PERORAL INTESTINAL BIOPSY

ANALYSIS OF RESULTS IN 134 PATIENTS*

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Thomas Gee, in his classical description of coeliac disease in 1888, remarked that 'nothing unnatural can be seen in the intestines—but whether atrophy of the glandular crypts be ever or always present I cannot tell'.[1] This was hardly surprising, since laparotomy at that time was rarely carried out and jejunal biopsy by means of gastro-intestinal intubation, unknown. Findings from autopsy were of little use, since rapid postmortem autolysis of the intestinal mucosa vitiated adequate interpretation of the histological changes.

The first suggestion of histological abnormalities in steatorrhoea, at least in tropical sprue, came in 1924 from Manson-Bahr[2] who based his findings on material obtained immediately after death. It was only in 1954 that unequivocal evidence of small-bowel changes in idiopathic steatorrhoea was obtained at laparotomy by Paulley.[3] His findings have, since then, been amply confirmed by means of peroral jejunal biopsy. The latter has now become a routine and indeed indispensable technique for the investigation of small-bowel disease.

The peroral jejunal biopsy technique was pioneered by Shiner[4] who crusaded with a modification of Wood's gastric biopsy tube. Refinements in technique soon followed and most centres now employ the Crosby-Kugler biopsy capsule[5] or, more recently, multiple biopsy instruments[6, 7] which have the added advantage of retrieving 2 or more biopsies from all levels of the gastro-intestinal tract.

This paper presents our experience with intestinal biopsy over a 2-year period. The normal intestinal histology and the morphological changes occurring in disease states will be outlined and the specificity of these changes discussed. While we have carefully evaluated the appearance of the small bowel mucosa on inspection of the freshly cut specimen with the hand lens and particularly on histology, a brief resumé of the dissecting-microscopic and electron-microscopic findings will also be presented.

Method

All biopsies in this series were obtained with the Crosby-Kugler capsule. The patient swallowed the capsule after an overnight fast and was then instructed to lie in the right-lateral position. To facilitate swallowing and to prevent excessive gagging and regurgitation, mild local pharyngeal anaesthesia was achieved by sucking 2 nupercaine lozenges 10 minutes before the procedure.

Fluoroscopy at the end of 3 or 4 hours usually showed the capsule to be in the 3rd portion of the duodenum or in the upper jejunum just distal to the ligament of Treitz. When ileal biopsies were required the capsule was inserted the previous night. Failure of the capsule to negotiate the pylorus occurred infrequently and usually signified pylorospasm. The exact location of the capsule could be ascertained by injecting micropaque into the polythene tubing but this was rarely necessary. More recently, the polythene tubing has been inserted through a No. 16 Rüsch radio-opaque gastroduodenal tube and passed into the duodenum under fluoroscopic control. Using this technique a jejunal biopsy could usually be obtained within 10 minutes and repeated, if required, at varying levels of the small bowel in the same patient.

After recovering the capsule the specimen for biopsy was gently teased onto blotting paper with the mucosal surface uppermost and examined by naked eye inspection, under the hand lens and by the 10× objective of a microscope before histological sectioning was carried out. Careful attention to sectioning was of paramount importance, as tangential sectioning or histological findings obtained from the periphery of the specimen resulted not only in interpretive difficulties but it also simulated the morphological changes encountered in idiopathic steatorrhoea. To minimize these errors, we have adopted the procedure recommended by Rubin[8] and have sectioned the specimen through its central core after careful orientation of the biopsy.

There were no complications with the technique of the biopsy in this series. It should be stressed, however, that we have had little occasion to investigate paediatric cases, partly owing to the apparently low incidence of coeliac disease in the South-Western Cape. The majority of recorded complications, of which haemorrhage and perforation of the gut predominate, have occurred in children, or after numerous specimens have been taken from the same adult patient.[9]

THE NORMAL INTESTINAL MUCOSA

Macroscopic Appearance

Naked-eye or hand-lens inspection of the freshly cut specimens for biopsy readily reveals the 'furry' nature of the normal mucosa. The fine, slender normal villi are usually clearly evident and imply that, in all probability, nothing abnormal will be found on histological examination.

Under the dissecting microscope these villi may display 2 distinct patterns. The type most commonly encountered consists of numerous finger-shaped projections arising from the mucosa, rounded at their tips and exhibiting a hazy transparency through which the capillary network in the lamina propria can be visualized (Fig. 1). The second normal variant consists of shorter and broader villi, which have been aptly described as 'leaf shaped' by Booth *et al.*[10] (Fig. 2). The fine intravillous capillary network is perhaps more evident in the leaf shaped villi. This appearance occurs most frequently in biopsies taken from the duodenum and decreases more distally in the small bowel. These 2 variants tend to be identical on histological examination.

Histological Appearance

Low-power histology. The general low-power appearance of the mucosa shows the long, tapering, finger-like villi arising from the underlying glandular (non-villous) mucosa (Fig. 3). Despite variations in the length and width of the villi in controls and occasionally from different levels of the gastro-intestinal tract there should be little difficulty in recognizing the normal histology. Broader and slightly plumper villi tend to occur in duodenal and ileal biopsies and occasionally the villi overlying Brunner's glands or lymphoid follicles may appear flattened and simulate partial villous atrophy. The glandular (non-villous) layer of the normal mucosa is narrow and contains the crypts of Lieberkuhn which penetrate the glandular layer to varying depths and open as pits immediately adjacent to the corresponding villous, and sparse mucosal glands.

