966a), the staining pattern of this localization should have revealed the activity of these cells and their approximate numbers within the colonic mucosa.

As expected, it was possible to trace the emigration of these cells from the submucosal vessels shortly after the establishment of the edematous response in the mucosa and submucosa. Massive infiltration and localization of these cells in the muscularis mucosae was the point at which the development of the lesion became irreversible. This black band of alkaline phosphatase localization became progressively larger, spreading into the mucosa and superficial aspect of the submucosa and indicating a massive polymorph infiltration. This material could eventually be seen sloughed into the lumen during the development of the large band of granulation tissue forming at the base of these lesions, and it was carried upward along with all the other necrotic, cellular debris.

Mucosal segments which had undergone an edematous inflammatory swelling for 14 days also exhibited moderate infiltration of these cells via this localization. These cells were generally localized around the edges of the lesions, the lamina propria in more distant regions of the inflamed mucosa not exhibiting as dense an accumulation of polymorphs.

Other workers have noted increased mast cell numbers in colitis (McGovern & Archer, 1957; McAuley & Sommers, 1961) as well as increased histamine content (Binder & Huidberg, 1967), particularly in cases of eosinophilia. Watson and Roy (1960) claimed that Paneth cells, which were not normally present in the colonic crypts of Lieberkuhn, appeared to reside there in ulcerative colitis in large numbers.

Colonic Motor Activity in Feline Experimental Colitis

The highly lymphatic nature of the rat colon, coupled with its relatively thin bowel wall led to consideration of the cat as a more suitable species for the induction of this experimental mucosal colitis. A carnivore such as the cat has a short colon devoid of sacculations resulting from the absence of taenia coli, a uniform longitudinal muscular coat, and a vestigial cecum. Although the human colon is architecturally closer to that of the herbivores, the cat has a similar neuronal organization, except for the direct adrenergic fibers innervating the longitudinal muscle (Daniel, 1975).

Reported alterations in colonic motor activity in patients with ulcerative colitis (Almy, 1961; Code et al., 1952; Kern et al., 1951;

Spriggs et al., 1951) stimulated interest in recording the motor activity before and after the induction of this experimental colitis by the present method. The motor activity of the cat colon has been extensively studied (Christensen, 1975; Daniel, 1975), and its size facilitated attachment of monitoring devices such as the contractile force strain gauge transducer.

The normal, spontaneous colonic activity in the cats observed in the present experiments corresponded well with results obtained by other workers (Christensen, 1975; Christensen et al., 1971). The most common wave type found was the phasic contraction, occurring in groups or singly and sometimes superimposed upon the longer tonic contractions which varied in amplitude. The relationship between colonic electrical activity, presenting as slow waves and spike-bursts, and contractions is still not clearly defined in the cat. The descending colon however, is the region in which the predominant activity is the migrating spike-burst - believed to be correlated with contraction. These waves produce strong tonic contractions resulting in a luminal flow in a caudal direction (Christensen, 1975; Christensen & Hauser, 1971a, 1971b).

The colonic motor activity of normal individuals has been well established (Connell, 1968; Ritchie, 1971) and a general lack of activity has been reported in patients with ulcerative colitis (Code et al., 1952). Over 60% of colitis patients exhibited significantly reduced phasic activity (loss of type I, II and III waves), defined as "straight line" activity. Increased occurrence of propulsive, type IV waves, or scattered type IV waves coupled with practically no other visible activity has also

been reported in these patients (Code et al., 1952). Occurrence of a modified type II wave with increased duration and amplitude has also been described (Almy, 1961), while extensive investigation of 72 ulcerative colitis patients revealed altered motility ranging from straight line, greatly reduced, and normal, to hyperactive, the diminishing frequency of the findings occurring in this order (Kern et al., 1951).

