Clinical, histochemical, and electron microscopic study of colonic histiocytosis

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EDITORIAL COMMENT These studies indicate that colonic histiocytosis is unrelated to Whipple’s disease and emphasize that the presence of P.A.S.-positive histiocytes in the lamina propria of rectal mucosa cannot, by itself, be considered diagnostic of Whipple’s disease.

Infiltration of the lamina propria of the mucosa of the small intestine with ‘foamy’ macrophages (histiocytes) which give a strongly positive cytoplasmic reaction with the periodic acid-Schiff (P.A.S.) stain is considered diagnostic of Whipple’s disease (Black-Schaffer, 1949; Chears, Hargrove, Verner, Smith, and Ruffin, 1961; Farnan, 1958; Plummer, Russi, Harris, and Caravati, 1950; Sieracki and Fine, 1959); other features, such as clubbing and fusion of the villi and the presence of lipid-filled spaces in the lamina propria, are variable in degree and occasionally absent. Similar infiltration with P.A.S.-positive histiocytes is frequently observed in mesenteric lymph nodes, and less commonly in extra-abdominal tissues (Chears, Smith, and Ruffin, 1959; Enzinger and Helwig, 1963; Eyler and Doub, 1956; Farnan, 1958; Koudouris, Stern, and Utterback, 1963; Sieracki and Fine, 1959; Sieracki, Fine, Horn, and Bebin, 1960; Smith, French, Gottsmann, Smith, and Wakes-Miller, 1965). These cells have been found in the lamina propria of the large bowel in several reported cases of Whipple’s disease in which the diagnosis was confirmed by histological examination of other tissues (Caravati, Litch, Weisiger, Ragland, and Berliner, 1963; Fleming, Yardley, and Hendrix, 1962; Sieracki and Fine, 1959). The absence of P.A.S.-positive histiocytes in the lamina propria of the mucosa of the large bowel in proven cases of Whipple’s disease has been seldom reported (Martel, Batsakis, and Bolt, 1963; Smith et al., 1965). There have been two recent reports advocating the use of rectal biopsy to diagnose this disease (Caravati et al., 1963; Fleming et al., 1962). P.A.S.-positive histiocytes have not been found in significant numbers in the lamina propria of rectal mucosa obtained from normal individuals or from various patients with diarrhoea not due to Whipple’s disease (Caravati et al., 1963; Flick, Voegtlin, and Rubin, 1962; Martel et al., 1963). Rowlands and Landing (1960), however, reported the post-mortem finding of P.A.S.-positive histiocytes in the lamina propria of colonic mucosa of two children; since these cells were not found in the jejunal mucosa, lymph nodes, or other tissues, this finding could have represented an early stage of Whipple’s disease, a related metabolic disorder, or an entirely different pathological process. These authors used the term ‘colonic histiocytosis’ to describe this finding.

Recently we have investigated chronic diarrhoea in a 7-year-old girl and in a 37-year-old woman, both of whom showed infiltration of the lamina propria to the rectal mucosa with large numbers of P.A.S.-positive histiocytes. Clinical, histochemical, and electron microscopic studies were carried out in an effort to determine if this pathological picture presented an early and localized form of Whipple’s disease. The results of these studies are presented in this report.

CASE REPORTS

CASE 1 This 7-year-old Caucasian girl was readmitted to the Babies Hospital for further evaluation of chronic watery diarrhoea and faecal incontinence which had been present since birth. Aside from the diarrhoea and previous urinary tract symptoms thought to be secondary to cystitis, the patient had been in excellent health. Her growth and development were normal. During a previous admission attempts to elucidate the aetiology of the diarrhoea, including barium studies of the oesophagus, stomach, and small and large intestinal tracts, were

