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we must explore the time-consuming task of looking for variants of known pathogens (HAV, HBV, HCV). Mutant viruses of HBV have already been linked to fulminant liver failure in a small number of HBsAg-positive patients.[22,23] The ultimate proof of an infectious agent lies in transmissability of infection. These important studies are underway. Is there an unidentified infectious agent responsible for fulminant NANB hepatitis? I used to think "yes"; I now believe that this is a syndrome caused by a heterogenous group of hepatotoxins and known hepatotropic viruses, both wild-type and mutant.

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References

1. Feray C, Gigou M, Samuel D, Reyes G, Bernuau J, Reynes M, Bismuth H, Brechot C. Hepatitis C virus RNA and hepatitis B virus DNA in serum and liver of patients with fulminant hepatitis. Gastroenterology 1992;103:549–555.
2. Liang TJ, Jeffers L, Reddy RK, Silva MO, Cheinquer H, Findor A, DeMedina, Yarbough PO, Schiff ER. Fulminant or subfulminant non-A, non-B viral hepatitis: hepatitis C and E viruses. Gastroenterology 1992;103:556–562.
3. Wright TL, Hsu H, Donegan E, Feinstone S, Greenberg H, Read A, Ascher NL, et al. Hepatitis C virus not found in fulminant non-A, non-B hepatitis. Ann Intern Med 1991;115:111–112.
4. Wright TL, Mamish D, Combs C, Kim M, Donegan E, Ferrell L, Lake J, Roberts JP, Lake JR. Hepatitis B virus and apparent non-A, non-B hepatitis. Lancet 1992;339:952–955.
5. Sallie R, Tibbs C, Silva AE, Sheron N, Eddelston A, Williams R. Detection of hepatitis "E" but not "C" in sera of patients with fulminant NANB hepatitis. Hepatology 1991;14:68A.
6. Yanagi M, Kaneko S, Unoura M, Morakami S, Kobayashi K, Sugihara J, Chuishi H, Muto Y. Hepatitis C virus in fulminant liver failure. N Engl J Med 1991;324:1895–1896.
7. Saboor SA, Johnson NM, McFadden J. Detection of mycobacterial DNA in sarcoidosis and tuberculosis with polymerase chain reaction. Lancet 1992;339:1012–1015.
8. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's disease. N Engl J Med 1992;327:293–301.
9. Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS. The agent of bacillary angiomatosis–an approach to the identification of uncultured pathogens. N Engl J Med 1990;323:1573–1580.
10. O'Grady JG, Alexander GJM, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology 1989;97:439–445.
11. Lidofsky SD, Bass NM, Prager MC, Washington DE, Read AE, Wright TL, Ascher NL, Roberts JP, Scharschmidt BF, Lake JR. Liver transplantation for fulminant hepatic failure: patient selection and the role of intracranial pressure monitoring. Hepatology 1992;16:1–7.
12. Lugassy C, Bernuau J, Thiers V, et al. Sequences of hepatitis B virus DNA in the serum and liver of patients with acute benign and fulminant hepatitis. J Infect Dis 1987;155:64–71.
13. Sacher RA, Melpolder JJ, Alter HJ. Hepatitis C and fulminant liver failure (letter). Ann Intern Med 1991;115:984–985.
14. Hsu HH, Wright TL, Luba D, Martin M, Feinstone SF, Garcia G, Greenberg HB. Is hepatitis C virus in human secretions? Hepatology 1991;14:763–767.
15. Kaneko S, Feinstone SM, Miller RH. Rapid and sensitive method for the detection of serum hepatitis B virus DNA using the polymerase chain reaction technique. J Clin Microbiol 1989; 27:1930–1933.
16. Hu K-Q, Yu C-H, Vierling JM. Direct detection of circulating hepatitis C virus RNA using probes from the 5′ untranslated region. J Clin Invest 1992;89:2040–2045.
17. Alter HJ. New kit on the block: evaluation of second-generation assays for detection of antibody to the hepatitis C virus. Hepatology 1992;15:350–353.
18. McHutchinson JG, Person JL, Govindarajan S, Valinluck B, Gore T, Lee SR, Nelles M, Polito A, Chien D, DiNello R, Quan S, Kuo G, Redeker AG. Improved detection of hepatitis C virus antibodies in high-risk populations. Hepatology 1992;15:19–25.
19. Todo S, Demetris AJ, Van Thiel D, Teperman L, Fung JJ, Starzl TE. Orthotopic liver transplantation for patients with hepatitis B virus-related liver disease. Hepatology 1991;13:619–626.
20. Wright TL, Donegan E, Hsu HH, Ferrell L, Lake JR, Kim M et al. Recurrent and acquired hepatitis C viral infection in liver transplant recipients. Gastroenterology 1992;103:317–322.
21. O'Grady JG, Williams R. Liver transplantation for viral hepatitis. Br Med Bull 1990;46:481–491.
22. Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med 1991;324:1699–1704.
23. Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath E. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. N Engl J Med 1991;324:1705–1709.

