STRUCTURES OF THE SMALL INTESTINAL MUCOSA IN NORMAL AND ABNORMAL STATES.

by

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"Knowledge is proud that he has
learned so much
Wisdom is humble that he knows
no more."

William Cowper.

PART I.

INTRODUCTION.

With the advance of microscopic methods in the investigation of the gastro-intestinal tract (i.e. improved technique with dissecting, light and electron microscopes) it is now realised that no one method alone is capable of giving a complete morphological picture of the small intestinal mucosa. The dissecting microscope gives a three dimensional view of the mucosa and allows a much wider view but its value is limited to the gross external appearance. The optical microscope exposes the structural details but its low resolving power and the inability to give a three dimensional picture limits its value. So long as the microscopist requires only to limit his search into the gross structural details or to follow the sequence of changes in the mucosa which occur in a disease process, the optical microscope suffices. But when the lesion is very early, that is, limited to the delicate intracellular components, such as the mitochondria, microvilli and terminal web which are beyond the resolving power of the optical microscope, only the electron microscope will expose any structural changes. The electron microscope has its own limitations too. By this method only a very small part of the mucosa can be viewed and the procedure is too elaborate and time consuming to have a use in routine work. Because of the complementary values of these microscopic methods, throughout the present work, the mucosa of the small intestine has been simultaneously examined by the dissecting, light and electron microscopes to get a

composite picture.

One of the major advances in the field of gastro-enterology is the increased knowledge of the structural details and the functional significance of the absorptive surface of the epithelial cell of the small intestine. The significance of the striated appearance of this surface has been a subject of great debate during the past century. Some held the opinion that it is due to rows of cilia whereas others thought that the appearance is due to canals between bars, through which the nutrients enter the body. Granger and Baker (1950) with the aid of the electron microscope showed that these striations are cytoplasmic prolongations to increase the absorbing surface of the cell and called them microvilli, thus solving the long standing dispute. Next, the packing of the microvilli stood as a subject of controversy. Although Granger and Baker thought that the microvilli are arranged in long rows, Palay and Karlin (1959) demonstrated that they are arranged in regular hexagons. In the present work (Part II, sec. B., Chapter 9) the disposition of microvilli in hexagonal array is shown to be a direct consequence of their general arrangement in multi-directional rows.

In the section of experimental pathology it is shown how in the presence of total ischaemia to the small intestine, epithelium can be preserved for a longer period simply by washing of the intestinal contents. It is also

shown under electron microscopic examination that dilatation of the microvilli is one of the earliest autolytic changes in the epithelial cell of the small intestine. In the same section it is shown how the protein deficiency state (in rat) causes subtotal atrophy of the mucosa. A study is also made on the mode of infection with v. cholerae. It is demonstrated how they stick to the striated surface of the mucosa of ileum of rabbit and exert their pathological effect from there. There is no evidence that these pathogens enter the mucosa of the intestine.

The study of the jejunal mucosa in steatorrhoea has been the major activity. In primary malabsorptive disease the electron microscopic evidence of the pathogenesis of microvillous atrophy is shown and the relative values of different microscopic methods in the diagnosis of this disease are discussed. The pathogenesis of steatorrhoea is discussed in great detail and it is suggested that the molecular distortion of the apical part of the cell is a contributory factor.

The present work includes completely diverging aspects of the study of the small intestinal mucosa, for which reason the thesis is divided into different chapters. To give some clarity to the whole subject, the broad headings of each chapter are mentioned at the beginning of the thesis and their details are mentioned at the beginning of the respective chapters. There is some unavoidable

repetition of some references, but this is felt justified since it facilitates an easier insight into the subject.

CHAPTER 1.

MATERIALS AND METHODS.

The present concensus of opinion is that to obtain an accurate picture of the small intestinal mucosa in normal and diseased states, the conventional examination under the light microscope is inadequate. The wider view is that the three dimensional impression of the mucosa under the dissecting microscope gives more accurate information than the two dimensional picture under the light microscope. At a very early stage of a pathological process, when the mucosal damage is only minimal, dissecting and light microscopes are unable to detect the lesion, which is only detected by electron microscopy. So, in order to obtain a full picture of the mucosa at various degrees of a pathological process the tissues should be examined under the dissecting, light and electron microscopes.

Materials.

In the case of human material, the specimen was usually obtained with a Crosby capsule (Crosby and Kugler, 1957). Sometimes the specimen was obtained at operation. In the case of experimental animals the specimens were obtained when the animal was still alive under anaesthesia. In some cases a blow was given to the head of the animal to make it unconscious. In one experiment where a sheep was used (Part II, Sec., A., Chapter 3) the animal was shot from a captive bullet of the humane killer.

When a specimen was obtained by the preoral capsule,

it was blown out of the capsule with air, or a piece of gauze was used to dislodge it from the knife edge. If these methods failed the pointed edge of a knife was used to dislodge it. Forceps were never used for this purpose because it might damage the tissue under the pressure of its blades. Sometimes post mortem contraction of the muscularis mucosae causes a curling up of the tissue which therefore needs to be orientated before processing for light microscopic examination (Rubin et al, 1960), but I have not come across such difficulties during the present work. In animal experiments, the perfusion of a loop of intestine with 10% formal saline before the specimen was taken avoided this difficulty.

With all the specimens obtained for electron microscopic examination, meticulous care was taken to minimise the time between obtaining the specimen and putting them in the fixative in order to avoid cytological changes due to delayed fixation. This was achieved within one minute in the case of tissues from experimental animals and within 2-3 minutes in the case of human materials. In animal experiments where a loop of small intestine was to be perfused, the specimen for electron microscopy was always obtained first and then the adjacent loop was ligated and perfused.

Some material was obtained before this present study began (Part II, Sec. B, Chapter 7). For viewing under the dissecting microscope the old paraffin blocks containing the tissue were obtained and the paraffin

trimmed off as much as possible with the help of a sharp knife. Then the tissue, thus denuded of paraffin, was put into xylol and left in an incubator at 56°C. The xylol was changed twice at half hourly intervals. Then the tissue was passed through various grades of alcohol (e.g. absolute alcohol, methylated spirits, 50%, 30% and 10% methyl alcohol) being left for half an hour at each stage. Then the tissue was ready for examination under the dissecting microscope and was reprocessed for light microscopic examination if required.

Methods.

A. Dissecting microscope. The dissecting microscopic examination was always done before the tissue was processed for the light microscopic examination. For viewing under the dissecting microscope the tissue was put on a watch glass, with just enough formol saline fixative to wet the mucosal surface, but not to float the specimen, and examined under the lowest power (x 2.5 objective) of a Watson's binocular dissecting microscope. In the case of primary malabsorptive disease, very often a thick layer of mucus is seen to envelope the mucosal surface and obscures the detail. Such specimens were treated with a mild mucolytic agent. A small bottle was half filled with equal parts of glycerine and normal saline. The specimen was transferred into the solution and the bottle is fitted to a blood cell suspension mixer "Matburn Limited" and spun for 10-15 minutes. At the end of this time the mucolytic agent was carefully poured out

and the bottle was half filled with normal saline and allowed to spin for 1-2 minutes. The specimen was removed again to the watch glass, containing formol saline fixative. This process removes the layer of mucus from the surface of the mucosa and allows a clear view. Then the tissue was processed for light microscopic examination.

B. Light microscopy.

Fixation of tissue: The foundation of all good histological preparation is adequate and complete fixation. Adequate fixation preserves the structure of the tissue in as natural a state as possible and allows the tissue to take up brilliant and selective staining. This stage is very essential because a fault in fixation cannot be remedied at a later stage. To obtain ideal conditions for fixation the tissue is fixed as soon as possible after death or removal, from the body. The volume of fixative in the jar is always at least 10 times the bulk of the tissue. The intestinal tissue is always thin for adequate penetration of fixative. 10% formol saline was used as a preliminary fixative for 24 hours and this was then followed by corrosive formol overnight. Post fixation with corrosive formol has been found to increase the intensity and selectivity of stains used in cytology.

