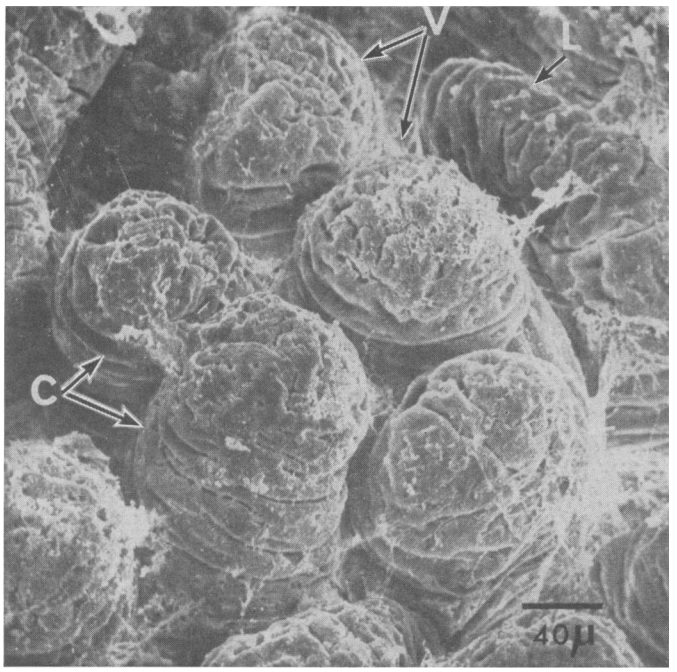

FIG. 1.—Low-power scanning electron micrograph of normal intestine. Finger-like villi (V), leaf-like villus (L), corrugations (C).

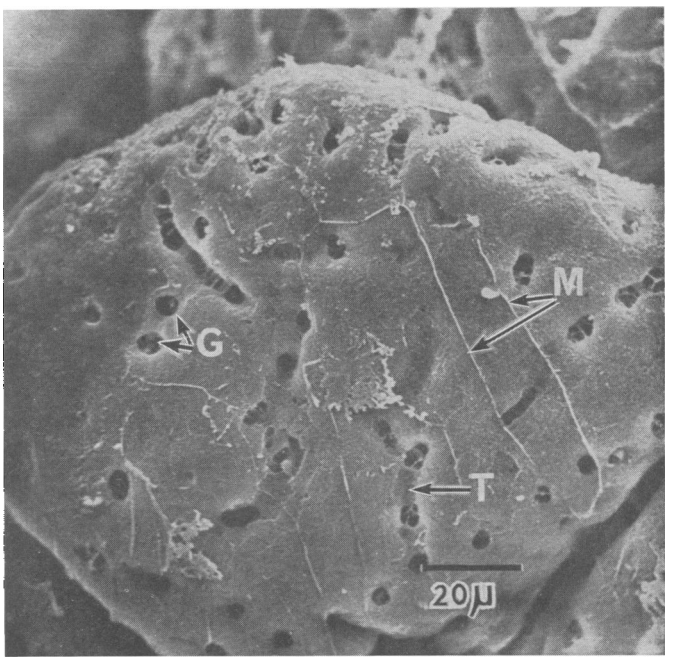

FIG. 2.—Finger-like villus of normal intestine at intermediate magnification Goblet cell orifices (G), shallow interconnecting troughs (T), strands of mucus (M).

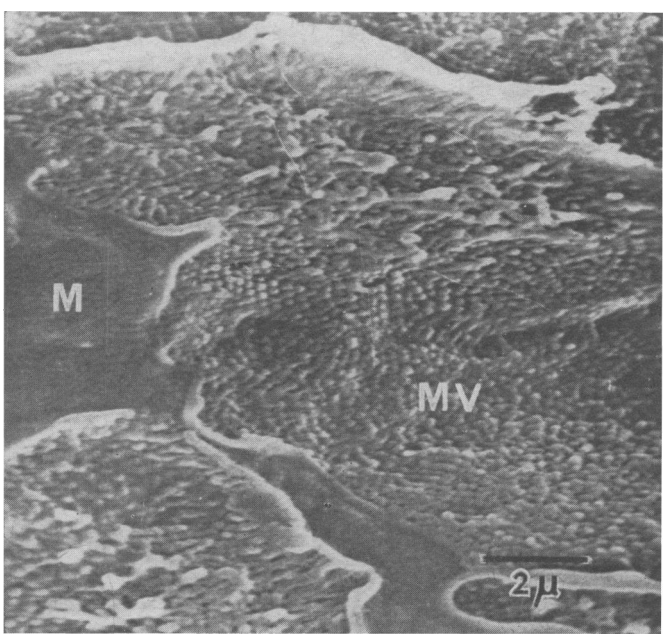

FIG. 3.—Normal villus surface at high magnification. Microvilli (MV), mucus (M).