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Causation of Crohn's Disease: The Impact of Clusters

Van Kruiningen et al.[1] describe the clustering of Crohn's disease in two families in northern France, the first in Valenciennes (population 39,276) and the second in Lecluse (population 1674), just 35 km away. In the first family, the father, mother, and all children, three sons and one daughter, developed Crohn's disease. One of the sons who developed Crohn's disease in 1974 met a girl 3 years later whom he subsequently married in 1983. She developed Crohn's disease in 1991. In the second family, neither the mother nor the father had Crohn's disease; however, four of the five sons and three of the six daugh-

ters developed Crohn's disease. These events represent the most concentrated clustering of Crohn's disease ever reported.

The investigations could hardly have been more thorough. There is certainly no doubt about the diagnosis. Although pedigree reviews and DNA analyses have yet to be completed, there was no antecedent family history, no consanguinity, and no evidence of HLA haplotype linking. Renewed microscopy of tissues, Ziehl–Neelsen staining, immunocytochemistry, stool culture using relevant specific methods and media, and antigen-capture enzyme-linked immunosorbent assay for viruses were all performed. These did not reveal acid-fast organisms, *Mycobacterium tuberculosis, Mycobacterium paratuberculosis, Yersinia, Mycoplasma, Campylobacter*-like organisms, *Borrelia,* spirochetes, *Rickettsia,* bovine coronavirus, or Breda virus (a torovirus). One of the 15 stool samples tested was positive for group A human rotavirus antigen; the other 14 were negative. Serology showed no evidence of *Yersinia,* brucellosis, influenza A and B, or bovine coronavirus, nor the agents of malignant catarrhal fever (a herpesvirus), bovine virus diarrhea (a pestivirus), equine viral arteritis (an arterivirus), or several other enteric animal viruses.

Throughout this in-depth study, there were two positive findings of potential significance. The first was elevated titers of antibody to Breda virus in 6 of 10 patients and in 2 of 5 unaffected family members. The second was that of the total of 13 cases of Crohn's disease between the two families, there seemed to be a distinct periodicity in disease emergence; 7 cases from 1970 to 1974 (4 in one family within 10 months from 1972 to 1973) and 4 cases from 1982 to 1984. The authors conclude that the outbreak of Crohn's disease in these families suggests a causative infectious microorganism with a considerable latency interval between exposure and the emergence of clinical disease.

It is difficult to find a major issue in contemporary gastroenterology in which views are more divergent or deeply entrenched than the causation of Crohn's disease. Let us for the moment set controversy aside and consider the case that chronic enteritis in humans and chronic mycobacterial enteritis in animals share a common cause. Thus, in humans, Crohn's disease could be paratuberculosis, a small group of closely related long-term inflammatory diseases caused by a group of specific chronic enteric pathogens of which *M. paratuberculosis* is at present best known and characterized. Might there be a larger pool of these specific pathogens, particularly in our farm animals,[2] than previously recognized? Might they be conveyed in some foods, especially milk,[3] or by water supplies? And might enteric cohabitation after a long latency interval[4] convert to parasitism and clinical disease?

