HISTOLOGICAL CHANGES IN THE DUODENAL MUCOSA IN COELIAC DISEASE

REVERSIBILITY DURING TREATMENT WITH A WHEAT GLUTEN FREE DIET

BY

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From the examination of biopsy material Sakula and Shiner (1957), Rubin, Brandborg, Phelps and Taylor (1960a) have demonstrated histological changes in the duodenal mucosa of patients with coeliac disease or wheat gluten intolerance and shown that these changes are identical with those demonstrated by themselves and others in adult patients with idiopathic steatorrhoea. The changes recorded by all observers in both conditions are very similar but vary in severity in individual patients. However, the interpretation of the nature of these changes and whether they are primary anatomical ones or secondary to the effect of ingested food substances has not been clearly demonstrated and is still a matter for conjecture.

Shiner and Doniach (1960) in their most recent publication state that the histology suggests defective formation of villous epithelial cells and describe the picture as villous atrophy of varying grades. They suggest that the changes are primary and irreversible and the harmful effect of wheat gluten is superimposed on this primary atrophy. Rubin, Brandborg, Phelps, Taylor, Murray, Steimler, Howry and Volwiler (1960b) also suggest that the lesion is an irreversible one and that it may persist throughout life; they agree, however, that their evidence for this is incomplete, as in two patients the lesion appeared to improve with treatment.

The present study demonstrates the histological changes in the duodenal mucosa in biopsy material from a group of newly diagnosed young coeliac patients and the complete or partial disappearance of these changes during the first year of treatment in patients of this group from whom subsequent biopsies have already been obtained.

Material and Method:

Clinical Material. From January, 1959 to April, 1960, a single duodenal mucosal biopsy was obtained from each of 17 children newly diagnosed as cases of coeliac disease or wheat gluten intolerance. All children were subsequently treated by excluding wheat gluten from the diet and were followed carefully. In 11 of these patients biopsies have been repeated at certain intervals during treatment.

Criteria for initial diagnosis were rigid and only children showing the features of clinical history and physical examination recorded in previous publications (Anderson, Frazer, French, Gerrard, Sammons and Smellie, 1952b; Anderson, 1959) were included. Laboratory investigations demonstrated steatorrhoea by fat balance, normal quantities of pancreatic enzymes in duodenal fluid using the methods described previously (Anderson et al., 1952b), normal sodium and chloride content of sweat determined by the method of Anderson and Freeman (1958). Gastro-intestinal infection and tuberculosis were excluded by relevant tests. All patients improved rapidly and showed normal clinical progress on a wheat gluten free diet.

During the period of treatment the diet was carefully checked at each visit. At the time of each subsequent biopsy, a three-day stool collection was examined for total fat excretion; haemoglobin, weight, height and clinical progress were recorded.

Collection of Biopsy Material. Biopsies were obtained by the multipurpose suction biopsy tube of Brandborg, Rubin and Quinton (1959) without the mercury containing balloon attached. Patients were fasted overnight and the tube passed orally while the patient was under basal anaesthesia with rectal bromethol (100 mg./kg.). The end of the tube was manoeuvred into the duodenum under fluoroscopy using an image intensifier and television screen. Radiation table dose was between 0.5 and 1 roentgen in young children and did not exceed 2 roentgens in the older children; the procedure was abandoned after these levels had been reached, if the tube was not in position. Specimens were taken from the second, third or fourth part of the duodenum and were about 3-5 mm. in diameter, consisting of mucosa and muscularis mucosae with a minimum of submucous tissue.
It has not been found easy with this type of tube, within the limits of radiation dosage and with the tube passed by mouth instead of nose, to obtain specimens beyond the duodenojejunal flexure in young children. Therefore in this paper the duodenal mucosal changes only are described.

No post-biopsy complications were encountered. The use of basal anaesthesia helped in the ease of the procedure and in abolishing any unpleasant memories of the event from the minds of the children, allowing follow-up material to be obtained more readily. A single biopsy specimen only from each child was examined as success in obtaining more than one specimen at a time was very limited.

The method for orientation of the biopsy specimens, fixing and H. and E. staining followed substantially those described by Rubin et al. (1960a) and, as these authors recommend, sections from the central core of the specimens were examined to avoid tangential artefacts. The sections were examined, and compared with reference to particular features by the author, who was aware of their origin and also by two pathologists of the Royal Children’s Hospital, Melbourne, Dr. A. Williams and Dr. L. Taft, independently, and without knowledge of their origin. Quantitative counting of villi as described by Rubin et al. (1960a) was not carried out.

