Subepithelial collagen in intestinal malabsorption

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SUMMARY Intestinal biopsies from 146 patients with adult coeliac disease and 13 patients with intestinal villous atrophy of different aetiology were assessed for the presence of subepithelial collagen and compared with a group of 20 control subjects.

Subepithelial collagen was a common and non-specific finding observed in intestinal biopsies from patients suffering from adult coeliac disease (36%) and tropical sprue.

In adult coeliac disease the described subepithelial changes usually regress following treatment, though marked subepithelial collagen deposition may indicate a poor prognosis.

The study showed that the presence of marked subepithelial collagen in a flat jejunal biopsy does not define a separate clinical entity.

The term 'collagenous sprue' has been used to describe extensive collagenization of the lamina propria in the flat jejunal mucosa of a patient with malabsorption unresponsive to a gluten-free diet (Weinstein, Saunders, Tytgat, and Rubin, 1970). Similar histological findings were reported previously in other patients (Schein, 1947; Hourihane, 1963; Cooke, Fone, Cox, Maynell, and Gaddie, 1963; Neale, 1968). More recently Holdstock and Oleesky (1973) have reported malabsorption and subtotal villous atrophy with extensive subepithelial collagen in a patient who responded to combined therapy with a gluten-free diet and corticosteroids. The question arises therefore as to the specificity of this change in a flat jejunal biopsy. The purpose of this paper is to assess the significance of subepithelial collagen and its prognostic value.

Materials and Methods

INTESTINAL BIOPSIES

Histological sections of 338 jejunal biopsies were studied from 146 patients suffering from adult coeliac disease. All patients had evidence of malabsorption and a flat jejunal biopsy and responded initially to treatment with a gluten-free diet either clinically or histologically. Mucosal biopsies from seven patients with tropical sprue, four patients with Whipple's disease, one patient with hypogamma-

globulinaemia, and one patient with strongyloidiasis were also studied. Three ileal biopsies from three patients with chronic tropical sprue were included in the study.

CONTROLS

The 20 control subjects whose jejunal biopsies showed normal dissecting microscope and histological appearances consisted of healthy laboratory workers and patients being investigated for gastrointestinal symptoms.

STAINING TECHNIQUE AND GRADING OF BIOPSIES

All sections were stained with haematoxylin and eosin (H & E) and haematoxylin van Gieson (HVG). The latter stain was used in order to confirm that a pink subepithelial zone visualized with H & E staining represented collagen and not serous exudate. They were then examined independently by two

Grade	Subepithelial Collagen	
	Amount	Staining Pattern (HVG)
1	None	Thin linear pattern confined to basement membrane
2	Present but slight	Thick linear pattern with involvement of vascular basement membrane
3	Moderate to marked	Thick irregular band extending into lamina propria to variable depths

Table I *Grading system*

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Fig 1 Jejunal biopsy from control patient, showing a normal grade 1 staining pattern. The basement membrane can be defined as a thin but distinct line (arrows) below the villous absorptive cells. HVG $\times 200$.

observers (K.H. and R.B., neither of whom was aware of the clinical diagnosis) and the amount of subepithelial collagen present noted.

A grading system from 1 to 3 was devised to assess the presence and amount of subepithelial collagen (table I). In grade 1, stainable collagen was either absent or strictly confined to the vicinity of the basement membrane. Thus the staining pattern is that of

a thin, distinct line related to the epithelial basement membrane (fig 1). In grade 2 there is a definite zone of subepithelial collagen which now involves the subepithelial vessels, and which results in a thickened linear staining pattern (fig 2). Grade 3 represents extension of the fibrotic process into the lamina propria with formation of an irregular band of subepithelial collagen which may be very thick

Fig 2 Jejunal biopsy from a patient with adult coeliac disease showing grade 2 changes. The mucosa is flat and the surface epithelial cells are reduced in height. There is subepithelial collagen formation (arrow) and involvement of the subepithelial vessels. HVG $\times 200$.

Fig 3 Jejunal biopsy from a patient with adult coeliac disease with severe (grade 3) changes. Below the surface epithelium of the flat mucosa there is a thick, irregular band of collagen due to the extension of the fibrotic process into the lamina propria. HVG $\times 200$.