Dehydration and embedding: The removal of water from the tissue is essential before it is embedded in paraffin wax. For this purpose, after adequate fixation, the tissue is passed through increasing concentrations of alcohol (e.g. 50%, 75%, 90% and then two changes of absolute

alcohol). After dehydration the tissue is treated with chloroform for clearing the alcohol. Then the tissue is impregnated with paraffin wax at 48°C. A vacuum bath is used to ensure complete removal of the clearing agent and thorough impregnation with wax. The tissue is embedded in a wax with a melting point of 54°C and is embedded with its long axis horizontal in a suitable container.

Cutting of paraffin sections: The wax in the embedding mould is cooled rapidly to obtain small wax crystals. The excess of paraffin is trimmed off leaving a pyramid, the upper face of which contains the tissue. It is then mounted in a chuck. Sections 5-6 μ thick are cut with a Cambridge rocker microtome. The sections are floated onto water, kept at approximately 10°C lower than the melting point of the wax. The crease-free sections are now mounted on clean glass slides (3"/1"). The mounted slides are held in a slide-rack and left in an incubator at 56°C for a minimum of one hour.

Staining procedure: The sections are deparaffinised with xylol for 2-3 minutes and then hydrated through the descending grades of alcohol (i.e. absolute alcohol, methylated spirits) and brought to water. The sections are then treated with iodine and hypo sequence to remove mercuric deposits from the sections and thereafter thoroughly washed with water to remove hypo salts. The sections are first treated with Meyer's acid alum haematoxylin for five minutes, and then washed with water. To ensure that the staining is satisfactory the slide is

checked under the low power of microscope. Sections requiring differentiation are treated with 1% acid alcohol for a short period and then 'blued' in running tap water or treated for a very short time (½ min.) with an alkaline solution (e.g. Scott's tap water solution of magnesium sulphate and sodium or lithium carbonate). Then the sections are allowed to stand in running tap water for 5 minutes. Next the sections are stained with eosin for 5-10 minutes washed in water and differentiated in methylated spirit. The sections are then dehydrated in absolute alcohol, cleared in xylol and mounted with DPX. In addition to the above standard staining technique the following were occasionally employed, - periodic acid-Schiff; Masson trichrome; red and yellow stain; reticulin stain.) Sometimes special stains were used to demonstrate mucin, and argentaffin cells.

Micrometry: Micrometry has been routinely used in most of the present work. With the help of this instrument it is possible to estimate variations in the height of different components of the mucosa in different species of animals, and the normal range of variation in the height of villi and the glandular layer of the mucosa in humans. Atrophy of villi which is a very prominent feature of the mucosal change in primary malabsorptive disease, and subtotal atrophy of the mucosa as seen in the protein malnutrition state can be correctly ascertained by such measurements.

The standard unit of measurement in micrometry is a

micron (μ). It is equivalent to one thousandth of a millimetre. To measure a microscopic object the micrometer is provided with an eyepiece scale and a stage scale. Both the scales are divided into 10 broad divisions and each division is again graduated into 10 small divisions. 100 small stage dividions are equal to 1000 μ or each small stage division is equal to 10 μ.

To measure a microscopic object first of all the eyepiece micrometer is placed inside the Huygenian eyepiece resting on the field stop. It is then calibrated against the stage micrometer at a particular magnification of the light microscope. The stage micrometer is then removed and the microscopic object is measured. In the microscope used in this study when the eyepiece lens magnification was x 8 and the objective lens magnification x 10, one small division of the eyepiece scale was equal to 10 μ and a big division was equal to 100 μ. At this magnification the thickness of the mucosa, the height of the villi and the height of the glandular layer were measured. To measure the height and nuclear size of the columnar cell, a higher magnifying lens (x 40) was used. At this magnification one small division of the eyepiece micrometer scale was equal to 2.50 μ.

C. Electron microscopy. It is not necessary for biologists to have a detailed knowledge of the theory of the electron microscope. But they need an adequate knowledge of the preparation of specimens for electron microscopy and the factors which influence image formation (e.g. resolving

power and contrast), so that they can appreciate the steps required for obtaining ideal results.

(i) Fixation: Fixatives which are used for electron microscopy should be simple. Its chemical property and effects on the tissue should be clearly known. Several fixatives are now available to us for fixing biological material for electron microscopic examination. 1% isotonic buffered osmium tetroxide has been constantly used as a fixative throughout the present work. The fixative is prepared as follows:-

Solution A - Buffer stock solution

2% potassium dichromate (The solution is brought to pH 7.2 with addition of potassium hydroxide)	- 1 volume
1.7% sodium chloride	- 1 volume

Solution B - 10% sucrose solution

(The stock solution is made with distilled water. The bottle is checked from time to time. The solution is discarded if there is the slightest evidence of contamination).

5 cc. of solution A and 5 cc. of solution B are pipetted out and put in an amber coloured glass bottle. An ampule of osmium tetroxide (0.1 g.) is soaked with water to remove its label. It is dried with a towel and dropped into the bottle containing the mixture of solutions A and B. With the aid of tissue forceps the ampule is broken and left inside the mixture. During this procedure, the mouth of the bottle is covered with a duster to prevent splashing of the fixative. A label stating the date of

preparation is fixed to the outer surface of the bottle. The bottle is wrapped with silver paper (to prevent access of light) and is kept inside a refrigerator. The fixative should be prepared at least 6 hours before use. A fixative which is more than 5 days old, or if the colour of the solution is changed from yellow to amber colour, is discarded. The temperature of the fixative at the time of use is also another important factor. Tissue fixation is maximum within the first one minute if the temperature of the fixative is maintained to near 0°C (Pease, 1960). When the fixative is carried to other hospitals, solid carbon dioxide is put inside a cardboard box along with the fixative bottle to maintain the temperature of the fixative. Just before use 1-2 cc. of fixative is pipetted out into a small bottle wrapped with silver paper. The fixative is never poured into the small bottle, to prevent the transfer of glass particles (from the broken osmium tetroxide capsule) which would get mixed with the tissue. The tissues are left for 1-1½ hours in fixative. When immediate dehydration and impregnation are impossible i.e. the specimen is obtained late in the afternoon or evening, it is fixed for at least 2 hours and then left in 10% alcohol overnight.

(ii) Dehydration: The tissues, after the scheduled period of fixation, are dehydrated as follows at room temperature.

1. 10% ethyl alcohol - 3 changes (30 min. each change)

2./

2. Absolute alcohol - 3 changes (30 min. each change) (standing on silica gel).

3. 1:2 epoxypropane - 2 changes (10 min. each change). (from refrigerator).

An adequate volume of fluid and the number of changes are as important as the time the tissue is left in the alcohol or epoxy propane.

The tissue is now ready for impregnation with Araldite. For embedding in methacrylate, after the last change with absolute alcohol the tissue is left in equal part of absolute alcohol and methacrylate.

(iii) Embedding: Araldite was constantly used as an embedding material, but when the tissues could not withstand Araldite, as in the protein deficient intestinal tissue of rat and the intestinal tissue of rabbit after infection with v. cholera, the tissues were embedded in methacrylate.

(A) With Araldite:

(1) Embedding mixture =

mixture (a):	Araldite (Ciba) 212	- 1 part
	Araldite (Ciba) 964B	- 1 part
mixture (b):	Araldite accelerator (Ciba) DY064	- 1 part
	Di-n butyl phthalate (BDH)	- 4 parts

Final embedding mixture (preferably made up the day before it was required): 19 ml. of (a)
1 ml. of (b)

This is poured into a universal container, fitted to

a mixture (1 revolution per minute) and left overnight. Usually at the end of this time, air bubbles collect inside the Araldite mixture which come up to the surface when the bottle is allowed to stand for 10 minutes. These air bubbles can be removed by passing a hot knife blade over the Araldite mixture or by leaving the bottle in an incubator (56°C) for 10-15 minutes. Freshly prepared Araldite is ideal for impregnating the tissue. Any mixture older than two weeks is normally not used for embedding.

