Evidence for a transmissible factor in Crohn's disease¹

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SUMMARY The injection of rabbits' ileum with homogenates of both normal and Crohn's affected human bowel tissue gave Crohn's-like changes in 11 of 27 animals after six months, but 12 months after injection the rabbit bowel had reverted to normal. The addition of ampicillin to the homogenates prevented the appearance of these Crohn's-like changes in 12 out of 12 rabbits. These results are interpreted as providing evidence for a transmissible factor present in both normal and Crohn's affected bowel in the aetiology of Crohn's disease.

Since it was first described (Crohn *et al.*, 1932), many attempts have been made to elucidate the aetiology of Crohn's disease, all with equal lack of success. In recent years, efforts have been made to demonstrate the presence of a transmissible factor. The inoculation of mouse foot pads with a homogenate prepared from the bowel of a patient with Crohn's disease (Mitchell and Rees, 1971) gave sarcoid-like granulomata in 13 of 56 foot pads, whereas with control animals injected with normal lymph node tissue only one out of 153 foot pads showed similar granulomata. Similar positive findings have also been reported (Taub and Siltzbach, 1974); five of 16 experimental mice developed foot pad granulomata using similar methods. The injection of homogenates of Crohn's affected and normal bowel into the wall of the terminal ileum of rabbits has also been made (Cave *et al.*, 1973). None of their controls showed any response, but all six of the homogenate injected animals developed the microscopic features of Crohn's disease. But negative findings with similar methods have also been reported (Bolton *et al.*, 1973; Heatley *et al.*, 1975). In this paper, the results of injecting rabbit ileum with normal and Crohn's affected human bowel and the effects of adding ampicillin to the homogenates are reported.

Methods

New Zealand white rabbits were used—56 have entered the experiment to date. The tissue for

injection was obtained at laparotomy. Normal human bowel was taken from hemicolectomy specimens resected because of a carcinoma; the tissue used was at least 10 cm from any tumour and grossly and microscopically appeared normal. Crohn's disease tissue was also examined microscopically to confirm the diagnosis (Morson and Dawson, 1972a) and stains for acid fast bacilli were negative. Crohn's material was from ileum in two cases, colon in one, and from cutaneous tissue affected by fistulous Crohn's disease in the fourth case. The homogenates were made as follows. The biopsy materials were washed in sterile normal saline, cut into small pieces, frozen with dry ice and alcohol, and stored at -4°C for a period of four days to two weeks. After thawing, the pieces were mixed with an equal volume of normal saline to give a total volume of 5 ml and homogenised five times. Ampicillin was added to two of the homogenates to give a solution of 125 000 units per ml and incubated for 24 hours at 37°C . Two millilitres of the homogenate were then injected subserosally into the terminal 5 cm of ileum of each rabbit in multiple blebs of 0.2 ml each. As controls, rabbits were given 2 ml injections of normal saline. Biopsies were taken at six and 12 months after injection. The whole of each biopsy was cut as multiple paraffin sections at 4μ (mean 19 sections per biopsy) and stained with H and E. The slides were examined by T.M.H. without knowledge of the material injected. The findings on microscopy were classified as normal, slight non-specific inflammation, or transmural inflammation with or without granulomata consistent with a diagnosis of Crohn's disease, using standard criteria (Morson and Dawson, 1972a).

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Results

Necropsies with microscopy were made on all rabbits who died. By the time of the first biopsy at six months three animals had died from causes unrelated to the injection of the homogenate. At

12 months 11 more animals had died, again from unrelated causes. The results in the surviving animals are given in the Table. In animals injected with normal saline no gross or microscopic lesions were found at six months. Biopsies at 12 months were not made. Eleven of 27 rabbits injected with

Table Results of homogenate injection in surviving animals

Rabbits (no.)	Homogenate	Findings					
		Six months			12 months		
		Survivors*	Normal or non-specific inflammation	Crohn's-like changes	Survivors*	Normal or non-specific inflammation	Crohn's-like changes
13	Normal saline	13	13	0	13	No biopsies	No biopsies
12	Normal human bowel	11	4	7	6	6	0
19	Human Crohn's tissue	16	12	4	11	11	0
6	Normal human bowel and ampicillin	6	6	0	5	5	0
6	Human Crohn's tissue and ampicillin	6	6	0	6	6	0

*The four animals dying before six months and the additional 11 animals dying before 12 months all showed no gross or microscopic evidence of Crohn's-like changes.

Figure Biopsy of rabbit ileum six months after injection of human Crohn's tissue showing transmurular inflammation with granulomata. H and E, $\times 40$.

normal or Crohn's diseased human bowel showed evidence of Crohn's-like changes; at laparotomy the injected bowel showed gross thickening and adhesions and microscopically transmural inflammation was present together with noncaseating epithelioid cell granulomata in nine of the 11 animals (Figure). While homogenates prepared from four patients suffering from Crohn's were injected, homogenates from only two of these patients induced Crohn's-like changes in the recipient rabbits. As outlined in the Table, homogenate prepared from normal bowel also induced Crohn's-like changes in the recipient rabbits. The addition of ampicillin to both normal and Crohn's tissue homogenates resulted in a failure to produce any Crohn's-like changes in the injected rabbits.