(fig 3). Observer agreement in grading the sections exceeded 95%.

Results

SUBEPITHELIAL COLLAGEN IN SMALL INTESTINAL BIOPSIES

The results are shown in table II. The amount of stainable collagen in biopsies from control subjects never exceeded grade 1. Thus grade 1 changes were accepted as within normal limits. In 94 of 146 patients with adult coeliac disease (64%), the appearances were similar (grade 1). In 41 patients (28%), however, the jejunal biopsy showed definite subepithelial collagen formation in the form of a thickened linear staining pattern (grade 2). Extensive subepithelial collagen (grade 3) was seen in 11 coeliac patients (8%).

Mild subepithelial collagen (grade 2) was found in intestinal biopsies from two patients with tropical

sprue. In one of these patients similar changes were present in the ileum as well as the jejunum (grade 2). The ileal biopsy from a third patient with tropical sprue showed marked (grade 3) subepithelial collagen formation. The biopsies from patients suffering from other diseases showed no abnormal changes (table II).

The tendency of the epithelial layer to strip off over collagen bands and the focal nature of the fibrotic process even within a single biopsy were conspicuous histological features. In one biopsy fibrin exudation around subepithelial vessels caused a homogeneous eosinophilic band in an H&E-stained section resulting in an identical appearance to that seen in subepithelial collagenization. However, the HVG stain for collagen was negative.

EFFECT OF TREATMENT

The effect of treatment with a gluten-free diet on subepithelial changes was studied in follow-up

	No. of Patients	Grade		
		1	2	3
Adult coeliac disease	146	94 (64%)	41 (28%)	11 (8%)
Tropical sprue	7	4	2 ¹	1 ¹
Whipple's disease	4	4		
Strongyloidiasis	1	1		
Hypogammaglobulinaemia	1	1		
Control subjects	20	20		

Table II Subepithelial collagen in jejunal biopsies

¹Includes one ileal biopsy

Grade of Biopsy in Untreated Patients	No Change	Disappearance of Subepithelial Collagen	Appearance of Subepithelial Collagen
1	29	—	1 (grade 3)
2	3	12	0
3	3 (2 ¹)	3	—

Table III Effect of a gluten-free diet on subepithelial collagen in jejunal biopsies in coeliac disease

¹Died of unremitting malabsorption.

Fig 4 *Jejunal biopsy from a patient with adult coeliac disease (a) on diagnosis and (b) following gluten withdrawal. Note the disappearance of the subepithelial collagen together with reversion to a normal morphological appearance. HVG $\times 200$.*

Fig 4b

jejunal biopsies of 51 adult coeliac patients who had taken a gluten-free diet for at least six months (table III). Appearances remained unchanged in 29 patients whose pretreatment biopsies were grade 1, ie, normal. One patient in this group, however, showed

irregular, marked (grade 3) subepithelial collagen in some of her follow-up biopsies, despite increase in villous height following gluten withdrawal. Twelve of 15 biopsies showing grade 2 changes improved to normal, most of them within six months of treat-

ment. Mild subepithelial collagen persisted in the remaining three in this group despite an increase in villous height in response to treatment. No deterioration from grade 2 to 3 was observed in this group.

In the group with marked subepithelial changes (grade 3) there were three patients who showed a full recovery in every respect and whose biopsies showed disappearance of the subepithelial collagen (figs 4a and b). No improvement occurred in three patients whose biopsies showed grade 3 changes despite prolonged gluten withdrawal. Two of these patients, who have been described in clinico-pathological conferences (Neale, 1968; Dowling and Henry, 1972), died of unremitting malabsorption following initially good clinical responses to a gluten-free diet. In each patient relapse appeared to follow inadvertent gluten ingestion.

Four further coeliac patients with marked subepithelial collagen in the pretreatment jejunal biopsies are not included in table III because there are no follow-up biopsies. Two showed a good clinical response to a gluten-free diet for one to one and a half years, but relapse was associated with Addison's disease in one and pulmonary tuberculosis in the other. Both died from complications of these diseases despite intensive treatment. The histological response in the two other patients could not be assessed as they did not adhere strictly to a gluten-free diet but both are alive and well after being followed up for four and 15 years.

