control. When the original rapid passage of this 'bolus' ceased in the ileum, up to 2 pints of 0·75 % Prepacol was injected to flush it through the whole of the small bowel. The Micropaque-coated walls of the small intestine were thus distended by a radiolucent bolus of Prepacol, producing an excellent 'double contrast' effect. In the majority the examination was completed within thirty minutes. The only ill effect encountered, in a few, was nausea associated with the passage of the tube.

Findings
The pathological conditions found comprised 8 examples of Crohn's disease, 2 of steatorrhœa, one of radiation ileitis, and 2 of multiple diverticulosis, not fully shown by the routine barium follow through. The normal appearances are shown in Figs 1–3, and three pathological cases in Figs 4–7.

Conclusion
This technique, in most instances, possesses certain diagnostic advantages over the routine follow-through examination, and possibly over Scott Harden's method. Since the technique has been perfected we have obtained consistently good results.

Its advantage is that the whole of the small bowel can be thoroughly examined at one session relatively quickly with little, if any, increase in the amount of radiation. The chances of missing a lesion appear to be considerably reduced, and the lines of demarcation between normal and abnormal bowel are exaggerated because of the mild distension involved. In most cases a double contrast mucosal pattern of the whole of the small intestine is obtained, and local conditions such as polypi and diverticula are more readily shown than by the usual oral 'follow through' method.

When, during the investigation, the bowel remained narrow and contracted, one could conclude that fibrotic or infiltrative changes were present and elasticity lost.

We do not infer that this technique should supplant the routine oral method but should supplement it, as it often gives additional information. A more detailed comparison of the two methods will follow later.

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The Histology of Intestinal Biopsies

Intestinal biopsies obtained by the per-oral route have been widely used in the diagnosis of malabsorption states, since Shiner (1956) introduced a tube biopsy method.

Idiopathic steatorrhœa almost invariably shows mucosal abnormalities (Doniach & Shiner 1958, Rubin *et al.* 1960) and the present survey concerns the results of 57 biopsies from 44 patients.

A normal biopsy (Fig 1) shows villi which project from the mucosa into the lumen of the gut. These vary in shape and size, and branching is common. The villi are covered by tall columnar epithelium (Fig 2), with parallel basal or suprabasal nuclei, eosinophilic cytoplasm, and a thin, refractile line on the luminal surface. This is the brush-border, shown on electron microscopy to be composed of parallel folds of cytoplasmic membrane – microvilli.
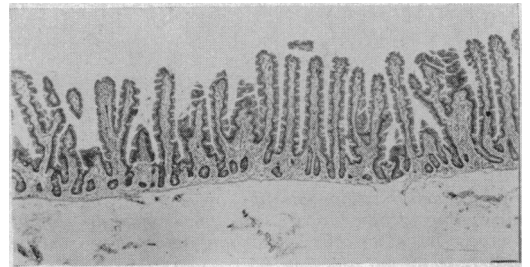

Fig 1 *Normal jejunal biopsy. Several branching villi are present, and the serrated villous outline is clearly shown. Hæmatoxylin and eosin.* × 22
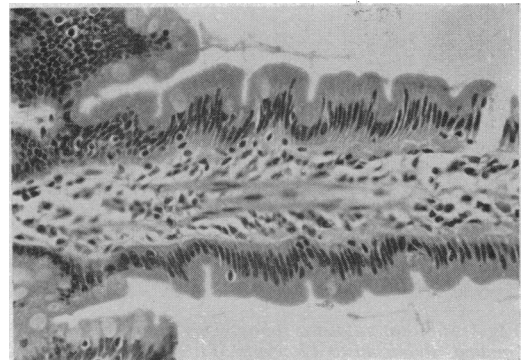

Fig 2 *Normal villus. The serrated outline is again clear. The columnar surface cells have parallel oval nuclei. The brush border can be seen as a pale refractile line on the free surface of the epithelium, and two lymphocytes are seen within the epithelium in the lower part of the photograph. The muscle around the central lacteal, slightly out of focus, is present in the centre of the villous stroma. H & E.* × 187

The outlines of the villi are serrated, said to be due to the retraction of the smooth muscle fibres which arise from the muscularis mucosæ, surround the central lacteal of the villus and end at the villus tip by attachment to the basement membrane (Figs 1 & 2) of the surface epithelium.

The lamina propria of normal villi contains a variable number of mononuclear cells (Fig 2), with plasma cells and eosinophils.