(2) Process of embedding: Prior to embedding the specimens, the bottle containing the Araldite mixture is left in the incubator at 56°C for 15 minutes, during which time the Araldite is converted to a less viscous state and is ready for impregnation of the tissue. 1-2 cc. of it is poured into small plastic boats (ice cube containers). The epoxy propane is poured off completely from the tissues, which are then allowed to drop on the surface of the Araldite mixture. They soon sink to the bottom of the boat and are left overnight. On the following day fresh Araldite (after it has been brought to a less viscous state) is poured into small gelatin capsules which are filled two thirds full. The capsules are held vertical in small holes drilled in a small wooden board. Then with the aid of a small spatula the tissues are removed from the embedding boat and put into these capsules. Care is taken to transfer as little of the Araldite from the impregnation boat along with the tissue. The tissues slowly sink to the bottom of the capsule. The capsules are left at 56°C for

48 hours to harden.

(B) Methacrylate:

(1) Embedding mixture = The Methacrylate embedding mixture comprises butyl methacrylate (ICI) and methyl methacrylate (BDH) in the proportion of 9:1. This combination seems ideal for embedding biological tissues. 270 cc. of N-butyl methacrylate and 30 cc. of methyl methacrylate are mixed in a separating funnel. 100 cc. of 20% sodium hydroxide is added to it and shaken well. When the mixture is allowed to stand sodium hydroxide (as a brown fluid) settles to the bottom of the funnel and is run off. The funnel is shaken again, allowed to stand and the final bit of brown fluid is drained off. The methacrylate mixture is washed with water six times. Each time 100 cc. of distilled water is added to the methacrylate mixture, shaken well and the above procedure is repeated. 6 grams of Benzol peroxide (dried between the layers of filter paper) is added to the methacrylate in a stoppered bottle and is left in a refrigerator overnight to dissolve. It is then filtered through 3 layers of filter paper. Silica gel (indicator) is added to it and it is stored in a refrigerator.

(2) Process of embedding: After the last change of absolute alcohol the tissue is allowed to stand for half an hour in a solution of equal parts of absolute alcohol and methacrylate and then treated with two changes of methacrylate allowing half an hour for each period. The tissues are transferred into gelatin capsules two third

filled with methacrylate. The mouths of the capsules are sealed with gelatin caps and are left in an incubator at 37°C for 48 hours to harden.

Section cutting: Following in the manner of light microscopy preparations when the electron microscope came into use there was a tendency to use steel microtome knives for cutting ultra thin sections. The disadvantagesof a steel microtome knife are in[illegible] achieving and maintaining sufficient sharpness and this involves considerable time. Glass has uniform homogenity, and hardens without brittleness,and it provides an excellent cutting edge. Diamond knives are very useful for cutting ultra thin sections, But their high cost (£80) is a great drawback for its routine use. Latta and Hartman (1950) first described a technique to make glass knives. Since this time various methods have been reported describing improvements in the technique (Cameron, 1956; Gelber, 1957; Tokuyasa and Okamura, 1959).

Glass knife: It is made by breaking the glass at an optimum angle 45° (Mercer and Birbeck, 1960). For making a glass knife, a jig, a glass cutter (tungsten carbide wheel) a pair of pliers, a ruler, and a suitable plate of glass are required. First of all a piece of clean ¼ inch glass plate 8 inches by 5 inches is scratched for 1 inch perpendicular to the glass edge with a glass cutter. The scored line is held with pliers whose blades are padded with elastoplast to exert uniform force on either side of the score. By applying a rapid but steady opposing

pressure on the blades of the pliers the glass is broken along the score mark. Thus long strips of glass (1¼ inches wide and 8 inches long) are obtained. These long strips are again divided into three small bits at least 2½ inches long. Thus the final strip of glass from which the knife is made is 2½ inches long and 1¼ inches wide. An ideal piece of glass should not show any stress marks. These are seen through the width of the glass as many arcs. The knife should be of a particular shape to fit the knife holder of a Huxley microtome. To achieve this, a template is provided along with the microtome. The glass is scored according to the shape of the template leaving 0.5 cm. from the edge which is to be used as a knife. With the aid of the pliers the piece of glass is broken into the shape of the knife. The cutting edge of the knife is held against an illuminating point of the light under the x 2.5 objective of the dissecting microscope. This procedure reveals the area with stress marks which portion is unsuitable for cutting sections. In general, the knife with smaller edge angle cuts better sections and produces less compressing effects on the section than the knives with longer edge angles. The cutting edge has a straight and a curved part. The portion of the straight part which is devoid of stress marks is the part most suitable for cutting thin sections. Metal boats are supplied along with the Huxley microtome. These section baths are fitted to the glass knife with hot paraffin wax with the aid of a brush, making sure that the junctional cracks are made water-tight. The knife is now

ready for use. In an average 4-6 knives are required for one day's cutting.

Microtome: The Huxley ultra microtome has been constantly used throughout this work. It has the advantage that it cuts sections of 10-100 mμ thickness with consistent accuracy. The microtome is provided with the following:-

(a) specimen holder

(b) the advance mechanism with a dial indicating the degree of advance

(c) the displacement mechanism

(d) the knife holder

(e) oil fitted dash pot

(f) a viewing microscope

The bar carrying the specimen and the lever which advances the bar are supported by a flexible steel grip. When the operating arm is lifted the specimen holder advances forward, the distance indicated on the dial;at the same time it lifts up a metallic plunger in the oil fitted dash pot. When the operating arm is allowed to fall the plunger in the oil pot has a smooth motion downwards and allows a uniform movement of the specimen against the cutting edge of the knife. When the specimen holder advances by 400 Å or more ribbons of sections can be obtained. When the instrument is set to advance less than 300 Å there is a tendency for sections to be cut alternately thick and thin.

The Huxley microtome has an advantage over the Porter-

Blum in that it has a mechanical device for bringing the tissue uniformly and smoothly against the knife edge, but this machine has some disadvantages too. The operating mechanism is too elaborate. The block cannot be trimmed in situ. Any attempt to do this will damage the suspension spring of the microtome. Therefore it has to be done separately on a collet and vice type chuck and holder assemblies. The microtome does not provide a lighting arrangement for the section bath. Above all, the specimen holder is thermal sensitive. Therefore on a hot day, or on a very cold day if a heater is kept close to the bench, the metallic specimen holder is affected and therefore the cutting of ultra thin sections is not possible unless some means are taken to adjust the room temperature.

Specimen grids: The grids required to hold the ultra thin sections are made up of copper 3.05 mm. diameter. These Smethrust High-light limited ATHENE (regd) grids are of two types. 'Type 483' has a siece of circular holes and is used to hold Araldite sections. 'Type new 200' is used for Methacrylate sections. It has big square shaped holes, too big to hold the sections. Formvar or collodion films are put over them first and then these films are carbonised (by evaporation in vacuo of carbon from the carbon arc, (Bradley, 1960)) before they are used for holding Methacrylate sections.

Trimming of block and cutting sections: The gelatin capsule containing the block is left in hot water (from the tap) for 2-3 minutes. This dissolved and removes the

gelatin from the block. The block is then dried with a towel and left for an hour. It is then held with a collet and vice type chuck and holder assemblies and put under the low power of the dissecting microscope. A villus is isolated in the central position of the block and the excess of the embedding material is trimmed off with the aid of a razor blade. Thus embedding material, in the shape of a pyramid, is left around the villus. The top face of the pyramid is trimmed off very carefully by a very sharp edge of the razor keeping it horizontal to the base of the holder. The hand trimming is stopped at the first indication of the razor cutting into the tissue. The block is now put back into the specimen holder of the microtome and thick (0.5μ) sections are cut. These thick sections are stained with Toluidine blue.