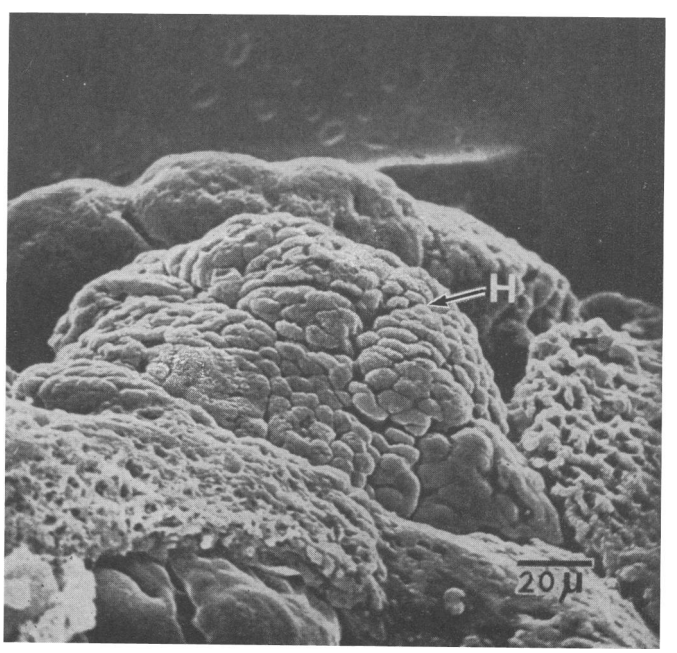

FIG. 4.—Intestine in coeliac disease. The micrograph illustrates a stunted villus in the centre composed of large hemispherical projections (H).