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At the normal. At the time of readmission the watery diarrhoea and faecal incontinence were still present and were the only symptoms. Physical examination revealed no abnormalities. Urine analysis was normal. The haemoglobin was 14.6 g. per 100 ml; the white cell count was 9,800 cells per mm.\(^2\) and the differential count was normal; platelets were adequate on smear. The faeces were liquid and did not contain occult blood, fat globules, fatty acid crystals, ova, or parasites. Barium studies of the oesophagus, stomach, and small and large intestinal tracts were again normal. Other laboratory investigations, including erythrocyte sedimentation rate, serum electrolyte concentrations, serum amylase activity, liver function tests, serum protein electrophoresis and scanning, oral glucose tolerance test, xylose excretion test, \(^{131}\)I-labelled triolein and \(^{131}\)I-labelled oleic acid absorption tests, assay of proteolytic activity in duodenal secretions, prothrombin time, urinary excretion of 5-hydroxy-indole acetic acid, faecal excretion of lactic acid, and assay of sodium and chloride concentrations in the sweat, all yielded normal results. A jejunal mucosal biopsy was obtained with Crosby-Kugler peroral intestinal biopsy capsule. The histology of the specimen was normal. There were no P.A.S.-positive histiocytes in the lamina propria. Bone marrow aspiration and peripheral lymph node biopsy were performed and the cytology and histology found to be normal. To complete the evaluation, sigmoidoscopy was performed. The rectal and sigmoid colonic mucosa was noted to have a slightly granular appearance. A rectal biopsy was performed and large numbers of P.A.S.-positive histiocytes were observed in the lamina propria (Figs. 1 and 2). Sigmoidoscopy and rectal biopsy were repeated to obtain material for histochemical and electron microscopic examination.

Clinical study Because of the beneficial effects of 'broad-spectrum' antibiotics described in patients with Whipple's disease (Davis, McBee, Borland, Kurtz, and Ruffin, 1963; England, French, and Rawson, 1960; Kent, Layton, Clifton, and Schedl, 1963; Perez, Schapira, Pellegrino, Rybak, and Larreeha, 1963), it was decided to treat the patient with tetracycline. She was given tetracycline, 250 mg. four times daily by the oral route, for 21 days. There was no effect on the diarrhoea either during or after administration of the drug. Subsequently she was given prednisone, 10 mg. three times daily, for three months, also with no effect on the diarrhoea. Repeat sigmoidoscopy and rectal biopsy following treatment with the drugs revealed no change from previous examinations. The patient was then given 2,2-diphenyl-4-(4-carboxyloxy-4-phenyl-1-piperidino) butyronitrile hydrochloride (diphenoxylate hydrochloride, Lomotil) with atropine sulphate, 2.5 mg. three times daily. The diarrhoea and faecal incontinence ceased and stools became formed several days after the drug was begun. Attempts to stop the drug have resulted in a return of the patient's diarrhoea.

CASE 2 This 37-year-old woman was admitted to the Presbyterian Hospital for evaluation of chronic diarrhoea, decreased appetite, weight loss, and fatigue. Twelve years before admission an attack of uveitis of undetermined origin resulted in total blindness in the right eye. The symptoms which prompted this admission began insidiously four years previously, and had been evaluated one year after onset at another hospital. Investigations at that time, including barium studies of the oesophagus, stomach, and small and large intestinal tracts, gave normal results.

Physical examination revealed a thin (weight 39 kg., 86 lb.) Caucasian woman with total blindness in the right eye, which had a fixed pupil and an opaque lens. The remainder of the physical examination was unremarkable. Urine analysis was normal. The haemoglobin was 14.5 g. per 100 ml; the white cell count was 6,120 cells per mm.\(^2\) and the differential count was normal; platelets were adequate on smear. The faeces were liquid to semi-formed and did not contain occult blood, fat globules, fatty acid crystals, ova, or parasites. Barium studies of the oesophagus, stomach, and small and large intestinal tracts were normal. Additional investigations, including erythrocyte sedimentation rate, serum electrolyte concentrations, serum amylase activity, liver function tests, serum protein electrophoresis and scanning, oral glucose
tolerance test, xylose excretion test, $^{131}$I-labelled triolein and $^{131}$I-labelled oleic acid absorption tests, assay of proteolytic activity in duodenal secretions, prothrombin time, urine excretion of 5-hydroxy-indole acetic acid and Schilling test without intrinsic factor, were normal. Mucosal biopsies of the proximal and distal jejunum were obtained with a Crosby-Kugler peroral intestinal biopsy capsule. The histological appearance of each specimen was normal. No P.A.S.-positive histiocytes were seen in the lamina propria. Tissue obtained by percutaneous biopsy of the liver showed mild periportal fibrosis. Sigmoidoscopy was performed and the rectal and sigmoid colon mucosa appeared slightly granular. A rectal mucosal biopsy was obtained and the histology showed large numbers of P.A.S.-positive histiocytes in the lamina propria (Fig. 3). Sigmoidoscopy and rectal mucosal biopsy were repeated to obtain material for histochemical and electron microscopic examination.

