CLINICAL MICROBIOLOGY REVIEWS, Jan. 1989, p. 90–117
0893-8512/89/010090-28$02.00/0

Vol. 2, No. 1

Crohn's Disease and the Mycobacterioses: a Review and Comparison of Two Disease Entities

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WHAT IS CROHN'S DISEASE?

The disease that Crohn, Ginzberg, and Oppenheimer described in 1932 was a chronic low-grade inflammation of the terminal ileum (59). Earlier cases may have been documented (196, 299), but the authors failed to receive recognition for describing a new disorder. These earlier cases were called nonspecific granulomata and were separated from those that had previously been termed hyperplastic tuberculosis of the intestine. Although Crohn's disease was first described as a segmental disease of the small intestine, in 1960 it was recognized that the same disorder affected the colon and had been confused with ulcerative colitis (161). In recent years, the lesions of Crohn's disease have been recognized in the mouth, larynx, esophagus, stomach, skin, muscle, synovial tissue, and bone (17, 141, 143, 152, 171, 182, 206, 298). Thus, Crohn's disease may be considered a newly recognized disease, with a defined clinical and pathologic description dating back only to the 1960s. Although the terms Crohn's disease, Crohn's colitis, Crohn's ileitis, and regional ileitis have been with us longer, there is uncertainty as to the accuracy of these diagnoses prior to 1960. To this date, Crohn's disease and ulcerative colitis continue to be confused clinically, and the term inflammatory bowel disease (IBD) was developed to include both diseases.

Patients afflicted with this disorder generally suffer from chronic weight loss, abdominal pain, diarrhea or constipation (obstruction), vomiting, and general malaise. Between 70 and 80% of Crohn's disease patients require surgical resection of the diseased intestine (84, 102, 120, 243). Difficulties usually are not ended by surgical intervention, and most patients will suffer recurrences and require further surgical procedures (71, 109, 123, 161, 181, 292). Generally, patients live with chronic pain, in and out of hospitals, throughout their lives. Mortality is approximately 6% (84, 239). Perhaps more important than mortality is the quality of life of Crohn's disease patients. Less than 50% of patients consider their quality of life to be "good"; suboptimal psychosocial function is recorded in 30 to 54% of patients (84, 230, 292). Patients with IBD (an estimated 2 million, 200,000 of whom are children, in the United States alone) represent a very unhappy population with little prospect for relief. The etiology remains obscure and medical treatment is supportive at best (102, 238, 243). Twenty-five years after the original description, Crohn and Yarnis wrote (61): "From this small beginning we have witnessed the evolution of a Frankenstein monster that, if not threatening to life, frequently results in serious illness, often prolonged and debilitating."

IS CROHN'S DISEASE AN INFECTIOUS PROCESS?

In their original disease description, Crohn and coworkers (59) attempted unsuccessfully to infect guinea pigs, rabbits, and chickens with diseased intestine and lymph nodes. Van Patter (Ph.D. thesis, University of Minnesota, Minneapolis, 1952) inoculated 131 animals, including guinea pigs, rabbits, cats, rats, and chickens, with diseased tissues from 43 patients; all remained normal. Mitchell and Rees (189, 190) and others (42, 43, 270) claimed to have transmitted a granuloma-inducing agent to the footpads of mice by inoculation of diseased-tissue filtrates. Others have reported that they transmitted ileitis to rabbits by intraserosal inocu-

lation of tissue filtrates (257). These studies and others could not be reproduced and did not withstand reexamination. The results could not be confirmed by others; granulomas were sometimes produced with filtrates from control tissues, and many of the granulomas were found to contain foreign material such as bone, hair, and synthetic fibers (5, 26, 240, 272). In a multicenter study in which various investigators exchanged material, a transmissible histologic abnormality could not be reproduced (272). In recent years, Das et al. (69) have described the production of lymphomas or plasma cell hyperplasia in nude mice by the injection of Crohn's disease tissue. Also, these authors claim that sera from Crohn's disease patients react with certain cells or cell types in these murine lymphomas as demonstrated by the fluorescent-antibody technique (13, 301, 310). This quite fascinating, and unexplained, phenomenon is currently under investigation and still awaits judgment from other laboratories. Thus, over the years, definitive evidence of an infectious (or at least transmissible) agent has not been forthcoming.

Nevertheless, despite the lack of any clear-cut evidence of an infectious etiology, investigators continue to seek agents in diseased tissues, and every few years, a new putative agent emerges. This continued pursuit probably is related to the lack of acceptance of other theories, including a role for allergy, autoimmune disease, and dietary factors. Also, the pathology of the disease, the multiple familial occurrences, and the multiple remote lesion sites all suggest an infectious process. Viruses (10, 98, 104, 108, 222, 223, 294, 295, 306) and L-form bacteria (19, 139, 261, 295) have been incriminated most commonly, but in recent years these all have been discarded as candidate agents. To date, the etiology of Crohn's disease, being infectious, transmissible, or not, has eluded the scientific community. Now, over 55 years since its distinction from tuberculosis, attention has been driven back to the beginning: "Is Crohn's disease a mycobacterial disease after all?" (96).

MYCOBACTERIA AND CROHN'S DISEASE: A HISTORICAL PERSPECTIVE

Medical historians suggest that Crohn's disease may first have been described as early as 1682 to 1771, or perhaps earlier (142). Reports of diseases suggestive of Crohn's disease have appeared in 1806, 1813, 1828, 1875, 1907, 1908, 1909, and 1913 (142). Whether these cases actually were Crohn's disease will remain unknown. Mycobacteria were not discovered until 1874 when Hansen described acid-fast bacilli in leprosy patients (118). The organism causing tuberculosis, which would be confused with Crohn's disease for years to come, was not discovered until 1882 (149), and intestinal tuberculosis was not recognized until several years later. Nevertheless, a disease was described in the early 1900s that was similar to intestinal tuberculosis, but acid-fast organisms could not be isolated. Dalziel (64), in 1913, described several patients with chronic intestinal enteritis which, although very similar to intestinal tuberculosis, was believed to be a new disorder. He drew attention to a recently described disease in cattle called pseudotuberculosis (now known as paratuberculosis) "in which the histological characters and naked-eye appearances are as similar as may be to those we have found in man." Dalziel goes on to state: "In my cases the absence of acid-fast bacilli would suggest a clear distinction, but the histological characters are so similar as to justify a proposition that the diseases may be the same." Also in 1913, contrary to the views of Dalziel, Ignard (cited in reference 197) wrote: "In many cases of hyperplastic tuberculosis of the intestine, no tubercles, giant cells or bacilli are found. The lesion consists of a mixture of variable proportions of tuberculous and inflammatory elements. In certain cases, the last only exists. Nevertheless, these inflammatory tumors should be classified among the tuberculous." The view of Ignard predominated, and these unusual intestinal diseases became known as hyperplastic tuberculosis.

By the 1920s, the belief was fading that intestinal tuberculosis occurred without acid-fast bacilli or caseous necrosis, and a disease known as "nonspecific granulomata" emerged (197, 299). In these cases, as well as in those described by Crohn et al. (59) in later years, the authors each discussed "the remarkable resemblance" of these cases to intestinal tuberculosis. The landmark article by Crohn, Ginzberg, and Oppenheimer (59) recognized regional ileitis as a separate and unique disease entity and displaced the long-held belief of a mycobacterial etiology. We now know that hypertrophic intestinal tuberculosis (3) and tuberculosis without caseation or demonstrable acid-fast bacilli (297) do exist, as does a distinct disease known as Crohn's disease. Nevertheless, over the years, the notion recurs that Crohn's disease might in fact be mycobacterial in origin.

THE SEARCH FOR A MYCOBACTERIAL ETIOLOGY

Cultural Data

Attempts to isolate mycobacteria from Crohn's disease patients date back to before the time that this disease was recognized as a distinct entity. During these periods, as discussed, cases of intestinal tuberculosis were documented in which the tubercle bacillus could not be visualized or isolated from diseased tissues. Even after the recognition of Crohn's disease, investigators continued to seek mycobacteria in diseased specimens, without success. These attempts were made almost exclusively with Lowenstein-Jensen media and sought *Mycobacterium tuberculosis* almost exclusively. As knowledge was gained about the cultivation of mycobacteria, investigators came to realize that routine methods and media were not appropriate for all mycobacteria, and many species failed to grow under these conditions. Because the clinical and pathological similarities between Crohn's disease and intestinal tuberculosis continued to suggest some form of relationship, researchers adapted different bacteriological methods and immunologic tests in attempts to find an elusive *Mycobacterium* species responsible for Crohn's disease.

Perhaps the first concerted effort to isolate mycobacteria from Crohn's disease patients was presented by Van Patter (Ph.D. thesis). In these studies, Van Patter reported the results of 1,762 cultures from 43 patients with Crohn's disease. By using seven different types of media and incubation periods up to 15 months for some cultures, he isolated acid-fast organisms from three patients (7%) after 6, 7.5, and 8 months of incubation. These organisms could not be subcultured and were never formally identified.

For the next 25 years, not a single report appeared on the attempt to isolate mycobacteria from Crohn's disease patients. Undoubtedly, attempts were made over the years, but such data were presented only as laboratory information related to a case report to rule out the possibility of intestinal tuberculosis. In 1962, Golde and McGill (101) addressed the issue of atypical mycobacteria (more appropriately called mycobacteria other than tuberculosis) in Crohn's disease and suggested searching for these organisms rather than for

M. tuberculosis alone. The ability of these organisms to produce chronic intestinal disease and the similarities of Crohn's disease to tuberculosis and Johne's disease (paratuberculosis) were also noted in their report. It was another 16 years before an original article appeared implicating mycobacteria as etiologic agents in Crohn's disease.

In 1978, a revival of the notion that mycobacteria might be related to Crohn's disease occurred with the articles by Burnham et al. (33, 34). These authors described the isolation of *M. kansasii* from the lymph node of a single patient with Crohn's disease and pleomorphic material, suggestive of cell-wall-deficient (CWD) organisms, from 22 of 27 Crohn's disease patients, 7 of 13 ulcerative colitis patients, and 1 of 11 controls. It was proposed that CWD forms of *M. kansasii* played an etiologic role in both Crohn's disease and ulcerative colitis; however, this theory was short-lived.

The most damaging evidence to the theory of *M. kansasii* as an etiologic agent in Crohn's disease was the failure of Burnham et al. (33, 34) to identify the CWD forms and their assumption that they were forms of *M. kansasii*. This organism is recognized as an opportunistic pathogen causing disease predominantly in individuals with underlying chronic disease (105, 215, 244, 285). It is not a primary pathogen in healthy individuals and is generally nonpathogenic in animals; a few strains may be pathogenic for mice. Although the natural reservoir of *M. kansasii* is not known (it is not found in soil or dust), it has been isolated from a variety of water sources (122, 241) and healthy animal tissues, including lymph nodes (136, 303). Thus, *M. kansasii* was not a good candidate for consideration as a primary pathogen. The pleomorphic organisms, however, continued to be investigated. Stanford (Proc. 2nd Int. Workshop Crohn's Dis., 1981, p. 274–277) cultured patient lymph nodes on many bacteriological media, in addition to Lowenstein-Jensen and Robertson's cooked-meat media, and reported the isolation of irregular acid-fast masses from 42 of 76 patients with Crohn's disease, 14 of 27 patients with ulcerative colitis, and 3 of 41 control lymph nodes. Although these masses resembled CWD forms, they could not be identified. In an attempt to indirectly support the notion that these masses were CWD mycobacteria, Stanford chemically induced CWD forms of *M. kansasii*, filtered them through 0.45- and 0.22-μm filters, and the inoculated the filtrates onto culture media. After a period of time, abnormal acid-fast forms appeared which were visually indistinguishable from those observed in IBD tissues. Attempts to isolate classical mycobacteria from these experimentally induced acid-fast masses were unsuccessful. In an accompanying paper, White (Proc. 2nd Int. Workshop Crohn's Dis., 1981, p. 278–282) presented additional data suggesting that this acid-fast material was of mycobacterial or corynebacterial origin. An examination of culture material by serology and thin-layer chromatography revealed that most material containing these coryneform bacteria or acid-fast forms reacted with highest titers to *M. kansasii*-related mycobacteria and *Corynebacterium* antisera. White also found evidence of mycolic acids in some of the acid-fast masses. From their data, Stanford and White concluded that IBD may be associated with or caused by an organism from the *Mycobacterium-Corynebacterium* axis.

The efforts of Stanford et al. were reviewed and updated at a recent symposium (266). Since 1974, these investigators have examined over 200 surgical specimens and have isolated pleomorphic, variable acid-fast organisms from 42 of 76 (55%) Crohn's disease patients, 17 of 27 (52%) ulcerative colitis patients, and 3 of 41 (7%) controls. The organisms remain unidentified, although efforts are in progress to classify them more precisely. Although antisera prepared against *M. kansasii* bind strongly to these pleomorphic organisms, mycobacterial genetic probes failed to produce restriction-fragment-length polymorphism patterns similar to any *Mycobacterium* species examined to date (J. J. McFadden, J. Thompson, E. Green, S. J. Hampson, J. Stanford, J. Haagsma, R. Chiodini, and J. Hermon-Taylor, Gastroenterology **94**:A294, 1988).

In 1984, there was another surge of interest in the role of mycobacteria and Crohn's disease, involving yet a different *Mycobacterium* species. From this period on, there have been more reports on mycobacteria and Crohn's disease than in the last 50 years; as expected, the data are equally conflicting and controversial. In 1984, Chiodini et al. published a series of papers describing the isolation of two strains of an *M. paratuberculosis*-like organism from 11 patients with Crohn's disease but not from 3 with ulcerative colitis or 3 with other bowel diseases (56). Detailed techniques and characteristics of the isolates (53) as well as antimicrobial susceptibility profiles (55) were reported, in addition to animal susceptibility studies. The isolates were pathogenic for mice by the intravenous or intraperitoneal route, but not for chickens, guinea pigs, rats, or rabbits. Oral inoculation of one of the strains into a newborn goat produced a granulomatous ileocolitis without observable acid-fast bacilli after 5 months. Immunologic studies on this animal failed to show seroconversion, except for an early immunoglobulin M (IgM) response which rapidly subsided. Although the authors presented data suggesting skin test reactivity in this animal to *M. paratuberculosis* purified protein derivative (PPD) but not *M. tuberculosis* PPD, the level of observed reactivity would not be considered positive in a clinical setting.

The authors concluded that their isolates were strains of *M. paratuberculosis* or a biovariant of that species and suggested that this organism plays an etiologic role in at least some cases of Crohn's disease. An editorial that accompanied some of these papers (96) suggested that these studies "provide the most intriguing evidence yet generated regarding a possible cause of this important illness" and that "scientists have come closer than ever to fulfilling Koch's postulates and developing a test system for Crohn's disease." On the other hand, this editorial also recognized some of the pitfalls of these studies, the need for further research, and that skepticism would and should exist.