Intestinal biopsies from three patients suffering from chronic tropical sprue showed subepithelial collagen formation. Grade 2 changes in one tropical sprue patient disappeared after folic acid therapy. No follow-up biopsies were available from the remaining two tropical sprue patients.

The results of this study confirm that collagenous subepithelial thickening, sometimes extending deeply into the lamina propria of jejunal biopsies, is a common finding in adult coeliac disease. These subepithelial changes were present in 36% of patients with adult coeliac disease in this series, a frequency similar to that reported by Hourihane (1963) and by Cooke *et al* (1963). In addition, identical changes were present in both jejunal and ileal biopsies in tropical sprue. Subepithelial collagen is therefore a non-specific finding probably related to an inflammatory reaction occurring in the region of the basement membrane. After gluten challenge in childhood and adult coeliac disease (Schmerling and Shiner, 1970; Doe, Henry, Holt, and Booth, 1972) jejunal biopsies showed thickening of basement membrane and subepithelial deposition of collagen fibrils as early as 48 hours after gluten challenge.

When studying the effect of a gluten-free diet on the subepithelial changes in adult coeliac patients, it became apparent that the majority of biopsies improved to the normal range, usually within two to six months. This improvement was observed even in cases where marked subepithelial collagen formation had been present, though it seemed to take much longer. However, in two of 11 coeliac patients with marked (grade 3) changes in their jejunal biopsies, severe relapse occurred, usually after introducing gluten into their diet, leading to death from unremitting malabsorption despite hospital treatment with corticosteroids and a gluten-free diet. The finding of marked subepithelial collagen formation in a flat jejunal biopsy may, therefore, indicate a poor prognosis in adult coeliac disease. It is not clear, however, why these patients became unresponsive to gluten withdrawal.

In answer to the initial question of whether marked subepithelial collagenization in a flat jejunal biopsy represents a separate disease entity as suggested by Weinstein *et al* (1970), the results of this study show that this histological feature is non-specific, in as much as it occurs in tropical sprue and quite frequently in otherwise typical adult coeliac disease. Furthermore, in coeliac disease the lesion, which is often focal in nature, may be reversible on treatment with a gluten-free diet when improvement parallels clinical and morphological recovery.

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References

- Cooke, W. T., Fone, D. J., Cox, E. V., Meynell, M. J., and Gaddie, R. (1963). Adult coeliac disease. *Gut*, **4**, 279-291.
- Doe, W., Henry, K., Holt, L., and Booth, C. C. (1972). An immunological study of adult coeliac disease. *Gut*, **13**, 324-325.
- Dowling, R. H., and Henry, K. (1972). Non-responsive coeliac disease. *Brit. med. J.*, **3**, 624-631.
- Holdstock, D. J., and Oleesky, S. (1973). Successful treatment of collagenous sprue with combination of prednisolone and gluten-free diet. *Postgrad. med. J.*, **49**, 664-667.
- Hourihane, D. O'B. (1963). The histology of intestinal biopsies. *Proc. roy. Soc. Med.*, **56**, 1073-1077.
- Neale, G. (1968). A case of adult coeliac disease resistant to treatment. *Brit. med. J.*, **2**, 678-684.
- Schein, J. (1947). Syndrome of non tropical sprue with hitherto undescribed lesions of the intestine. *Gastroenterology*, **8**, 438-460.
- Schmerling, D. H., and Shiner, M. (1970). The response of the intestinal mucosa to the intraduodenal instillation of gluten in patients with coeliac disease during remission. In *Coeliac Disease: Proceedings of an International Conference*, edited by C. C. Booth and R. H. Dowling, pp. 64-74. Churchill, Livingstone, Edinburgh and London.
- Weinstein, W. M., Saunders, D. R., Tytgat, G. N., and Rubin, C. E. (1970). Collagenous sprue—an unrecognised type of malabsorption. *New Engl. J. Med.*, **283**, 1297-1301.