The surface epithelium of the villus is continuous with the epithelium which dips down towards the muscularis mucosæ to line the straight, blind-ended tubule – the crypt of Lieberkühn. Mitoses are restricted to the upper part of the crypt in the normal intestine, and the
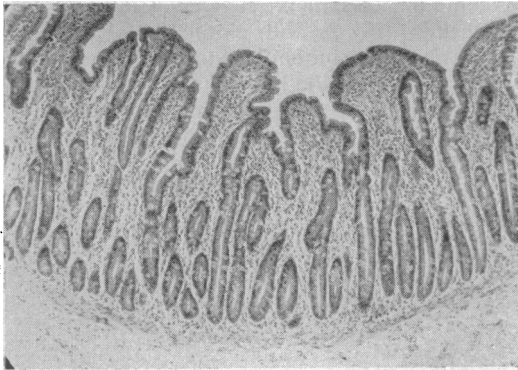

Fig 3 *Partial villous atrophy of jejunum. Thickened projections from surface of thick mucosa. Slight increase in goblet cells and stromal inflammatory cells. H & E. × 58*
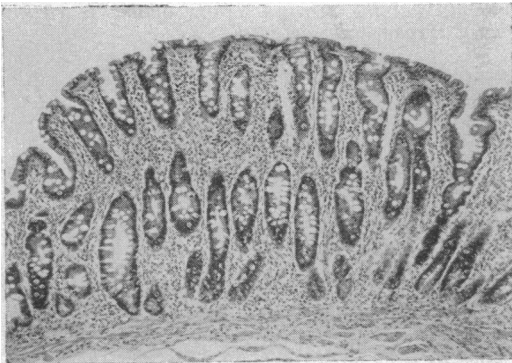

Fig 4 *Subtotal villous atrophy. This picture of a single 'mound' shows up to 12 crypts of Lieberkühn, arranged in parallel, and all within the 'mound'. Several crypt openings can be seen on the surface and the crypts do not appear to be obstructed, although apparently lengthened. There is a diffuse increase in stromal inflammatory cells, and in goblet cells within the crypts. These appearances suggest formation of 'mound' by fusion of groups of villi, the fusion occurring around the openings of the crypts. Such fusion would lead to the apparent lengthening of crypts, and shortening of villi. H & E. × 58*

newly formed cells are pushed upwards (like an escalator) towards the villous tip, from which the normal cells are desquamated into the intestinal lumen after a total life of two to three days (Leblond & Stevens 1948, Bertalanffy & Nagy 1958). Paneth cells and argentaffin cells are found in the lower third of the crypt, and goblet cells with their contained mucin are found scattered singly in the crypt and on the villous surface.

In idiopathic steatorrhœa, the total mucosal thickness is not much reduced, but the villi are greatly thickened and shortened (Fig 3), with apparent lengthening of the crypts of Lieberkühn. There is an increase in the number of goblet cells, and an increase in the inflammatory cells in the lamina propria (Fig 4). Paneth and argentaffin cells are normal. Doniach & Shiner (1958) divided the appearances into degrees of villous atrophy – partial (Fig 3) and subtotal (Fig 4); these probably represent different stages of the same process, as both may be found in different biopsies from the same patient.

Examination of fresh biopsies with hand lens or dissecting microscope reveals an interesting picture (Holmes *et al.* 1961). The normal intestine (Fig 5) shows the finger-shaped villi, separate from each other, but with occasional leaf-shaped forms. In a case with severe (subtotal) villous atrophy, no villi can be seen (Fig 6). The surface is divided into 'mounds', on which can be seen the discharging pits of the crypts of Lieberkühn. These 'mounds' can be seen histologically (Fig 4); study of them reveals that they may have arisen through fusion of neighbouring villi. A normal villus has an average width of 110 μ, and the 'mounds' of severe villous atrophy vary in width from 300 to 1,200 μ; when examined histologically each shows multiple, parallel crypts of Lieberkühn (Fig 4) from 8 to 12 in number, the total width of the 'mound' corresponding to fusion of 8–12 villi. The surface of the 'mound' describes an even curve and shows the serrated appearance of the epithelium seen in normal villi. Parallel muscle strands are found also near the surface, supporting the concept of fusion of villi. Histochemical findings support the concept of incorporation of villi into the apparent crypt (Padykula *et al.* 1961).