The height of the villi varies from 320 - 570 μ and the mucosal thickness ranges from 120 - 265 μ. A rapid assessment of normality may thus be derived by comparing villous length

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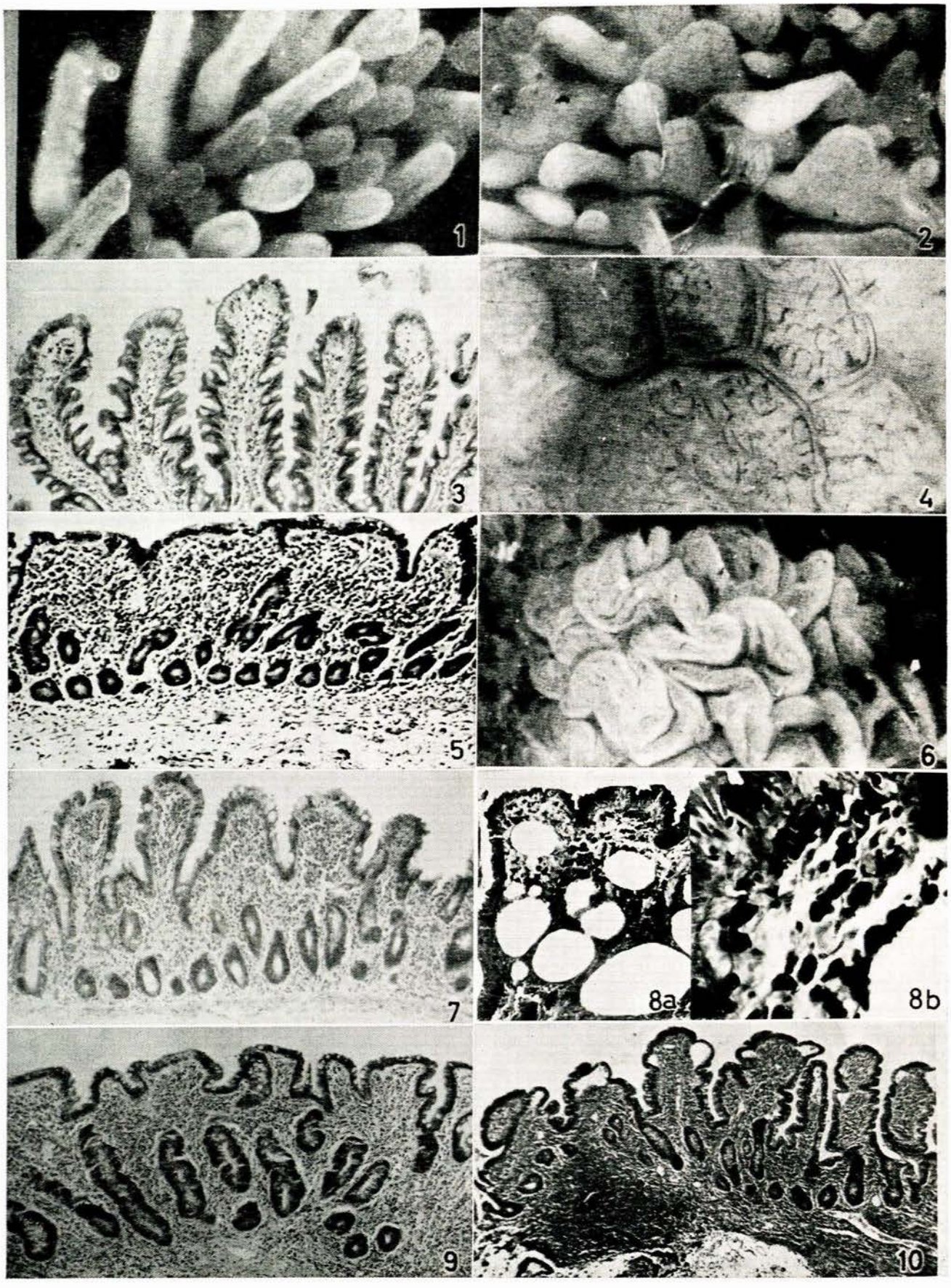
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with mucosal thickness in which the ratio should be at least 2:1. A similar but more refined technique has been applied by Cameron *et al.*[11] who defined a histological index based on these 2 parameters. The relative frequency of fragmentation of individual villi during histological preparation prompted Rubin to accept 4 normal villi occurring in a row as indicative of normality.

High-power histology. The surface epithelial layer of columnar cells is perhaps the most important single histological feature. The cells are tall and narrow (29 - 41 μ), with a regular row of basal rounded and slightly elongated nuclei (Fig. 3). Nuclear mitosis is infrequent and cellular infiltration of the epithelial layer is confined to a few small lymphocytes. The fine brush border of microvilli may be recognizable on high power. An uninterrupted 'pencil-line' basement membrane connects the epithelial cells and the intercellular borders are distinct. The columnar-cell layer is interrupted at frequent intervals by rounded or oval mucous-secreting goblet cells which stain with periodic-acid Schiff reagent.

The epithelial cells of the crypts of Lieberkuhn differ only slightly from the villous columnar cells. Apart from being somewhat squatter, nuclear mitosis is frequent and at the base of the crypts Paneth and argentaffin cells may be distinguished.