Toluidine blue	0.5 g.
Borax ($Na_2B_4O_7.10H_2O$)	1 g.
Distilled water	100 ml.

The thick sections are lifted out of the section bath by means of a hair loop and put on an albumised slide and allowed to dry in an incubator at 37°C (for Methacrylate sections there is no need to leave the slide in the incubator as these are collected on acetone which evaporates quickly at room temperature). Then a few drops of Toluidine blue is put over the section and left at room temperature for 20-30 minutes or at 56°C for 4-5 minutes. The Toluidine blue is then washed off with tap water, dried and mounted with Canada balsam (DPX decolourises Toluidine blue). By this method the block is orientated. The

face of the block is again trimmed with meticulous care to keep the upper and lower margins sharp. Any unevenness with these two margins fails to hold sections together.

The block is now ready for cutting ultra thin sections. A new glass knife is held tightly in place. The 'section bath' is filled with distilled water (for Araldite sections) or 5% acetone (for Methacrylate sections) until a light reflex is seen against the edge of the knife. Filling the boat with fluid in addition to floating the sections has a stretching effect and thus the creases are minimised. Finally the sections are stretched a little further by moving a rod dipped in chloroform (for Araldite sections) or xylol (for Methacrylate sections) over the sections. The sections of desirable thickness are collected on the appropriate grid. For this the grid is held with watch maker's forceps and after the non-glazed surface of the grid touches the ribbon of sections it is lifted up together with the sections. Silver coloured sections (600-900 Å thickness) are suitable for most purposes. Grey coloured sections are those suitable for high resolution work (Peachey, 1958).

Watson (1958) demonstrated that heavy atoms can be introduced into these ultra thin sections and can give better contrast when the tissue is viewed under the electron microscope. Although this is an important development it is only partially exploited. Methacrylate embedded specimens obtain and retain enough contrast from

the fixative (i.e. osmium tetroxide) and from the evaporation of the embedding medium, and therefore there is no need for further staining of the sections. But the Araldite embedded tissues carry a poor contrast of their own and therefore they need to be stained. Various solutions are available to stain these ultra thin sections, viz. potassium permanganate, uranyl acetate, lead hydroxide, and phosphotungstic acid. It is found from the present work that intestinal epithelium embedded in Araldite gives the best contrast when stained with potassium permanganate.

Potassium permanganate stain:

(A) Stock solution: (stored in refrigerator 6-8 months.

Potassium permanganate crystals	1 g.
Distilled water (analar)	100 ml.

(B) Staining solution: (gets oxidised and loses its potency therefore has to be freshly made every 4-6 weeks).

Stock solution	10 ml.
Uranyl acetate	0.25 g.

The bottle should be tightly corked to avoid oxidation and left at room temperature. The addition of uranyl acetate to potassium permanganate causes a precipitation of manganese particles which are in suspension and thereby prevents inducing artefacts.

The face of the grid containing the sections is allowed to float on the potassium permanganate solution on a watch glass for 15-20 minutes. It is then held with watchmaker's forceps, pushed under the surface of water,

moved to and fro for a few seconds and removed at an acute angle to the surface of water. The section is then treated in the above way with acidulated water for 10 seconds (5 drops of 5% acetic acid in 5 ml. of water) and finally rinsed with clean water and dried over filter paper. Any dirt in the rinsing water will induce artefacts. The section is now ready for viewing under the electron microscope.

Electron microscopy: The electron microscope 6 (AEI) has been used for this entire work. The accelerating voltage used is 50 KV, providing an electron beam with a wavelength of 0.05 Å. Occasionally an accelerating voltage of 75 KV has been used.

The instrument is first correctly aligned. The specimen chamber is locked up completely and then the door is opened to the exterior. The grid with the side holding the sections facing upwards, is put into the specimen holder with meticulous care to avoid contamination with dust or loose matter. The grid is put squarely across the aperture and then screwed home firmly. Failure in this respect results in an irregular drifting movement of the image. The door is now locked and the specimen chamber is connected with the water pump. When evacuation of the air is complete the connection between specimen chamber and water pump is closed and the specimen chamber is opened to connect with the vacuum chamber of the microscope. The section is now ready for viewing. An electron beam is then obtained,the illumination intensity

of which is set at the minimum consistent with good visibility at a chosen magnification. The intensity of illumination should only be varied by means of the electron gun bias control. It is a good plan to reserve one of the three objective apertures for screening the section for suitable fields of view, and for alignment of the beam. The second and third apertures are kept clean for low and high resolution micrographs respectively. Before taking an electron micrograph of the image astigmatisum is corrected. The image is focussed using 10 x telescope. The image can be received on $3\frac{1}{4}$ x $3\frac{1}{4}$ inch plates. The recording camera holds six of these plates.

Measurements in electron microscopy.

1 mm.	=	1000 μ
1 μ	=	1000 mμ
1 mμ	=	10 Å

$$\text{Size of an object} = \frac{1000 \times 1000 \times 10}{\text{magnification}} \times X \text{ in mm.}$$

(X = size of the object on screen or on electron micrograph).

Magnifications in common use

		Size of object in Å
2,500	=	X x 4000
4,000	=	X x 2500
8,000	=	X x 1250
10,000	=	X x 1000
13,000	=	X x 769
16,000	=	X x 625
20,000	=	X x 500
40,000	=	X x 250

REFERENCES.

1. Associated Electrical Industries Limited. (1961). Instruction Manual for Electron Microscope, Type E.M.6.

2. Bahr, G.H. (1954). Exp. Cell Res., 7, 457.

3. Bradly, D.E. (1960). J. roy. micros. Soc., 79, 101.

4. Cameron, D.A. (1956). J. biophys. biochem. Cytol., 2, Suppl. 57.

5. Crosby, W.H. and Kugler, H.W. (1957). Amer. J. digest. Dis., 2, 236.

6. Grelber, D. (1957). J. biophys. biochem. Cytol., 3, 311.

7. Huxley, A.F. and Pattern. (1961). Ultra-microtome pamphlet.

8. Latta, H. and Hartman, J.F. (1950). Proc. Soc. exp. Biol., 74, 436.

9. Mercer, E.H. and Birbeck, M.S.C. (1960). Electron Microscopy. A Handbook for biophysics. Blackwell Scientific Publications, Oxford.

10. Peachey, L.D. (1958). J. biophys. biochem. Cytol., 4, 233.

11. Pease, D.C. (1960). Histochemical techniques for Electron Microscopy. Academic Press, New York and London.

12. Rubin, C.E., Brandborg, L.L., Phelps, P.C., and Taylor, H.C. Jr. (1960). Gastroenterology, 38, 28.

13. Sabour, M.S. (1962). Studies in Renal Pathology, Ph.D. Thesis, Edinburgh University.

14. Tokuyasu, K., and Okamura, S. (1959). J. biophys. biochem., Cytol., 6, 305.

CHAPTER 2.

NORMAL MICROSCOPIC ANATOMY OF SMALL INTESTINAL MUCOSA.

Introduction.

Digestion and absorption are the two main physiological functions of the small intestine. Therefore the structural details of its mucosa has drawn the attention of morphologists over the past century. In recent years, with a better understanding of the morphological details of the small intestine, there is a tendency to interpret the morphology of the intestinal mucosa in terms of the dynamic function it serves. In this chapter attempts will be made to present the appearances of the normal mucosa of the upper part of the small intestine of mammals under the dissecting, light and electron microscopes and to evaluate these in terms of function.