In less severely affected cases (Fig 7) the appearances are more complex. The surface may be composed of apparently continuous, convoluted ridges or of discontinuous groups of projections from the surface, probably formed by lateral fusion of neighbouring villi.

Of 57 biopsies in this study, 29 showed severe (subtotal) villous atrophy and 21 showed mild (partial) atrophy. Biopsies from ileum tended to be less severely affected than jejunum and might be normal when jejunum was abnormal. Five
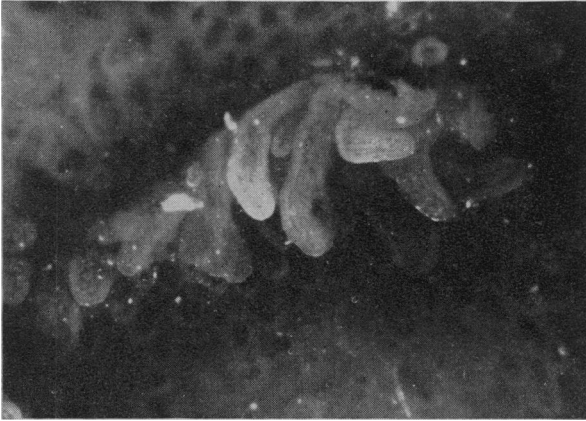
Fig 5 *Unstained specimen viewed with dissecting microscope. Normal finger-shaped villi. The surface epithelium is transparent unless in profile, and it is then seen as a pale surface layer. Unstained.* × 24
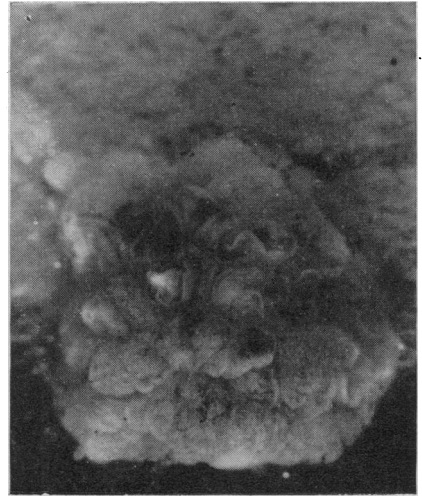

Fig 7 *Partial villous atrophy of jejunum, showing discontinuous ridges in upper part, with suggestion of 'mounds' in lower part. Unstained.* × 11·5

Fig 6 *Dissecting microscope view of subtotal villous atrophy. The surface is devoid of projections, and is divided into multiple, contiguous 'mounds', upon which a delicate vascular pattern is discernible. Openings of crypts of Lieberkühn can be indistinctly seen on the surface, between the blood vessels, and at the periphery is a layer of surface epithelium in profile. The 'mounds' are unusually small in this case. Unstained.* × 21

Fig 8 *Short surface epithelial cells show irregular disposition of nuclei. The surface epithelium has been torn from basement membrane in right-hand side of picture. The basement membrane is seen as a thick, pale zone, which stains as collagen. H & E.* × 320

ileal biopsies were normal and 2 jejunal specimens were inadequate for assessment.

The surface epithelium showed abnormalities in the severe cases, with short cubical cells and irregularly situated pyknotic nuclei. Beneath the epithelium the basement membrane, which is normally a continuous, thin line staining weakly as collagen, fr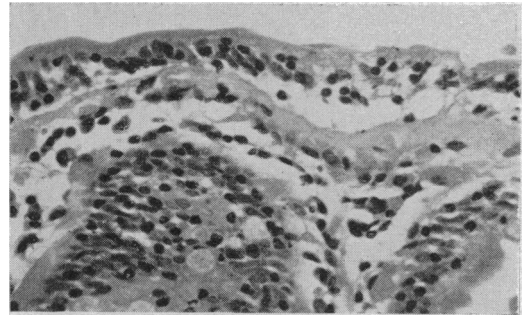equently showed collagenous thick-ening (Fig 8); this thickening of basement membrane, situated as it is between surface epithelium and underlying capillaries, may easily affect transport of substances into the blood and lymphatics; it was present in 35% of the biopsies of this survey and was usually focal in distribution, being most severe beneath the surface projections.