Mononuclear cells, plasma cells and eosinophils constitute the major histological components of the lamina propria. The cellular population varies considerably and moderate increase in these cells does not necessarily imply disease. Lymphatic channels and intracapillary red cells are usually readily discernible within the lamina propria of the villous.

Electron-microscopy. Although used largely as a research tool, electron-microscopy has been of value in defining the brush border and the intracytoplasmic and nuclear composition of the epithelial cell. The microvilli of the brush border protrude from the columnar cell as small erect rods which are remarkably uniform both in height and width. Variations in size of the microvilli in controls implies that the biopsy has not been sectioned in a plane perpendicular to its surface and represents tangential artifacts.

SUBTOTAL VILLOUS ATROPHY (TOTAL VILLOUS ATROPHY, FLAT MUCOSA)

Macroscopic Appearance

Naked-eye or hand-lens examination of the fresh biopsy specimen shows a 'bald' mucosa, devoid of villi and contrasts sharply with the 'furry' appearance presented by the normal mucosa. Indeed, without previous fluoroscopic positioning of the biopsy capsule it is difficult to tell whether the specimen for biopsy has been obtained from the oesophagus, the stomach or the jejunum. Examination under the dissecting microscope confirms the flat, featureless mucosa with a total absence of villi (Fig. 4). Occasionally the surface presents a mosaic appearance with prominent though irregular vasculature.

Fig. 1. Dissecting-microscopic appearance of normal jejunum showing finger-like villi. (By permission of Dr. C. C. Booth.)
Fig. 2. Dissecting-microscopic appearance of normal jejunum showing 'leaf-shaped' villi. (By permission of Dr. C. C. Booth.)
Fig. 3. Histology of jejunum showing normal tall and tapering villi.
Fig. 4. Dissecting-microscopic appearance of flat mucosa with mosaic appearance from a patient with idiopathic steatorrhoea. (By permission of Dr. C. C. Booth.)
Fig. 5. Total villous atrophy (flat mucosa) from a patient with idiopathic steatorrhoea. Note the absence of villi, cuboidal-surface epithelium and cellular infiltration in the lamina propria and surface epithelial layer.
Fig. 6. Dissecting-microscopic appearance of partial villous atrophy showing a convoluted-type of villous pattern. (By permission of Dr. C. C. Booth.)
Fig. 7. Partial villous atrophy. The villi are stunted and clubbed with a reversal of the normal villous:mucosal (non-villous) ratio.
Fig. 8a. Jejunal biopsy obtained from a patient with Whipple's disease showing villous atrophy, large lipid spaces and numerous PAS-positive histiocytes (stain black).
Fig. 8b. Magnification of lipid space and PAS-positive histiocytes scattered throughout the lamina propria
Fig. 9. Severe partial villous atrophy from a patient with lymphoma of the mesenteric glands. Atypical lymphocytes were present in the lamina propria muscularis mucosa and submucosa.
Fig. 10. Dilated lymphatics distorting the villi with intense cellular infiltration of the lamina propria from a patient with lymphoma of the mesenteric glands.

Histologic Appearance

Low-power histology. Fig. 5 shows the histological counterpart of the flat mucosa seen under the dissecting microscope and characteristic of idiopathic steatorrhoea. There is a total loss of villi and the crypts of Lieberkuhn open onto the surface at irregular intervals. The mucosal glandular layer is thickened and although the crypts are usually normal they may show evidence of hyperplasia, elongation, corkscrew appearance and may be arranged in a disorderly fashion. When the crypts open at regular intervals along the surface of the mucosa the intervening mucosa may give an erroneous impression of villi and the lesion may be interpreted as partial villous atrophy.

High-power histology. The columnar cells of the surface epithelium show distinctive changes. The cell height is reduced and approximates cuboidal or flat epithelium (Fig. 5). Vacuolation of the cytoplasm with a 'cobweb' appearance may be evident and the basement membrane and intracellular membrane is indistinct, fragmented or absent. Nuclear irregularity of the surface epithelial cells is marked. The nuclei vary in size and shape, many appearing triangular, crenated and pyknotic and an increase in mitotic figures may be demonstrated. Apart from the changes in configuration the nuclei also show variations in the degree of staining properties. Rubin has described the presence of 'rotten cells' in the surface epithelium between crypt spaces and suggests that they represent 'old' cells which have not been replaced by cells migrating up from the widely spaced crypts.[12] A homogeneous acellular area just below the surface epithelial cell layer has been noted by Cooke *et al.*[13] but its significance is uncertain. An infiltration of mononuclear cells or eosinophils is usually found in the surface epithelial layer and the brush border cannot be defined.

In direct contrast to these striking changes the epithelium of the mucosal crypts do not deviate materially from normality and cellular infiltration is absent. Despite the wide quantitative changes found in controls there is usually little doubt of the increased cellularity in the lamina propria, in which a marked infiltration of lymphocytes, plasma cells and eosinophils has occurred.

Electron-microscopy. To date the findings with electron-microscopy have been relatively unrewarding as a diagnostic measure. Although shortening, irregularity, fragmentation and complete loss of microvilli have been found[14] and changes in the intracytoplasmic organelles described, simple microscopy invariably shows the more gross changes associated with villous atrophy.