M. N. MARSH *ET AL.*: STUDIES OF SMALL-INTESTINAL MUCOSA WITH THE SCANNING ELECTRON MICROSCOPE

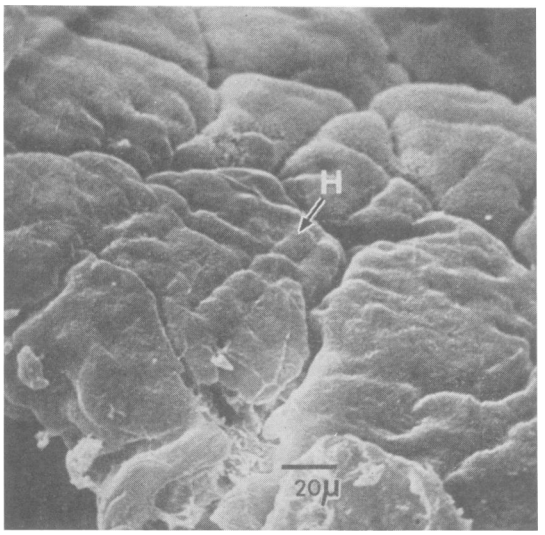

FIG. 5

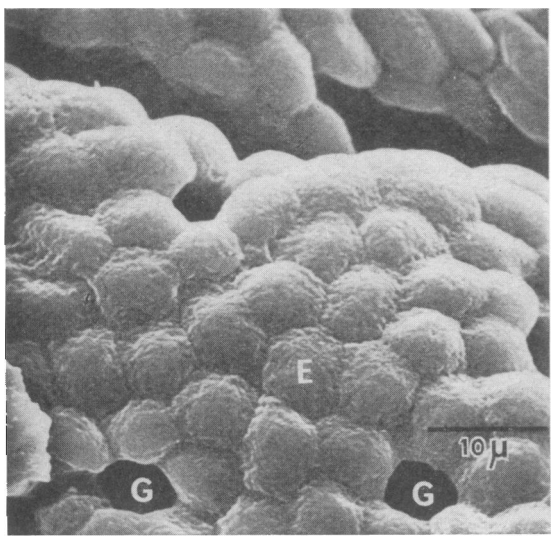

FIG. 6

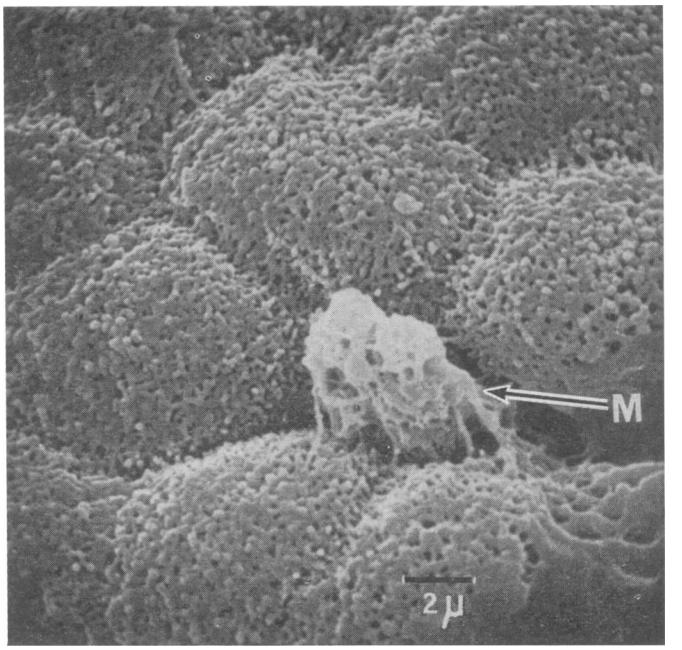

FIG. 7

FIG. 5.—Intestine in coeliac disease. This micrograph illustrates the cracked surface of an apparently villus-free zone. Hemispherical projections are also evident (H).

FIG. 6.—Top surface of a stunted villus in coeliac disease. The surface is apparently covered by spherical epithelial cells (E). Goblet cell orifices (G).

FIG. 7.—Similar area to Fig. 6 but illustrating epithelial cells covered by swollen microvilli. A mucous plug (M) is evident at a goblet cell orifice.

G. VAN ROS *ET AL.*: HAEMOGLOBIN STANLEYVILLE II

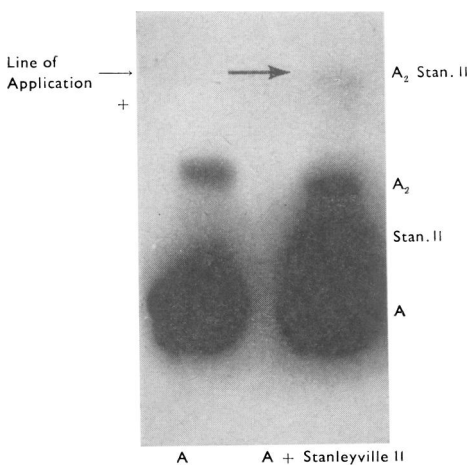

FIG. 1.—Demonstration of two Hb A_2 fractions in the specimen containing Hb A and Hb Stanleyville II (for details see text). On starch-gel electrophoresis at *p*H 8.6 Hb A and Hb A plus Hb Stanleyville are compared. Only one Hb A_2 is seen in the control. When Hb Stanleyville II is also present its variant α-chains give rise to a second Hb A_2. The gel had to be overloaded to visualize the two Hb A_2 fractions, and therefore separation between Hb A and Hb Stanleyville II is poor.

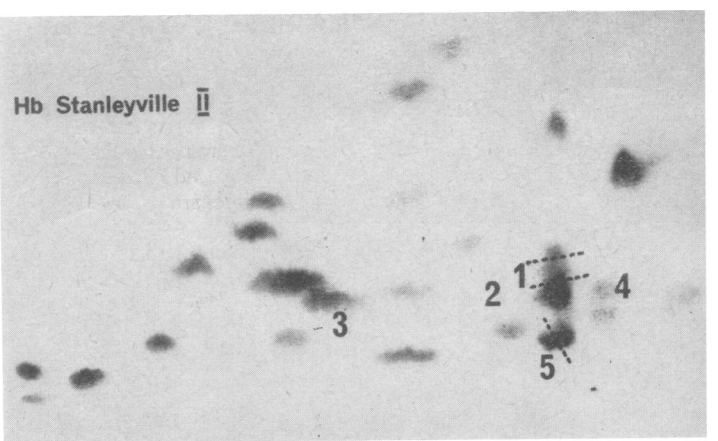

FIG. 2.—The "fingerprint" of Hb Stanleyville II. 1, The area where αTpIX (residues 62–90) of Hb A is absent. 2, The area where αTpVIII-IX (residues 61–90) of Hb A is absent. 3, αTpIX-b of Hb Stanleyville II (residues 79–90). 4, αTpIX-a of Hb Stanleyville II (residues 62–78). 5, A methionine staining peptide which is presumed to be αTpVIII-IX-a of Hb Stanleyville II (residues 61–78).

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Preliminary Communications

Studies of Small-intestinal Mucosa with the Scanning Electron Microscope

[WITH SPECIAL PLATE]

Brit. med. J., 1968, 4, 95–96

Summary: Preliminary observations indicate that scanning electron microscopy is a useful method for studying the surface of the small intestine.