Conversion may be catalyzed by numerous cofactors such as an inherited susceptibility, repeated viral infections,[5] or microbial cooperation,[6] as exemplified by the known specific emergence of Crohn's disease after an incidental enteric infection. Psychological and stress factors, whose mechanisms are as yet poorly defined on a molecular basis but probably operate through the immune system, are manifestly involved. Each of these factors has its parallel among paratuberculosis in animals.[7]

Although dependent on DNA-based methods that have only recently become available, evidence for the involvement of *M. paratuberculosis* in a substantial majority of Crohn's disease patients is accumulating.[8–14] Other polymerase chain reaction studies performed blind on long-term in vitro cultures established from Crohn's disease tissues and maintained for 2–6 years[15] show that *M. paratuberculosis* from humans is in a form that hardly replicates in culture, if at all.[16] The same is found in some examples of paratuberculosis, particularly in sheep.[17] However, important differences exist between species in the overall susceptibility to *M. paratuberculosis* infection and the nature of the tissue response.[4] The chronic enteritis due to *M. paratuberculosis* found in some (but by no means all) animals is characterized in its extreme state by the presence of millions of organisms in the affected intestinal tissues and macrophages but with a modest additional immune response. The disease in humans is the opposite, characterized by a tissue microbial abundance at or near the limit of detection by our present methods[14] and a major immunological reaction. In another mycobacterial context, this is reminiscent of the well-known extremes represented by the lepromatous and tuberculoid forms of leprosy.

How does this case compare with that of two French families? There is a strong probability that the unpasteurized milk that both families drank was at some time heavily contaminated with *M. paratuberculosis.*[3] After a latency interval, well known in animals and indicated in this case, this would result in the coincident emergence of the clinical manifestations of enteric disease in each genetically related group. Conventional stool culture for *M. paratuberculosis* from these patients, dependent on the achievement of the bacillary form of these versatile organisms, could be predicted to be negative. The involvement of both parents in family 1 but not in family 2 could reflect differences in their individual susceptibilities. It could also have

been determined by whether or not they habitually drank the milk.

Because clinically or subclinically infected animals may shed millions of *M. paratuberculosis* organisms in their feces, it is highly likely that ground and river waters contaminated with animal waste may be a source of human exposure. This has not been possible to test experimentally because of the need hitherto to rely on the uncertainties of culture. Family 2 from time to time drank farm spring waters; they were open to exposure by this route. This mechanism is also likely to have been responsible for the previously described clustering of Crohn's disease in a Cotswold village, England,[18] that was centered in an area where paratuberculosis was prevalent in the farm animals. (R. N. Allan, personal communication, November 1992).

An additional example of Crohn's disease clustering that has so far escaped reasoned explanation but that demands examination against the present case is that identified in Cardiff,[19] a city on the coastal plain of South Wales beside the sea. North of the city lie the Brecons, steep upland pastures that are heavily grazed by sheep and cattle that may shed trillions of *M. paratuberculosis* organisms onto the thin topsoil. Heavy rains from the Atlantic falling on the Brecons will wash these organisms into the spate rivers. One of these rivers, the Taff, runs through the middle of Cardiff. In 1978, Mayberry and Hitchens[19] showed a highly significant ($P < 0.001$) concentration of cases of Crohn's disease (but not of ulcerative colitis) in 11 of the Cardiff city wards. Of these, 8 directly bordered the river Taff, and the 3 wards that did not were immediately adjacent to the north and east. This is the direction in which aerosols would be carried by the prevailing southwesterly winds. The incidence of Crohn's disease among the people of South Wales,[20] as that of Northeast Scotland,[21] has risen to the proportions of an epidemic.

The most difficult question to answer is how does the present case compare with the generality of Crohn's disease in some but not all human populations? It is easy to conceive that *M. paratuberculosis* is more prevalent in temperate regions of the globe with adequate rainfall and ground waters.[22,23] It is also possible to imagine how different farm animals may affect the local abundance, phenotype, and distribution of these organisms. But what about the people with Crohn's disease who live in towns and cities and have never been in contact with farm animals, potentially contaminated waters, or unpasteurized dairy products? To reach these people, *M. paratuberculosis* would have to persist in a product such as milk distributed on a regional (or even national or international) basis and be capable of surviving pasteurization.[24,25] Studies we have performed on milk samples widely obtained throughout southern England so far suggest that this may occur. A previously unsuspected exposure of the population to these enteric pathogens is supported by the identification of *M. paratuberculosis* in the normal colon (but not normal small intestine) of a small proportion of people without inflammatory bowel disease.[14]