PARTIAL VILLOUS ATROPHY

Macroscopic Appearance

The naked-eye or hand-lens appearance depends largely on the degree of villous atrophy; slight blunting or clubbing of the villi presents the normal 'furry' mucosa while more severe atrophy imparts a 'convoluted' type of appearance. Booth *et al.* have shown these features with a dissecting microscope and stressed its diagnostic value.[10] The mucosa is thrown into folds, presents a whorled appearance and the normal villi cannot be identified. The term 'convoluted' mucosa has been aptly applied to these findings (Fig. 6) and virtually excludes tangential sectioning as the cause of stunted villi on histological examination.

Low-power histology. The villi in partial villous atrophy are stunted, flattened, clubbed and thickened and measure 100 - 300 μ in height (Fig. 7). The degree of villous change varies but does not appear to correlate well with the severity of the patient's symptoms. Although thickening of the mucosal-glandular layer and hyperplasia of the crypts are rarely present, the finding of villous-glandular (non-villous) ratio less than 2:1 and a low histological index is perhaps the best guide in the diagnosis of partial villous atrophy. It is stressed that the villi overlying Brunner's glands and lymphoid follicles may exhibit varying degrees of shortening and thickening and should not be misinterpreted as villous atrophy.

High-power histology. The morphological alterations in the surface columnar cells occupy an intermediate position between normal and flat mucosa. Little change may be seen or the cells may show varying degrees of cuboidal change, the nuclei

usually appear normal, mitosis is infrequent and the basement membrane and intercellular borders are distinct. Mononuclear-cell infiltration of the epithelium may or may not be evident. The degree of histological change is often dependent on the underlying aetiology. We have not been able to find any change in the cells of the crypt-surface epithelium.

It is our experience that the finding of partial villous atrophy on histological examination should prompt a meticulous scrutiny of the cell population in the lamina propria. An excess of lymphocytes, plasma cells and eosinophils and, in particular, the presence of abnormal lymphocytes or atypical histiocytes may provide the only clue to an underlying reticulosis or infiltrative lesion. The diagnostic lesions of Whipple's disease, carcinoid, Crohn's or Peutz-Jegher's disease will obviously depend on whether the specimen for biopsy is taken at the site of the localized pathology.

Electron-microscopy. The microvilli in partial atrophy vary from complete normality to some reduction in their size and number. To our knowledge there have been no authenticated changes in the intracytoplasmic organelles and nucleus.

Minor Histological Changes

While it is difficult to furnish convincing evidence for changes other than subtotal or partial villous atrophy as constituting abnormal histological findings, certain minor abnormalities in the mucosa may be consistent with disease.

Initially in this series certain minor abnormalities were observed in retrospect after the pathology was confirmed. With increasing awareness of these changes a more confident opinion could be given or at least the findings were regarded with suspicion.

1. *Interstitial oedema* of the lamina propria is notoriously difficult to appraise and only deserves comment when it is marked and leads to secondary widening of the villous. We have found this soon after infective diarrhoea and in disseminated lupus erythematosus with the accent on gastro-intestinal symptomatology.

2. *Increased vascularity* or capillary dilatation in the villi may suggest portal cirrhosis.

3. *The dilatation of a lymphatic channel,* occasionally present at the tip of a villous in apposition to the surface epithelium, is compatible with normal. However, the finding of such changes in numerous villi particularly in association with dilatation of the central lacteal should alert one to the possibility of an obstructive lesion of the lymphatic channels. This lymphectasia has also been recorded in protein-losing enteropathy.[15]

4. *A preponderance of one cell type* in the lamina propria, particularly if the cell population is judged to show a quantitative excess, may provide a clue to gastro-intestinal allergy or increased histamine release in the case of eosinophils, or a reticulosis when lymphocytes or plasma cells predominate. Polymorph infiltrations suggest an infective lesion or superficial mucosal ulceration.

5. It is not known whether *flattening of the surface epithelial cell* or deficiency in the brush border alone, in association with otherwise normal villi, may be regarded as abnormal. While there is little doubt that surface epithelial cells may bear the brunt of the damage in idiopathic steatorrhoea, the question whether such changes may occur in the absence of any other abnormality of the villous remains open. However, selective involvement of the surface epithelial cells manifesting as extreme cytoplasmic vacuolization has been claimed in acanthocytosis.[16]

MATERIAL

Table I shows the primary indications for 144 jejunal biopsies in 134 patients, the number of biopsies in each group and the incidence of abnormal histological findings in these groups. The vast majority of patients examined complained of non-specific diarrhoea or frank steatorrhoea, postgastrectomy diarrhoea, abdominal pain or anaemia. Apart from intestinal biopsy a full small bowel work-up comprising glucose- and xylose-tolerance tests, vitamin-B_{12} uptake, barium meal and follow-through, urinary-FIGLU, radioactive and chemical-faecal fat and pancreatic-function tests were done to confirm or refute malabsorption and to delineate the level of a localized lesion in the gastro-intestinal tract. Jejunal biopsies were also carried out on a number of patients with pancreatic or hepatic steatorrhoea in an attempt to determine whether long-continued steatorrhoea *per se* could produce mucosal

TABLE I. INDICATION FOR JEJUNAL BIOPSY IN 134 PATIENTS

Indications	*Number of Biopsies*	*Number Abnormal*
Diarrhoea—		
1. ? Small-bowel lesion	69	18
2. ? Carbohydrate intolerance	9	2
3. Postgastrectomy	22	1 + ?1
4. Pancreatic	12	?1
5. Cirrhosis	3	1
Abdominal pain	15	2
Anaemia	9	1
Dermatitis	5	0
	144	27*

* Constitutes 17 patients.

abnormality, and in 5 patients with exfoliative dermatitis, in view of recent reports of the possible role of malabsorption in the aetiology of dermatological disorders.