INTRODUCTION

The development of a commercial scanning electron microscope (Stereoscan, Cambridge Instrument Company, Cambridge, England) has made possible direct examination of the mucosal surface of the human small intestine. With this method large pieces of tissue up to 12 mm. in diameter may be examined rapidly at low and high magnifications (from ×20 to ×10,000). Resolutions of the order of 0.05 micron (μ) are obtained, permitting ready identification of microvilli, and great depths of focus may be achieved, so that parts of the specimen at different levels are clearly focused. The depth of focus of the scanning electron microscope is at least 300 times that of the optical microscope. Specimens may be viewed in almost any orientation. Indeed, by tilting the specimen, stereo-pair electron micrographs may be obtained, facilitating the interpretation of surface topography.

In this preliminary report we describe the scanning electron microscope appearances of the jejunum in normal subjects and in those with adult coeliac disease.

METHODS

Fresh jejunal mucosa was obtained either at laparotomy from four control subjects or by biopsy capsule (Crosby and Kugler, 1957) located just beyond the duodenojejunal flexure in two individuals with adult coeliac disease. The specimens were orientated, carefully pinned out, and washed with normal saline. They were fixed in 5% glutaraldehyde (buffered with 0.1 M phosphate at *p*H 6.8) for four hours at 4° C., then washed and stored in 0.1 M phosphate buffer, *p*H 6.8 (containing 0.1 M sucrose), at 4° C. until required.

Specimens were subsequently washed in three changes of distilled water at 4° C. and then rapidly frozen in liquid N_2-cooled Arcton 12 for 30 seconds before transfer to the previously cooled stage of a Speedivac-Pearse vacuum tissue drier. Tissue was freeze-dried for 18 hours at −50° C. and then placed in clean glass bottles containing anhydrous calcium chloride until ready for vacuum-coating with metal.

The freeze-dried specimens were glued with Durofix to small 12-mm. diameter aluminium mounting stubs and coated with a layer of carbon about 100 Å thick and then with a layer of silver about 500 Å thick in an Edwards 12E6 vacuum-coating unit, the specimens being rapidly rotated during this process.

The coated specimens were examined directly in a Cambridge Stereoscan Mk II scanning electron microscope operated at an accelerating potential of 20 kV. Photographs of the specimen were recorded from the 1,000 line television display screen of the microscope on Ilford 35-mm. FP3 film.

CONTROL SMALL INTESTINE

The majority of villi of small intestine from control subjects appeared as finger-shaped projections of about 0.12 mm. diameter (Special Plate, Fig. 1). Occasionally leaf-shaped villi about 0.1 mm. thick and up to 1 mm. long were observed.

The surface of each villus was broken up by numerous transverse corrugations and pitted with several small holes approximately 5–6 μ in diameter, some of which were connected by shallow furrows (Special Plate, Fig. 2). These holes may represent the orifices of underlying goblet cells.

At higher magnifications the villus surfaces were partly covered by strands and irregular blobs of mucus. In areas where mucus was absent relatively well organized arrays of tiny nodules (about 0.1–0.15 μ diameter) were seen. These appear to be the tips of the microvilli (Special Plate, Fig. 3), for their dimensions correspond to those of microvilli seen in transmission electron micrographs of sectioned intestine (Fawcett, 1966).

ADULT COELIAC DISEASE

In general, the appearances of the mucosal surface were strikingly different from the normal. There was also marked variability in the surface morphology between adjacent areas; no particular area could be taken as wholly representative.

At low magnifications, short, stubby, and relatively flat-topped villi could be recognized whose surface consisted of close-packed, almost hemispherical projections (Special Plate, Fig. 4). However, in other areas the surface was flatter and deeply creviced (Special Plate, Fig. 5) and irregularly studded with similar hemispherical projections.

At intermediate and higher magnifications (Special Plate, Figs. 6 and 7) the surface of these hemispherical projections was covered with smaller projections of 6–8 μ diameter which were taken to represent epithelial cells. In some areas these cells had a somewhat blurred granular surface structure (Fig. 6) while in

others the cell surfaces were covered apparently by swollen, stunted microvilli of about 0.3 μ diameter.

As in control jejunum, holes or openings could also be seen in these specimens (Fig. 6), and may represent the orifices of subjacent goblet cells. Sometimes, as in Fig. 7, a plug of mucus-like material could be seen at these openings. The corrugations which had been seen running round the villi in control small intestine were not evident in the coeliac biopsies.