Shortly thereafter, Chiodini et al. reported that in primary isolation, their putative agent occurred in a CWD form or as a spheroplast (54). On primary culture these organisms appeared as non-acid-fast coccobacillary forms that had the ultrastructural appearance of spheroplasts and, after several months of incubation, transformed into characteristic *M. paratuberculosis*-like organisms. By using genetic techniques, i.e., restriction polymorphism of the 5S ribosomal ribonucleic acid genes, spheroplasts were found to be identical to the parent bacillary *M. paratuberculosis*-like forms (54) and were identified as strains of *M. paratuberculosis*. These workers also isolated spheroplasts, four of which transformed into *M. paratuberculosis*, from 16 of 26 patients with Crohn's disease (61%), but not from 13 patients with ulcerative colitis or from 13 patients with other bowel disorders. Although these spheroplasts remain unidentified, 7 of 10 tested seroagglutinated with specific *M. paratuberculosis* antisera, suggesting that these unidentified forms were also *M. paratuberculosis*. Some of these CWD forms required up to 1.5 years of incubation for primary emergence of colonies. The authors indicated that the presence of CWD

forms could account for (i) the inability to demonstrate acid-fast bacilli in patients' tissues; (ii) the failure to demonstrate a strong and consistent immunologic response because CWD mycobacteria are generally of low immunogenicity; and (iii) the previous failure to isolate these organisms because of the caustic nature of most other techniques for processing mycobacteriologic specimens. On the basis of available information about mycobacterial spheroplasts, which suggests that only bacillary forms are pathogenic, they postulated that a very slow rate of reversion with subsequent local hypersensitivity-type immunologic responses could account for the chronicity of Crohn's disease.

In a series of studies, the organisms were identified definitively as strains of *M. paratuberculosis*. By both restriction polymorphism of ribosomal 5S genes (R. J. Chiodini and T. J. Yang, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, U-20, p. 122; R. J. Chiodini, Proc. 21st Joint U.S.-Japan Leprosy Tuberc. Conf. 1986, p. 8–12; R. J. Chiodini, Proc. 22nd Joint U.S.-Japan Tuberc. Conf. 1987, p. 47–51) and studies of random gene sequences (175, 176), restriction patterns of the Crohn's disease isolates and *M. paratuberculosis* were found to be identical. These papers not only served to confirm the identification of these isolates, but also described the first genetic technique capable of separating the pathogenic *M. paratuberculosis* from its close relatives of the environmental *M. avium-M. intracellulare* (MAI) complex. Previously applied techniques, i.e., deoxyribonucleic acid (DNA)-DNA hybridization, failed to separate this closely related group of organisms (174, 307). Although taxonomically it has been proposed that these related organisms be classified into an *M. avium-M. intracellulare-M. paratuberculosis* complex (Chiodini, Proc. 22nd Joint U.S.-Japan Tuberc. Conf. 1987), sufficient genetic divergence is present to maintain *M. paratuberculosis* as a distinct species.

At a research conference on paratuberculosis in Melbourne in 1986, Coloe et al. (P. J. Coloe, C. R. Wilks, D. Lightfoot, and F. A. Tosolini, Aust. Microbiol. **7**:188, 1986) reported the isolation of *M. paratuberculosis* from 1 of 30 patients with Crohn's disease. The organism was isolated from colonic material after 16 weeks of incubation; cultures of the draining lymph nodes were negative. This isolate was identified by biochemical criteria and cellular fatty acid profiles. This work represented the first confirmation of the isolation of *M. paratuberculosis* from a Crohn's disease patient. At present, Coloe et al. have cultured biopsies from approximately 50 Crohn's disease and 50 control (ulcerative colitis, normal tissue) patients. Although some acid-fast growth has been observed on some cultures from Crohn's disease patients, these slow-growing isolates have not yet been characterized (P. J. Coloe, personal communication).

Whitehead examined the serologic activity of serum from the Crohn's disease patient from whom this Australian isolate was obtained, as well as sera from a few additional patients (J. Whitehead, Proc. 2nd Int. Colloq. Paratuberc., in press). Using an immunoblot technique with patient sera on *M. paratuberculosis* antigens separated by polyacrylamide gel electrophoresis, this investigator found that identical antigen bands were recognized by the sera from the Crohn's disease patients examined and cattle naturally infected with *M. paratuberculosis*.

Graham and co-workers in 1987 reported their results of culture and DNA hybridization (106). They described the isolation of mycobacteria from 47.6% of 105 specimens, including those from Crohn's disease, ulcerative colitis, and control patients. Mycobacteria were isolated from 9 of 59 patients with Crohn's disease, 9 of 19 with ulcerative colitis, and 18 of 27 non-IBD controls. Most isolates were of the MAI complex and *M. fortuitum* complex, with a single *M. kansasii* isolate and one they claimed to be similar to *M. paratuberculosis* isolated from an ulcerative colitis patient (genetic studies conducted after publication suggested that this isolate was not *M. paratuberculosis*). They did not find any specific association between mycobacteria and Crohn's disease and brought to light the widespread occurrence of mycobacteria in diseased tissues. Yoshimura et al. (307) failed to find any association between mycobacteria and Crohn's disease by DNA-DNA hybridization. Application of this technique to 31 biopsy specimens revealed mycobacterium-related sequences in 10 of 19 patients with Crohn's disease, 2 of 6 with ulcerative colitis, and 1 of 6 controls. Again, the results did not support the notion that mycobacteria are etiologically related to Crohn's disease. The authors recognized that their findings did not rule out mycobacteria as etiologic factors in Crohn's disease, but the results may have been limited by low sensitivity of the methods used (42% positive rate is equal to the sensitivity they achieved by cultural methods). In their article, Yoshimura et al. (307) presented additional data supporting the identification of the Crohn's disease-isolated mycobacteria reported previously (53) as *M. paratuberculosis*.

The culture results of Graham and co-workers (106) provide some useful and important information and illustrate the ubiquitousness of some *Mycobacterium* spp. These workers applied tissue-processing techniques of lower stringency than recommended for the isolation of *M. paratuberculosis*, and these methods probably account for their results. Although the authors suggest that their method, using 0.1% hexadecylpyridinium chloride, is that recommended by the National Animal Disease Center, this laboratory actually recommends 0.1% benzalkonium chloride (280) or, more recently, 0.75% hexadecylpyridium chloride (187). At concentrations of <0.75% hexadecylpyridium chloride, contaminants, which include environmental mycobacteria, commonly overgrow cultures from clinical specimens. Since organisms of the MAI and *M. fortuitum* complexes are widespread in the environment, processing techniques of low stringency would result in the isolation of these species from a variety of sources. The results of Graham et al. (106) are comparable to those obtained from environmental sources. MAI complex can be isolated from 26 to 63% of soil samples, 50% of tap water samples, 13% of dust samples, and 35% of air samples (27, 91, 122, 241, 290). *M. fortuitum* can be found in 39% of soil samples, 63% of dust samples, and 25% of air samples (137, 241, 285).

An interesting feature of the culture results of Graham et al. (106) is the specimen type from which mycobacteria were isolated. Except for a single strain of *M. fortuitum* complex, mycobacteria could not be isolated from resected tissues, but 35 strains of mycobacteria (predominantly MAI and *M. fortuitum*) were isolated from biopsy specimens of aphthous ulcers. These ulcers provide a suitable microenvironment for the propagation of such environmental organisms. On the other hand, Graham et al. (106) isolated as yet unidentified spheroplasts primarily from resected tissues of Crohn's disease patients rather than from aphthous ulcers. Retrospectively, these culture data appear to support, rather than refute, a CWD mycobacterial etiology. Thus, as in all other diseases, the area from which material is obtained for culture is of great importance, as are the techniques applied to tissue processing.

At about the same time, Haga (115) briefly reported that he

was unable to isolate mycobacteria from 17 fecal, 5 bowel resection, or 9 biopsy specimens from Crohn's disease patients, although he did report the isolation of acid-fast coccoid bodies from a Crohn's disease patient which could not be identified or subcultured. S. R. Pattyn, F. Portaels, and Y. Van Maercke presented their culture results at a meeting of the International Working Group on Mycobacterial Taxonomy held in Bithoven, The Netherlands, in September 1987 (S. R. Pattyn, personal communication) and later published their data in the form of a letter (L. J. Colemont, S. R. Pattyn, P. P. Mitchielsen, J. H. Pen, P. A. Pelckmans, Y. M. Van Maercke, and F. Portaels, Letter, Lancet **i**:294–295, 1988). These workers examined tissues from 32 patients with Crohn's disease and demonstrated acid-fast bacilli in 11 (34%) by acid-fast staining. Cultivation attempts yielded two strains of *M. chelonei*, which were said to be mycobactin dependent; mycobacteria could not be isolated from the remaining nine cases in which acid-fast bacilli were observed. The authors acknowledged that their processing technique, i.e., 0.15% benzalkonium chloride–0.5% NaOH, may have been harmful to other acid-fast bacilli; *M. paratuberculosis* is known not to survive exposure to NaOH decontamination.

In 1986 and 1987, a few more reports on mycobacteria and Crohn's disease appeared, but they were not research papers. Tytgat and Mulder (279) presented a review on the etiology of Crohn's disease and were the first to report, in other than abstract form, the isolation of *M. paratuberculosis* from a patient in The Netherlands. Data published in this review represented the first independent duplication of previous efforts and confirmation that *M. paratuberculosis* may be isolated from some cases of Crohn's disease. While all active theories on the etiology of Crohn's disease were addressed, the authors considered that "a microbial aetiology, particularly mycobacterial, seems the most promising."

Further data from some of the previously mentioned investigators were recently presented at an international research symposium sponsored by the National Foundation for Ileitis and Colitis, Inc., late in 1987.

Haagsma et al. presented data on cultivation of mycobacteria from patients with Crohn's disease (112) and the presence of *M. paratuberculosis* antibodies in Crohn's disease patients (113). They cultured 66 surgical specimens and isolated *M. paratuberculosis* from 1, *M. fortuitum* from 1, and acid-fast material from 2. Colonies of *M. paratuberculosis* emerged after 11 and 16 months of incubation on Herrold egg yolk and Ogawa media, respectively. These studies, however, had two major flaws: the processing protocol changed sometime during the study, and control tissues were not cultured. Their isolates of *M. paratuberculosis* were found to be genetically identical to those isolated by Chiodini in the United States (49). These investigators have recently isolated an additional strain of *M. paratuberculosis* from a Crohn's disease patient, bringing the number of Crohn's disease-associated *M. paratuberculosis* isolates to 2 of 88 specimens examined (J. Haagsma, personal communication).

Yoshimura et al. (308) presented data on characterization of some of their previous isolates (106). Their isolates were predominantly of the MAI complex, probably serovar 19, but some remain unidentified by biochemical or genetic methods. Many of their isolates could not be fully characterized due to their slow growth, but, of those examined, none were *M. paratuberculosis*.

Gitnick et al. (97) described their efforts to isolate mycobacteria from resected Crohn's disease tissues and inoculation of animals with their organisms. These authors cultured tissues from 27 patients with Crohn's disease, 29 with ulcerative colitis, and 26 with other bowel diseases. Three strains of mycobacteria were isolated, of which two remain uncharacterized. One isolate from a patient with Crohn's disease was identified as *M. chelonei*, another was said to be similar to *M. paratuberculosis*, and the third isolate (from a cancer patient) remains uncharacterized. The two isolates from Crohn's disease required 3 and 12 months of incubation. Acid-fast spherules were isolated from a few Crohn's disease patients as well as from controls. The *M. chelonei* isolate was inoculated orally into newborn goats which subsequently developed a transient diarrhea. Three animals died 5 to 10 days postinoculation. Intestinal lesions were limited to mild inflammation and colonic infiltration with polymorphonuclear cells. Animals receiving the uncharacterized *M. paratuberculosis*-like organism remained clinically and pathologically normal. Of interest is the apparent acute diarrheal disease produced by *M. chelonei* since this organism is generally associated with immunocompromised hosts or with traumatic wounds (31, 110, 111, 204, 263). An acute intestinal disorder produced by *M. chelonei* could have significant meaning to both the veterinary and medical professions. However, the authors did not adequately rule out other neonatal diseases of goats as a possible cause of the observed diarrhea and acute bowel inflammation. The latest data from this laboratory indicate the isolation of mycobacteria from 3 of 27 patients with Crohn's disease, 1 of 31 ulcerative colitis patients, and 1 of 27 controls. Two of the three isolates from Crohn's disease are *M. paratuberculosis* (one genetically confirmed); the other is the strain of *M. chelonei* reported above. The strains from controls and an ulcerative colitis patient are slow growers, as yet unidentified, and do not appear to be *M. paratuberculosis* (G. Gitnik, personal communication).

At the American Gastroenterological Association meeting in May 1988, the abstracts related to mycobacteria and Crohn's disease were only genetic studies on some of the isolates. McFadden et al. (Gastroenterology **94**:A294, 1988) presented data showing that the *M. paratuberculosis* organisms isolated by Chiodini et al. (53–56), an *M. paratuberculosis* organism isolated independently in The Netherlands (112), an *M. paratuberculosis* strain isolated from primates (172), and wild-type *M. paratuberculosis* associated with disease in ruminants were identical. They also found that spheroplasts isolated from Crohn's disease and other patients by Stanford (266) were not *M. paratuberculosis* but a heterologous group of organisms. Hampson et al. (S. Hampson, J. J. McFadden, J. Thompson, E. Green, M. Moss, F. Portaels, and J. Hermon-Taylor, Gastroenterology **94**:A170, 1988) used DNA probes to examine mycobacteria isolated from acquired immunodeficiency syndrome (AIDS) patients, patients with atypical mycobacteriosis, and healthy individuals. They used a specific *M. paratuberculosis* DNA probe and were unable to identify this species in any of their material. They concluded that the absence of *M. paratuberculosis* in their study population confirms that this organism has only been isolated from Johne's disease and Crohn's disease, two pathologically similar disease processes.

Last, the Bovine Pathology Laboratory of the Lyon Veterinary School in France isolated a strain of *M. paratuberculosis* from a 45-year-old woman with Crohn's disease. This isolate was identified by numerical taxonomic methods at the Laboratoire Central de Recherches Veterinaires. Descos and Perard of the Lyon-Suds Hospital and Lyon Veterinary School, respectively, have initiated a study to attempt iso-

TABLE 1. Isolates of mycobacteria from Crohn's disease patients and control populations[a]

Investigator(s)	No. of isolates/no. of patients (%)		Identification
	Crohn's disease	Controls	
Van Patter	3/43 (7)	0	Unidentified
Burnham et al.	1/27 (4)	0/24	*M. kansasii*
	[22/27 (81)]	8/24 (33)	[Unidentified CWD]
Chiodini et al.	4/26 (15)	0/26	*M. paratuberculosis*
	[16/26 (61)]	0/26	[Unidentified CWD]
Gitnick et al.	1/27 (4)	0/55	*M. chelonei*
	2/27 (8)	0/55	*M. paratuberculosis*
	1/27 (4)	1/55 (2)	Unidentified
Pattyn et al.	2/50[b]	0/0	*M. chelonei*
Graham et al.	9/59 (15)	27/46 (59)	*M. fortuitum*
			M. kansasii
			M. avium complex
Haagsma et al.	2/88 (2)	0/0	*M. paratuberculosis*
Haga et al.	[1/14 (7)]	0/0	[Unidentified CWD]
Coloe et al.	1/30 (3)	0/0	*M. paratuberculosis*
Thorel et al.	1/NK[c]	0/NK	*M. paratuberculosis*

[a] Bracketed results indicate that mycobacterial identity was not confirmed.
[b] Number of patients includes controls.
[c] NK, Not known.

lation from fecal and biopsy specimens from approximately 50 patients with Crohn's disease (M.-F. Thorel, personal communication).