There were 27 (19%) biopsies classified as abnormal by 2 independent observers in 144 specimens obtained. These 27 specimens for biopsy were obtained from 17 patients; the incidence of abnormality in the series thus being 13%. As expected, the number of biopsies showing abnormal histology was greatest in patients investigated for diarrhoea but there were 3 abnormal biopsies in patients presenting only with abdominal pain or anaemia.

HISTOLOGICAL FINDINGS

Subtotal Villous Atrophy (Total Villous Atrophy, Flat Mucosa)

Six biopsy specimens in this series showed the classical histological changes of subtotal villous atrophy (Table II). These were obtained from 4 patients and *all were ultimately proved to have idiopathic steatorrhoea,* (Table II), or gluten-induced enteropathy. A secondary cause for the steatorrhoea

TABLE II. AETIOLOGICAL DIAGNOSIS OF 27 BIOPSIES WITH ABNORMAL FINDINGS

Histology	*No.*	*Diagnosis*
Subtotal (total) villous atrophy	6	All idiopathic steatorrhoea
Partial villous atrophy	14	Whipple's, Crohn's, lymphoma, mastocytosis, duod.-colic fistula, TB, ? *formes fruste* idiopathic steatorrhoea, recovering idiopathic steatorrhoea
Oedema; distorted villi	2	Lymphoma; DLE
Cellular infiltration and other changes	5	Postgastrectomy, cirrhosis, pancreatitis, lymphoma

6 additional biopsies showed doubtful changes.

was excluded by laparotomy in 2 patients and the remaining 2 responded immediately to dietary gluten restriction.

It is of interest that prior to jejunal biopsy the diagnosis of idiopathic steatorrhoea had not been entertained in 3 of the 4 patients; and they were considered to have functional diarrhoea, idiopathic hypoproteinaemia and 'bread intolerance' respectively.

Response to gluten-free diet. The effect of gluten withdrawal on subtotal villous atrophy was assessed by repeat biopsies in 1 patient at 3 months and in 1 at 3 and 6 months. Diarrhoea had been present for more than 20 years in both patients and the first jejunal biopsies showed a flat mucosal pattern. In the first patient the jejunal biopsy was unchanged at the end of 3 months despite marked symptomatic improvement and reversion of the gross fat, B_{12}, carbohydrate and mineral malabsorption to satisfactory levels. The second patient exhibited just the reverse. At 3 months subtotal villous atrophy was still present but at 6 months villous regeneration was noted despite lack of response to gluten withdrawal. The *histology* in this patient suggested that villous regeneration was gradual and

that it passed through a phase in which the mucosal appearance was identical with partial villous atrophy. Regeneration of the surface epithelial cells occurred concomitantly with villous restoration. The cells became progressively more columnar and the previously irregular and pyknotic nuclei returned to normal. Mucosal-cellular infiltration gradually receded. The intercellular borders and basement membrane were restored to distinct outlines.

Effect of gluten readministration. The villous alterations following gluten readministration was studied in 1 patient after a symptomatic cure had been obtained with gluten restriction for 5 months. A biopsy at this time showed abundant, though somewhat stunted, villi despite slightly impaired B_{12}, folic acid and xylose absorption. Steatorrhoea was re-established within days of adding gluten to the diet and a repeat biopsy obtained after 1 week showed the villous-surface epithelial and nuclear changes of subtotal villous atrophy.

Partial Villous Atrophy

Partial villous atrophy was present in 14 of the biopsy specimens and comprised a variety of pathological states (Table II).

(a) *Whipple's disease.* The typical histological features of Whipple's disease are shown in Fig. 8a; apart from the villous atrophy there were large lipid-filled spaces in the lamina propria of the villi with giant cells at the periphery of these spaces. The mucosal layer contained numerous macrophages with PAS-positive intracellular granules some of which were typically 'sickliform' in shape (Fig. 8b). In addition extracellular aggregates of PAS-positive material were evident. The epithelial-cell layer was cuboidal in some areas with a lymphocytic infiltration. The patient had been admitted to hospital suffering from loss of weight and steatorrhoea, but went into a spontaneous remission symptomatically shortly after. A biopsy was done during this latter period. The characteristic histology was present at all levels of the jejunum and ileum when an autopsy was carried out 6 months later. PAS-positive macrophages were also demonstrated in the heart, brain and other organs but it is of interest that the rectum was spared.

(b) *Mesenteric glandular lymphoma.* Two biopsies were taken from a patient with abdominal pain and megaloblastic anaemia and low B_{12}-uptake but normal faecal fat. In addition to partial villous atrophy the lamina propria of the villi and mucosa was infiltrated with small lymphocytes which extended into the muscularis mucosa and submucosa (Fig. 9). A few of these lymphocytes were abnormal and showed infrequent mitotic figures. At laparotomy large glands were scattered throughout the mesentery. Histological section showed the features of a lymphoma but classification of the type was difficult.