I. Dissecting microscopic appearance.

The villi are the diagnostic features of the small intestine of mammals and birds (Kendall, 1940). These villi serve to increase the absorptive surface of the small intestine. To increase the mucosal surface area further, the mucosa of the distal part of the duodenum and the first part of the ileum is provided with additional circular folds known as valvulae connieventes or valves of Kerkring (Finerty and Cowdry, 1960). Wilson (1962) determined that these mucosal folds increase the surface area by three times in man.

Shape of the villi: The dissecting microscopic appearances of the villi in different species present a

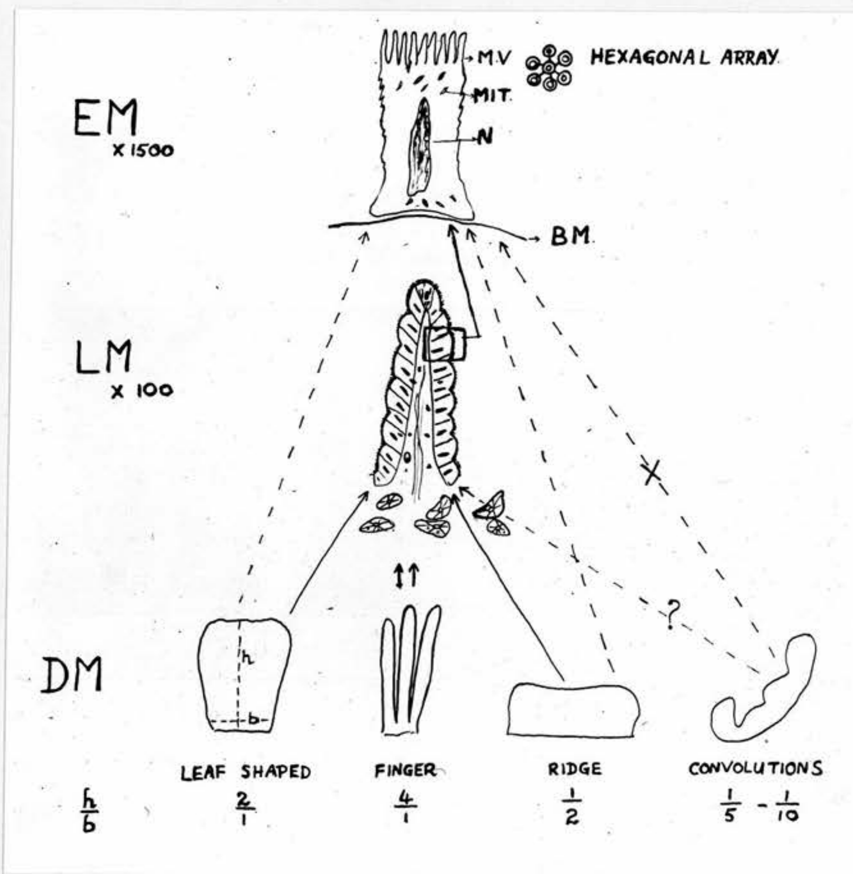

Fig. 1. The illustration shows the different types of villi as seen under the dissecting microscope (DM), a diagramatic representation of the structure of the villus as seen under the light microscope (LM) and the region selected for electron microscopic (EM) viewing. The diagram also shows the characteristics of an epithelial cell under the electron microscope.
MV = microvilli - note their hexagonal array in T.S.
Mit = mitochondria; N = nucleus; BM = subepithelial basement membrane.

variety of pictures. In man these villi are mostly long and finger-like. About a fifth of the villi are flattened from side to side and take the form of a leaf. Baker and coworkers (1962) noted that ridge shaped villi comprised 20% and convolutions comprised 27% of normal healthy south Indians. In this present study, although an occasional ridge shaped villus is found in the small intestinal mucosa of normal British people, any person who had predominantly ridges or convolutions were among those diagnosed as primary malabsorptive disease. The villi of the cat and sheep also present finger-like villi. But in rodents (mouse, rat, guinea pig, rabbit) the villi are mostly flat and leaf shaped, except over the upper quarter of the small intestine (duodenum and upper jejunum) of the rat where the villi are represented by parallel ridges which zig-zag. Distally over the lower half of the jejunum the ridges are replaced by leaves which have a broad base and a deep groove over their surface. Over the lower ileum the villi are again found to be finger shaped.

Verzar and McDougall (1936) mentioned in their monogram, the very early work of Heidenhain and Weidermann in which attention was drawn to the difference between the villi of carnivorous and herbivorous animals. The former have finger shaped villi and the herbivorous animals have flat, leaf shaped villi. Although the first part of the observation is correct, finger shaped villi are not confined to the carnivorous animals. Some of the herbivorous animals like sheep, have finger shaped villi. Whereas some rodents (mice and rats) which are not rigidly

herbivorous have leaf shaped villi as well. In rats it is noted that the new born suckling animals have only finger shaped villi (Verzar and McDougall, 1936; Baker et al, 1963), but as the rat grows older, the villi of the upper part of the small intestine change first to leaf shaped and then to ridge shaped villi. Verzar and McDougall suggested that such physiological changes are due to the type of diet that adult rats take. The suckling animals have finger like villi to cope with a liquid diet. Baker and coworkers (1962) consider the change to be due to the absorption of toxic substances in the food or in the intestinal juice, to which the villi of the upper intestine are more frequently exposed.

Architecture of the villi: The diameter at the base of a finger like villus is about a fifth of their height. The blood vessels in the core of the villi are occasionally seen through the covering epithelial cells. The leaf shaped villi have a broader base whose diameter is about a third of their height. These leaf shaped villi have flat dorsal and ventral surfaces and the latter holds a deep groove in the centre along its long axis. In the case of ridges, the diameter at the base is greater than the height. These features are quite evident in sections examined under the light microscope, where it has been possible to cut a villus through its entire thickness and length. But in the case of convolutions their irregular contours make the study under light microscope impossible. Their diameter is estimated from the dissecting microscopic

appearance to be 4 or 5 times the height. (Figs. 2 - 8).

Movement of villi: In addition to the gross movement of the intestine (segmental peristalsis) the villi also show separate movements. Hambleton (1914) observed the movements of the intestinal villi of the dog by means of a binocular microscope. He noticed that the individual villi and villi in groups show various movements. There is a shortening and lengthening of the villi in the long axis and a swaying movement laterally. Wells and Johnson in 1934 noted similar movements of the individual villi from freshly cut jejunal mucosa of dog. According to them the rhythmic movements of the villi are myogenic rather than neurogenic in character. Verzar and McDougall (1936) described how the finger like villi of man show a rhythmic pumping movement in their long axis, whereas the flat leaf shaped villi of rodents do not show any such movement. Contrary to the view of Wells and Johnson who hold that absorption is maximum in resting villi, Verzar and McDougall (1936) suggested that the shortening and lengthening of the villi empties the lacteals and thereby plays an active role in the absorptive process. They also thought that local irritation or swelling of the villi impede the venous and possibly lymphatic drainage. The 'villous pump' idea is accepted by many. By this mechanism the intestinal contents are effectively stirred and fresh fluid is allowed to come in contact with the mucosa of the intestine thus facilitating absorption.

II. Light microscopic appearance.

The villi are seen to project from the surface of the

mucosa. In man, over the upper part of the small intestine, these villi vary in their length, from 350-550μ a finding confirmed by the work of Doniac and Shiner (1957). There are about 30 villi per square millimetre of intestine (Bourne, 1960). The surface mucosa of the villi is thrown into a series of folds or plicae. The contraction of the villus makes these folds more prominent (Verzar and McDougall, 1936). Under the low power of the light microscope the absorptive surface of the villi are found to be covered with columnar epithelial cells. These cells rest on the subepithelial basement membrane. Goblet cells are found to be scattered among these columnar cells. The lamina propria forms the core of the villus. At the bases of the villi simple tubular glands (the glands of Lieberkühn) extend down to the muscularis mucosae. These glands communicate with the surface of the mucosa by the crypts of Lieberkühn. Paneth cells and argentaffin cells are situated at the bases of these glands. The lamina propria, in addition to its contribution to the formation of the core of the villi fills up the space between the glands of Lieberkühn. Groups of mucus forming glands called Brunner's glands are a special feature of the duodenal mucosa and groups of lymphoid nodes called Peyer's patches are the special histological feature of the ileum.