A unique opportunity of studying the distribution of mucosal lesions was offered by a single necropsy examination. In this case, of idiopathic steatorrhœa, areas of subtotal villous atrophy alternated with partial villous atrophy but the small intestine, which was studied in its entirety by 'Swiss-roll' preparations, was abnormal from pylorus to ileocæcal valve (Fig 9). In some areas (Fig 10) hyaline collagen had completely replaced the villi and crypts, an appearance not yet seen with biopsy material.
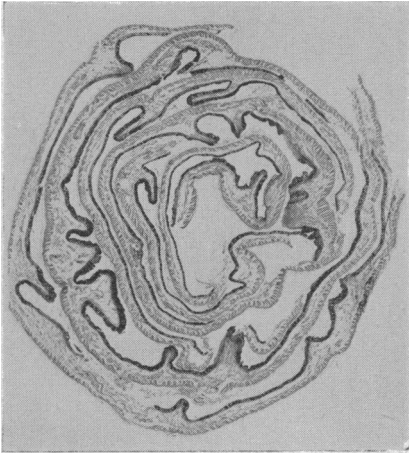
Fig 9 *'Swiss-roll' preparation of upper jejunum. The mucosa is present as an even dark zone, in which some folds of Kerckring but no villi can be seen. PAS.* × 2·3
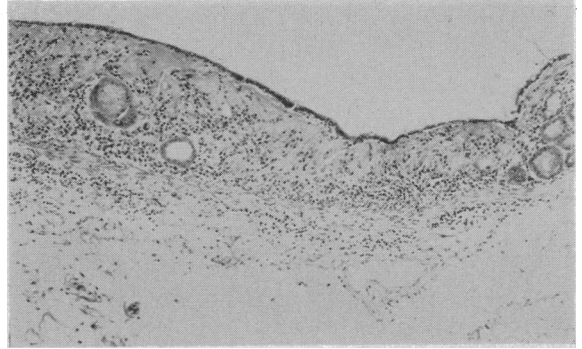

Fig 10 *Part of ileum from same case as Fig 9. The surface is flat, and the crypts of Lieberkühn and lamina propria have been replaced by hyaline material, which stains weakly as collagen. Hæmatoxylin and Van Gieson.* × 67
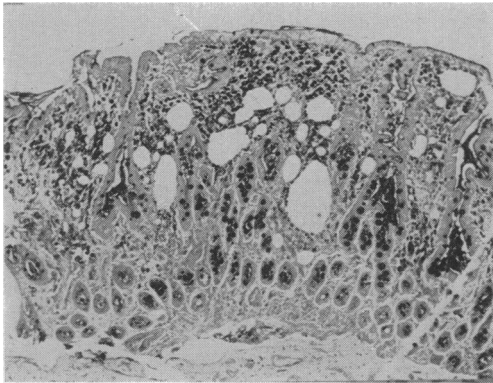

Fig 11 *Whipple's disease. The jejunal mucosa is distended with histiocytes, with PAS-staining bodies in their cytoplasm. The reaction to PAS is more intense near the surface, and the cells are more frequent here. Several clear, fat spaces are also present. PAS.* × 46
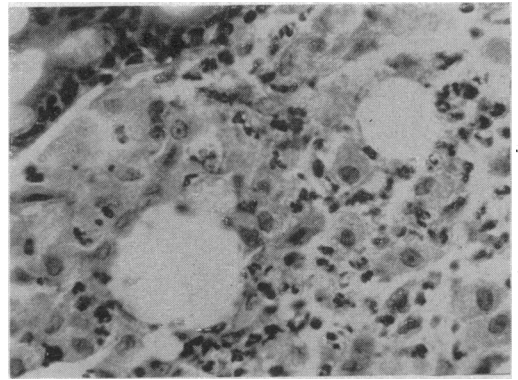

Fig 12 *Fat spaces in jejunal mucosa in Whipple's disease, surrounded by polymorphonuclear leucocytes and foamy histiocytes. H & E.* × 445

Although study of biopsies has increased knowledge and understanding of the lesions of idiopathic steatorrhœa, the mechanism of development of the lesions remains obscure.

Butterworth & Perez-Santiago (1958) suggested that in tropical sprue (in which comparable mucosal lesions are found) the intestinal cells might have a shortened life-span and that this might produce the picture seen. Williams (1961) has produced lesions similar to those of idiopathic steatorrhœa by feeding rats with antimetabolite drugs.