(c) *Systemic mastocytosis.* Three biopsies taken from a patient with steatorrhoea and the skin, bone and reticuloendothelial lesions of systemic mast-cell disease, revealed partial villous atrophy with marked eosinophil infiltration of the mucosa and surface epithelial cell layer. An increase in plasma cells and small lymphocytes was present. The histological appearance of the jejunum had remained unchanged over the 5-year period of observation.[17]

(d) *Crohn's disease.* Partial villous atrophy with superficial mucosal ulceration occurred in 1 specimen of a biopsy from a patient with subacute obstruction and steatorrhoea due to localized cicitrizing enteritis. These morphological alterations were probably dependent on stagnation and superimposed infection rather than proximal extension of the disease, since pseudotubercles could not be identified in the biopsy specimen.

(e) *Miscellaneous causes.* Partial villous atrophy was also observed in long-standing tabes mesenterica with ascites and steatorrhoea; tuberculosis in the abdominal lymph glands; in the recovery phase of idiopathic steatorrhoea (see above) and in 1 patient with gross and protracted steatorrhoea owing to a gastro-jejunocolic fistula. Two patients with partial villous atrophy of the jejunal mucosa were difficult to classify into any group of diseases; in 1, diarrhoea had been present for 20 years, a barium meal showed flocculation in the ileum but small-bowel function was normal during a period of relative constipation. The other had intermittent abdominal pain, vomiting and diarrhoea, and although faecal fat was increased to 8·4 G/day the small bowel was normal radiologically as were other tests of intestinal function. Whether these patients represent a *formes fruste* idiopathic steatorrhoea can only be a conjecture as the period of follow-up is too short.

Minor Changes Other than Villous Atrophy

The intestinal biopsies in 7 patients in this series showed histological changes other than subtotal or partial villous atrophy (Table II).

(a) *A moderate quantitative increase in mononuclear cells* in the lamina propria of the villi and mucosa associated with excess iron pigment in the villi was found in 1 patient after a total gastrectomy and in 1 with pancreatic steatorrhoea. The latter was of particular interest in that iron absorption has been shown to be increased in pancreatic disease.[18] This patient did in fact over-absorb ^{59}Fe on testing.

(b) *Oedema* of the lamina propria leading to widening of villi was the only abnormality in a patient with diarrhoea and abdominal pain, in whom disseminated lupus erythematosus was presumably the cause of the gastro-intestinal symptoms. Submucosal oedema was found at an operative jejunal biopsy by Bruce and Sircus in a patient with DLE but the villi were apparently normal.[19]

(c) *A striking increase in vascularity* of the villi was seen in a patient with combined hepatic and pancreatic cirrhosis with oesophageal varices. This probably represents the effect of pressure transmitted to the intestinal micro-circulation consequent on partial hypertension.

(d) Two patients with *lymphoma of the mesenteric glands* were particularly instructive and the case histories are recorded in detail.

Case 1. A 23-year-old non-White female presented with progressive oedema, mild abdominal pain, post-prandial distension, intermittent diarrhoea and amenorrhoea. Physical examination revealed oedema of the ankles, a smooth tongue, clubbing of the fingers grade 1 and abdominal distension. The liver could be palpated 1 cm. below the costal margin. There was no lymphadenopathy or splenomegaly and the other systems were normal. Special investigation showed that she had a mild iron-deficiency anaemia and a leukocytosis of 14,000/cu.mm. and the sedimentation rate was 6 mm./hr. Albumin ranged between 1·8 and 2·9 G per 100 ml. and the globulin between 2·2 - 3·8 G per 100 ml. The faecal fat was 8·7 G/day but the xylose and glucose-tolerance tests and $CO^{60}B_{12}$ uptake were normal. A barium series showed thickened mucosal folds in the small bowel but segmentation and flocculation was absent. A diagnosis of idiopathic hypoproteinaemia was made and she was discharged on a high-protein diet and diuretics. She presented 6 months later with increasing oedema, abdominal pain and diarrhoea. The physical findings and special investigations were essentially unchanged and the faecal PVP excretion was 1·1% of the administered dose. A jejunal biopsy (Fig. 10) at this stage showed distended lymphatics at the tip of numerous villi with mononuclear cell infiltration. The findings were compatible with Waldmann's intestinal lymphangiectasis and supported a diagnosis of enteropathic hypoproteinaemia. Laparotomy, carried out in view of persistent abdominal pain and virtually normal PVP excretion showed diffuse fleshy mesenteric glands. The histological examination of these glands showed a loss of architecture of the gland with occasional 'Dorothy-Reed cells' and reticulum cells. The histology was most suggestive of Hodgkin's lymphoma.

Case 2. A 19-year-old non-White male was admitted with a right hemiparesis and left 3rd-nerve palsy. The past history showed a history of mild diarrhoea for 2 months. Apart from the neurological signs, examination showed only marked clubbing of the fingers which had been present for 4 years. His haemoglobin was 12·5 G/100 ml. blood, sedimentation rate 8 mm./hr. and white-cell count 8,120/cu.mm. The serum electrophoretogram and stool fats were normal. Xylose excretion was 2·2 G in 5 hours and $CO^{60}B_{12}$ uptake was grossly impaired and not correctible by the administration of intrinsic factor. The glucose-tolerance test was frankly diabetic and the pancreatic-function test was normal. A barium meal was reported as showing a moderately widened duodenal loop

with distinct duodenitis and prominent folds. A *duodenal biopsy* taken from the area of maximal radiological deformity showed an intense accumulation of plasma cells and an infiltration of histiocytes of indeterminate origin in the mucosa. The villi were normal. These findings were thought to reflect a reaction to inflammation or neoplasia in close proximity to the lesion. Laparotomy confirmed the presence of a diffuse reticulosis of the mesenteric glands with almost complete replacement of the body and tail of the pancreas by tumour tissue.