Motor activity recorded at the period of greatest colonic ulceration (3 days post-acid) in the cat exhibited a pattern in the unstimulated condition similar to the straight line activity of colitis patients. Absence of motility tracings in these patients was correlated with the severity of diarrhea, the pattern returning to normal when the patients were free of diarrhea (Kern et al., 1951). This alteration was also noted in the cat, the lack of activity occurring when the animal presented with diarrhea 3 days following induction of the experimental colitis. Cats afflicted with summer diarrhea reportedly exhibited an altered pattern of slow waves (Christensen, 1975), and disappearance of this activity was also observed in patients with functional diarrhea (Almy et al., 1950) and in experimentally induced diarrhea in dogs (Galapeux et al., 1938).

Kern et al. (1951) reported no relationship between the amount of fibrosis and the character of motility tracings from patients with ulcerative colitis, and concluded the absence of phasic activity was not the result of rigidity or fibrosis of the colon. This was also demonstrated in the cat colon, as generally any rigidity and fibrosis occurred later in the experiment (7 to 21 days). This phenomenon was clearly unrelated to the degree of anatomical change. The thickening of the muscularis

externa observed in patients with chronic colitis might well be the result of sustained muscle spasm or contraction causing hypomotility of the bowel (Morson & Dawson, 1972).

The stimulating effect of bethanechol chloride (Urecholine) on smooth muscle is well known. Although its clinical use is restricted to subcutaneous injection, none of the contraindications were observed after sub-serosal injection in any of the cats during the recording periods, or thereafter. Kern et al. (1951), reported an increase in response of his patients with ulcerative colitis to Urecholine, while that of the cat 3 days post-acid administration was greatly reduced and much less than normal. Increased motor activity and tension in the proximal and distal canine colon in response to the administration of bethanechol chloride has also been noted (Rinaldo et al., 1971). A greater response was elicited in the longitudinal muscle of the distal colon than in the proximal region. The motor activity of the feline colon appeared to remain depressed in the unstimulated colon after 7 days following induction of these lesions, but exhibited contractions of greater amplitude and duration than those seen in control animals following administration of urecholine. By the later stages (day 21) of the experiment, the unstimulated and Urecholine-stimulated activity was greatly increased over that of the controls.

The return of phasic activity after blocking colonic, autonomic stimulation with drugs like Banthine and tetraethylammonium chloride suggest that the colon may be subject to constant autonomic, probably parasympathetic stimulation (Kern et al., 1951). Kyosola et al. (1976)

claimed that decreased colonic activity in ulcerative colitis was the result of a decrease in the number of enterochromaffin cells and an exaggerated adrenergic innervation with associated increased levels of noradrenaline in the axons of these patients. Investigators working on the etiology of this disease correlated neural control of the musculature with control and supply of blood to the colonic mucosa (Wolf, 1966). Fasth and Hulten (1975) linked the control of both colonic motility and blood flow in cats through antagonistic nervous mechanisms. Here, the sacral parasympathetic nerves actively induced contraction and emptied the colon as well as induced mucosal vasodilation and increased mucus secretion.

Healing of the Lesion:

The histopathological picture of healed colitis varies in ulcerative and granulomatous colitis. In ulcerative colitis there are various degrees of mucosal atrophy with irregular branching of the tubules and a gap between the base of the crypts and the luminal aspect of the muscularis mucosae. The epithelium exhibits reactive hyperplasia in attempts to repair defects with a general decrease in the number of goblet cells and with enlargement and hyperchromatism of the nuclei of absorptive cells. In granulomatous colitis a patchy mucosal atrophy is also exhibited but is coupled with a fibrosis of the muscularis mucosae and an excess of fibrotic tissue in all layers of the bowel wall with scattered aggregations of lymphoid tissue (Morson & Dawson, 1972).

Eastwood (1977), in a recent review of the literature on gastro-

intestinal epithelial renewal, stated that in patients with ulcerative colitis, rectal biopsies have shown that enhanced cell proliferation and accelerated migration were characteristic of active colitis. It was also demonstrated by Bleiberg et al., (1970) and Eastwood and Trier (1973) that the proliferative zone for colonic epithelium renewal appeared to have expanded upward in these cases, perhaps giving a clue as to why patients with this disease may develop cancer.