Duodenal biopsies from children without organic disease have been obtained under identical conditions and examined in the same way. The appearance described by Shiner and Doniach (1960) and Rubin et al. (1960a) for normal duodenum have been confirmed, but for the sake of brevity are not illustrated again in this paper.

**Results**

**Initial Biopsy Specimens.** The duodenal mucosa was abnormal in all patients. Low magnification microscopic examination showed a characteristically flat surface to the mucous membrane. In most cases villi were completely absent, replaced by broad flat areas between crypts (Figs. 1, 3, 7 and 11). In a few, flattened stumpy villi were present but these did not show branching nor project above the surface (Fig. 2). The chief characteristic was the straight flat edge of the mucosal surface. In the majority, surface epithelial cells were altered, the single layer of tall columnar cells with basal nuclei being replaced by flattened cells with irregularly placed nuclei, sometimes several layers of cells giving a stratified appearance. Usually very few goblet cells were present on the surface, but in the crypts epithelial cells had a more normal appearance and goblet cells were present. The lamina propria was considered of normal thickness but in some cases a deceptive increase in thickness was suggested because the lamina was not drawn up into the villi. The lamina propria contained many plasma cells, some eosinophils and occasional polymorphonuclear cells, but there was considerable variation in the density of cell population among individual cases, this being often about the upper limits of normal, but sometimes greater than normal.

As only one biopsy specimen was obtained from each patient a strict quantitative assessment of the mucosal changes seemed unwise since it is probable that these changes vary from place to place in the gut. However, the biopsies did show individual variation and the degree of severity of the changes was rated according to a series of pluses (+), by the three observers, independently, with very minor disagreement. In Table 1 this rating is compared with certain clinical findings. Only the most characteristic features were considered in determining the rating; for instance, degree of

![Fig. 1.—Example of duodenal biopsy (× 37) from Group I. Case 5—moderately severe (+ + +) changes—long flat areas between crypts—irregularity of surface epithelium.](http://adc.bmj.com/first-published-as/10.1136/adc.35.183.419)

![Fig. 2.—Example of duodenal biopsy (× 35) from Group II. Case 14—straight edge to mucosal surface—short stumpy villi—minimal change in epithelial cells.](http://adc.bmj.com/first-published-as/10.1136/adc.35.183.419)
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TABLE 1
CLINICAL FINDINGS IN 17 CASES OF COELIAC DISEASE COMPARED WITH SEVERITY OF DUODENAL MUCOSAL CHANGES IN BIOPSY SPECIMENS

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age in Years</th>
<th>Approximate Duration of Symptoms</th>
<th>Weight (kg.)</th>
<th>Hb g./100 ml.</th>
<th>Fat Excretion (g./day) Average 5 days</th>
<th>Severity of Histological Changes in Duodenal Mucosa</th>
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<td>Most severe—disorganization of surface epithelium with stratification, no villi.</td>
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<td>Occasional flattened villi, otherwise as above.</td>
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<td>Surface epithelium less severely disorganized, no stratification. Occasional flattened villi.</td>
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<td>Least severe, surface epithelium still tall columnar with some flattened areas. Villi stumpy and poorly differentiated from crypts. Straight edge to mucosa.</td>
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TABLE 2
CLINICAL FINDINGS IN 11 TREATED CASES OF COELIAC DISEASE (FROM TABLE 1) AND DEGREE OF IMPROVEMENT IN DUODENAL BIOPSY

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Duration of Treatment</th>
<th>Strictness of Diet</th>
<th>Weight (kg.)</th>
<th>Hb g./100 ml.</th>
<th>Fat Excretion (g./day)</th>
<th>Biopsy—Degree of Improvement</th>
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+ Villi present but still stumpy, surface epithelium tall columnar cells.

+ + Villi longer but not quite normal, surface epithelium normal.

+ + + Villi and surface epithelium within normal limits.

Fat Excretion. Initial: Average of five days. In-patient. Follow-up: Average of three days. Usually out-patient.

Diet: As far as could be assessed by comprehensive questioning of mother.

disorganization of surface epithelium and state of the villi.