We are grateful to Professor C. C. Booth, whose patients we have studied, for his interest and helpful criticisms.

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Medical Memoranda

Primary Peritoneal Pregnancy

Brit. med. J., 1968, **4**, 96–97

For many years there has been disagreement about the validity of primary peritoneal pregnancy. Some authorities believe that all peritoneal implantations are secondary to ovarian or ruptured tubal pregnancies, but most would agree with Jeffcoate (1967) that while the majority are secondary a few primary implantations have probably occurred. Since Studdiford (1942) suggested criteria for primary peritoneal pregnancy, cases have been reported occasionally which claim to fulfil these criteria—for example, Baldwin (1954), Myles (1954), Ahnquist and Lund (1955), Rodriguez and Siegel (1960), Baccarini (1961), Tow (1961), and Kemp (1964). In a review of the literature Kroupa and Bleicher (1955) found 15 cases which were acceptable, but in a review of cases at the Charity Hospital, New Orleans, Beacham *et al.* (1962) concluded, "never have we said that the primary type cannot occur but we have pointed out that it is very rare." The evidence is now becoming increasingly strong, and a case is reported here which strengthens the conviction that primary peritoneal implantation does in fact occur.

CASE REPORT

A 20-year-old Chinese woman was admitted to the casualty department of the West London Hospital on 13 August 1967 with an eight-hour history of intermittent lower abdominal pain, without vomiting, but with slight vaginal bleeding for two days. Her previous period, lasting five days, finished 21 days earlier; up to that time her periods had been regular.

On initial examination she was not shocked but had slight lower abdominal tenderness. Rectal examination was normal, but after this she had her bowels open followed by tenesmus, and over the next hour there was a rapid increase in pallor and shock with the development of generalized abdominal guarding and rebound tenderness. A vaginal examination was not performed at this stage but a diagnosis of ruptured ectopic gestation was made, intravenous therapy was started, and preparations were made for immediate operation.

At laparotomy 3 pints (1.7 l.) of blood and clots were found in the peritoneal cavity, but the tubes and ovaries (and spleen) were normal. Further search revealed three raw areas in the pouch of Douglas each about 1.5 cm. in diameter; one was on the floor of the pouch with some trophoblast attached and was bleeding, the second on the posterior aspect of the uterus, and the third on the anterior surface of the rectum (Figs. 1 and 2).

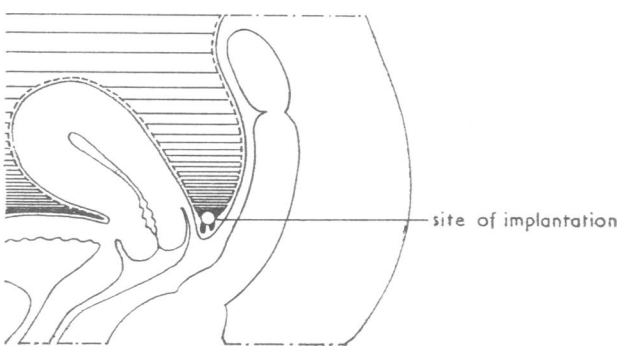

FIG. 1.—Diagram of sagittal section of pelvis to show site of implantation.

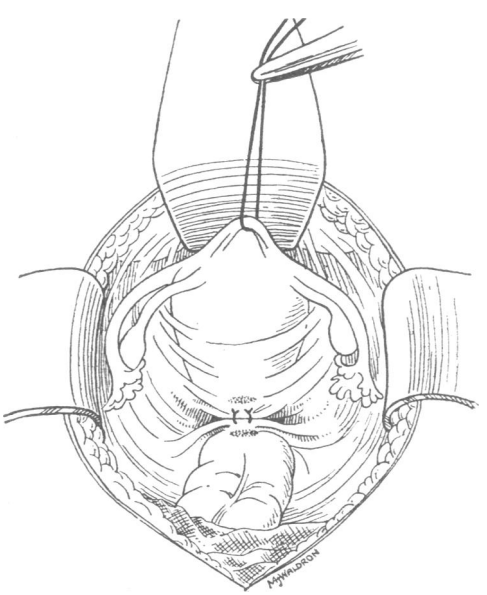

FIG. 2.—Drawing from operation sketch. The bleeding area has been oversewn and the adjacent raw areas are represented by stippling.

When traction was released from the uterus and rectum the three areas lay adjacent to each other, forming a small hemispherical cavity. The bleeding area was oversewn with catgut. Trophoblast, but not the embryo, was found among the blood clot. Vaginal examination under anaesthesia postoperatively showed no evidence of injury or instrumentation. Possibly the rectal examination had produced the final rupture.