Discussion. It is now clear that a host of different mycobacteria can be isolated from Crohn's disease patients, as well as from control populations, and that diseased tissue may be a suitable microenvironment for colonization of some of these species (Table 1). Most of these organisms are environmental opportunists (Table 2), although a few investigators have isolated the pathogenic *M. paratuberculosis* (Table 3). Unfortunately, in all studies reported to date, different methods have been used (Table 4); consequently, many different results have been obtained. This is true not only for cultural studies, but also for immunological studies. Thus, no consistent data are available that support the role of mycobacteria in Crohn's disease.

This controversy is perhaps heightened by the evidence that *M. paratuberculosis* may be an etiologic agent in Crohn's disease because the organism itself is controversial. *M. paratuberculosis* has never been subjected to numerical taxonomic methods and has been ignored by the International Working Group on Mycobacterial Taxonomy. This organism is the slowest growing of the culturable mycobacteria and has a variety of sensitive growth requirements for cultivation (48, 50–52). It generally takes years to become fully proficient at working with this species. Often even the most experienced mycobacteriologists have difficulty growing it because conventional methods are not appropriate. In addition, some laboratory and other strains of *M. paratuberculosis* that are being studied are actually MAI. The lack of any previous suggestion that *M. paratuberculosis* had public health significance has also added to its being disregarded by medical mycobacteriologists. These workers are now studying *M. paratuberculosis* but lack the background to cope successfully with the peculiarities of this species. The experience and expertise are in the hands of veterinary mycobacteriologists who generally do not have access to human tissue. It is interesting to note that all investigators who have been successful in isolating *M. paratuberculosis* from Crohn's disease patients were trained originally in veterinary mycobacteriology and had years of experience dealing with this peculiar species.

TABLE 3. Isolation of pathogenic *M. paratuberculosis* from patients with Crohn's disease

Investigators	Country of isolation	No. of strains
Chiodini et al.	United States (Connecticut)	4
Gitnick et al.	United States (California)	2
Coloe et al.	Australia	1
Haagsma et al.	The Netherlands	2
Thorel et al.	France	1

The microbiologic data on mycobacteria and Crohn's disease are similar to those observed on leprosy. Although leprosy is caused by *M. leprae*, a host of other mycobacteria have been associated with the lesions. Such mycobacteria, which are termed leprosy-associated mycobacteria (LAM) or armadillo-derived mycobacteria, can be isolated from leprosy skin lesions of humans and armadillos (72, 74, 200, 224–226). Isolation rates in armadillos average about 50% for naturally infected, experimentally infected, and noninfected animals. Organisms isolated include MAI, *M. scrofulaceum*, *M. gordonae*, *M. terrae*, and several groups of unclassified, difficult to grow mycobacteria (72, 224). Draper (74) suggested that infection with *M. leprae* favored the multiplication of environmental and other culturable mycobacteria within the lesions. These organisms are considered by most to be "insignificant" or "contaminants" (74, 224).

In addition to LAM or armadillo-derived mycobacteria, leprosy lesions are associated with large numbers of coryneform bacteria, termed leprosy-derived corynebacteria (LDC) (58, 135, 232, 234). Although it was once thought that these organisms were non-acid-fast forms of *M. leprae*, it is now known that they are not related to mycobacteria and probably play a role similar to that played by LAM (74, 232). Unlike the LAM, LDC are primarily associated with the lesions and are rarely isolated from noninfected tissues. Some investigators believe that LDC and LAM have a symbiotic relationship with *M. leprae*, while others believe these organisms represent opportunistic superinfection of the leprosy lesion. Regardless of which view is correct, it is clear that LAM and LDC have no significance to the disease; their isolation in culture should be disregarded, and they

TABLE 2. Pathogenic characteristics of mycobacteria isolated from Crohn's disease patients and controls

Organism	Classification	Infection associated with:	Natural reservoir
M. chelonei	Opportunist	Immunocompromised state/traumatic wounds	Environment
M. fortuitum	Opportunist	Immunocompromised state	Environment
M. avium (MAI)	Opportunist	Immunocompromised state	Environment
M. intracellulare (MAI)	Opportunist	Immunocompromised state	Environment
M. kansasii	Opportunist	Underlying chronic disease	Environment
M. paratuberculosis	Animal pathogen	Disease	Diseased animals

TABLE 4. Methods used for isolation of mycobacteria from Crohn's disease tissues[a]

Investigator(s)	Decontamination with:	Media used[b]	Media on which isolation made[b]
Van Patter	5% Oxalic acid	Egg yolk nutrient, pea extract-egg yolk, modified Minett, modified Dorset & Henley	Egg yolk nutrient, pea extract-egg
Burnham et al.	ND[c]	Modified LJ, Robertson cooked meat, modified Sauton	ND
Chiodini et al.	0.1% BC, 0.75% HPC	HEYM	HEYM
Coloe et al.	ND	HEYM	HEYM
Graham et al.	0.1% HPC	HEYM, LJ, 7H10 & -11	Various
Gitnick et al.	0.25% HPC or 0.75% HPC or 0.1% BC	HEYM, modified 7H9, BYE	HEYM
Colemont et al.	0.15% HPC and 0.5% NaOH	Ogawa	Ogawa
Haagsma et al.	4% NaOH and 5% oxalic acid or 0.75% HPC	Smith-Dubos, LJ, modified Ogawa, Stonebrink, HEYM, Coletso, 7H10	HEYM, Ogawa

[a] Investigators cited in text.
[b] LJ, Lowenstein-Jensen; BC, benzalkonium chloride; HEYM, Herrold egg yolk medium; HPC, hexadecylpyridinium chloride (cetylpyridinium chloride); BYE, Barile-Yarguchi-Eveland agar.
[c] ND, Not defined.

should be considered contaminants. It is also relevant to point out that, even with the large numbers of *M. leprae*, LAM, and LDC present in leprous tissues, many cultivation attempts are negative.

A similar phenomenon occurring in Crohn's disease would clearly account for the numerous environmental mycobacteria isolated from Crohn's disease and control tissues and also for the coryneform bacteria isolated from Crohn's disease patients (S. White and J. Stanford, Proc. 2nd Int. Workshop Crohn's Dis., 1981). Because $>10^6$ viable bacteria are required to yield a single colony when some mycobacteria are subcultured in vitro (presumably more bacteria are needed for primary culture) (226), low numbers of a pathogenic strain or species overgrown by environmental mycobacteria and coryneform bacteria would be difficult to isolate, particularly if the organism has peculiar in vitro growth requirements. In addition, organisms present in numbers of $<10^6$/g of tissue are not detectable by acid-fast staining and light microscopy. If Crohn's disease patients are infected with low numbers of *M. paratuberculosis* or some other *Mycobacterium* species and superinfected with organisms similar to LAM and LDC, it could account for the data generated.

Immunological Data

The use of immunologic responsiveness to specific antigens is a well-recognized method of determining the etiology of infectious disease. Generally, these determinations are based on the demonstration of rising antibody titers, but in some diseases, particularly chronic conditions, such is often not demonstrable. Diagnostic assays of chronic disease are therefore more generally based on cell-mediated immunity (CMI) or delayed-type hypersensitivity (DTH) rather than humoral responses. Despite the appropriate evaluation of cellular immunity in chronic conditions, such as Crohn's disease, most studies to date have examined humoral immunity, and these have been quite limited. Except for a few scattered reports, immunologic studies related to mycobacteria and Crohn's disease either have been conducted in direct response to bacteriologic data (see preceding section) or involved the use of mycobacterial antigens in accessing general immunologic functions.

Morganroth and Watson (195) examined delayed cutaneous reactions and precipitating antibodies in Crohn's disease patients to antigens of atypical mycobacteria of Runyon groups I, II, and III, as well as standard PPD. No increased incidence of sensitivity to these antigens was detected in 22 Crohn's disease patients compared with controls. Unfortunately, the authors did not describe the species of mycobacteria examined or the nature of the antigens used. Thayer et al. (275) examined skin test reactivity of Crohn's disease patients to tuberculin PPD, in addition to several other nonmycobacterial antigens, to evaluate anergy in Crohn's disease. These authors also failed to find an increased skin reaction to PPD in Crohn's disease patients and found no evidence of anergy as assessed by skin test reactivity. Bird and Britton (23) also failed to find increased responses to *M. tuberculosis* in Crohn's disease patients by the lymphocyte blastogenesis assay. Matthews et al. (168) examined sera from 24 Crohn's disease patients in an agglutination assay with antigens from *M. paratuberculosis* and *M. avium*, in addition to antigens from nonmycobacterial microbes. As antigen, these authors used phenol-killed whole cells of the mycobacteria which displayed wide cross-reactivity. The majority of sera from Crohn's disease patients (79 to 96%) agglutinated *M. paratuberculosis* and *M. avium* cells, but such reactivity was also observed in an equal number of controls. Thus, there was no clear difference observed between Crohn's disease patients and controls.

In conjunction with their isolation of *M. kansasii* in culture, Burnham et al. (33, 34) determined that in skin tests with antigens prepared from *M. kansasii* a high proportion of Crohn's disease patients showed an increased response compared with controls. No differences in reactivity between controls and Crohn's disease patients were noted with antigens prepared from 16 other *Mycobacterium* species. White et al. (293), in Burnham's study group, also found increased reactivity of Crohn's disease patients to *M. kansasii* antigens. By using an indirect fluorescent-antibody technique, positive responses were found in 9 of 11 sera from patients with Crohn's disease but not in any of 33 control sera. Based on these and their cultural data, these authors suggested CWD *M. kansasii* as an etiologic agent in Crohn's disease. During the same year, however, Whorwell et al. (295) reported their inability to demonstrate *M. kansasii* in tissues by immunofluorescence, and by 1980, members of Burnham's group reported that they were unable to duplicate their original immunologic findings. Although increased responsiveness to skin tests with *M. kansasii* antigens was still observed in Crohn's disease patients, they also found increased responsiveness in their control population (77). Also, in 1980, Grange et al. (107) reported increased IgA and

IgM antibodies to *M. tuberculosis* in patients with Crohn's disease. They noted that responses of tuberculosis patients were predominantly of the IgG class rather than IgA and IgM as found in Crohn's disease.

In conjunction with the isolation studies reported by Chiodini et al. (53, 56), these authors (274) presented data suggesting increased serologic reactivity to *M. paratuberculosis* antigens in Crohn's disease by enzyme-linked immunosorbent assay. Patients with Crohn's disease had a statistically significant increase in antibody titer to a protoplasmic antigen of *M. paratuberculosis* compared with controls. Examining cross-reactivity between antigens, these authors found 52.5 and 39% cross-reactivity of their antigen with *M. kansasii* and *M. tuberculosis*, respectively. As a result, a significant proportion of Crohn's disease patient sera also reacted to *M. kansasii* antigens. These results have not been duplicated in any other laboratory.

Cho et al. (57) examined seroreactivity of Crohn's disease and control patients to common mycobacterial antigens and a species-specific glycopeptidolipid of *M. paratuberculosis*. Increased reactivity of Crohn's disease patients versus controls was not observed with either antigen. They concluded that, as in paratuberculosis, seroreactivity is not a reliable method of examining the relationship between Crohn's disease and mycobacteria. Haga et al. (116) also failed to duplicate the results of Thayer et al. (274) and reported that the antibody titers to *M. paratuberculosis* of 32 Crohn's disease patients, 37 ulcerative colitis patients, or 48 non-IBD controls did not differ in any immunoglobin class.

Cho et al. (57) recognized that these inconsistencies in seroreactivity did not necessarily contradict the ongoing theories, particularly when organisms related to the MAI complex were involved. The MAI group is so widespread that all individuals, healthy and diseased, are likely to be exposed to these organisms and their antigens. Seroreactivity of the general population to MAI antigens is expected, not only because of their ubiquitous nature, but also because antigens in the order *Actinomycetales* are highly conserved. Common antigens, particularly those of major cellular components, exist between all families of the order, including *Streptomycetaceae*, *Nocardiaceae*, *Actinomycetaceae*, and *Mycobacteriaceae* in addition to *Corynebacteriaceae*. Thus, when unpurified antigens, such as sonicated whole cells, are used, a wide range of reactivity among normal and diseased populations would likely be found. Some studies have shown that 40 to 60% of the general public react to MAI antigens, probably related to their constant exposure to these agents in the environment (211, 303). Most of the "common" mycobacterial antigens, such as lipoarabinomannan, are cell wall components which would be lacking in a CWD form. Unless a specific antigen can be located that will not cross-react with MAI and related organisms, a difference among populations is not likely to be noted. Although Cho's use of a species-specific antigen from *M. paratuberculosis* (57) was the proper approach, animals naturally infected with *M. paratuberculosis* do not respond to this antigen and this antigen has not been found in wild-type strains isolated from clinical cases, suggesting that this antigen does not exist or is not expressed in wild-type strains (37). Furthermore, this species-specific antigen was later found not to be species specific but identical to that of MAI serovar 2 (38), and some recent data suggest that the laboratory strains in which this specific antigen was detected are not *M. paratuberculosis* but rather MAI (Chiodini, Proc. 22nd Joint U.S.-Japan Tuberc. Meet. 1987).

Jiwa et al. (138) described IgG serum antibodies to mycobacterial PPDs in Crohn's disease patients. These investigators examined seroreactivity to PPDs prepared from *M. tuberculosis*, *M. kansasii*, *M. phlei*, *M. paratuberculosis*, and *M. smegmatis* and found that Crohn's disease patients have elevated antibody titers to all species examined. Serologic studies conducted with a crude antigen and three antigenic fractions of *M. paratuberculosis* also showed a slightly but insignificantly increased antibody titer in Crohn's disease patients compared with controls. Such widespread reactivity to PPD, probably based on a ubiquitous cross-reactive antigen, is highly indicative of sensitization by environmental organisms gaining immune access through a defective mucosal barrier.