Luminal border of the intestinal epithelial cell: Under the high power of the light microscope the luminal border of the cell has a refractile appearance. In some sections vertical striations are seen through the refractile surface which resembles the bristles of a brush, hence it is

popularly known as the brush border. There has been much controversy during the past one hundred years, regarding the morphological details of the luminal border of the epithelial cell of the small intestine. Granger and Baker (1950) with the aid of the electron microscope solved this long standing controversy. These strands are tube like cytoplasmic projections from the apical part of the cell and are called microvilli.

<u>Epithelial cell:</u> A single layer of tall columnar epithelial cells lines the surface of the villi. In man these cells vary in height from 35-45μ. The nucleus of the cell is oval shaped, 10-14μ in length and 2-3μ in width. The supranuclear cytoplasm is always present in excess of the infranuclear cytoplasm. The infranuclear region presents vacuoles in the cytoplasm. Occasionally mononuclear cells are found in between the basal part of these cells. The cells at the apex of the villi are morphologically and in staining characteristics distinct from the cells at the base of the villi. Over the apical region they are taller, the cytoplasm is acidophilic (with basophilic strands) and the Golgi region is more conspicuous, whereas the cells over the base of the villi are shorter and take up a basophilic stain.

<u>Goblet cells:</u> Goblet cells are distributed in between the tall columnar absorptive cells, of the epithelium. They are unicellular mucous glands. The mucus is discharged over the luminal surface of the absorptive cells and forms a protective covering. When they are filled with

a mucigen droplet they resemble a wine glass, the distal cytoplasm being distended and bulged out by the clear mucus. It was due to this appearance of the full cell that they were given the name "goblet" cells (Gobel - old French for conical cup). In the small intestine its fundamental characteristics is in its full condition. A less filled goblet cell presents a more elaborate ergastoplasm and Golgi complex. The nucleus of the cell is situated over the smaller basal part which is joined to the rest of the cell by a narrow stem. They are mostly distributed along the middle third of the villus and vary in number from 8-20 per villus. Butterworth and Perez-Santiago (1958) mentioned that an average villus contains 5-30 goblet cells. The number actually varies according to the length of the villus and to the site of the mucosa. Goblet cells are less in number over the villi of the upper half of the small intestine and their number steadily increases towards the distal end of the ileum. In the human jejunum they are rather empty and smaller over the apical part of the villus, because the increased activity of the cell in this region causes more mucus to be discharged than is formed.

The discharged mucus, in addition to its protective function is a lubricant helping the passage of the intestinal content down the gut. Mucus contains both protein and carbohydrate. It is probable that the protein is formed in the endoplasmic reticulum of the goblet cell. This protein material is transferred to the sacs of the Golgi apparatus where the sulphated carbohydrate element

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is added forming individual droplets of mucus. The larger mucus droplets accumulate in the apical part of the cell and are finally released into the lumen of the intestine (Wyburn, 1962). Sometimes the cells are found empty and a few discrete granules occupy the Golgi region of the cell just above the nucleus. Florey (1962) suggested that in these cells the discharge of mucin has been faster than its synthesis. This state can be produced experimentally by applying an irritant to the surface of the mucosa.

Lamina propria: The lamina propria forms the core of the villi. A network of reticular fibres extends throughout the substance. It contains scattered lymphocytes, eosinophils, plasma cells, neutrophils, blood vessels and lymphatic channels, a plexus of nerves and smooth muscle fibres. Throughout the reticular network there are irregularly scattered branching cells with pale cytoplasm very similar to undifferentiated reticulo-endothelial cells. The reticular fibres unite with the subepithelial basement membrane and give supporting strength to the lamina propria (Ham and Leeson, 1961). There is a prominent lacteal over the central part of the core of the villus. The network of capillaries and smooth muscle fibres surrounds the central lacteal and fill up the core of the villus. The capillary network comprises arterioles, venules and lymphatic channels. There are smooth muscle fibres arranged around the central lacteal and some are located under the mucous membrane. The contraction of the former enables the villi to contract and expand and the contraction of muscle fibres under the mucous membrane

causes a local movement of the membrane. The submucosal nerve plexus (Meissner's plexus) sends twigs into the lamina propria. It is derived from the superior mesenteric plexus. In ordinary histological preparations it is difficult to identify these nerve fibres from connective tissue fibres.

Glandular layer: Between the bases of the villi the epithelium dips down to variable depths. These are the crypts of Lieberkühn. In man they are about 200μ in depth. In places they almost reach the muscularis mucosae. The majority of the cells lining these crypts are goblet cells. The other cells of these crypts secrete many enzymes. These glands are largely concerned with the regeneration of epithelial cell. In normal mucosae, mitosis is mainly confined to the cells of the crypt. The columnar epithelial cells, after having originated in these crypts become more mature as they glide along the sides of the villi, towards the apices, and from there they are shed into the lumen. Paneth cells are situated over the bases of these crypts. They are columnar cells which have a broad base and a narrow apex. The nucleus of the cell occupies the broad basal part of the cell. The supranuclear cytoplasm contains eosinophilic granules of unknown function. In addition, the intestinal glands have single flattened cells scattered over their bases. These cells contain granules which take up a silver stain, hence they are called argentaffin cells. It is suggested that these granules are concerned with normal haemopoiesis.

<u>Lymphatic tissue of the intestine:</u> Nodules of lymphatic tissue are found in the lamina propria of all parts of the digestive tract, but they are relatively more numerous in the lower part of small intestine (ileum). They are present either as solitary nodules or as large confluent masses called Peyer's patches. These Peyer's patches vary from 1-12 cm. in length and 1-2½ cm. in breadth. The villi are peculiarly absent over these areas. The smaller patches remain entirely confined to the lamina propria, but the larger ones (mostly over the lower part of the ileum) bulge through the muscularis mucosae into the submucosa. These lymphatic tissues tend to diminish and even disappear completely in old age. The tissues surrounding these nodules are infiltrated with the lymphocytes derived from these nodules. They are supposed to play a part in preventing the invasion of bacteria into the small intestinal wall.

<u>Submucosa:</u> Below the glandular layer of the mucosa, there is a layer of smooth muscle (muscularis mucosae) uniformly limiting the extent of the mucosa. The scattered smooth muscles of the core of the villus are derived from this muscle layer. Beneath this muscle layer is the submucosal layer which is found to be filled with connective tissue. Blood and lymphatic vessels and nerve plexuses are present in this connective tissue. Outside this submucous layer there is another zone of smooth muscle which is arranged in two layers. The inner circular layer is arranged in close spirals and the outer longitudinal layer is arranged in extended spirals. The muscle layer is

covered externally by a smooth serous membrane.

<u>Comparative morphology:</u> The morphology of the mucosa of the upper part of the small intestine in rodents is almost identical to those of humans. The villi of the mouse vary in height from 200-300μ and they get steadily taller in rat, guinea pig and rabbit. In rat the villi are 250-400μ in height, in guinea pigs 350-450μ and in the rabbit some of the villi are as tall as 700μ. The breadth and the plications of the villi show an increase in the same order. The subserosal muscle layer is not so well marked in the upper small intestine of mouse but it is very prominent in rat, guinea pig and rabbit.