DISCUSSION

The results of our study support the findings of previous workers with regard to the value of jejunal biopsy in the investigation of patients with steatorrhoea or isolated defects of absorption.[8, 20, 22] Jejunal biopsy has become established as an essential diagnostic procedure in patients suspected of having small-bowel disorder and is clearly the most important single investigation in the diagnosis of idiopathic steatorrhoea.

Subtotal villous atrophy, the histological description applied to the flat mucosa found characteristically in idiopathic steatorrhoea may be found in tropical sprue[21] but is excessively rare in other conditions.[22, 23] The term subtotal villous atrophy is perhaps confusing since it has been used to connote total rather than incomplete loss of villi.[22] We believe that a better histological description of the flat mucosa associated with complete loss of the villi found characteristically in idiopathic steatorrhoea would be *total villous atrophy*.

Variations in the localization of the small bowel mucosal change in idiopathic steatorrhoea also pose certain problems in diagnosis. Firstly, villous atrophy is usually more pronounced in the duodenum and upper jejunum than in the ileum and, secondly, it has been claimed that the changes of mucosal atrophy may be scattered rather than diffuse.[12] Although the latter has not been our experience, the observation of the gradient of mucosal change is of obvious importance with regard to selection of the biopsy site. The ideal biopsy site appears to be in the vicinity of the ligament of Treitz, preferably just beyond it in the upper jejunum. Similarly, repeat-biopsies for assessing the mucosal response to gluten restriction should be obtained from the same level of the upper jejunum.

Reversal of the mucosal changes has been adequately documented[20, 24-26] in patients with idiopathic steatorrhoea following gluten withdrawal. The villi usually reappear within 3 - 6 months, but regeneration has been recorded in as short a period as 3 weeks. In many patients, however, villous regeneration fails to occur despite clinical improvement and the converse was found in 1 of our patients who showed only a slight symptomatic response after 6 months treatment despite undoubted histological improvement. Rubin[12] has commented on the 'disquieting lack of correlation between the severity of the patient's biopsy and the activity of his illness'. The rapidity of onset of villous atrophy on challenging the relatively normal ileum with wheat in gluten-sensitive patients has also been demonstrated;[20] severe mucosal alterations were noted within hours of the instillation of wheat into the bowel. Villous atrophy and gross steatorrhoea was reproduced within days of reinstating gluten in the diet in another of our patients who had previously shown an excellent clinical and histological response to gluten restriction.

Attention has recently been focused on the morphological changes in the epithelial-cell layer of the villi as an earlier and perhaps more important index of the changes which may occur in idiopathic steatorrhoea. Yardley *et al.*[27] have shown sequential improvement in the villous surface epithelial cells within days of commencing gluten restriction; stretches of lessened cellular damage were observed within 3 days and an increase in cell height with a reduction in inter-epithelial inflammatory cell infiltration was noted within 6 - 10 days. Previously thinned and abnormal-looking surface epithelium became more normal irrespective of the degree to which villi returned. These findings were found to correlate well with the rapid onset of clinical recovery in patients responsive to gluten restriction and may afford an explanation for the apparent discrepancy between early clinical recovery and delayed, or indeed failed, villous restoration.

Partial villous atrophy, characterized by the presence of short and blunted villi with a clearly reduced villous: glandular-mucosal ratio, may be found in idiopathic steatorrhoea, tropical sprue or coeliac disease, but the change is then less specific than total villous atrophy. Partial atrophy may occur in a wide variety of conditions which in the present series included Whipple's disease, stagnation above intestinal strictures, long standing gastrocolic fistula, Crohn's disease, mesenteric lymphoma, mastocytosis and tabes mesenterica. Similar changes may occur after neomycin therapy[28] and in apparently healthy Thai people[29] in whom the chronic ingestion of betel-nuts has been suggested as a possible cause. Sprinz *et al.*[29] have shown experimentally that bacterial contamination of the small bowel may also produce partial villous atrophy. As expected, partial villous atrophy was also found during the recovery phase in 1 of our patients with idiopathic steatorrhoea. The fact that partial villous atrophy occurs in such a variety of small-bowel diseases should not detract from the value of the finding since it is clearly *not compatible with normality,* with the possible exception of the results obtained in the Thai. While it is difficult to refute Rubin's contention that partial villous atrophy is usually due to tangential artifacts or, possibly, to latent idiopathic steatorrhoea, the past histories and subsequent findings in our patients suggest that the mucosal changes are manifestations of, and have occurred concurrently with, the onset of their disease.