Table 1 shows that the cases fall into two groups, the first 11 in whom the changes were severe or moderately severe, and the remaining six in whom the changes were definitely milder, particularly the change in surface epithelium. The majority of the older children and therefore those in whom
symptoms had been present for the longest time, are in the first group. However, this correlation is not complete as there is one older child with mild changes (Case 12) and two quite young children (Cases 1 and 2) with very severe (+ + + +) changes. Among the first group the degree of malabsorption in most cases appears to be rather more severe than in the second group but this correlation is still not extremely close, Cases 12 and 15 showing moderately severe malabsorption and Cases 1, 9 and 11 showing a milder degree of malabsorption. The degree of clinical illness did not correlate with the changes, as some of the very young children of the second group showed quite severe symptoms, whereas the older children in the first group showed milder clinical symptoms which no doubt accounted for their late diagnosis. The two 10-year-old boys had been diagnosed in early infancy, treated for one or two years, and had then resumed a normal diet and were considered fairly well until readmitted for investigation of recurrent abdominal pain.

Biopsy Specimens During Treatment. In Table 2 the patients from Table 1 who had biopsies repeated during treatment are listed with records of fat excretion, haemoglobin and weight gain at those stages, together with some indication of the degree of improvement in the appearance of the mucosa. In all cases alteration and improvement towards normal were noted, but to a variable degree, resumption of a normal or near normal appearance of the surface epithelial cells being the most constant and marked feature. Tall columnar cells interspersed with goblet cells are again present. Villi reappeared in each case but were very variable in their size, shape and number. Again a rating has been given to each of the subsequent biopsies, according to the degree of improvement in the surface epithelium and the villi. In five (+ + + +) the biopsies were considered to be within normal limits; in four (+ +) the surface epithelium was normal in appearance but the villi were considered to be still somewhat shorter and wider than normal. In two the improvement was slight (+) villi being very short and blunt but there was definite improvement in the appearance of the surface epithelium which was of the normal tall columnar type. The degree of improvement did not correlate well with any particular clinical feature, but was present after three months’ treatment (Cases 10 and 12) to a similar extent, as after nine to 12 months’ treatment (Cases 6 and 7).

Dietary restriction was well carried out but in most patients occasional lapses took place, more particularly in the school age children who accepted offerings from schoolmates, or because the mother was unaware that certain prepared and tinned foods contained gluten. Absorption was not always quite normal when the biopsies were repeated and these dietary lapses may have contributed to this. Marked clinical improvement had taken place in each case as can be seen from the weight and haemoglobin gains, but the biopsy did not always show a comparable degree of improvement.

Figs. 3 to 14 give visual evidence of the improvement in three patients. Low magnification pictures show the overall appearance of the biopsy with reappearance of villi during treatment, and higher magnification illustrates the detail of epithelial cells and lamina propria. Figs. 3 to 6, from Case 6, illustrate the return to a normal or near normal appearance in the specimen taken 12 months after the beginning of treatment. Figs. 7 to 10, from Case 10, illustrate biopsies taken initially and after only three months of treatment and show a return to normal appearance of villi and surface epithelium. Figs. 11 to 14 from Case 1 illustrate a lesser degree of improvement of the villi, despite good clinical improvement, strict dietary regime and normal absorption; initial changes were of the severe type (+ + + +) with complete absence of villi and gross alteration of surface epithelial cells.

Discussion

Histological changes in the duodenal mucosa of patients with coeliac disease investigated in this study appear to be identical with those recorded by Sakula and Shiner (1957) and Rubin et al. (1960a). The variation in severity of the changes in the initial biopsy specimens parallels closely that observed by Rubin et al. (1960b) and the present findings agree with theirs, in that little correlation occurs between the severity of the lesion and the clinical symptoms; somewhat more correlation occurs with the degree of malabsorption; the closest degree of correlation is with the age of the patient. The most severe lesions occurred in the older children although again there were exceptions.

Changes in the mucosa of the small intestine in patients with coeliac disease and idiopathic steatorrhoea indicate a reduction in surface area exposed to digested foodstuffs and this is thought to be responsible for malabsorption. In the present study some of the patients with severe degree of malabsorption, as determined by fat balance, did show the most severe changes in histology of the duodenal mucosa. However, some of the children with milder lesions showed considerable malabsorption (Cases 12 and 15). Explanation of this is uncertain but only one biopsy
from each patient has been examined and the changes in the rest of the mucosa may have varied as shown by Rubin et al. (1960a). That clinical symptoms did not parallel biopsy changes is not so surprising since the degree of malabsorption does not always parallel severity of clinical symptoms. Observation of patients with coeliac disease during a number of years has shown that this latter finding is a common one. It is also characteristic that symptoms in later childhood may not be as severe as in early life, despite an apparently greater absorptive defect. This was clearly seen in Cases 3 and 4, two boys of 10 years, who were thought to have recovered from childhood coeliac disease and were taking a normal diet. Their absorptive defect was considerable despite only mild symptoms of recurrent abdominal pain, recurrent loose stools in one and constipation in the other. The author holds the view that the degree of malabsorption in coeliac disease does not fully explain the degree of illness nor malnutrition and that there is probably some generalized deleterious effect of the toxic fraction of wheat gluten, this effect being more serious in the young child.