Discussion

As in the present results Cave *et al.* (1973) have also produced experimentally Crohn's-like changes by the injection of Crohn's tissue. The factor in the homogenates which evokes Crohn's-like changes is unknown. The resultant reaction is not of foreign body type—doubly refractile particles or foreign body giant cells were not seen. The fact that ampicillin abolishes the reaction is in favour of a transmissible factor, as are the negative findings 12 months after injection; persistence of the factor or repeated exposure to it appear to be necessary for the persistence of Crohn's-like changes. The fact that treatment with ampicillin prevented a reaction also makes it unlikely that the factor is viral in nature. The treatment of the donor tissues by washing and freezing cannot be expected to have removed all the microbial agents present in such bowel tissues. Although the homogenates were incubated with ampicillin for 24 hours for bacteriocidal action to take place the incubation may also have altered other unknown non-microbial factors causing Crohn's-like changes in the rabbit, and perhaps this may explain why no changes resulted.

Immunological factors have been postulated (Morson and Dawson, 1972b) as important in the development and persistence of Crohn's disease but others (Bolton, 1974; MacPherson *et al.*, 1976) contend that immune depression in Crohn's disease has not been proven and that its role in pathogenesis is uncertain. In the present experiments immunity need not be invoked as a factor, although the reaction to a second injection of homogenate could bring immunological factors into play. Hypersensitivity as a result of repeated challenges by a transmissible agent may account for the chronicity and recurrence of human Crohn's disease (Shorter *et al.*, 1972). Although all four donor Crohn's

tissue homogenates showed the histological changes of Crohn's, it is interesting that only in the two tissues giving no reaction in the rabbit were granulomata present. The presence of granulomata in human Crohn's disease is thought to affect the prognosis favourably (Glass and Baker, 1976); it may be that patients with such a granulomatous response are reacting more effectively against the causative agent, which may therefore be present in suboptimal amounts in the homogenate injected. Unlike previous reports (Bolton *et al.*, 1973; Cave *et al.*, 1973; Cave *et al.*, 1975; Heatley *et al.*, 1975), Crohn's-like changes were noted in rabbits after injection of homogenate of normal human bowel. In view of these findings, it is suggested that the transmissible factor is present in normal, as well as Crohn's affected bowel. Previous results by Sachar *et al.* (1975) may be interpreted as supporting this hypothesis, and the findings of Cave *et al.* (1976) that a transmissible agent may be involved in the aetiology of ulcerative colitis is an interesting parallel observation.

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References

- Bolton, P. M., Owen, E., Heatley, R. V., Jones Williams, W., and Hughes, L. E. (1973). Negative findings in laboratory animals for a transmissible agent in Crohn's disease. *Lancet*, **2**, 1122-1124.
- Bolton, P. M. (1974). Immune competence in Crohn's disease. *Lancet*, **2**, 158.
- Cave, D. R., Mitchell, D. N., and Brooke, B. N. (1975). Observations on the transmissibility of Crohn's disease and ulcerative colitis. *Gut*, **16**, 401.
- Cave, D. R., Mitchell, D. N., and Brooke, B. N. (1976). Evidence of an agent transmissible from ulcerative colitis tissue. *Lancet*, **1**, 1311-1315.
- Cave, D. R., Mitchell, D. N., Kane, S. P., and Brooke, B. N. (1973). Further animal evidence of a transmissible agent in Crohn's disease. *Lancet*, **2**, 1120-1122.
- Crohn, B. B., Ginzburg, L., and Oppenheimer, G. D. (1932). Regional ileitis; a pathological and clinical entity. *Journal of the American Medical Association*, **99**, 1323-1328.
- Glass, R. E., and Baker, W. N. W. (1976). Role of the granuloma in recurrent Crohn's disease. *Gut*, **17**, 75-77.
- Heatley, R. V., Bolton, P. M., Owen, E., Jones Williams, W., and Hughes, L. E. (1975). A search for a transmissible agent in Crohn's disease. *Gut*, **16**, 528-532.
- MacPherson, B. R., Albertini, R. J., and Beeken, W. L. (1976). Immunological studies in patients with Crohn's disease. *Gut*, **17**, 100-106.
- Mitchell, D. N., and Rees, R. J. W. (1971). Sarcoidosis and Crohn's disease. *Proceedings of the Royal Society of Medicine*, **64**, 944-945.
- Morson, B. C., and Dawson, I. M. P. (1972a). *Gastrointestinal Pathology*, pp. 269-272. Blackwell: Oxford.

- Morson, B. C., and Dawson, I. M. P. (1972b). *Gastro-intestinal Pathology*, p. 262. Blackwell: Oxford.
- Sachar, D. B., Taub, R. N., and Janowitz, H. D. (1975). A transmissible agent in Crohn's disease? New pursuit of an old concept. *New England Journal of Medicine*, **293**, 354-355.
- Shorter, R. G., Huizenga, K. A., and Spenser, R. J. (1972). Inflammatory bowel disease. *Digestive Diseases*, **17**, 1024-1032.
- Taub, R. N., and Siltzbach, L. E. (1974). Induction of granulomas in mice by injection of human sarcoid and ileitis homogenates. In *Proceedings of the VI International Conference on Sarcoidosis, Tokyo*, pp. 20-21. Edited by K. Iwai and Y. Hosoda. University Park Press: Baltimore.