Little attention has been paid to the diagnostic value of changes in the lamina propria. This is due largely to the fact that the cell population of the lamina propria has been stated to vary quantitatively in neighbouring villi and that the cellularity of the specimen may vary with the intensity of suction exerted on the mucosa during biopsy. However, a marked increase in mononuclear cells in the lamina propria, with or without villous atrophy, should alert one to the possibility of an underlying reticulosis or infiltrative lesion, particularly if morphological abnormalities or the preponderance of 1 cell type is found. The preponderance of lymphocytes extending into the muscularis mucosa and submucosa coupled with minor atypicality provided the lead to the diagnosis of intra-abdominal disease in no fewer than 3 patients; all 3 patients were found to have enlarged mesenteric nodes due to lympho-

TABLE III. CAUSES OF MALDIGESTION OF FAT AND MALABSORPTION IN 115 PATIENTS*

Maldigestion of Fat (70)

Pancreatic:		*Post-gastrectomy†:*		*Hepato-biliary:*	
Alcoholic	25	Aff. loop stasis	4	Hepato-pancreatic	4
Biliary disease	2	Infection	3	Pancreatic carcinoma	6
Nutritional	1	Vagotomy	3		
Fibrocystic	1	Total gastrectomy	1		
Idiopathic	5	Unknown	15		
	34		26		10

Malabsorption of Fat or B_{12} (45)

Medical:		*Surgical:*		*Medico-surgical‡:*	
Idiop. steat	5	Fistulae —Duod. colic	2	Lymphoma	5
Ileo-colitis	1	Jejuno-colic	1	Idiop. dilatation	1
Crohn's	2	Ileo-colic	1	TB Adenitis	3
Diabetic	2	Adhesions—Jejunal	2	Stricture (? Crohn's)	3
Infective	3	Ileal	3	? Nutritional	1
Whipple's	1	Resections—Total	1	Unclassified	1
Vascular obstruction	1	Ileal	2	Excess HCl secretion	2
Systemic mastocytosis	1				
Phenindione	1				
	17		12		16

*Investigated over 3 years.
†Excludes postgastrectomy B_{12} or iron malabsorption.
‡Laparotomy usually necessary to establish the diagnosis finally.

ma. It is of interest that 2 of these 3 biopsies showed otherwise normal villi. A marked eosinophil infiltration in another patient was associated with mastocytosis.[17] Intestinal lymphangiectasia, considered to be diagnostic of enteropathic hypoproteinaemia,[15] was found to be due to proximal lymphatic obstruction in the mesenteric glands in 1 of our patients. Our findings in this lone patient justify a laparotomy to confirm or exclude a potentially treatable glandular disease, before ascribing mucosal lymphangiectasis to 'idiopathic hypoproteinaemia'.

The relatively large proportion of patients with small-bowel disorder not resulting from idiopathic steatorrhoea in our series has perhaps alerted us to the importance of changes other than villous atrophy alone. Table III shows the causes of steatorrhoea and malabsorption in 115 patients investigated at our clinic over a 3-year period. Coeliac disease, idiopathic steatorrhoea and gluten-enteropathy was found in only 5 patients and appears to be a relatively rare disease in Southern Africa. All 5 patients were females of British extraction. Idiopathic steatorrhoea has not been found in the Bantu in this series. While the rural Bantu may be protected by the fact that maize constitutes practically the sole dietary carbohydrate, this does not explain the low incidence in the urban Bantu where wheat gluten forms a significant dietary component.

A small-bowel profile incorporating intestinal biopsy is not usually necessary in the pancreatic, postgastrectomy or hepatobiliary groups as the diagnosis is nearly always established by the history, serum chemistry, glucose-tolerance and pancreatic-function test. However, pitfalls may occur even in these groups particularly when secondary pancreatic dysfunction supervenes after long-continued intestinal malabsorption.[30] Although minor villous changes have been found in pancreatic and postgastrectomy and hepatobiliary steatorrhoea in this series, we have not seen villous atrophy in these groups. Normal histology has been found in this series in patients with pernicious anaemia, iron-deficiency anaemia, duodenal diverticulosis, exfoliative dermatitis, after gastroenterostomy, scleroderma and in steatorrhoea owing to cystic fibrosis of the pancreas, gross gastric hypersecretion, diabetes, phenindione therapy, giardiasis, mesenteric vascular obstruction and entero-entero or entero-colic fistulae.

SUMMARY

Our experience with peroral intestinal biopsy over a 2-year period is presented and the gross, dissecting microscopic, histological and electron microscopic appearances of the normal and abnormal mucosal morphology is reviewed.

In this series, a flat mucosa or total villous atrophy (subtotal villous atrophy) was found to be *diagnostic of idiopathic steatorrhoea or gluten-enteropathy*. Reversal of these changes occurred with prolonged gluten restriction and conversely a rapid conversion from virtual normality to total villous atrophy could be induced by the readministration of gluten in a patient with gluten-enteropathy.

Partial villous atrophy occurred in a wide variety of pathological conditions including idiopathic steatorrhoea. This did not detract from its diagnostic value as it was clearly not compatible with normality. Partial villous atrophy was associated with an underlying reticulosis or infiltrative lesion in a number of cases in this series. Various histological aberrations in the vascular, lymphatic and cellular constituents in the lamina propria have been discussed. Although these findings have been referred to as 'minor villous changes', they may be the only intestinal indications of an underlying pathological state, and particularly reticulo-endothelial disease of the mesenteric glands.

The results of our present investigations suggest that idiopathic steatorrhoea, gluten-enteropathy and coeliac disease occurs relatively rarely in South Africa.

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