In the present series mucosal changes in the biopsies obtained during the patient’s treatment with a wheat gluten-free diet have altered, and a more normal and sometimes quite normal appearance is resumed. The improvement is consistent in type although variable in degree. This finding is at variance with that of Sakula and Shiner (1957), who report one case of coeliac disease, stating that improvement in mucosal histology did not occur after five weeks’ treatment. However, they give no detail of the rigidity of the gluten-free diet, nor the state of absorption at the time of the second biopsy. Rubin et al. (1960b), in their most recent communication, report two patients in whom they considered some improvement had taken place on a gluten-free regime, although they did not
discard the view that the histological lesion was a primary one, because other patients did not show such improvement.

Single biopsies only have been examined in the present study, the state of the rest of the small intestine at the time being unknown. It is perhaps unwise to make a dogmatic conclusion from this type of material but the fact that all the patients who were followed showed some degree of improvement in histology of the mucosa is enough evidence to conclude that the lesion is at least associated with the presence of wheat gluten in the diet.

The degree of improvement varied considerably, some subsequent biopsies being normal, others showing moderate improvement, others still less. However, all showed a surface epithelial cell which looked normal, in that it was tall columnar with a basal nucleus. Variation showed up particularly in the size, shape and number of villi. Interpretation of what is normal in this respect is difficult, as mentioned by Shiner and Doniach (1960) and observed by ourselves in controls. The fact that there can therefore be considerable variation in size, shape and number of villi in patients with normal intestinal absorption makes it not impossible that some coeliac patients may have normal or near normal villi. Moreover, in man, the small intestine is certainly longer than is necessary for maximal absorption, since considerable lengths may be removed surgically without altering absorptive capacity. Consequently a complete return to normality in histological appearances of the duodenum is not necessary before the child can be clinically well and show normal absorption. The degree of improvement may be sufficient, or other
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FIG. 11.—Initial biopsy ( x 28).

FIG. 12.—Biopsy after nine months’ treatment ( x 28).

FIG. 13.—Initial biopsy ( x 86).

FIG. 14.—Biopsy after nine months’ treatment ( x 86).

FIGS. 11–14.—Case 1—example of most severe changes (+ + +) in initial duodenal biopsy specimen, and moderate (+ +) improvement after nine months’ treatment with a gluten-free diet. Villi still short and stumpy—epithelial cells normal.

parts of the gut may have improved still more.

If the abnormal changes are directly associated with the presence of gluten in the diet, return to complete normality must also depend on the rigidity and persistence of the gluten-free dietary regime. Experience over a number of years has shown how difficult it is for active toddlers and school children to be accurately restricted in diet. The complexity and diversity of modern, prepared and tinned foods makes it difficult for a mother to know which foods contain gluten, often used as an additive. Until the toxic fraction of wheat gluten is recognized it will not be clearly known whether small amounts may occur in other foodstuffs, in amounts, for instance, too small to cause clinical symptoms but enough to prevent full normality of the mucosa. Rubin et al. (1960b) did not comment on the strictness of the diet in their patients who did not show improvement in duodenal histology, but it is perhaps significant that in the two patients who did show improvement, biopsies were obtained after only short periods of treatment. It is well known to those who treat these children that the diet is much more rigidly adhered to in the early stages of treatment than later when the child is well. The present study shows that improvement in histological appearances is evident quite early during treatment, even after only three months, at which time it may be as marked as after nine months.

From the data presented in this paper it can be seen that in very young coeliac patients who were kept as rigidly as possible to a wheat gluten-free dietary regime, the histological appearances of the duodenal mucosa improved significantly. Improvement was also noted in 6-year-old children who had had symptoms since toddler age. The two 10-year-old boys have not yet been re-investigated. The appearance of the mucosa in these older patients resembles the severity of change seen...
in adult patients. It is well known that adult patients do not show such a quick response clinically to a gluten-free dietary regime as do young children (French, Hawkins and Smith, 1957). This may have some relationship to the fact that Rubin et al. (1960b) do not demonstrate significant improvement in biopsy specimens from older patients. Have the changes been present too long for much improvement in structure to take place, or will it take longer, or depend on the replacement of other long-standing deficiencies in these patients?