The healed appearance of these acetic acid lesions resembled those of granulomatous colitis rather than ulcerative colitis with a great bulk of granulation tissue present in the submucosa and focal mucosal areas. The surface epithelium had extended out over this tissue mass and crypts invaded it, starting from the edges inward. Mucus cell staining intensity, as well as the number of cells in these crypts and surface epithelium adjacent to these scarring sites, was generally decreased. The mucosa in these healed areas often exhibited a varying degree of crypt disfiguration with tortuous branching crypts, sometimes dilated into large mucus-secreting vacuoles. In cases which had exhibited a longer duration and a more severe degree of ulceration, extending deeper into the bowel wall, the entire thickness of the bowel wall was a mass of granulation tissue except for the serosa and a few muscle fibers remaining from the once thick muscularis externa. In most of these cases there was little evidence of epithelial invasion except for the creeping of a superficial layer of cuboidal-like surface epithelium across the luminal aspect of this granulation tissue from the edges of the lesion.

showed a characteristic late phase of healing which again appeared very similar to that of the rat colon exposed to acetic acid. A progressive reduction in edema and hemorrhage allowed an increase in vascular channels and organization of the ulcer base. At the same time proliferating epithelium grew outward from the periphery of the lesion, differentiating rapidly from flat to cuboidal to columnar. This cellular layer was observed under the superficial inflammatory exudate along the granulation tissue. Rudimentary invaginations in this epithelial sheet produced columnar-lined crypts. The progression and extent of this regeneration was dependent upon the size, depth and duration of the lesion in the dog. The healing ulcer bases were composed of a superficial inflammatory exudate, large areas of well organized granulation tissue which were permeated with engorged capillaries and mixed inflammatory cells. The lamina propria of the healed mucosal regions in these animals appeared thicker and hypercellular while the muscularis mucosae was replaced by connective tissue with few cells or capillaries. The submucosa contained few or no inflammatory cells.

Previous studies had demonstrated the regeneration of colonic mucosa in dogs (Braucher & Kirsner, 1962), monkeys (Foley & Wattenberg, 1960) and cats (McMinn & Johnson, 1958), with or without preservation of the muscularis mucosa while the failure of mouse colonic mucosa to regenerate following chemical burn with silver nitrate (O'Connor, 1954) or excision biopsy (O'Connor, 1956) had also been outlined.

The rat colon however, has been shown in this study to be capable of recovering from severe ulceration and disruption of all four bowel wall

layers including fibrous and polymorph infiltration. Although in the more severe cases cicatrization was still evident at 60 days, extended experiments might indicate eventual healing similar to those areas less extensively or severely ulcerated and exposed to the optimal concentrations of the agent. In most cases the muscularis mucosae had regenerated and stretched across the defect, but in cases where the lesion had been extensive, the muscularis mucosae was not always present in its entirety the whole distance across the area where the lesion had been. The latter continued to display an irregular morphology.

The Effect of Therapeutic Agents on Lesion Development and Healing:

Over the past two decades a vertiable myriad of reports concerning the effectiveness of various compounds in the treatment of inflammatory bowel disease have accumulated in the literature. The current topics for discussion range from zinc sulfate (Dronfield et al., 1977), oral disodium cromoglycate (Mani et al., 1976), and elemental diets (Axelsson & Jarnum, 1977), to progress reports on the value of an immunosuppressive agent, Azathioprine (Rosenberg et al., 1975), a sulphonamide preparation, salicylazosulfapyridine (Dissanayake & Truelove, 1973) and corticosteroids such as prednisone (Lennard-Jones et al., 1965). Earlier, even psychotherapy had been suggested as a helpful therapeutic approach for inflammatory bowel disease (O'Connor et al., 1964). Now investigators are testing these more successful agents against one another in double-blind trials (Caprilli et al., 1975) and taking a closer look at the patients for possible side effects (West et al., 1974; Pounder et al., 1975).

Sulphasalazine (Salazopyrin) was first used for the treatment of ulcerative colitis by Svartz in 1942. Although it has become an accepted form of treatment for this disease, controlled therapeutic trials have shown that corticosteroid therapy was superior to sulphasalazine for treatment of acute attacks (Lennard-Jones et al., 1960; Truelove et al., 1962). However, the position is reversed upon consideration of maintenance treatment, as Truelove & Witts, (1959) and Lennard-Jones and associates (1965) have shown that cortisone and/or prednisone at 15 mg/day are ineffective in reducing exacerbations while daily doses of only 0.5 mg sulphasalazine significantly reduced relapse rate (Misiewicz et al., 1965).