Kobayashi et al. (147) sought antibodies to mycobacteria in Crohn's disease patients by enzyme-linked immunosorbent assay, using lipoarabinomannan and a protoplasmic antigen preparation of *M. paratuberculosis* as the antigen. These authors failed to find any significant elevation in IgA, IgG, or IgM antibody levels in Crohn's disease compared with controls. On the basis of their findings, these authors concluded that, since all chronic infections have an associated serologic response to the etiologic agent, their failure to find a response in Crohn's disease greatly diminishes the likelihood of mycobacteria as etiologic agents. This statement is not entirely correct. For example, patients with the tuberculoid form of leprosy by definition fail to mount a humoral immune response (46, 119, 158, 202). Also, although these authors attempted to address and correct errors made in other studies, their results with control antigens did not agree with those reported previously. Crohn's disease patients have a generalized increased antibody response to enteric organisms (11, 25, 86, 154), but in the study by Kobayashi et al. (147) antibody titers to lipid A were not increased. The use of lipoarabinomannan as a broad mycobacterial antigen may also be inappropriate because most normal individuals have demonstrable lipoarabinomannan titers, probably related to exposure to environmental mycobacteria. Although patients with mycobacterial diseases such as leprosy and tuberculosis generally have lipoarabinomannan titers higher than those of control groups, it is unclear whether other mycobacterioses produce similar responses. This study was also the first to attempt duplication of the serologic results of Thayer et al. (274) by using a similar protoplasmic antigen. Some questions have been raised regarding the nature of their preparation, however, since Kobayashi et al. reported that this antigen had at least 20 sodium dodecyl sulfate-polyacrylamide gel electrophoresis bands while Thayer et al. reported that their antigen contained only 5 such bands (W. R. Thayer, J. A. Coutu, R. J. Chiodini, and H. J. Van Kruiningen, Gastroenterology **88**:1613, 1986).

Markesich et al. (167) have studied the interaction of peripheral blood monocytes with *M. paratuberculosis* to determine whether monocytes from Crohn's disease patients react differently to mycobacteria compared with controls. These investigators found that macrophages from Crohn's disease patients inhibited growth more efficiently than those from controls and that the survival of *M. paratuberculosis* in Crohn's disease monocytes was significantly less than that in controls. The authors noted that their study was conducted with a limited number of patients, and the possibility or effect of increased activated macrophages in Crohn's disease patients (180, 207) was not assessed.

Das et al. (P. K. Das, J. L. G. Blaauwgeers, A. W. Slob, J. Spies, A. Chand, A. Kolk, and H. J. Houthoff, Gastroenterology **94**:A88, 1988) examined the possible relationship of

mycobacteria and Crohn's disease by using immunoblot analysis and a lymphoproliferative assay. They found that sera from patients with Crohn's disease reacted with various mycobacterial and gut-associated antigens and that many seroreactive epitopes were shared between mycobacteria and human gut tissue. Thus, they concluded that the pathogenesis of Crohn's disease could involve either cross-reactive epitopes or idiotypes, without the persistent presence of viable mycobacteria. Their lymphoproliferative assay showed that lymphocytes from five of six patients with Crohn's disease reacted specifically to *M. paratuberculosis* antigens, whereas those from control, ulcerative colitis, and bowel cancer patients did not. Other than a brief, inconclusive report by Thayer et al. (W. R. Thayer, J. A. Coutu, R. J. Chiodini, and H. J. Van Kruiningen, Gastroenterology **90**:1662, 1986), these preliminary studies represent the only investigation of CMI responsiveness to mycobacterial antigens in Crohn's disease.

Ajitsu et al. (S. Ajitsu, S. Mirabella, and H. Kawanishi, Gastroenterology **94**:A4, 1988) examined the immunologic responsiveness of murine intestinal tissues by studying the response of gut-associated lymphoid tissues to orally administered *M. paratuberculosis* antigens in young and old mice. They found that cells from old mice responded to *M. paratuberculosis* antigens to a much greater extent than those from young mice (stimulation index, >10 versus <3). Also, cells from old mice responded by producing IgG, IgM, and IgA, while cells from young mice produced only low levels of IgA. These authors concluded that the oral tolerance to *M. paratuberculosis* antigens in old mice is impaired, that the gut mucosal immunity in these older mice is hyperreactive, and that these age-associated features are due in part to impaired antigen-specific T suppressor cells with overreactive antigen-specific B and T helper cells. Although not suggested by the authors, their observation that young animals respond poorly to oral *M. paratuberculosis* challenge compared with aged mice could explain why *M. paratuberculosis* appears to infect successfully only young animals. The hyperreactive mucosal immunity in the aged mice could explain the age-dependent resistance to *M. paratuberculosis* infection (52).

Discussion. Crohn's disease patients do not have any consistent, reproducibly significant antibody response against mycobacterial antigens. Some studies have demonstrated responses in some patients, while others have not. Patients with mycobacterioses usually have a humoral immune response; therefore, its lack in Crohn's disease could be strong evidence against the etiologic role of mycobacteria in this disease. While some patients with pulmonary tuberculosis (45, 76, 78, 201) and, occasionally, lepromatous leprosy (46, 47, 119, 202) fail to elicit a humoral immune response, these cases are generally associated with bacterial overload and anergy. Immunologic nonresponsiveness could also be caused by advancing age (235, 284), debility (78), or malnutrition (78, 158). Such is not the case in Crohn's disease. However, during certain periods in mycobacterioses, a humoral immune response is not demonstrable. Primary immunity to mycobacteria is cell mediated. Only as the disease progresses, and there is an increasing bacterial load, does the humoral immune response become activated with the production of antibody. Patients with polar tuberculoid leprosy, in which there is a low bacterial load, fail to elicit a humoral response to *M. leprae* (119, 277). Most cattle with paratuberculosis have a low antibody response, but it is often not greater than that in noninfected animals (52). Cattle with overt clinical disease, unless anergic, do have a significant antibody response compared with control cattle; variable immune responsiveness occurs in animals during subclinical disease, which is perhaps more relevant to Crohn's disease. Clinical paratuberculosis (severe diarrhea and rapid weight loss) is considered the terminal stage of the disease since animals generally die within a few months after clinical onset (52). During this subclinical period, when animals appear normal but suffer subtle decreased productivity, weight loss, and increased susceptibility to other infections (51, 52, 151), immunologic responses are not readily distinguishable from those of noninfected animals (52). The level of immunity in these animals probably is masked by cross-reactive responsiveness to environmental mycobacteria and related organisms (2, 66, 95, 121, 155, 156, 177–179, 220, 233). Only in recent years has the use of purified antigens been successful in diminishing some of the nonspecific cross-reactive responses (1, 305) and shown that each animal species, i.e., cattle, sheep, and goats, responds serologically to different antigenic determinants (248, 249). In humans it may be necessary to examine tissue lymphoid cells rather than those of the peripheral blood (82, 134, 191, 192) to seek antigens which may be masked and therefore not demonstrable (252–255), to purify antigens to reduce nonspecific reactions due to environmental mycobacteria (2, 66, 95, 121, 155, 156, 177, 178, 220, 233), and/or to define antigenic determinants recognized by the human immune system since they may be different from those of ruminants.

At this time, insufficient information is available on the immune response of early paratuberculosis in cattle to judge whether a demonstrable immune response is present and how it is elicited. It is also unclear what type of immune response occurs in animals with tuberculoid-type paratuberculosis, in which acid-fast bacilli are not demonstrable but are culturable (32, 52). A tuberculoid response, i.e., DTH reaction to *M. paratuberculosis* antigens, would be expected in these animals, but more often than not they fail to mount any humoral, cellular, or DTH reaction (52). Likewise, no information is available on the immunologic responses in primary human intestinal tuberculosis. The only report on CMI responses in intestinal tuberculosis (22) does not define the disease as primary or secondary or specify whether concurrent pulmonary disease was present. Patient histories suggest that those with ulcerative or ulcerotrophic (secondary) tuberculosis generally respond to intradermal injection of tuberculin PPD, unless pulmonary disease is far advanced (anergy). This response would be expected because most patients with pulmonary tuberculosis are PPD reactive. Patients with primary intestinal tuberculosis caused by *M. bovis* likewise produce a positive response to PPD, but patients with hypertrophic intestinal tuberculosis are generally nonresponsive to PPD if the infection is caused by *M. tuberculosis* rather than *M. bovis* (3). Even in cases when *M. tuberculosis* has been isolated, the patients do not respond to skin tests. Thus, PPD reactivity is of no diagnostic value in primary hypertrophic intestinal tuberculosis if the causative agent is *M. tuberculosis* (3). The reason(s) for this lack of reactivity is unknown and has not been investigated. It would be important to determine both humoral and CMI responses in subclinical tuberculoid paratuberculosis and intestinal tuberculosis, particularly the hyperplastic types. Such information on defined mycobacterial disorders would be invaluable for understanding the lack of consistent immunologic reactivity in Crohn's disease if the etiology is related to a *Mycobacterium* sp.

Since Crohn's disease is a granulomatous disease, and therefore assumed to be DTH mediated, it would be appro-

priate to study CMI rather than humoral responses. If an infectious agent is present in Crohn's disease, it is present in low numbers and probably not in sufficient amount to stimulate a humoral immune response. The presence of granulomas in Crohn's disease is evidence of a functional and responsive CMI system, and such responses should be measurable. However, if the agent is similar to *M. paratuberculosis*, it may be difficult to sort specific responses from cross-reactive responses without using purified antigens or defined antigenic determinants recognized by the human immune system. Although an attempt to demonstrate a CMI or DTH response to mycobacteria in Crohn's disease is the obvious route of investigation, as yet only limited inconclusive studies have been performed.

Histochemical Data

The ultimate goal in determining the etiologic relationship of an organism to a disease state is to demonstrate the association of that organism with the lesions. Routine acid-fast staining has not been successful in Crohn's disease; the absence of acid-fast bacilli was a criterion for the classification of Crohn's disease as a distinct disease entity. There have been few reports on the search for mycobacterial antigens or acid-fast bacilli in tissues from patients with Crohn's disease, but no good evidence of tissue lesion-associated mycobacteria has been found.

The first published attempt to identify mycobacteria in tissue sections from patients with Crohn's disease was a report by Whorwell et al. (295) in 1978. These investigators sought evidence for the presence of *M. kansasii*, in addition to other pathogenic microorganisms, by immunofluorescence in tissues from patients with Crohn's disease and ulcerative colitis. No evidence of infection was found. Haga (115) also reported that he was unable to demonstrate *M. paratuberculosis* antigens in 18 Formalin-fixed tissues from Crohn's disease patients by immunohistochemistry, using anti-*M. paratuberculosis* antisera.

On the other hand (as discussed under cultural data), Yoshimura et al. (307), by liquid genomic DNA-DNA hybridization, were able to detect mycobacterium-related sequences in 53% of Crohn's disease patients, 33% of ulcerative colitis patients, and 17% of controls. None of these sequences, however, were identical to *M. paratuberculosis*, and whether or not the methodology was sensitive and specific enough to determine whether the sequences were even mycobacterial in origin is questionable.

Van Kruiningen et al. (283) used peroxidase-antiperoxidase immunohistochemistry to demonstrate mycobacteria in Crohn's disease tissues. They examined 50 Formalin-fixed, paraffin-embedded tissues from 15 patients with Crohn's disease and found positive staining in areas of submucosal inflammation in 3 patients. One of the positive specimens was considered possibly to represent phagocytized erythrocytes and another was possibly due to cross-reactions with non-acid-fast organisms around an abscess, but the third could not be accounted for by any artifact. Control, non-Crohn's disease tissues were not examined in this study. These authors acknowledged that their method, although successful in identifying organisms in positive control specimens, did not demonstrate mycobacteria in tissues from animals with experimentally induced intestinal mycobacterial infections. By conventional acid-fast staining, these tissues were either acid-fast negative or contained very few demonstrable organisms.

Kobayashi et al. (148) failed to demonstrate mycobacteria in Crohn's disease tissues by immunohistochemical methods. They examined 67 specimens (from 30 patients with Crohn's disease), fixed in Formalin or periodate-lysine-paraformaldehyde or not fixed and reacted with anti-*M. paratuberculosis*, anti-*M. tuberculosis*, and monoclonal anti-lipoarabinomannan antisera. Although staining was observed in control tissues, e.g., lymph node from an AIDS patient, pulmonary tissue from two patients with mycobacterial infection, and liver from rats infected with *M. kansasii*, *M. fortuitum*, *M. paratuberculosis*, or spheroplasts of MAI serovar 26, no staining was observed in any of the Crohn's disease specimens. These authors indicated that their negative finding "eliminates mycobacteria as causing Crohn's disease in any conventional way." They assumed, however, that experimentally induced CWD forms in a constant state of reversion (as suggested by the presence of acid-fast and non-acid-fast forms) are immunologically identical to naturally occurring CWD forms and that their antisera which reacted with these experimental forms would also react with naturally occurring forms. Likewise, the detection of lipoarabinomannan in experimentally induced unstable CWD forms does not indicate that lipoarabinomannan exists in stable, natural CWD forms. At least one of the antisera they used was previously shown to cross-react with naturally occurring *M. paratuberculosis* spheroplasts (54). Their methods could detect large numbers of acid-fast bacilli (e.g., in tissues from AIDS patients or experimentally inoculated animals) as well as a few organisms visualized by acid-fast staining. Staining methods generally reveal 10^5 to 10^6 organisms per g of tissue, i.e., reasonably large numbers. It has been shown that, in some experimentally induced mycobacterial intestinal diseases, mycobacteria could not be detected by immunohistochemistry even though acid-fast bacilli were known to be the cause of the lesion and could be cultivated. Demonstration of acid-fast bacilli by peroxidase-antiperoxidase immunohistochemistry was effective only in sections containing organisms visible by acid-fast staining (283; R. J. Chiodini, J. A. Erickson, H. J. Van Kruiningen, W. R. Thayer, and J. A. Coutu, Gastroenterology **90**:1372, 1987). It is unclear whether low concentrations of a *Mycobacterium* sp., e.g., 1,000, 100, or even 10 per g, are capable of producing disease in the gastrointestinal tract, but some documented cases of intestinal tuberculosis and paratuberculosis and experimental studies suggest that very low numbers of organisms can cause a progressive disease.

Colemont et al. (L. J. Colemont, S. R. Pattyn, P. P. Michielsen, J. H. Pen, P. A. Pelckmans, Y. Van Maercke, and F. Portaels. Lancet **i**:294–295, 1988; Pattyn, personal communication), using simple acid-fast staining techniques was successful in identifying mycobacteria in 11 of 32 (34%) specimens from tissues with Crohn's disease. Identification of these acid-fast bacilli was not possible and immunohistochemistry was not performed.

Das et al. (P. K. Das, J. L. G. Blaauwgeers, A. W. Slob, J. Spies, A. Chand, A. Kolk, and H. J. Houthoff, Gastroenterology, **94**:A88, 1988), using mycobacterial monoclonal antibodies, demonstrated that a particular B-cell subset was present predominantly in Crohn's disease patients. Although these antimycobacterial antibody-reactive subsets were also present in non-Crohn's disease tissues, they were present in lesser numbers and in a different distribution pattern.

Discussion. The inability to detect mycobacteria or their antigens in tissue from patients with Crohn's disease is perhaps the most damaging evidence against mycobacteria as etiologic agents; however, if an agent associated with Crohn's disease could be easily demonstrated, it would have been found years ago. It is also unclear why one investigator

failed to find any evidence of mycobacteria in 30 patients with Crohn's disease (148), another found evidence in 1 of 15 patients with Crohn's disease (282), and yet another found evidence in 34% of Crohn's disease specimens (Colemont et al. Letter, Lancet **i**:294–295, 1988). As Rubin and Pinner (237) have said about mycobacteria and sarcoidosis, "the failure to find tubercle bacilli in the majority of cases . . . is not a convincing argument against tuberculosis as an etiologic factor, nor is the occasional tubercle bacillus which is found positive proof that tuberculosis is the cause."