Clinical study The patient was treated with tetracycline, 250 mg. in 50 ml. distilled water given as a retentive enema four times daily. Following one week of therapy the watery diarrhoea became more severe and the drug was discontinued. She was treated subsequently with diphenoxylate hydrochloride with atropine sulphate, 2-5 mg. three times daily by mouth. On this therapy the diarrhoea became much less severe and stools were mostly formed. At the time of discharge from the hospital the patient was urged to increase the amount of food she ate. During the 12 months following discharge she gained 6-4 kg. (14 lb.) and had only occasional diarrhoea.

Cases 3-5 Jejunal mucosa was obtained by peroral biopsy from three untreated patients with Whipple's disease, showing characteristic histological features on light microscopy (Fig. 4). The clinical findings and brief reference to the jejunal lesions in two of these patients have been given in a previous report (Smith et al., 1965). Light microscopy of colonic mucosa obtained at necropsy from these two patients failed to demonstrate the presence of P.A.S.-positive histiocytes. A rectal mucosal biopsy was obtained from the third patient nine months after treatment with tetracycline and this showed a moderate infiltration with P.A.S.-positive histiocytes in the lamina propria.

FREQUENCY OF OCCURRENCE OF P.A.S.-POSITIVE HISTIOCYTES IN RECTAL MUCOSA

Rectal and colonic mucosal specimens obtained from 131 patients with and without disease of the large or small bowel were examined for the presence of P.A.S.-positive cells (Table I). Virtually all of the specimens contained some P.A.S.-positive plasma cells just beneath the layer of surface epithelium. An occasional P.A.S.-positive histiocyte was found in approximately 25% of the specimens. Moderate infiltration with P.A.S.-positive histiocytes was found in biopsies obtained from one patient with non-specific colitis and from one patient with

<table>
<thead>
<tr>
<th>Clinical State</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No known bowel disease</td>
<td>32</td>
</tr>
<tr>
<td>Normal mucosa adjacent to carcinoma of large bowel</td>
<td>30</td>
</tr>
<tr>
<td>Normal mucosa adjacent to benign tumour of large bowel</td>
<td>29</td>
</tr>
<tr>
<td>Non-specific colitis</td>
<td>18</td>
</tr>
<tr>
<td>Adult coeliac disease</td>
<td>8</td>
</tr>
<tr>
<td>Normal mucosa adjacent to diverticulitis</td>
<td>4</td>
</tr>
<tr>
<td>Rectal schistosomiasis</td>
<td>2</td>
</tr>
<tr>
<td>Regional enteritis</td>
<td>2</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhoea of undetermined origin</td>
<td>2</td>
</tr>
<tr>
<td>Intestinal lymphangiectasia</td>
<td>1</td>
</tr>
<tr>
<td>Acquired adult hypogammaglobulinaemia with diarrhoea</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>131</td>
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</table>
no bowel symptoms or disease. In neither of these specimens was the infiltration as heavy as that found in cases 1 and 2 of this report.

HISTOCHEMICAL STUDY

Standard methods (Pearse, 1960) were used on the rectal biopsies from cases 1 and 2 and on jejunal biopsies from the patients with Whipple's disease. The histochemical properties of the cytoplasm of the histiocytes in these specimens are given in Table II and are compared with the histochemical properties of the cytoplasm of the histiocytes in the colonic mucosa of the two cases of colonic histiocytosis reported by Rowlands and Landing (1960). The cytoplasm of the histiocytes in the rectal mucosa of cases 1 and 2 showed a strongly positive reaction with the mucicarmine stain, whereas the histiocytes in the jejunal mucosa of the patients with Whipple's disease gave a weakly positive reaction with this stain.

ELECTRON MICROSCOPIC STUDY

One half of each biopsy specimen was fixed immediately in iced 10% formol-saline and a small portion of this was frozen for staining for lipid. The remainder of the formol-saline-fixed tissue was subsequently embedded in paraffin for light microscopy. The other half of each biopsy specimen was fixed either in 1% phosphate buffered osmium tetroxide or in 6-25% glutaraldehyde in 0-1 M phosphate buffer at pH 7-2 followed by post-fixation with 1% osmium tetroxide. Fixation was followed by dehydration in increasing concentrations of ethanol and propylene oxide. The specimens were embedded in Epon 812, Methacrylate, or Araldite (Luft, 1961; Sabatini, Bensch, and Barrnett, 1963). Sections were cut with glass knives on a Huxley-Cambridge ultramicrotome and mounted on carbon-coated copper grids. Tissues were stained with lead citrate and/or a saturated solution of uranyl nitrate in 50% methanol (Watson, 1958; Zobel and Beer, 1961). Specimens were examined with an A.E.I. EM 6 electron microscope. Electron micrographs were taken at magnifications ranging between 1,500 and 50,000 on Kodak B.10 high-contrast plates. Further enlargement was obtained photographically.