The initial therapeutic trial with the acetic acid lesion utilized the corticosteroid drugs, Betnesol (in a retention enema) and Meticortelone. Pre-treatment and treatment for 3 days following induction of the lesion revealed no decrease in lesion severity compared to controls. The second effort utilized the maintenance drugs - Salazopyrin and Salazopyrin coupled with a Betnesol enema. The pre-treatment and post-treatment times were increased to seven days and upon sacrifice those treated orally with Salazopyrin showed a significant reduction in ulcer severity. Achord (1974) states that although salazopyrin is a weak antibiotic in its bacterially split active form it may exhibit an anti-inflammatory effect centered on the basement membrane of the epithelial lining of the gut. This however, has not been demonstrated. Donnellan (1966) however, claimed that focal necrosis of the basement membrane was one of the early histological changes in ulcerative colitis. Taking into account the

initial epithelial separation from its basement membrane induced in the colon following intraluminal administration of the acetic acid, this suggested mode of action for Salazopyrin could account for the significant difference exhibited by this group. However, histological verification is required. While those animals treated with Salazopyrin as well as the corticosteroid enema showed a slight reduction in severity, it was neither significant nor comparable to that achieved with the Salazopyrin alone. Explanation of these latter results is difficult without additional testing but it may be that the corticosteroid effected the bowel flora which are responsible for the action of this sulfanilamide compound. It is emphasized that these findings, while encouraging with respect to the utility of this animal model for the testing of possible therapeutic agents, are quite preliminary.

Acetic Acid and Vascular Phenomenon:

The possible interaction of acetic acid and the bowel microcirculation in the development of experimental lesions for inflammatory bowel disease might well maintain interest in this technique. Many workers, including Fairburn (1973) suggested that a vascular alteration might be responsible for the pathogenesis of this disease, resulting from a neural or hormonal control of the bowel musculature (Fasth & Hulten, 1973). Morson and Dawson (1972) claimed that early colitis appeared to be a vascular response, particularly of the capillaries and venules of the mucosa, to an unknown etiological agent - the histology of ulcerative colitis suggesting an allergic or hypersensitivity factor.

Pfeiffer and Sethbhakdi (1971) reviewed the controversial role of vascular impairment as a possible etiologic factor in peptic ulcer, and

subsequent workers investigated this theory using substances which altered blood flow, including acetic acid. Augur (1970) found that mucosal blood flow was initially increased with administration of acetic acid but subsequently declined with the appearance of damage to the gastric mucosal barrier. Skarstein et al., (1974) also noted changes in blood flow associated with the acetic acid gastric ulcer in cats. Here the mucosal flow was again found to be increased in areas exhibiting a one week old ulcer.

Amongst the most interesting data was that provided recently by Molster et al., (1976) on changes in the vascular permeability in acetic acid ulcers in the rat. They claimed that protein leakage was markedly increased in areas closest to the ulcer, which were associated with edema formation, and indicated that inflammatory mediators were released in a wide area around the lesion. The increase in albumen accumulation around the vessels was highly significant in the areas near the lesion, and it was demonstrated that this increase was due to the application of the acetic acid.

While these changes were observed in the acute lesion they must be of some significance in the further development of the ulcer into its chronic state and this model would be useful in studies concerning the inflammatory changes associated with the ulcer. Further colonic experiments using even less concentrated solutions of acetic acid administered more often might well selectively induce the capillaries of the mucosa into producing the secondary changes characteristic of the active human disease.

On the basis of exploring both serosal and intraluminal application of acetic acid for induction of an experimental colitis, it is concluded that the intraluminal application was a superior technique in that there was less damage to the muscularis externa and risk of complications including perforation. Histopathologically, the process is an acute, edematous inflammatory response either involving the mucosa or whole bowel wall, depending upon the mode of application and the severity of the insult. Although there were some similarities to human forms of non-specific colonic inflammation, the overall picture was more consistent with an acute edematous, erosive burn. The colon has been reported to respond in a similar manner to various types of injury. Therefore, the inflammatory regenerative response, as seen in this model, may be identical to that initiated by a wide variety of irritating agents.