The histological appearances would support an inflammatory process, perhaps hypersensitivity to gluten, with fusion of villi initially in one plane but eventually surrounding the openings of crypts of Lieberkühn (the crypts are never cystic and do not appear obstructed). That an inflammation may produce this appearance is shown by the occurrence of villous atrophy proximal to intestine affected by Crohn's disease (Shiner & Drury 1962). The villous atrophy may be a non-specific result of intestinal inflammation due to many causes, but gluten instillation into previously normal gut has been shown to induce atrophy in patients with idiopathic steatorrhœa (Rubin *et al.* 1962).

Anderson (1960) has shown partial regression of lesions towards normal, following therapy, in children but it was not possible to study this in the present series. Separate biopsies must be interpreted with regard to the variability in severity in different parts of the mucosa.

In addition to idiopathic steatorrhœa intestinal biopsy may be of diagnostic importance in other diseases, such as intestinal amyloidosis and Whipple's disease.

In Whipple's disease, although abnormal and pathognomonic lesions may be widespread in the body (Farnan 1959), the intestinal mucosa is always affected in a characteristic manner. Seven cases of Whipple's disease were studied, 6 of them with intestinal biopsy or necropsy material.

The lamina propria of the intestinal mucosa is distended with foamy histiocytes, which are most frequent immediately beneath the surface (Fig 11). Fat-filled spaces are also present, also more prominent towards the surface; these spaces may represent distended lacteals but in only one case can they be recognized as such; the majority appear to be interstitial in site.

The foamy histiocytes contain no neutral fat; numerous granules, rods and sickle-shaped particles (Haubrich *et al*. 1960), which stain intensely with Schiff's reagent following periodic acid treatment (PAS), are found in the cytoplasm; the reaction is more intense towards the epithelial surface and the number of positively staining bodies is also increased in this area.

In 5 intestinal biopsies from 4 patients with Whipple's disease, in which additional slides were available, diffuse blue staining of the cytoplasm of the histiocytes was obtained with Perl's reaction. In 4 of the biopsies brown granules of hæmosiderin were present in submucosal phagocytes. The diffuse reaction within the foamy histiocytes was associated occasionally with minute granules. In the single case studied at necropsy a similar diffuse reaction for iron was found within the foamy histiocytes in lymph nodes and was again associated with large granules of hæmosiderin within the surrounding phagocytes.

The significance of this reaction for iron is unknown. It is unlike the staining reaction for iron found in patients on oral iron therapy and its presence within lymph nodes suggests a close relationship with the disease, although the latter remains obscure.

Focal collections of neutrophil and eosinophil polymorphonuclear leucocytes are found in intestinal mucosa and lymph nodes (Fig 12); they appear to be related to the clear fat spaces and are also found immediately beneath the surface epithelium of intestine.

Increased quantities of brown lipofuscin pigment (not hæmosiderin) may be found within intestinal muscle in any case of chronic diarrhœa or malabsorption (Fullerton 1960) and such pigment was present in muscularis mucosæ in two of the biopsies from patients with Whipple's disease. This pigment may colour the small intestine so that it appears honey-brown to the naked eye.

The bodies and granules within histiocytes are probably composed of glycoprotein and some reports suggest that the bodies are altered mitochondria (Fisher 1962).

The nature of this disorder is obscure. Chears & Ashworth (1961) have suggested that some of the intracytoplasmic bodies may, in fact, be bacteria but I have been unable to demonstrate the Grampositivity reported by them. Bacterial cultures of intestinal biopsies should resolve this problem.

Thoracic duct obstruction in animals may lead to appearances similar to Whipple's disease in intestinal mucosa (Amman & Bockus 1961), but the systemic distribution of the characteristic foamy histiocytes and fat spaces in humans (Farnan 1959) and the patent thoracic duct demonstrated at necropsy (Christie & Galton 1952, Farnan 1959) suggest that this is a generalized disease, perhaps a metabolic defect.

In the material presented here, foamy histiocytes were found in duodenal and jejunal mucosa, in lymph nodes, both central and peripheral, in liver, in rectal mucosa and within small veins. The single necropsy case showed lesions in several heart valves, with thrombotic vegetations associated with them.

Very few studies have been published on the blood in Whipple's disease. Perhaps analysis of serum glycoproteins might be found of interest. Another approach, suggested by the finding of the characteristic histiocytes within small veins, is examination of peripheral blood smears for these cells. If the cells should be found, this would make diagnosis and research on this disorder more practicable, and would support the concept of a systemic disorder, perhaps comparable to some of the storage diseases.

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