RESULTS

JEJUNAL MUCOSA IN WHIPPLE'S DISEASE. The electron microscopic appearances were similar to those reported by others (Chears and Ashworth, 1961; Cohen, 1964; Kent et al., 1963; Kopecký, Malinský, Kodousek, and Maršálek, 1964; Kurtz, Davis, and Ruffin, 1962; Perez et al., 1963; Yardley and Hendrix, 1961). The lamina propria contained large round spaces filled with electron-dense material which is presumably lipid. Surrounding the round spaces were histiocytes which contained cytoplasmic inclusions, some of which appeared to be groups of phagocytosed bacilliform bodies (Fig. 5). Encapsulated, extracellular bacilliform bodies were present in the lamina propria (Figs. 6 and 7).

RECTAL MUCOSA FROM CASES 1 AND 2. Electron microscopic examination failed to reveal either encapsulated extracellular bacilliform bodies or histiocytes with inclusions similar to those found in histiocytes in jejunal mucosa of the patients with Whipple's disease. Clusters of predominantly rod-shaped electron-dense bodies intimately associated with the basal portion of occasional goblet cells were seen (Fig. 8); smaller numbers of spherical electron-dense bodies were also present in a similar location (Fig. 9). The exact position of these bodies was not clear. Some of them could have been in the cytoplasm of adjacent cells such as capillary endothelial cells. They did not resemble the granules of Paneth or Kultschitzky (argentaffine) cells but showed some similarity to the lipochrome pigment granules described in endothelial cells in the jejunal mucosa of patients with Whipple's disease (Kent et al., 1963).

The histiocytes in the lamina propria were found to contain various types of inclusions, some of which were relatively large and homogeneous on high magnification (Figs. 10a and 10b). Other smaller inclusions were aggregated into irregular masses and occasionally contained indistinct membranous particles demonstrated on high magnification (Figs. 10c and 10d). Although extracellular structures resembling the larger inclusions were found in the vicinity of one of the histiocytes, there was no convincing evidence of active phagocytosis. The inclusions were unlike those found in the cytoplasm of histiocytes in the jejunum in Whipple's disease.

### TABLE II

<table>
<thead>
<tr>
<th>Stain</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Whipple's Disease (Jejunal Mucosa)</th>
<th>Cases of Colonic Histiocytosis Reported by Rowlands and Landing (1960)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodic acid-Schiff (P.A.S.)</td>
<td>Strongly positive</td>
<td>Strongly positive</td>
<td>Strongly positive</td>
<td>Purple-red</td>
</tr>
<tr>
<td>P.A.S.-diastase digested</td>
<td>Strongly positive</td>
<td>Strongly positive</td>
<td>Strongly positive</td>
<td>Purple-red</td>
</tr>
<tr>
<td>(salivary diastase)</td>
<td>Strongly positive</td>
<td>Strongly positive</td>
<td>Weakly positive</td>
<td>Not reported</td>
</tr>
<tr>
<td>Mucicarmine</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive in cystic areas</td>
<td>Negative</td>
</tr>
<tr>
<td>Sudan IV (frozen section)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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FIG. 5. Electron micrograph: Whipple's disease of jejunal mucosa. Large numbers of extracellular bacilliform bodies are seen in the lamina propria. The margins of several histiocytes are also included. × 15,000.

FIGS. 6a and 6b. Electron micrographs: Whipple's disease of jejunal mucosa. Longitudinal and transverse sections through extracellular bacilliform bodies, demonstrating capsular membranes and internal structure. (Fig. 6a, × 70,000; Fig. 6b, × 140,000.)

FIG. 7. Electron micrograph: Whipple's disease of jejunal mucosa. An inclusion (arrows) in the cytoplasm of a histiocyte consists of a clump of phagocytosed bacilliform bodies. × 40,000.
FIG. 8. Electron micrograph (case 1) of rectal mucosa. Rod-shaped electron-dense bodies are present near the base of a goblet cell. LP = lamina propria. × 60,000.