SUMMARY

The results of this study can be best summarized in point form:

1. Colonic lesions were successfully reproduced with 100% reliability in the descending colon of rats using an intraluminal or serosal technique. Screening of various concentrations of acetic acid (10, 25, 35, & 50%), at intervals up to 60 days, revealed a dose-response curve which indicated that 10% acetic acid intraluminally and 35% serosally were optimal concentrations for lesion induction with these two techniques. These lesions proved to be semi-chronic, non-

relapsing, and exhibited a minimal frequency of complications.

2. Intraluminal, per rectum, administration of 50% acetic acid induced a similar lesion in the distal colon of the cat. These lesions exhibited histopathological features similar to those of the rat, but were of a less severe nature, usually healing within 21 days.

3. Histological features exhibited by this lesion included: initial mucosal edema, infiltration of inflammatory cells into the lamina propria, surface epithelial sloughing, degeneration of crypt epithelium, reduction in mucosal thickness, submucosal edema and inflammatory cell infiltration, excess collagenous fiber establishment, total ulceration of the mucosa down to and occasionally past the muscularis mucosae, occurrence of polypoidal-like structures, reduction in mucus production, edematous separation of the components of the muscularis externa followed by infiltration of inflammatory cells, disruption of the serosa and the formation of an inflammatory exudate on its visceral aspect, organization of granulation tissue occupying the submucosa and mucosa with mucosal regeneration, and distorted configuration of the crypts in regenerated mucosal areas.

4. Monitoring the mucus content of goblet cells with PAS staining and measurement of crypt depths showed both parameters became decreased as the mucosa degenerated, while alkaline phosphatase

exhibited increasing density as the inflammatory response developed. Histopathological and gross morphological criteria were also established to evaluate the severity of these lesions sampled at the various screening intervals.

5. Thin and ultrathin plastic sections successfully elucidated the mechanism of this lesion. Lifting of epithelial cells from the basement membrane on the surface and extending down into the crypts was an initial observation. These cells exhibited necrotic appearance as well as inclusion of cytosomes, opaque bodies, reduction and shortening of the microvilli and cytoplasmic vacuolation. Invasion of the lamina propria with plasma cells, lymphocytes, erythrocytes and polymorphonuclear leucocytes was observed. This cellular infiltration burst through into the lumen and open ulcerations were evident.

6. Colonic motor activity was studied in the cat before and after induction of the acetic acid lesion and revealed a significant reduction in both unstimulated and urecholine-stimulated activity 3 days after induction of the lesion, the point at which the animals experienced greatest ulceration and presented with diarrhea. Within 21 days post-acid, the colonic motor activity of these animals in the unstimulated condition had attained a hyperactive state while that of the stimulated condition appeared more or less within the normal range.

7. Pilot trials with various therapeutic agents used in the

treatment of ulcerative colitis, revealed that pre- and post-treatment of rat colon with Betnesol (betamethasone disodium phosphate) enemas at a daily dosage of 0.5 mg. and Meticortelone (prednisolone as sodium hemisuccinate) enemas at a daily dosage of 5 mg., both corticosteroid compounds, had no significant effect on the development of the lesion. A second trial utilizing Salazopyrin (salicylazosulfapyridine) at a daily dosage of 250 mg. resulted in a significant decrease in lesion severity after a 7 day pre- and post-treatment period. Salazopyrin coupled with a Betnesol enema however, did not result in a significant decrease in lesion severity, the reason not being clear.

5. Examination of human mucosal biopsies excised from patients afflicted with ulcerative colitis in a number of disease stages, revealed few direct comparative points with the rat and cat lesions except for features exhibited in general non-specific colonic inflammations.

6. The colonic mucosal response and healing pattern was very similar to that documented for edematous burn phenomenon and reaction to agents such as formalin. This indicates that the colonic mucosa of many species responds in a similar manner to injury from many varying etiologic agents.

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