The proposition that chronic enteritis in humans and chronic mycobacterial enteritis in cattle may be caused by the same organisms is nearly a century old.[26] Since then, *M. paratuberculosis* has been shown to cause chronic enteritis in many other species, including primates.[27] Its involvement in chronic enteritis in humans should not come as too much of a surprise. We should commit resources to finding out whether *M. paratuberculosis* causes Crohn's disease. If the case is proven to be incorrect, a small amount of money and time will have been added to the huge effort in research that has so far conspicuously failed to elucidate Crohn's disease. However, if the case is mostly correct, carefully designed studies should give a clear picture within a few years. The combined effect of measures that could then be taken, by analogy with other diseases, may reverse the major problems of Crohn's disease, though the need for vigilance would as always be there.[28] Savings in the form of farm and health care costs are calculable[29]; those in the form of human and animal suffering are not.

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References

1. Van Kruiningen HJ, Colombel JF, Cartun RW, Whitlock RH, Koopmans M, Kangro HO, Hoogkamp-Korstanje JAA, Lecomte-Houcke M, Devred M, Paris JC, Cortot A. An in-depth study of Crohn's disease in two french families. Gastroenterology 1992;103:351–360.
2. Chiodini RJ, Van Kruiningen HJ. The prevalence of paratuberculosis in culled New England cattle. Cornell Vet 1986;76:91–104.
3. Taylor TK, Wilks CR, McQueen DS. Isolation of *Mycobacterium paratuberculosis* from milk of a cow with Johne's disease. Vet Rec 1981;109:532–533.
4. Chiodini RJ, Van Kruiningen HJ, Merkal RS. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet 1984;74:219–262.
5. Ekbom A, Adami HO, Helmick CG, Jonzon A, Zack MM. Perinatal risk factors for inflammatory bowel disease: a case-control study. Am J Epidemiol 1990;132:1111–1119.
6. Tashiro M, Ciborowski P, Klenk H-D, Pulverer G, Rott R. Role of staphylococcus protease in the development of influenza pneumonia. Nature 1987;325:536–537.