If an infectious agent is present in Crohn's disease, it is either localized in very small foci or scattered in small numbers, because immunohistochemical studies performed with polyclonal antisera should have identified any *Mycobacterium* spp. present. If mycobacteria are present, they are below the level demonstrable by the methods applied. Immunohistochemical techniques intensify staining of acid-fast-positive sections in general, but their ability to detect mycobacteria in acid-fast-negative tissue is questionable (Chiodini et al., Gastroenterology **90**:1372, 1986). Such techniques need to be developed and standardized and shown to work in tissues with low numbers of acid-fast bacilli, e.g., in culture-positive, acid-fast stain-negative cases of intestinal tuberculosis or paratuberculosis. Even more sensitive methods may need to be applied, such as DNA hybridization; however, these techniques also have limitations. Specific genetic probes need to be obtained to prevent hybridization with complementary sequences of other microbes and eucaryotic cells. Liquid genomic DNA-DNA hybridization is probably not specific or sensitive enough to detect low numbers of organisms, and in situ hybridization is an extremely difficult technique which only a few laboratories are equipped to use. If the current etiologic theory is correct, additional problems become inherent. Can or will tissue DNA extractions liberate mycobacterial DNA since mycobacteria are so difficult to lyse? Application of standard tissue methods are not likely to liberate DNA from intact mycobacteria. Exposure of these organisms to chloroform (used in DNA extraction) generally makes the mycobacterial cell wall more rigid and even more difficult to lyse. If the organisms exist in a CWD form, what is their stability after tissue death and what influences do exogenous eucaryotic deoxyribonucleases have on their genome? These factors need to be addressed and circumvented to adequately and convincingly conduct DNA hybridization studies. Again, acid-fast-negative, culture-positive cases of intestinal tuberculosis or paratuberculosis would be the ideal model system for developing these techniques, and unless techniques are shown to be effective in these circumstances, the data generated will not be conclusive. These diseases provide a model of progressive granulomatous intestinal infection caused by a very few mycobacteria which cannot be seen by acid-fast-staining methods.

Animal Model Data

Of all the putative agents of Crohn's disease that have been isolated, until recently none have been shown to be pathogenic for laboratory animals, humans, or, specifically, the gastrointestinal tract. Because it has been assumed that its etiologic agent would show preference for gastrointestinal tissues, appropriate animals models have been sought to demonstrate the pathogenic potential of agents isolated from the tissues of Crohn's disease patients. In addition, such studies have been conducted as an indirect means of fulfilling Koch's postulates and thereby convincingly identifying the etiology of Crohn's disease.

In 1984, using infant goats, Chiodini et al. (56) described the first successful production of a granulomatous ileocolitis, or Crohn's disease-like infection, in experimental animals with a putative etiologic agent. Further, more detailed studies with this goat animal model were later published in 1986 (282). Oral inoculation of goats with the putative agent, later identified as *M. paratuberculosis*, produced intestinal disease in approximately 5 to 6 months. The earliest lesions occurred within Peyer's patches of the ileum and consisted of noncaseous granulomatous clusters of epithelioid cells which often occurred in a mantle of lymphocytes between germinal centers and the muscularis mucosae, quite similar to the early lesions of Crohn's disease. Other features of the disease included tuberculoid granulomas without caseation, confluence of granulomas, ulcerations of the mucosa, and lymphocytic lymphangitis. Several animals had no demonstrable acid-fast bacilli, although the bacillary organisms were isolated from all except controls. The authors concluded that the lesions produced in these animals were distinctly similar to those occurring in Crohn's disease.

Gitnick et al. (97; personal communication) inoculated infant goats with a strain of *M. chelonei* subsp. *abscessus* isolated from a Crohn's disease patient, but failed to produce a granulomatous intestinal disease. These animals developed a transient diarrhea; intestinal lesions were limited to mild inflammation and colonic infiltration with polymorphonuclear cells. Others lesions seen in these animals were also present in controls, and the findings were complicated by the presence of parasites. These investigators also inoculated infant goats with a strain of *M. paratuberculosis* isolated from a Crohn's disease patient, but again, failed to produce a granulomatous intestinal response; all animals remained normal during the 5-month observation period, and lesions were not detected at necropsy.

The failure of these investigators to produce disease in ruminants with a human isolate of *M. paratuberculosis* is surprising because this organism is the etiologic agent of Johne's disease and there are no known nonpathogenic strains of *M. paratuberculosis*. In our original goat studies (53, 282), animals were fed viable organisms, while Gitnick et al. inoculated animals by stomach tube. Although the latter method would presumably be more precise than feeding, we also failed to produce disease by this method (unpublished data). Some physiologic change could occur by feeding *M. paratuberculosis*, which is essential to pathogenicity and which plays a role in the natural morbidity of this infection. Experimental infection studies with *M. paratuberculosis* in animals have always used natural feeding (52); to my knowledge, no attempt has been made previously to infect animals by using a stomach tube.

Recent data on natural *M. paratuberculosis* infection in animals have relevance to the animal model studies described above. McClure et al. (172) described an epizootic of naturally occurring paratuberculosis in subhuman primates, stump-tailed macaques. Prior to this time, *M. paratuberculosis* was considered innocuous in primates, and this paper raised concerns regarding the potential public health implications of *M. paratuberculosis* infection. Several interesting features of paratuberculosis in subhuman primates were brought out by this article. Even though tissues containing large numbers of acid-fast bacilli were submitted to several laboratories for culture, none of the laboratories were successful in isolating the organism and they reported either unculturable mycobacteria or unidentified acid-fast bacilli. The organism was not isolated until tissues were processed for *M. paratuberculosis* 2 years after the initial clinical case.

Although the entire animal colony was thought to be infected on the basis of cultural data, no animal responded to tuberculin PPD or *M. paratuberculosis* antigens (johnin). Antibodies to *M. paratuberculosis* were present in all animals except those with clinical disease. Thus, it was noted that neither cell-mediated nor humoral immune responsiveness could be used to determine epidemiologic or diagnostic data. The authors concluded that either the *M. paratuberculosis* strain isolated had unique features that allowed it to infect subhuman primates or the stump-tailed macaque was a susceptible host that had previously not been exposed to the organism. Finally, several severely ill animals were successfully treated with an experimental antimycobacterial agent, rifabutin (Adria Laboratories, Columbus, Ohio), in combination with kanamycin, with subsequent disease remission. This was the first report of successful treatment of paratuberculosis and paved the way for drug trials in humans with Crohn's disease (to be discussed later).

Czuprynski et al. (63) described the interaction of *M. paratuberculosis* with bovine macrophages and the experimental infection of gnotobiotic mice with *M. paratuberculosis*. They found that intracellular growth of *M. paratuberculosis* could be restricted or enhanced by monocyte treatment with various cytokines. Intracellular replication was restricted by crude interferon or recombinant alpha interferon, and enhanced growth resulted when monocytes were treated with a crude cytokine preparation obtained from an immunized animal. They concluded that production of this cytokine required the presence of both immune cells and *M. paratuberculosis*, suggesting that its production was dependent on specific lymphocytes (311). Experimental intragastric inoculation of euthymic and athymic mice with *M. paratuberculosis* was also reported by these authors. In euthymic mice, a persistent low-level colonization occurred, while in athymic mice, progressive multiplication and consistent fecal shedding of *M. paratuberculosis* occurred. Organisms were recovered from the ilea of both animal groups, but were more abundant in athymic mice which also had demonstrable focal acid-fast clusters and occasional granulomas. *M. paratuberculosis* did not replicate in the intestinal lumen, even in the absence of competing microbes, but multiplication occurred in the mucosa. These findings are in agreement with those of others who demonstrated the inability of *M. paratuberculosis* to replicate outside the intestinal tissues and associated lymph nodes of its host (146, 184, 185).

Momotani et al. (193) studied the mechanism of *M. paratuberculosis* infection in ligated ileal loops of calves. Within 5 h after inoculation, *M. paratuberculosis* had penetrated the intestinal lining and acid-fast bacilli could be visualized within subepithelial macrophages. By 20 h, >50 bacilli per section were within subepithelial macrophages and in the supranuclear cytoplasm of M cells. Specific antisera seemed to enhance entry of *M. paratuberculosis*. It was concluded that *M. paratuberculosis* invades the intestines through the ileal M cells and that the subepithelial and intraepithelial macrophages secondarily phagocytize bacilli or bacterial debris which are expelled from the M cells.

Some recent animal experiments conducted at the University of Wisconsin in Madison yielded some unexpected results which also have relevance to animal models of Crohn's disease (H. A. Mokresh, S. Hurley, C. Czuprynski, and D. Butler, personal communication). Newborn rabbits were orally inoculated with *M. paratuberculosis* and necropsied at various time periods to determine whether intestinal lesions could be produced in these animals. Some developed a transient diarrhea and some developed a few intestinal granulomas. Interestingly, culture and prolonged incubation (11 to 15 months) of fecal and ileal tissue homogenates from some rabbits resulted in the growth of very small translucent colonies. The organisms stained poorly and, on electron microscopic examination, were found to be morphologically identical to the mycobacterial spheroplasts described previously in Crohn's disease (54). These CWD forms could not be subcultured. Should these results be repeated, they would represent the first successful in vivo transformation of mycobacteria into CWD forms. They may also represent an animal model for the physiologic and morphologic changes in *M. paratuberculosis* which may be occurring in the human intestine. These preliminary results have inspired further efforts currently in progress.

Discussion. Perhaps as a follow-up to the suggestions first made by Dalziel (64) in 1913, and later by Golde and McGill (101) and Patterson and Allen (218), Morgan (194) recently published a theoretical paper comparing Crohn's disease and Johne's disease (paratuberculosis) and suggested that, on the basis of previously reported experimental and epidemiologic data, these two diseases had similar etiologies. He noted the difficulties encountered in experimentally transmitting Johne's disease, not only to other cattle, but also to laboratory animals, as well as a host of other remarkable similarities. This author believed that the similarities and disease histories were too remarkable to be coincidental.

It has been disputed that the experimental production of a granulomatous ileocolitis in goats with human isolates of *M. paratuberculosis* has little meaning and does not support an etiologic role of this agent in Crohn's disease (D. Y. Graham, D. C. Markesich, and H. H. Yoshimura, Letter, Dig. Dis. Sci. **33**:251–252, 1988). Since the putative agent of Crohn's disease has been identified as *M. paratuberculosis*, the experimental infection is not Crohn's disease-like, but, expectedly, is only Johne's disease. Such a conclusion, while taxonomically correct, should not be viewed as a simple distinction based on disease classification and thereby separating two similar diseases. If Crohn's disease is not caused by *M. paratuberculosis*, then clearly the granulomatous ileocolitis produced in ruminants by human strains of *M. paratuberculosis* is Johne's disease. But, if Crohn's disease is caused by *M. paratuberculosis*, then the experimental infection produced in animals does represent Crohn's disease, even if the appropriate classification of the infection in animals is Johne's disease.

The isolation of a known animal pathogen from human patients with Crohn's disease has more implications to etiology than the isolation of a new or unknown species with no defined pathogenic characteristics. That *M. paratuberculosis* is not an environmental organism and cannot replicate in the environment, readily penetrates and has a predilection for the gastrointestinal tract, produces a noncaseating granulomatous intestinal disease in animals, and has been isolated from the diseased tissues of patients with Crohn's disease must at least raise questions of coincidence and cause investigators to be suspicious.

Treatment Data

Reports on the treatment of Crohn's disease with antimycobacterial agents are sporadic and generally not double-blinded or well controlled with placebo treatment. Many are individual case reports (J. B. Warren, H. C. Rees, and T. M. Cox, Letter, N. Engl. J. Med. **314**:182, 1986; A. Picciotto, G. P. Gesu, G. C. Schito, R. Testa, G. Varagona,

and G. Celle, Letter, Lancet i:536–537, 1988; C. Prantera, R. Argentieri, and R. Pangiarotti, Letter, Lancet i:536, 1988) or involve few patients (216, 217, 273, 302). Antimycobacterial drugs have been selected randomly, on the basis of their effectiveness in tuberculosis and not on efficacy with other mycobacterial species. Nevertheless, most studies have shown improvement, often long term, in Crohn's disease patients following administration of antimycobacterial chemotherapeutic agents. These long-term effects, usually in severely ill patients, help rule out a placebo effect, but do not eliminate the possibility of spontaneous disease remission.

The only double-blinded study of antimycobacterial agents in Crohn's disease used rifampin and ethambutol with sulfasalazine and steroids versus sulfasalazine and steroids alone (245). Pursuing the possibility that *M. kansasii* was etiologically related to Crohn's disease, in 1984 these investigators reported on a 2-year, randomized, double-blind, crossover, controlled trial with 27 patients. Thirteen patients were withdrawn before completion due to poor compliance, the need for surgical intervention, or adverse effects. Of the 14 patients who completed the trial, 4 required surgery, 5 were withdrawn because of poor compliance, and 5 were withdrawn because of drug side effects. Thus, results were based on few subjects and shorter treatment periods than originally planned. Nevertheless, analysis of the data suggested that there was no significant difference in response to the antimycobacterial drugs compared with sulfasalazine and steroids when expressed in terms of the Crohn's disease activity index or any other clinical indicator of disease activity, Also, there was no consistent pattern of change in the requirement for steroids in patients receiving antimycobacterial drugs. From their experience and data, these authors concluded that rifampin and ethambutol have no place in the treatment of Crohn's disease and that it was unlikely that *M. kansasii* was etiologically significant (245).

Recently, there has been a surge of interest in the treatment of Crohn's disease with antimycobacterial agents, which undoubtedly has been precipitated by the suggestions of *M. paratuberculosis* as the etiologic agent of this disease. Of the studies recently conducted or in progress, most have used rifabutin, a rifampin derivative, either alone or in combination with other drugs. The interest in rifabutin as the antimycobacterial drug of choice is due in part to its high in vitro activity against *M. paratuberculosis* (unpublished data) and its successful use in the treatment of paratuberculosis in subhuman primates (172). The manufacturer of rifabutin has been very supportive of its use as a chemotherapeutic agent in Crohn's disease. Nevertheless, the data accumulated to date have been poorly organized and difficult to interpret.

Basilisco et al. (G. Basilisco, T. Ranzi, M. C. Campanini, L. Piodi, and P. A. Bianchi, XIII Int. Congr. Gastroenterol. 1988, abstr. no. 637; G. Basilisco, personal communication) reported on the use of rifabutin in a randomized double-blind trial in 24 patients with active Crohn's disease. Twelve patients received 300 mg of rifabutin daily and 12 received a placebo for 6 months. Nine patients (5 from the treated group and 4 from control group) dropped out before completion. Of the remaining patients, five from the rifabutin-treated and six from the placebo-treated groups improved transiently, but in neither group was there any evidence of long-term improvement in lesions or disease course. There was a high incidence of drug-related side effects, described as a flulike syndrome, which precluded further studies.