<u>Turnover of intestinal epithelium:</u> The epithelial cells lining the mucosa are formed in the crypts of Lieberkühn. This assumption is drawn from the large number of mitoses seen in the cells lining the crypts of Lieberkühn. After these cells are formed they migrate from the crypt along the sides of the villi in orderly sequence till they reach the tip of the villi. At the tip there is a distinct cleft in the epithelium called the extrusion zone from where the cells are shed into the lumen of the intestine. (Fig. 11). There is a controversy concerning the fate of these cells at the villous tip. Some hold the view from the electron microscopic evidence that these older cells undergo dissolution and very little of the cell constituent is lost into the lumen of the gut (suggested by Curran and Creamer, 1963). The life span of the epithelial cells of the villi is found in several

species to be 1-3 days. Autoradiographic studies have made it possible to determine their speed of migration over the surface of the villi. At 24 hours they reach the base and at 72 hours they reach the tip of the villi (Hooper, 1961). As the cells migrate upwards they become matured structurally and functionally and become efficient in coping with the physiological need of absorption and metabolism of the food.

III. Electron microscopic appearance.

The free surface of the intestinal epithelial cell is found to have finger like projections called microvilli. Due to improved methods of cutting ultra thin sections for examination under the electron microscope fine structure of the small intestinal epithelial cell has come to light. Various authors have described the intestinal epithelial cell both in animals (Granger and Baker, 1950; Dalton, 1951; Palay and Karlin, 1959(a)) and in man (Zetterquist and Hendrix, 1960; Hartman et al, 1960; Ashworth et al, 1961; Padykula et al, 1961; Brown, 1962).

The microvilli: The microvilli which constitute the striated surface of the intestinal epithelial cell vary in their size and number per cell, from animal to animal. In man, they are on the average 1μ in height and 0.1μ in width. They are uniform in their dimensions. In longitudinal section they are found to lie parallel to the long axis of the cell. A section passing transversely through the microvilli shows that they are arranged in long rows and that they are packed in close hexagons. (Described in more detail in Chapter 9, p. 157.

Brown (1962) made an extensive study of the microvilli at various levels of the villi of human jejunum. The microvilli are taller over the villous crest, where they are about twice the size of the microvilli of the cells of the crypt. Over the crest of the villi there are about three times as many microvilli per micron of cell surface than over the crypts. The cytoplasm of the apical part of the cell is continuous with the core of the microvilli as fine fibrillar framework. This framework prevents collapse of the luminal surface during active muscular movement of the mucosa associated with digestion (Wyburn, 1962).

Plasma membrane: Each microvillus is enveloped by a lipoprotein membrane. This plasma membrane under the high resolution of the electron microscope presents two opaque zones which include an intermediate uniform zone of low optical density (originally reported by Weiss, 1955). From the present work it was found that each of the darker zones was 20Å thick separated by a light zone of 30-35Å width. An almost identical finding was reported by Robertson in 1959. At the junction between the adjacent cells of the epithelium the plasma membranes present complex foldings and interdigitations and at the apical regions of the cells they fuse to form the terminal bar. Palay and Karlin (1959a) thought that these interdigitations of the plasma membrane form a complex mechanical joint or locking device all round the cell surface. Thus it helps to coordinate the activity between the adjacent cells. But in actual fact, the

epithelial cell needs an excess of cell membrane to accomodate its increased size following absorption of water or hypotonic fluid. The excess cell membrane is folded up to be accomodated within the least space. These folds of the membrane are lost following the drinking of water. (Ruska, 1960; Wynn Williams, 1963). The lipoprotein membrane keeps the individuality of the cell with relation to the surrounding fluid and it acts as a highly specialised and selective diffusion membrane.

Terminal web and terminal bar: Over the apical part of the cell, the cytoplasm is devoid of mitochondria and vesicles of the endoplasmic reticulum. The fibrillar element of this part of the cell spreads like a web throughout the entire terminal part of the cell. Hence it is called the terminal web. To this web the cores of the microvilli are anchored and become continuous with the endoplasm of the cell. Improper dehydration of the tissue seems to have a special damaging effect on this region of the cells. In a two dimensional view the terminal bars present a wedge shaped delineation. But in a three dimensional reconstruction of the distal part of the cell it presents as a cementing substance over the superficial part of the cell just below the attachment of the microvilli. In the present day with the improved technique of cutting thin sections details of this cementing substance have come to view. The cementing substance is actually desmosomes, fibrillar electron dense bodies present along the entire course of the terminal part of the cell. The desmosomes are also found between

the cell membranes of adjacent cells and they hold the cells together at these sites. In general, the terminal web gives extra support to the terminal part of the cell, and helps in the coordination of the action of the cell.

Cytoplasm: The cellular cytoplasm is divided into supranuclear, nuclear and infranuclear zones. The supranuclear cytoplasm is always present in excess of the infranuclear cytoplasm. It contains the endoplasmic reticulum and cytoplasmic organelles namely mitochondria, the Golgi apparatus, centrosomes, microsomes, dense bodies, fat droplets and granules of various metabolic products. Agranular cytoplasm (ectoplasm or sub-cuticular region) separates it from the microvilli.

(a) Endoplasmic reticulum: The central part of the cell has a vast network of an intercommunicating cytoplasmic membrane system called the endoplasmic reticulum. (Endoplasm means the central part as against ectoplasm or the peripheral part). It comprises vesicles of different shape, some round and some flat. Some of these vesicles have a smooth wall (devoid of RNA granules) and some are rough walled being studded with numerous dense particles or rhibosomes. These smooth walled vesicles are found more over the superficial part of the endoplasmic reticulum. Some of them are small and the others are large in size. The smaller vesicles show features like invagination, suggesting that they are concerned with the pinocytotic activity and carry absorbed food material from the surface of the cell to its interior. The larger, smooth surfaced membranous structures are concerned with the

metabolism of glycogen. In other vesicles RNA granules are studded on the walls and give it a rough appearance. These granules constitute the ground substance for the manufacture of protein (Roodyn, 1962).

(b) Golgi apparatus: This region of the cell comprises smooth walled membranous sacs and minute vesicles. Some of the smooth walled sacs are distended to form large vacuoles which are usually arranged in groups. Other sacs are flattened and are very closely packed giving a lamellated appearance. (Fig. 16). Its exact role in the intracellular synthesis of triglyceride is not known, but this much is certain that lipid molecules lie clustered within the dilated cisternae of the Golgi comples. (Palay and Karlin, 1959a).

(c) Mitochondrial morphology: The mitochondrium is a cell organelle bounded by a double layered surface limiting membrane and by this feature it is distinguished from the other cytoplasmic organelles. The surface membranes divide the mitochondria into two chambers (Rouiller, 1960). The outer chamber is enclosed by two membranes and the inner chamber is bounded by an inner membrane. These membranes act as a semi-permeable envelope separating the mitochondrial matrix from the surrounding cytoplasm which has a different osmotic concentration. The inner limiting membrane folds on itself to form cristae (Palade, 1953). (Fig. 14). By this feature the inner surface of the mitochondria can be increased. The number of cristae per mitochondrial volume varies according to the species, tissues and

organs (Rouiller, 1960). The density of the mitochondrial matrix varies in different animals (Palade, 1955). Sometimes dense granules are seen in the mitochondrial matrix. In vitro the mitochondrion is the major site for the incorporation of aminoacids into insoluble lipoproteins (Roodyn, 1962). The mitochondria in the supranuclear cytoplasm of the intestinal epithelial cell appear to be displayed parallel to the long axis of the cell (Palay and Karlin, 1959b). This may be more apparent than real. No such arrangement is seen in the infranuclear region.

(d) Centrosome: The centrosome is a tiny cytoplasmic organelle. As its name implies, it lies very close to the centre of the cell, close to the Golgi region but is not considered as a part of the Golgi apparatus. Under the electron microscope it presents longitudinally arranged fibrils grouped around a central lumen. It is supposed to be concerned with organising the fibrillar material of the cell, which appears between the two centrioles in a dividing cell.

(e) Other cell organelles:

Intracytoplasmic fat is usually seen in the supranuclear region of the cell as minute droplets. They carry a membrane around them (Palay and Karlin, 1959b).