7. Chiodini RJ. Crohn's disease and the mycobacterioses: a review and comparison of two disease entities. Clin Microbiol Rev 1989;2:90–117.
8. Chiodini RJ, Van Kruiningen HJ, Merkal RJ, Thayer WR, Coutu JA. Characteristics of an unclassified mycobacterium species isolated from patients with Crohn's disease. J Clin Microbiol 1984;20:966–971.
9. Van Kruiningen HJ, Chiodini RJ, Thayer WR, Coutu JA, Merkal RS, Runnels PL. Experimental disease in infant goats induced by a mycobacterium isolated from a patient with Crohn's disease. Dig Dis Sci 1986;31:1351–1360.
10. McFadden JJ, Butcher PD, Chiodini R, Hermon-Taylor J. Crohn's disease–isolated mycobacteria are identical to *Mycobacterium paratuberculosis*, as determined by DNA probes that distinguish between mycobacterial species. J Clin Microbiol 1987;25:796–801.
11. Yoshimura HH, Graham DY, Estes MK, Merkal RS. Investigation of association of mycobacteria with inflammatory bowel disease by nucleic acid hybridization. J Clin Microbiol 1987;25:45–51.
12. Green EP, Tizard MLV, Moss MT, Thompson J, Winterbourne DJ, McFadden JJ, Hermon-Taylor J. Sequence and characteristics of IS900, an insertion element identified in a human Crohn's disease isolate of *Mycobacterium paratuberculosis*. Nucleic Acids Res 1989;17:9063–9073.
13. Hermon-Taylor J, Moss MT, Tizard MLV, Malik Z, Sanderson J. Molecular biology of Crohn's disease mycobacteria. In: Ciclitira PJ. Balliere's Clin Gastroenterology. London: Bailliere Tindall, 1990:23–42.
14. Sanderson JD, Moss MT, Tizard MLV, Hermon-Taylor J. Mycobacterium paratuberculosis DNA in Crohn's disease tissue. Gut 1992;33:890–896.
15. Markesich DC, Graham DY, Yoshimura HH. Progress in culture and subculture of spheroplasts and fastidious acid-fast bacilli isolated from intestinal tissues. J Clin Microbiol 1988;26:1600–1603.
16. Moss MT, Sanderson JD, Tizard MLV, Hermon-Taylor J, El-Zaatari FAK, Markesich DC, Graham DY. Polymerase chain reaction detection of *Mycobacterium paratuberculosis* and *Mycobacterium avium* subsp *silvaticum* in long term cultures from Crohn's disease and control tissues. Gut 1992;33:1209–1213.
17. Collins DM, Gabric DM, DeLisle GW. Identification of two groups of mycobacterium paratuberculosis strains by restriction endonuclease analysis and DNA hybridization. J Clin Microbiol 1990;28:1591–1596.
18. Allan RN, Pease P, Ibbotson JP. Clustering of Crohn's disease in a Cotswold village. Q J Med 1986;229:473–478.
19. Mayberry JF, Hitchens RAN. Distribution of Crohn's disease in Cardiff. Soc Sci Med 1978;12:137–138.
20. Mayberry JF. Recent epidemiology of ulcerative colitis and Crohn's disease. Int J Colorect Dis 1989;4:59–66.
21. Kyle J. Crohn's Disease in the northeastern and northern isles of Scotland: an epidemiological review. Gastroenterology 1992; 103:392–399.
22. Kazda JF. The principles of the ecology of mycobacteria. In: Ratledge C, Stanford J, eds. The biology of the mycobacteria. Volume 2. London: Academic, 1983:323–324.
23. Sonnenberg A, McCarty DJ, Jacobsen SJ. Geographic variation of inflammatory bowel disease within the United States. Gastroenterology 1991;100:143–149.
24. Chapman JS, Bernard JS, Speight M. Isolation of mycobacteria from raw milk. Am Rev Respir Dis 1965;91:351–355.
25. Sanderson JD, Hermon-Taylor J. Mycobacterial diseases of the gut: some impact from molecular biology. Gut 1992;33:145–147.
26. Dalziel TK. Chronic interstitial enteritis. BMJ 1913;2:1068–1070.
27. McClure HM, Chiodini RJ, Anderson DC, Swenson RB, Thayer WR, Coutu JA. *Mycobacterium paratuberculosis* (Johne's disease) in a colony of stump-tail macaques (*Macaca arctoides*). J Infect Dis 1987;155:1011–1019.
28. Bloom BR, Murray CJL. Tuberculosis: commentary on a reemergent killer. Science 1992;257:1055–1064.
29. Benedictus G, Dijkhuizen AA, Stelwagen J. Economic losses due to paratuberculosis in dairy cattle. Vet Rec 1987;121:142–146.

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Will the Real Cholesterol-Nucleating and -Antinucleating Proteins Please Stand Up?

Articles in this issue by Abei et al.[33] and Ohya et al.[36] describe different proteins that influence cholesterol crystal nucleation. One of these proteins accelerates cholesterol nucleation, whereas the other delays nucleation. These are the latest in a line of important contributions on the subject of cholesterol gallstone pathogenesis by Holzbach and his colleagues from Cleveland. Gallstone disease is of great interest not because it is so injurious but because it is so frequent.[1] It is a major health care problem that costs $4.5 billion yearly in this country. Understanding how to prevent stone formation or stone recurrence is a prime health field goal.

Cholesterol is very sparingly soluble in water. Its solubility is dependent on other solutes, bile salts, and phospholipids with which it complexes to form molecular aggregates. The study of cholesterol gallstone pathogenesis attained a good scientific footing about 25 years ago. Then, almost simultaneously, Hegardt and Dam[2] and Small et al.[3] provided quantitative methods of assessing cholesterol solubility in bile. Both evaluated cholesterol solubility as part of a water-bile salt-phospholipid-cholesterol system. The triangular coordinate system and its derivative the cholesterol saturation index[4] prevailed because it was easier to grasp and because its description was followed