Rutgeerts et al. (P. Rutgeerts, G. Vantrappen, J. Van Isveldt, and K. Geboes, Gastroenterology **94**:A391, 1988) reported on the use of rifabutin and ethambutol for 6 months in 16 patients with Crohn's disease. In this trial, patients were evaluated based on pretrial and follow-up ileocolonoscopy and biopsies. Six patients withdrew from the trial due to the flulike syndrome, and of the remaining 10, 7 completed the trial. No improvement was noted by endoscopy during the trial period, and it was concluded that rifabutin-ethambutol treatment had no effect on the lesions of Crohn's disease. These investigators also noted the high occurrence of flulike symptoms in patients on rifabutin and suggested that this high incidence might be due to an inherent disorder in Crohn's disease.

Mulder (personal communication) initially performed a 6-month open trial with rifabutin and ethambutol in eight patients with Crohn's disease. Two patients improved clinically and are still doing well after 1 year. Of the remaining six, all of whom declined the recommended surgery before entering the trial, no effect was noted in three and a temporary improvement was observed in two, but surgical intervention was required on all. The eighth patient developed intestinal scarring, but otherwise improved with complete ulcer healing. These studies prompted the initiation of a small double-blind placebo trial in 15 patients, 7 receiving rifabutin and ethambutol and 8 receiving placebo. Although some improvement has been noted in the treated patients, the data are not very convincing. Two patients on placebo and one on drugs required surgery; laboratory parameters improved in one of the drug-treated patients as opposed to improvement in none and deterioration in three placebo-treated patients; the Harvey, Bradshaw, and Scope Crohn's disease index improved in three and deteriorated in one drug-treated patient versus improvement in none and deterioration in three placebo-treated patients; and steroid dependence was reduced in one and increased in one drug-treated patient versus no reduction and increased dependence in two placebo-treated patients. Although there may be a minor effect on drug-treated patients, the data are not very encouraging.

Thayer et al. (W. R. Thayer, J. A. Coutu, R. J. Chiodini, and H. J. Van Kruiningen, Gastroenterology **94**:A458; unpublished data) used rifabutin in combination with streptomycin in an open trial with 12 patients with Crohn's disease. Streptomycin (1 g) was given intramuscularly 5 days a week for 2 to 4 months, and rifabutin was given orally at 300 mg/day for a minimum of 6 months or until drug withdrawal was elected. All patients were treated on a compassionate basis due to severe refractory Crohn's disease or because of extensive or repetitive fistularization and abscess formation. All patients have reportedly improved clinically, commonly with prednisolone withdrawal, healing of fistularization, and marked improvement in their Crohn's disease activity index. Of the 12 patients, 8 (66%) have completely withdrawn from steroids, 2 of 2 (100%) have withdrawn from 6-mercaptopurine or other drugs, 7 of 7 (100%) no longer have rectal bleeding, 4 (33%) have endoscopic healing of lesions, and 1 of 2 (50%) have radiographic improvement. These improvements were generally not noted until after at least 4 months of treatment and were most prominent after 6 months. Some patients failed to respond until after 6 months of treatment, thereby illustrating the need for long-term therapy in this chronic disease.

Hampson et al. (S. J. Hampson, M. C. Parker, S. H. Saverymuttu, J. J. McFadden, and J. H. Taylor, Gastroenterology **94**:A170, 1988) treated 17 Crohn's disease patients with quadruple antimycobacterial chemotherapy. The antimicrobial agents used in combination were rifampin, ethambutol, isoniazid, and pyrazinamide, with clofazimine replac-

ing pyrazinamide in a few cases. Of the 17 patients, 12 (71%) had a statistically significant improvement in their Crohn's disease activity index and 9 of 10 (90%) had been completely withdrawn from steroids. To date, these investigators have treated 20 patients with quadruple therapy, and after 9 months of treatment, 11 of 20 (55%) are considered to be in disease remission. Based on indium-111 scans, objective evidence of improvement after 1 year of treatment was found in 14 of 20 (70%) patients (S. A. Hampson, personal communication).

The flulike syndrome (which may or may not be associated with leukopenia) frequently observed in patients with Crohn's disease receiving rifampin or its derivatives is not understood. Other patients receiving rifampin or rifabutin, including patients with tuberculosis, leprosy, atypical mycobacteriosis, or AIDS, do not develop these symptoms; the flulike syndrome appears unique to Crohn's disease patients. Although this syndrome was considered grounds for withdrawal from therapy in some studies, most consider that the severity is not sufficient to warrant drug withdrawal. Patients receiving steroids at the time rifabutin therapy is initiated fail to develop flu symptoms; i.e., steroids prevent the flulike syndrome. Thus, this condition is probably either related to drug toxicity or, considering the intervention with steroids, some form of immune phenomenon.

Discussion. Although a mycobacterial etiology of Crohn's disease has been considered for well over 50 years, few studies have been conducted and little conclusive data are available on the effects, beneficial or not, of antimycobacterial chemotherapy. Often studies have been performed with little forethought and without supportive laboratory data.

Shaffer et al. (245) failed to show any clear benefit of rifampin and ethambutol chemotherapy in Crohn's disease; however, several recent investigators chose and used rifabutin and ethambutol rather than evaluate other drug combinations. While rifampin and its derivatives show high in vitro activity against *M. paratuberculosis*, ethambutol does not (55) and should be considered a poor choice for dual therapy. Also, the use of rifabutin monotherapy would be expected to fail since the combination of rifampin and ethambutol is not effective and monotherapy is rarely, if ever, effective for treating mycobacterial diseases. The only studies, other than case reports, in which Crohn's disease patients have apparently shown improvement following antimycobacterial chemotherapy have been those that use rifabutin in combination with an aminoglycoside (which shows high in vitro activity) or quadruple therapy. Unfortunately, most antibiotics to which *M. paratuberculosis* strains are susceptible in vitro are not available in oral preparations, leading to more difficulty in obtaining patient compliance and approval for experimental human use.

If Crohn's disease is caused by an organism similar to *M. paratuberculosis*, treatment schemes would need to follow current recommendations for other mycobacteria other than tuberculosis infections. General guidelines for the treatment of pulmonary mycobacterioses in nonimmunocompromised patients include at least quadruple drug therapy and treatment durations of 2 to 3 years (6, 14, 129, 130, 278). Although the efficacy of treating pulmonary disease caused by MAI (closely related to *M. paratuberculosis*) has not been clear, current evidence suggests that treatment may be effective after prolonged periods (6, 14, 130, 278). In at least one recent study, clinical improvement of pulmonary MAI infection required 3.6 ± 0.5 years of continuous chemotherapy (129). Such examples need to be evaluated in considering antimycobacterial chemotherapeutic regimens in the treatment of Crohn's disease.

In addition to the multiple-drug regimens and prolonged therapy, whether or not antimycobacterial chemotherapy would be effective in Crohn's disease even if the etiology was mycobacterial also needs to be considered; chemotherapy is generally not effective in any known intestinal mycobacteriosis. In the treatment of hypertrophic ileocecal tuberculosis, surgical intervention is generally required since chemotherapeutic drugs alone are ineffective (3, 41). Some investigators consider that chemotherapy in hypertrophic intestinal tuberculosis is not necessary and should be provided, if at all, only as an adjunct to surgical intervention (41). Although paratuberculosis has been known as an intestinal mycobacterial disease since 1895, it has yet to be successfully treated despite the use of a wide range of antimicrobial agents (9, 52, 93, 94, 183, 230, 231). Even the prophylactic treatment of animals with antimycobacterial agents does not prevent experimental intestinal infection (231). Thus, the efficacy of treating intestinal mycobacterial diseases in general needs to be considered.

Perhaps with the advent of new generations of drugs, and the recent successful treatment of intestinal paratuberculosis in subhuman primates with rifabutin in combination with kanamycin (172), effective chemotherapeutic drugs will become available. Nevertheless, data on the effects of antimycobacterial chemotherapy in Crohn's disease are needed and further evaluations of antimycobacterial drugs are warranted, particularly in view of the many case reports suggesting their efficacy. Future treatment studies, however, need to be supported with solid laboratory data rather than just random selection of antibiotics. These studies should include not only in vitro susceptibility of organisms to individual antibiotics, but also antagonistic and synergistic effects of multiple drugs. Ideally, in vitro susceptibility profiles should be evaluated for treatment efficacy in an animal model system. Without background data on which to base human treatment schemes, such efforts will be greatly hindered. Last, one must consider that, because antimycobacterial agents have broad activity, their effectiveness in Crohn's disease, should it exist, is only supportive and is not conclusive evidence of a mycobacterial etiology.

SIMILARITIES BETWEEN CROHN'S DISEASE AND OTHER MYCOBACTERIAL DISEASE

As has been noted since the first description of Crohn's disease in 1932, the similarities between Crohn's disease and mycobacteriosis are remarkable (59). Since that time, Crohn's disease and mycobacteria have been pushed apart so far that many of the common features have become obscure. Many consider that Crohn's disease received a too enthusiastic and uncritical acceptance as a unique disease entity and that the diagnosis of primary intestinal mycobacteriosis was too lightly discarded. Primary hypertrophic intestinal tuberculosis does occur, and although early investigators thought that this disease was Crohn's disease, the two have been distinguished. In the Western world, intestinal tuberculosis is generally misdiagnosed as Crohn's disease, and such cases are properly diagnosed only postsurgically (3, 39). On the other hand, in underdeveloped and those developing countries in which tuberculosis is common, cases of granulomatous intestinal disease are generally diagnosed as tuberculosis. The similarities and dissimilarities of Crohn's disease and the mycobacterioses will be difficult to understand fully in the near future. A literature search from

1966 to September 1987 retrieves 7,661 reports on Crohn's disease or IBD and 31,429 on mycobacteria, tuberculosis, or leprosy. Comparisons must be made almost exclusively between Crohn's disease and tuberculosis or leprosy, because relevant data are limited to these human diseases. The similarities and differences are summarized below.

Pathology

The pathologies of Crohn's disease and intestinal tuberculosis, as well as those of other intestinal mycobacterioses, have been thoroughly reviewed and compared (128, 247, 269, 271, 297). While tuberculosis produces a characteristic and almost pathognomonic disease in most cases, in others the disease is much less defined. Intestinal tuberculosis arising secondary to pulmonary disease is readily diagnosed by radiography of the thorax, presence of abundant acid-fast bacilli, and caseation necrosis. On the other hand, primary intestinal tuberculosis, i.e., intestinal infection without disease in other sites, may display nonspecific pathologic features.

Three types of intestinal tuberculosis are recognized which depend, among other factors, on the virulence of the organism, resistance of the host, and the extent of illness. The ulcerative type is the most common and is generally associated with intestinal infection. It is always associated with pulmonary involvement. The ulcerohypertrophic type may occur as a result of pulmonary disease or as a primary infection and results in ulcer healing with fibrosis and stenosis of the lumen. The hypertrophic form, which is rare and has been called pseudotuberculosis, is always a primary infection and is characterized by intense fibroblastic reactions in the submucosal and serosal layers of the bowel (3). Primary intestinal tuberculosis, particularly the hypertrophic form, is uncommon, which probably explains why intestinal disease is difficult to produce experimentally with *M. tuberculosis* (39, 128). Hypertrophic tuberculosis may have a range of histopathological appearances from the frank presence to a complete absence of caseation necrosis, but its diagnosis is generally based on the demonstration of caseation necrosis, primarily in draining lymph nodes. Cultural efforts and the microscopic demonstration of acid-fast bacilli often are negative.

Crohn and Yarnis (60) believed that the vast majority of hypertrophic tuberculosis cases were not tuberculosis, but rather examples of regional ileitis. Others have disagreed (40, 128, 131, 219, 269, 287), and if we accept *M. tuberculosis* as a strict pathogen, then hypertrophic intestinal tuberculosis without caseation necrosis or demonstrable acid-fast bacilli does exist. Paustian and Bockus (219) established that at least one of their four criteria is needed to make a diagnosis of intestinal tuberculosis: (i) positive culture or guinea pig disease after inoculation; (ii) microscopic demonstration of acid-fast bacilli in tissues; (iii) presence of tubercles with caseation in diseased tissue; or (iv) caseous granulomata in draining lymph nodes. All of these criteria are seldom satisfied in hypertrophic tuberculosis, but at least one is generally accepted as sufficient for diagnosis (219). Acid-fast bacilli generally are not demonstrated and cultural attempts are positive in only a portion of cases. Adams and Holden (3) failed to demonstrate acid-fast bacilli in 55% of patients with primary intestinal tuberculosis. Hoon et al. (128) detected acid-fast bacilli in only 33% of 58 cases examined. Stains for acid-fast bacilli in or around tubercular fistulae are always negative. Shah (247) examined 20 cases of hypertrophic intestinal tuberculosis and successfully isolated *M. tuberculosis* in only 7 (35%) cases. Wig et al. (297) were successful in cultivating organisms in only 26 (35%) of the 69 cases of hypertrophic tuberculosis they examined. In other cases, diagnosis was made based on the presence of caseation necrosis of the diseased tissues. Such necrosis, however, is generally absent in intestinal tissues and is only observed in the draining lymph nodes. Thus, the importance of lymph node histopathology in the diagnosis of primary hypertrophic tuberculosis of the intestine has been stressed and the lack of caseation necrosis does not exclude the diagnosis of tuberculosis. Caseation necrosis of draining lymph nodes may be present in as few as 24.4% of culture-positive cases of intestinal tuberculosis (62). Among culture-positive cases of hypertrophic tuberculosis, Wig et al. (297) found 10 cases in which there was no caseation and the lesions were limited to nonspecific inflammation. Thus, the ability to demonstrate acid-fast bacilli in cases of hypertrophic tuberculosis of the intestine is poor, as is the ability to culture *M. tuberculosis*, and in some cases caseation necrosis is absent. There are no documented cases of intestinal tuberculosis in which acid-fast bacilli were not demonstrable, cultures for *M. tuberculosis* were negative, and no caseation necrosis was seen. Such cases most likely would be diagnosed as Crohn's disease, if pulmonary radiographs were normal.

TABLE 5. Clinical similarities between Crohn's disease and mycobacterioses[a]

Clinical feature	Occurrence in:		
	Crohn's disease	Intestinal tuberculosis	Paratuberculosis
Diarrhea	Yes	Yes	Yes
Intermittent diarrhea	Yes	Yes	Yes
Abdominal pain	Yes	Yes	NA[b]
Weight loss	Yes	Yes	Yes
Obstruction	Yes	Yes	No
Ileac region mass	Yes	Yes	No
Blood in stool	Rare	Rare	Rare
Vomiting	Yes	Yes	No[c]
Quiescent periods	Yes	Yes	Yes

[a] References cited in text.
[b] NA, Not available; domestic animals generally fail to display chronic pain.
[c] Vomiting (regurgitation) is a normal function of ruminants.