Interpretation of the mechanism of the deleterious effect of gluten on the duodenal mucosa is still a matter for conjecture and no real evidence for this is presented in this paper. Present theories of the toxic mechanism of gluten in coeliac disease postulate the production of a harmful peptide in the intestine due to incomplete proteolytic digestion of gluten (Weijers and Kamer, 1955; Frazer 1956; Alvey, Anderson and Freeman, 1957). If this is so, is this peptide directly irritative to the mucosal surface or is it absorbed, exerting its toxic influence generally on the patient and more particularly on the intestinal mucosa. Anderson, Astley, French and Gerrard (1952a) have shown from barium studies that the small intestine in coeliac disease is dilated and lacks the normal fine feathery patterning of the mucosa. They have suggested that this may be evidence of generalized loss of tone in the gut wall or that the muscularis mucosae is hypotonic. The dilatation disappears on recovery and reappears during relapse. It is well known that the young child with coeliac disease shows hypotonia of skeletal musculature out of proportion to the degree of malnutrition and that the child is temperamentally depressed. These features also disappear with treatment. It is possible that the harmful fraction of gluten may in some way cause hypotonia and that the effect of this on the gut is to depress the activity of smooth muscle and muscularis mucosae, perhaps altering the appearance of villi and mucosa. Verzar and McDougall (1936) have described and recorded the movements of the villi and their control by the muscularis mucosae. Johnson (1913) has shown, in animals, that when the small intestine is dilated the villi become shorter and blunter, the surface flatter and that alterations appear in the tall columnar cells. Is there villous atrophy as described by Shiner and Doniach (1960) or failure of formation of villi by alteration of villous mechanics?

It is possible that toxic influences other than gluten may produce similar changes in the gut and mucosa. Clinically and biochemically tropical sprue closely resembles adult idiopathic steatorrhoea and coeliac disease, but does not respond to a gluten-free dietary regime (French, et al., 1957). Butterworth and Perez-Santiago (1958) among others, have reported similar changes in the intestinal mucosa in patients with tropical sprue but these are milder in nature, resembling the less severe cases in the present series, and they are apparently reversible. The gut in this condition also has the same dilated hypotonic appearance (Ardran, French and Mucklow, 1950). Shiner and Doniach (1960) have reported similar mild reversible changes in biopsy specimens from patients with post-gastrectomy steatorrhoea. Rubin et al. (1960a) show good evidence that this mucosal lesion is specific to coeliac disease and adult idiopathic steatorrhoea among the series of patients they investigated. The present study shows no evidence for or against the specificity of this lesion owing to inadequacy of material. However, biopsy material from children with other disturbances of gastrointestinal function and motility, temporary as well as chronic, with or without associated malabsorption, must be studied before the specificity of this lesion, particularly in its milder grades, is unquestioned.

Summary

Histological changes in the duodenal mucosa in biopsy material obtained from 17 patients with newly-diagnosed coeliac disease are described. These changes are of a consistent pattern but vary in severity.

The abnormal changes disappeared completely or partially in biopsy specimens obtained from all 11 of these children who were followed during their first year of treatment with a diet free from wheat gluten.

The histological changes in the intestinal mucosa of patients with coeliac disease are considered to be directly associated with the ingestion of wheat gluten.

The histopathology of the lesion is briefly discussed.

Many people have helped in various phases of this work and to them I owe thanks: Dr. Rubin and his colleagues in Seattle for supplying me with an early model of the biopsy tube and personal instruction in the preparation of sections; the physicians of the Royal Children's Hospital for generously referring clinical material; the registrars, nursing staff and radiology staff who have helped in obtaining the biopsies; Miss Mavis Freeman and the Clinical Research Unit laboratory for the laboratory investigations; Miss Jean Allan for supervising the diets; the Pathology Department technicians for cutting and staining the sections; and lastly to the two pathologists, Dr. A. Williams and Dr. L. Taft, for reviewing the sections with me.
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REFERENCES

Addendum
Since this paper has been sent to the press, Rubin, Brandborg, Flick, Parmentier, Phelps and Van Niel (1960) have demonstrated in two treated adult sprue patients that instillation of wheat into the ileum was followed by changes in the mucosa. These changes were those characteristic of the sprue lesion prior to treatment with a gluten-free diet. These authors state that this demonstration of intestinal mucosal damage by wheat implicates gluten exposure as the precursor to anatomic changes in idiopathic sprue.

REFERENCE