FIG. 9. Electron micrograph (case 2) of rectal mucosa. Mainly spherical electron-dense bodies near the base of a goblet cell (GC). The lamina propria (LP) is at the lower left of the micrograph. × 13,000.
**FIG. 10a.** Electron micrograph (case 1) of rectal mucosa. Histiocytes (H) in the lamina propria. Large inclusions (1) and aggregates of small inclusions (2) are present in the cytoplasm. The cells are surrounded by connective tissue (CT). × 3,000.

**FIG. 10b.** Electron micrograph (case 1) of rectal mucosa. Higher magnification of the histiocytes (H) shown in the lower portion of Figure 10a. Large inclusions (1) and aggregates of small inclusions (2) are present in the cytoplasm. The cells are surrounded by connective tissue (CT). × 12,000.
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DISCUSSION

The results of these studies support the view that these two patients with chronic diarrhoea and P.A.S.-positive histiocytes in the lamina propria of the rectal mucosa probably had the disorder previously described as colonic histiocytosis (Rowlands and Landing, 1960) and did not have classical Whipple's disease. Treatment with tetracycline failed to relieve the diarrhoea. Histochemical comparison of the cytoplasm of these histiocytes with similar P.A.S.-positive histiocytes in the lamina propria of the jejunal mucosa of patients with Whipple's disease revealed a difference of affinity for the mucicarmine stain. Finally, electron microscopic examination of rectal mucosa from these patients failed to reveal either extracellular encapsulated bacilliform bodies or histiocytes with cytoplasmic inclusions similar to those found in the histiocytes present in jejunal mucosa of patients with Whipple's disease.

The nature and significance of the electron-dense bodies found at the base of some of the goblet cells in the rectal mucosa of the two patients is not clear, and we can do no more than record their presence. It is also difficult to account for the cytoplasmic inclusions found in the rectal histiocytes of the two patients. The failure to observe evidence of active phagocytosis does not necessarily indicate that these inclusions were formed within the cytoplasm of the histiocytes.

The reason for the infiltration of the rectal mucosa with P.A.S.-positive histiocytes and the relation of this infiltration to the chronic diarrhoea has not been determined. Nevertheless, our observations indicate that such infiltration is not necessarily diagnostic of Whipple's disease, which is more reliably diagnosed by peroral jejunal biopsy. The frequency and relevance of rectal or colonic histiocytosis can only be determined by the routine use of rectal biopsy with P.A.S. staining of the tissue in patients with chronic diarrhoea of obscure aetiology.

The therapeutic effect of diphenoxylate hydrochloride in the control of the diarrhoea is a result of its chemical similarity to morphine. The successful use of this drug with atropine sulphate in the two patients reported here presumably reflects only the
effectiveness of the anti-peristaltic activity of the drugs.

**SUMMARY**

Light microscopy of the rectal mucosa taken from two patients with chronic diarrhoea showed infiltration of the lamina propria with P.A.S.-positive histiocytes similar to those found in the jejunal mucosa of patients with Whipple's disease. Light microscopy of jejunal mucosa, obtained from the two patients by peroral biopsy, was normal. Tetracycline had no effect on the diarrhoea, which was subsequently controlled with diphenoxylate and atropine sulphate. The affinity of the cytoplasm of the rectal histiocytes for the mucicarmine stain was found to be much stronger than that of the cytoplasm of the jejunal histiocytes in Whipple's disease. Electron microscopic examination of the rectal mucosa demonstrated the presence of the histiocytes in the lamina propria and electron-dense bodies at the base of some goblet cells. The electron microscopic appearances did not resemble those observed in jejunal mucosa of three patients with Whipple's disease. Our findings support the contention that colonic histiocytosis is unrelated to Whipple's disease and emphasize that the finding of P.A.S.-positive histiocytes in the lamina propria of rectal mucosa cannot, of itself, be considered diagnostic of Whipple's disease.

**ADDENDUM**

Fisher and Hellstrom (1964) have recently described electron microscopic findings of histiocytes in the rectal mucosa of patients with normal jejunal mucosal biopsies. Their findings are similar to those reported here, although only homogeneous cytoplasmic bodies were observed in the histiocytes. It is these authors’ opinion that the cytoplasmic inclusions represent ceroid or lipofuscin.

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