Every clinical, radiologic, endoscopic, and pathologic feature of Crohn's disease may occur in primary intestinal tuberculosis or some other mycobacterioses, and they are indistinguishable (Tables 5 to 7). Both occur most frequently in the ileocecal region, and both may occur anywhere in the gastrointestinal tract from mouth to anus. In the United States, where ileocecal tuberculosis is rare, such cases are generally diagnosed only after surgical resection for Crohn's disease (3). When the features of these two diseases are compared, the only distinguishing criteria are the presence of caseating granulomas and acid-fast bacilli in tuberculosis. Thus, the absence of caseation necrosis and failure to isolate or demonstrate mycobacteria are the chief if not sole criteria for the diagnosis of Crohn's disease. As discussed, these are not reliable criteria. Taylor (271), in his study of intestinal tuberculosis and Crohn's disease, concluded that "it is impossible on the basis of clinical features or morbid anatomy to distinguish between these two conditions." Cattel and Mosely (41) shared this view and stated that ileocecal tuberculosis and Crohn's disease "may be virtually impossible" to distinguish. Even Crohn himself, in a discussion of

TABLE 6. Pathologic similarities between Crohn's disease and mycobacterioses[a]

Pathologic feature	Occurrence in:			
	Crohn's disease	Intestinal tuberculosis	Paratuberculosis	Other mycobacterioses
Segmental distribution	Yes	Yes	Yes	Leprosy
Strictures	Yes	Yes	No	NK[b]
Obstruction	Yes	Yes	No	NK
Skin lesions	Yes	Yes	Yes	Leprosy
Perforations	Yes	Yes	NK	NK
Stenosis	Yes	Yes	Yes	NK
Abdominal mass	Yes	Yes	No	NK
Fibrosis	Yes	Yes	No	NK
Ulcerations	Yes	Yes	Yes	Leprosy
Transmural inflammation	Yes	Yes	Yes	NK
Abdominal edema	Yes	No	Yes	NK
Fissures	Yes	Yes	No	NK
Fistulae				
Internal	Yes	Yes	No	Various
External	Yes	Yes	No	Various
Sinus tracts	Yes	Yes	No	NK
Lymphoid hyperplasia	Yes	Yes	Yes	Leprosy
Pseudopolyps	Yes	Yes	No	NK
Granulomas	Yes	Yes	Yes	All
Noncaseating granulomas	Yes	Yes (25%)	Yes	Various
Nonspecific inflammation	Yes	Yes	Yes	Various
Giant cells				
Foreign body	Yes	Yes	Yes	NK
Langhans	Yes	Yes	Yes	All

[a] References cited in text.
[b] NK, Not known or not applicable due to site specificity.

the paper of Watson et al. (288), conceded that "any pathologist would have difficulty in differentiating the pathology of so called pseudotuberculosis, of defining histologically sarcoidosis, ileitis, or ileojejunitis." Without doubt, certain cases of hypertrophic tuberculosis of the intestine are difficult, if not impossible, to differentiate pathologically from Crohn's disease. Some investigators have compared intestinal tuberculosis and Crohn's disease and have provided detailed pathological descriptions that allow their differentiation (269). These reports, however, did not describe hypertrophic tuberculosis but dealt primarily with secondary intestinal disease or the ulcerohypertrophic variety.

TABLE 7. Systemic similarities between Crohn's disease and mycobacterioses[a]

Systemic manifestation	Occurrence in:			
	Crohn's disease	Intestinal tuberculosis	Paratuberculosis	Other mycobacterioses
Arthritis	Yes	Yes	Yes	Leprosy
Erythema nodosum	Yes	Yes	Yes[b]	Yes
Amyloidosis	Yes	Yes	Yes	Leprosy
Granulomatous hepatitis	Yes	Yes	Yes	Leprosy
Nephrolithiasis	Yes	No	Yes	NK[c]
Oral ulcers	Yes	Yes	NK	NK
Ocular	Yes	NK	Yes	Leprosy

[a] References cited in text.
[b] Skin lesions often exhibited as alopecia.
[c] NK, Not known or not applicable due to site specificity.

Crohn's disease and mycobacterioses share not only the features of primary intestinal disease, but also extraintestinal manifestations. In Crohn's disease, arthritis, iritis, erythema nodosum, and amyloidosis are occasionally encountered and are considered to be important extraintestinal manifestations (28, 66, 124, 143, 152, 212, 300). Arthritis is a well-known complication of mycobacterial infections (126), and in recent years it has been shown that arthritis can be produced by mycobacterial antigens alone (127, 281). Erythema nodosum has its counterpart in leprosy, a condition known as erythema nodosum leprosum (117, 289). Amyloidosis may occur in intestinal tuberculosis (62, 128), leprosy (70, 286), and paratuberculosis (Johne's disease) of animals (32, 52, 199). Ocular lesions occur in leprosy (264) and are occasionally encountered in paratuberculous animals (199).

Comparisons of Crohn's disease pathology have been made almost exclusively with tuberculosis, yet *M. tuberculosis* most likely is not the etiologic agent of Crohn's disease. The major distinguishing feature between Crohn's disease and primary intestinal tuberculosis is the presence of caseation necrosis and pulmonary lesions, features of disease produced by the *M. tuberculosis* complex but not necessarily by other mycobacteria. Therefore, if Crohn's disease is caused by some other *Mycobacterium* sp., caseation necrosis need not be present. In addition, *M. tuberculosis* intestinal infections are not readily produced experimentally, suggesting that this is not a preferred site of the organism. In contrast, *M. paratuberculosis*, a more likely candidate as the etiologic agent, has a strict preference for the gastrointestinal tract and does not produce caseation necrosis.

Epidemiology

The population epidemiologies of Crohn's disease and the mycobacterioses are not readily comparable because of the manner in which these studies have been conducted. Whereas the epidemiology of tuberculosis is well defined and based on total population studies, that of Crohn's disease is not. There are no methods for population surveillance (such as PPD reactivity), and prevalence or incidence data are determined regionally through hospital records. Also, Crohn's disease population epidemiology is hampered by a long lapse between onset of clinical symptoms and diagnosis and an unequal precision in the use of diagnostic criteria in different study centers. In a proportion of studies it has been determined that approximately 20% of the study group are misclassified and do not have Crohn's disease (36). Thus, epidemiology will be addressed briefly.

Crohn's disease occurs most often in the United States, the United Kingdom, and Scandinavia. It is less frequent in Central Europe and rarely is reported in Africa, Asia, and South America. The disease is seldom reported in underdeveloped or developing countries (35, 36). The incidence is between 3.1 and 13.5 per 100,000 population in the United States and between 0.3 and 7.3 in other countries that have reported the disease (35, 36). Reports are conflicting, but the incidence of Crohn's disease in the United States and in other countries has been increasing, particularly in certain regions (35, 36, 92, 208, 262). Generally, the prevalence of disease appears to have stabilized in most countries. In contrast, tuberculosis (and leprosy) occurs with highest frequency in those areas where Crohn's disease is rarely seen and with low frequency where Crohn's disease is most frequent.

TABLE 8. Epidemiologic features of Crohn's disease (CD), ileocecal tuberculosis (TB), and paratuberculosis (PTB)[a]

Disease	Female preponderance (%)	Ileocecal disease (%)	Primary age incidence (yr)	Incidence in under-40 age group (%)	Bimodal age incidence	Familial association
CD	30–75	85	15–25	84	Maybe	Yes
TB	70–75	85	15–24	65–85	Maybe	Yes
PTB	Unknown[b]	Majority	Prime of life	Majority[c]	Unknown[d]	Yes

[a] References cited in text.
[b] Females constitute the major population in domestic livestock such that preponderance cannot be determined.
[c] Age designation beyond lifespan of domestic livestock. Paratuberculosis rarely occurs in older animals.
[d] Normal agricultural life of livestock not long enough to determine.

The incidence of tuberculosis in the United States has been decreasing for the last few decades, but in recent years has begun to increase. Some individuals have suggested that the apparent increase of Crohn's disease in the Western world is related to the decreasing incidence of tuberculosis, because infection or immunization with one *Mycobacterium* species provides protection against infection with another species. For example, BCG and other mycobacterial antigens provide some cross-protection against leprosy and other mycobacterial diseases (160, 210, 250, 265); *M. avium* vaccination protects cattle from *M. paratuberculosis* infection (52). The American black population had an increased incidence of Crohn's disease at about the same time the reported tuberculosis rate was decreasing (44). The prevalence of Crohn's disease has now appeared to stabilize in the United States, again corresponding with the current rise in *M. tuberculosis* infection. Although the protection afforded by endemic tuberculosis may be less common in the Western world, most likely the misclassification of disease accounts for the geographic distribution of Crohn's disease. Nevertheless, from 1960 through 1980, the prevalence of tuberculosis decreased 55% in the United States (163) and Crohn's disease increased 38 and 61% in Baltimore, Md., and Olmstead County, Minnesota, respectively (36).

Epidemiologic data about Crohn's disease, ileocecal tuberculosis, and paratuberculosis are compared in Table 8. In women of English or northern European descent, the incidence rate of Crohn's disease is 30% greater than in age-matched males (36). The age incidence of Crohn's disease shows a bimodal distribution. The primary incidence mode occurs at ages 15 to 25, followed by a second mode at ages 55 to 60 (36). Of the 121 Crohn's disease patients studied by Schoffield (243), 84% were under the age of 40 and 75% were females. While pulmonary tuberculosis has a greater frequency in males, primary ileocecal tuberculosis is predominant in females, approximately 70% of cases (247, 297). The maximum age incidence of intestinal tuberculosis is also 15 to 24 years (297), with 65 to 85% of patients being under the age of 40 (247). If we assume that Crohn's disease and human intestinal tuberculosis occur at the prime of life (15 to 25 years of age), then a similar maturity incidence occurs in animals with paratuberculosis. The maximal age incidence of paratuberculosis in cattle is 3 to 5 years, during their prime of life and period of maximum productivity (52). Since animals in the cattle industry are primarily female, a preponderance for disease in females cannot be assessed. The only feature that is not almost identical between Crohn's disease and intestinal tuberculosis is the presence of a secondary age incidence mode, which is not invariable (36). Insufficient cases of primary intestinal tuberculosis have been examined to determine whether a secondary mode exists. Because a secondary age mode is well documented in pulmonary tuberculosis, it is likely that it also occurs in the ileocecal disease. In pulmonary tuberculosis, this secondary age incidence mode arises as a result of degeneration of a Ghon lesion acquired earlier in life (196). Perhaps a Ghon-type lesion occurs in the gastrointestinal tract.

There is a known familial association of Crohn's disease (83, 144, 157, 170, 258, 291), which suggests a genetically linked increased susceptibility to the disease or, alternatively, a common exposure to an etiologic agent. There is a low incidence of Crohn's disease in married adults, but these rare occurrences have yet to be explained (228, 296; R. Bennett, P. H. Rubin, and D. H. Present, Gastroenterology **94**:A611, 1988). A genetic link, as assessed by HLA typing, has not been found (88), but genetic predisposition is likely. There is a 30 times greater rate of Crohn's disease in siblings and 13 times greater incidence in first-degree relatives (83, 85). Such a familial association, the occurrence of Crohn's disease in siblings and mono- and dizygotic twins (including those living apart since early childhood), and the rarity of Crohn's disease in half-siblings (21, 29, 85, 145, 205) indicate a genetic susceptibility or predisposition occurring as a recessive trait.

Morgan (194) has proposed an alternative explanation based on the epidemiology of *M. paratuberculosis* infection in animals. In this disease, animals are infected with *M. paratuberculosis* during early childhood (before 30 days of age) but disease becomes manifested later in adult life. An age-dependent resistance develops such that adult animals not exposed to the agent during early life rarely become infected, even experimentally. Thus, Morgan postulated that early exposure to an infectious agent (*M. paratuberculosis*) would account for the occurrence of Crohn's disease in siblings and mono- and dizygotic twins and the rarity of Crohn's disease in half-siblings. He felt that the case for early infection was particularly supported by the disease occurrence in twins separated since childhood. Since paratuberculosis is rarely transmitted to adult animals due to age-dependent resistance, a low incidence in adult married couples would be expected. Morgan proposed a time-space clustering study of Crohn's disease patients during their first 5 years of life to address these issues.

Immunology

As would be expected in a chronic granulomatous disease, there is no consistent humoral immune dysfunction in Crohn's disease. Although a few reports have described an intrinsic B-cell defect and dysfunction (165, 251), these have not been found by other investigators (79, 165). There may be an increased number of IgM-bearing cells in the intestinal mucosa (165). Perhaps the only humoral immune finding in Crohn's disease is an increased number of spontaneous immunoglobulin-secreting cells and a decrease in responsiveness to B-cell mitogens during active disease (165), suggesting an in vivo polyclonal B-cell activation.

A high proportion of Crohn's disease patients have autoantibodies against gastrointestinal tissue and other self-antigens (8, 260). Although these anticolon antibodies may arise due to cross-reactivity with *Escherichia coli* O14:K1 antigens (260), the documentation is rather weak. Circulating immune complexes are also observed in Crohn's disease (67, 99, 153). Although they are not consistently detected, it is now known that their presence correlates with episodes of clinical disease. Both of these manifestations are probably secondary to the disease state even though they may have clinical relevance. Autoantibodies are commonly found in mycobacterial infections and are thought to arise as a result of the potent mitogenic and adjuvant activities of mycobacteria. Autoantibodies have been investigated most thoroughly in leprosy, in which anti-skin, anti-DNA, anti-neuron, and a host of other self-antibodies arise as secondary phenomena of infection (81, 90). In leprosy primarily, but also in tuberculosis (65, 169, 259), circulating immune complexes frequently occur and are commonly associated with episodes of erythema nodosum leprosum (89, 90, 207, 229). However, the presence of low-level autoantibodies and circulating immune complexes are common manifestations of chronic disease in general.

Studies on CMI function in Crohn's disease are conflicting. Although many studies have shown dysfunction or intrinsic cell defects (15, 73, 75, 80, 209, 251, 268, 304), others have found no abnormalities (23, 30, 79, 86, 134, 150, 164). Decreased cytotoxicity has been observed in Crohn's disease (15, 16), as well as in ulcerative colitis, but this effect is believed to be caused by an increased number of immature monocytes in the peripheral blood as a result of rapid turnover rate. Increased or defective suppressor cell activity has also been observed in Crohn's disease (75, 87, 103), and although reports conflict (134, 221), increased suppressor cell activity is common in chronic disease. The only consistent finding is a reduced number of T cells in peripheral blood (213), but in general there are no imbalances or dysfunctions of helper or suppressor T cells and no alterations in T-cell immunoregulation or function in the peripheral blood or in the intestinal mucosa of Crohn's disease patients (30, 82, 100, 173, 214, 221, 309). The failure to detect consistently a humoral or CMI dysfunction may mean only that none yet has been demonstrated. Crohn's disease patients elicit an abnormal and exaggerated immune response in the gastrointestinal tract characterized by a DTH reaction. Because so little is known and understood about the immunology of Crohn's disease, comparisons with the immunology of other mycobacterioses cannot be made (45, 46, 52, 119, 158, 202).

Chemotherapy

In many respects, Hippocrates was correct when he said, "Diarrhoea attacking a person with phthisis is a mortal symptom" (4). While people with pulmonary tuberculosis (phthisis) occasionally did improve, secondary infection of the gastrointestinal tract was always fatal. This was also true for patients with primary ileocecal tuberculosis; advances in abdominal surgery, not the advent of chemotherapeutic agents, provided relief. Primary ileocecal tuberculosis, especially the hypertrophic type, is not responsive to drug therapy alone and surgical resection of the bowel is required (20, 41). Chemotherapy is only an adjunct to surgical intervention and is often not necessary (3, 41).

Chemotherapeutic treatment of Crohn's disease generally involves the use of prednisolone or sulfasalazine or both (236), with surgical intervention needed in 60 to 80% of patients (102). The mechanism of action of prednisolone is known; that of sulfasalazine is less clear. Sulfasalazine is cleaved by colonic bacteria into sulfapyridine and 5-aminosalicylic acid (12), which are believed to be the active products. A variety of other drugs has been evaluated and occasionally used, but currently no chemotherapeutic drug or regimen has provided a cure for Crohn's disease. Treatment and disease management are supportive.

The current data on the use of antimycobacterial chemotherapy in Crohn's disease have been discussed previously. Some data, particularly case reports, strongly suggest a beneficial effect, but larger studies have not been as encouraging. Sufficient data, however, are not available to precisely define the effects of these agents on Crohn's disease. Nevertheless, an understanding of the use of chemotherapy in mycobacterioses is essential to appreciate the potential application of this form of therapy.

Any discussion of chemotherapeutics in Crohn's disease must consider the placebo effect and spontaneous clinical remission. Several large studies (166, 267) have shown that 25 to 40% of patients receiving placebo improve enough during the first 3 to 4 months of treatment to be considered to have gone into clinical remission. About 20% of placebo-treated patients remain well after 1 year and 10% do so after 2 years. Such remissions may even be accompanied by radiographic improvement (188). Drug toxicity occurs in 6 to 8% of patients on placebo medication. The effect of placebo on maintaining clinical remission is even more striking. Of 20 patients achieving remission while on placebo (267), 15 (75%) remained well for at least 1 year. Of 11 patients followed for a second year, 7 (63%) remained in remission. Thus, any data presented on the treatment of Crohn's disease which is not performed in a double-blinded placebo fashion or is performed only over a short period of time must be interpreted with the knowledge that 42% of ill patients may get better without any specific therapy during the first 3 to 4 months (166, 188, 267). The placebo effect may be reduced after 1 to 2 years, after which continued improvement is evident in only 20 and 10% of patients, respectively.

The use of antimycobacterial therapy in mycobacterial disease is a well-established therapeutic approach, but it is not always effective, particularly in certain diseases or disease types. Chemotherapeutic drugs alone are ineffective in the treatment of hypertrophic ileocecal tuberculosis. This disease state requires intestinal resection (as in Crohn's disease), with antimicrobial agents provided only as adjunct therapy. Paratuberculosis, a well-recognized intestinal mycobacterial disease of ruminants, has yet to be successfully treated despite the use of a wide range of antimicrobial agents (52). Prophylactic treatment of animals with antimycobacterial agents does not even prevent experimental intestinal infection (231). Therefore, it must be appreciated that, although antimycobacterial agents are effective in classical diseases such as tuberculosis and leprosy, they are not effective in any known intestinal mycobacterioses. These drugs would probably have limited efficacy in Crohn's disease even if the disease was caused by a *Mycobacterium* species.

The use of steroids in Crohn's disease patients has been a major argument against a mycobacterial etiology. Steroids and other immunosuppressive therapy are considered contraindicated in pulmonary tuberculosis, exacerbating the disease. However, the detrimental effects of immunosuppressive drugs on mycobacterial infections are not as pronounced as believed. Steroids in combination with antimi-

crobial agents have been used for treatment in leprosy (133, 227, 246) and in tuberculosis and other mycobacterial infections (125, 203). Retrospective studies have now established that corticosteroids do not cause reactivation of pulmonary tuberculosis (114) as case reports had long suggested, especially if only small numbers of bacilli are present. Reactivation of disease due to steroid therapy is more likely caused by the chronic condition that mandated steroid therapy than the metabolic effects of the steroid (114). Studies in cattle with paratuberculosis have shown that massive corticosteroid administration does not significantly influence the clinical manifestations or outcome of the disease, although it was expected to (7). Treatment of experimental *M. paratuberculosis* infection in rabbits with methotrexate, a powerful immunosuppressive drug, resulted in clinical improvement even though the bacillary load increased (186). In Crohn's disease, as in tuberculosis and leprosy, steroids are used to provide clinical relief, but neither disease can be cured by such treatment. Although disease remission sometimes occurs in Crohn's disease patients receiving corticosteroids, it is difficult to determine whether this remission is due to the steroid therapy, occurs spontaneously (placebo effect), or reflects a masking of the disease as occasionally observed in leprosy (227).

Nonsteroidal anti-inflammatory agents are known to activate quiescent Crohn's disease (140) and induce intestinal inflammation in other types of patients as well (24). Therefore, their use is contraindicated. Nonsteroidal anti-inflammatory drugs are also known to activate quiescent pulmonary tuberculosis (276) and are also contraindicated in mycobacterioses.

In summary, chemotherapeutic schemes offer several similarities between the mycobacterioses and Crohn's disease: (i) antimycobacterial agents appear effective in only a portion of Crohn's disease patients, as in intestinal mycobacterioses; (ii) corticosteroids offer clinical improvement in Crohn's disease, leprosy, and some cases of tuberculosis and other mycobacterioses; and (iii) nonsteroidal anti-inflammatory agents activate quiescent Crohn's disease and tuberculosis. Despite these similarities, the efficacy and appropriateness of antimycobacterial chemotherapy in Crohn's disease remain to be evaluated.

CONCLUSIONS

Experimental and comparative data have been presented on the association of mycobacteria and Crohn's disease. A large portion of this information is either preliminary or in abstract form and must be interpreted with caution. While no firm evidence clearly identifies mycobacteria as an etiologic agent, the notion is supported by suggestive and circumstantial data and by remarkable similarities to other known mycobacterial diseases. A consensus could probably be reached on the notion that, if the etiology of Crohn's disease is microbial in origin, it is most likely mycobacterial.

All major texts on gastroenterology and mycobacteriology make reference to the possible mycobacterial etiology of Crohn's disease. This is surprising, particularly in older texts, since the data which suggested such an association were sparse when these texts were written (Table 9). Crohn et al. (59) dismissed the notion of a mycobacterial etiology with their description of Crohn's disease in 1932. It was 20 years later that Van Patter presented his doctoral thesis seeking to associate mycobacteria and Crohn's disease. These efforts were never formally published; therefore, presumably this information was not generallv known.

TABLE 9. Time span between investigations seeking a mycobacterial etiology of Crohn's disease

Yr published	Time (yr) between investigations	Author(s)
1932	0	Crohn et al.
1952	20	Van Patter[a]
1978	26	Burnham et al.
1984	6	Chiodini et al.
1986	2	Coloe et al.
1987	1	Graham et al.
1987	0	Gitnick et al.
1987	0	Colemont et al.
1987	0	Haagsma et al.

[a] Ph.D. thesis which was never formally published; therefore, data are not widely known.

Twenty-six years later, Burhnam et al. (33) published their data on *M. kansasii* and Crohn's disease. Since Van Patter's work was not referenced by any text or article during this period, the first concerted effort to associate mycobacteria and Crohn's disease was in 1978, 46 years after Crohn dissociated the disease from mycobacteria. Recently, a concerted effort has again been made to investigate the possible association of mycobacteria and Crohn's disease, but the fact that texts written prior to 1984 considered mycobacteria as a possible etiologic agent of Crohn's disease indicates that the medical community has never dismissed this notion.

The relationship between Crohn's disease and mycobacteria is an old idea that has never been thoroughly investigated. Data are just now becoming available through active research efforts. The notion of a mycobacterial etiology of Crohn's disease should be viewed with skepticism and criticism, but the level of controversy surrounding this issue is exaggerated considering the data available prior to this latest surge of interest (Table 9).

Perhaps the biggest error in the study of Crohn's disease is the assumption that Crohn's disease is a single disease entity. The clinical and pathological criteria of Crohn's disease are not specific enough to ensure precise diagnoses. No unique features identify Crohn's disease except the inability to diagnose or associate the signs and symptoms with another disease. There is such great variability between patients that Crohn's disease probably reflects a variety of diseases grouped into one. The histologic hallmark of this disease, i.e., noncaseating granulomas, is found in only 40 to 60% of cases (198, 242), or even fewer (132). Based on epidemiologic data, it has been estimated that at least 20% of Crohn's disease diagnoses are misclassifications (36), but this value is probably conservative. Adding to the confusion is the grouping of Crohn's disease along with ulcerative colitis as IBD. Such data are nearly impossible to interpret as two distinct disease entities. If what we now know as Crohn's disease is not a single disease entity, statistically significant data cannot be achieved. Investigations need to be conducted with well-characterized patient populations and pathological material to limit the possible effects of misclassification and grouping together of several disease entities. Even if Crohn's disease is found to be caused by a mycobacterial agent, the disease would probably be confirmed in only a portion of the patients we now consider to have this disease.

If Crohn's disease has a mycobacterial etiology, the most likely agent would be *M. paratuberculosis*. This organism is

unique among the mycobacteria because the gastrointestinal tract is the only environment in which it can replicate in vivo. As *M. leprae* and *M. ulcerans* favor the skin, *M. paratuberculosis* favors the intestines and associated lymph nodes. It is incapable of survival (replication) in a variety of environmental materials and has never been found in any environmental source not associated with disease. By-products of its own environs are toxic to it; feces are bacteriostatic and urine is bactericidal. Because it is unable to live in the environment, it must be considered a strict pathogen (52). It is clearly suited to cause a chronic granulomatous ileocolitis in ruminants and subhuman primates and perhaps even in humans. Its disease remains incurable.

The question as to the role of mycobacteria as etiologic agents in Crohn's disease is likely to remain unanswered for years. Such an agent would need to (i) exist in an unculturable fashion (perhaps as a spheroplast); (ii) occur at concentrations below the microscopic detection level of current technology ($<10^4$ to 10^5 per g of tissue); (iii) fail to elicit a strong humoral immune response, as in polar tuberculoid leprosy; and (iv) remain at low concentrations even when steroid therapy is administered. Perhaps it would even fail to elicit a DTH skin reaction as in hypertrophic ileocecal tuberculosis; such a disease syndrome, if it exists, would be difficult to detect. The only circumstance in which these conditions could occur would be the development of local DTH reactions (as in tuberculoid leprosy) within the intestinal tissues. A competent immune system capable of hindering bacterial proliferation at the macrophage level could result in low bacillary loads. An unmetabolizable product that could induce DTH and occur either as part of the organism, e.g., certain components of the mycobacterial cell wall, or as a by-product of metabolism or degradation could produce a progressive chronic disease. Mycobacterial cell walls inoculated into the lungs of mice produce progressive hypersensitivity pneumonitis in the absence of viable organisms (18, 159, 179, 256). The *M. paratuberculosis* vaccine used in cattle results in a large granuloma which lasts for the lifetime of the animal. This vaccine, accidentally injected into humans, results in progressive granulomatous inflammation necessitating surgical amputation of the injection site (52). The same vaccine, given to subhuman primates, resulted in a severe progressive and disseminated disease, suggesting the presence of viable organisms (H. M. McClure, personal communication). Cultures of the vaccine were negative, as were the animal tissues at autopsy. Such potent immune modifiers could produce the syndrome known as Crohn's disease.

Future research direction is clear. Efforts need to be concentrated on techniques for demonstrating low concentrations of mycobacteria in tissues. Two possible routes exist: the use of specific genetic probes or the more readily available monoclonal antibodies. A variety of mycobacterial monoclonal antibodies react with genus-specific antigens and many react against cytoplasmic components. The latter monoclonal antibodies should detect CWD forms, although they would not identify the particular species. Important information about the role of mycobacteria and Crohn's disease could be gained. Species-specific genetic probes, particularly against *M. paratuberculosis*, need to be used, but the genus-specific probes could provide the framework for future efforts. Microbiologic efforts need to concentrate on improved cultivation and isolation techniques, transformation of CWD forms into classical bacillary forms, and methods to precisely identify these CWD forms.

Since Crohn's disease is a DTH-mediated disease, efforts need to concentrate on examining CMI and DTH in Crohn's disease patients. Such studies need to be performed not only on peripheral blood cells, but also on intestinal mucosal cells. Unfortunately, species-specific antigens are not available and are not likely to be available in the near future. Mycobacterial antigens have been exhaustively examined and many monoclonal antibodies have been developed, yet the species-specific antigens detected are few and far between (66). However, useful information can be obtained by using nonspecific antigens.

Areas of research of less importance include antigen purification, humoral immunity, and animal model studies. Sufficient data have been obtained to document that Crohn's disease patients do not have a consistently demonstrable antibody response to mycobacteria. Although this could very well be related to nonspecific mycobacterial antigens and cross-reactions with environmental mycobacteria, highly purified immunogenic antigens are not now available and probably will not be for many years. Studies seeking specific antigens are too time-consuming to warrant much effort now. If mycobacteria are established as the cause of Crohn's disease, then purified antigens for use in diagnostics would be an appropriate effort. Likewise, animal model studies are of limited value at this time. If mycobacteria are found not to be the cause of Crohn's disease, production of a granulomatous ileocolitis resembling Crohn's disease in goats or mice following inoculation of mycobacteria has little significance as an animal model. Such models need to be developed only after the cause is established. Some investigators seeking animal models to prove a mycobacterial etiology consider that the pathologic disease must be identical to that found in Crohn's disease (H. J. Van Kruiningen, Letter, Dig. Dis. Sci. **33**:251–252, 1988). While this situation would be ideal, it is unrealistic. Mycobacterial diseases are essentially immunologically mediated disorders; therefore, each species responds differently immunologically and identical pathologic diseases would not be expected. For example, *M. paratuberculosis* infection in cattle does not produce caseation necrosis, but 25% of goats develop caseating granulomas in response to infection with *M. paratuberculosis* (52). Humans are likely to respond differently. Microbiologic efforts to isolate mycobacteria must also be considered an area of less priority since such efforts have not been very productive in the past. If Crohn's disease is caused by a *Mycobacterium* sp., perhaps in a spheroplast form, current techniques are inadequate to ensure consistent isolation or propagation or both. New methods need to be developed.

It is highly unlikely that mycobacteria cause all cases of Crohn's disease, but available data suggest strongly that they do cause some. The mycobacterial etiology theory of Crohn's disease remains alive. Despite the negative data generated, the similarities of Crohn's disease and the mycobacterioses are too remarkable to dismiss as coincidental. Perhaps with the new wave of interest in this old idea, an answer to this persistent question will be forthcoming.

Regardless of the outcome of current studies, the pathologic findings, familial occurrences, extraintestinal manifestations, ectopic sites of disease often occurring concurrently with intestinal infection, and the recurrence of disease at resection margins all suggest an infectious etiology. Efforts to find that agent will undoubtedly continue.

ACKNOWLEDGMENTS

The support provided by research grants from the National Institute of Allergy and Infectious Disease, the National Institutes of

Health, and the National Foundation for Ileitis and Colitis, Inc., is acknowledged.

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