A lysosome although possessing a similar single membrane is distinct in its features from the intracytoplasmic lipid droplet. The lysosomes do not possess a round shape and cast a less dark shadow on the electron microscopic screen. Lysosomes are seen in cells

undergoing physiological and pathological lysis (Becker and Barron, 1961).

The microsomes are suggested to have their origin in the endoplasmic reticulum as pinched off vesicles. These vesicles bear ribosomes on their external surface (Palade and Siekevitz, 1956). In addition to the above cell organelles, one often notices dense bodies in the cytoplasm whose real identity is still to be determined.

Nucleus: The nucleus of the columnar epithelial cell under the electron microscope presents a double membranous envelope. (Fig. 16). These nuclear membranes are separated by a perinuclear space. The outer membrane is continuous with the endoplasmic reticulum (Watson, 1955). These nuclear membranes are discontinuous in places which appear as pores (Gall, 1959). There is a controversy as regards the structural details and function of these pores. Watson (1955) suggested that these pores in the nuclear membrane allow the selective transfer of substances between the nucleus and the surrounding cytoplasm. The nuclear membrane disappears during cell division. (Fig. 15).

Under the electron microscope, the nucleus of a non dividing cell presents a uniformly granular appearance. The nuclear substance is found to be comprised of a reticulum with tightly packed granules. The nucleolus is seen as a solitary body inthe nuclear substance (very occasionally more than one nucleolus is seen). During cell division the nucleolus gets smaller and less prominent, and eventually disappears altogether.

The basal part of the cell and the subepithelial basement membrane: The basal or foot processes of the cell is flat and triangular in shape. It rests on the subepithelial basement membrane. Sometimes triangular areas, of empty spaces, are included in between the basal parts of the cells. Sometimes mononuclear cells from the lamina propria are found to infiltrate into these spaces.

The subepithelial basement membrane is a regular structure formed of hyaline and amorphous material. It is seen as a continuous line of about 70 mμ thickness in man. It lines the external surface of the lamina propria and holds the structures of the lamina propria inside its boundaries. The loose connective tissue of the lamina propria separates the subepithelial basement membrane from the vessels, nerve fibres and various cells inside the lamina propria.(Figs. 17, 18).

Goblet cells: The nucleus and the cytoplasm of the goblet cell are more osmophilic which feature makes the goblet cell distinct from the absorptive columnar epithelial cells of the small intestine. The apical surface of the nonsecreting goblet cell presents small microvilli which are distinctly less in number than the microvilli covering the luminal surface of the columnar absorbing cell. The microvilli disappear when the cell is discharging its secretion (Fig. 13). The cell holds an elaborate endoplasmic reticulum (ribonucleoprotein granules are studded on these membranes). Between the prominent superficial part (goblet) of the cell and the nucleus is the Golgi complex - an abundant collection of

of smooth membranes. Between these membranes mucigen droplets are found which are oval or round in shape. Besides these, mitochondria are noted in the cytoplasm. Padykula (1962) remarked that the goblet cells over the apical part of the villus in human jejunum are empty. This suggests increased activity of the apical region of the villus. No morphological evidence is available of the passage of water through the goblet cell. It still remains as a puzzle, how water and salt are added to form mucin (Wyburn, 1962).

Lamina propria: The prominent structures in the lamina propria are capillaries, lymphatic channels, unmyelinated nerves, smooth muscle fibres, and various red and white blood cells. Eosinophils, lymphocytes and plasma cells are prominent among the cellular components. (Figs. 18, 19, 20).

The lymphatic capillaries (or lacteals) differ distinctly from the blood capillaries. The endothelial wall of the lymphatic capillaries is noticeably thicker (about 5 or 6 times that of blood capillary). There are no fenestrations in the walls of the lacteals, which feature is prominent in the blood capillaries. Moreover the endothelial cells of the blood capillaries overlap at their margins with adjacent cells. The pinocytotic vesicles in the walls of the lacteals are more prominent as they deal with the fat that is absorbed into the villus. Unlike the blood capillaries, the lacteals have a less definite basement membrane, which may be absent in places. There are demonstrable gaps at the junction of two

neighbouring endothelial cells through which fat particles are seen to enter into the lacteals. (Palay and Karlin, 1956b). Unmyelinated nerves are found underneath the subepithelial basement membrane. They are also seen in association with the blood vessels.

The eosinophils present a distinct ultra microscopic structure. The matured granules of these eosinophils show a central body which has a square or rectangular shape and occupies a variable part of the granule. (Fig. 21). The nucleus of the cell does not look very dark because the chromatin of the nucleus is not so densely packed as in neutrophil.

The lymphocytes have very little cytoplasm around the nucleus. The nucleus is round or ovoid with a small indentation on one side. The nucleus is more granular and the granules are more closely packed towards the periphery of the nucleus. The cytoplasm of the lymphocytes in addition to the mitochondria holds small membranous vesicles, the Golgi complex and RNA granules.

The plasma cells present a typical cytological feature. The cell is rich in rough surfaced vesicles similar to the endoplasmic reticulum of the cell. The abundance of ribonucleoprotein in the cytoplasm suggests that the manufacture of protein at these sites is meant for extra cellular purposes (Ham and Leeson, 1961). In addition to this highly developed ergastoplasm it has a well developed Golgi complex. It is an active site for anti-body production. (Thiery, 1960).

The gross morphological features of the small intestinal epithelial cells of humans differ very little from those of rat. In humans the villi are taller and the microvilli covering the absorbing surface of the intestinal mucosa are taller and more closely packed per micron of absorbing surface than in the rat. The eosinophil cells are more abundant in the lamina propria of rat intestinal villus. A more detailed comparative anatomy is beyond the scope of this present work.

REFERENCES.

1. Ashworth, C.T., Chears, W.C. Jr., Sanders, E., and Pearce, M.B. (1961). Arch. Path., 71, 13.

2. Baker, S.J., Mathan, V.I. and Cherian, V. (1963). Lancet, i, 860.

3. Baker, S.J., Mercy, Ignatious, Mathan, V.I., Vaish, S.K., and Chacko, C.C. (1962). In Ciba Foundation study group. No. 14. J. & A. Churchill Ltd., London.

4. Becker, N.H. and Barron, K.D. (1961). Amer. J. Path., 38, 161.

5. Bourne, G.H. (1960). An introduction to functional histology. 2nd Ed., J. & A. Churchill Ltd., London.

6. Brown, A.L. (1962). J. Cell. Biol., 12, 623.

7. Butterworth, G.E.J. and Perez-Santiago, E. (1958). Ann. intern. Med., 48, 8.

8. Curran, R.C. and Creamer, B. (1963). J. Path. Bact., 86, 1.

9. Dalton, A.J. (1951). Amer. J. Anat., 89, 109.

10. Doniac, I. and Shiner, M. (1957). Gastroenterology, 33, 71.

11. Finerty, J.C. and Cowdry, E.V. (1960). A textbook of histology, Henry Kimpton, London.

12. Florey, Sir Howard. (1962). Lectures on General Pathology, 3rd Edition. Lloyd-Luke Ltd. , London.

13. Gall, J.G. (1959). J. biophys. biochem. Cytol., 6, 115.

14. Granger, B. and Baker, R.F. (1950). Anat. Rec., 107, 423.

15. Ham, A.W., and Leeson, T.S. (1961). Histology, 4th Edition, Pitman Publishing Co. Ltd., London.

16. Hambleton, B.F. (1914). Am. J. Physiol., 34, 446.

17. Hartman, R.S., Butterworth, C.E. Jr., Hartman, R.E., Crosby, W.H. and Chirai, A. (1960). Gastroenterology, 38, 506.

18. Hooper, C.E.S. (1961). Meth. med. Res., 9, 326.

19. Kendall, J. (1940). Microscopic anatomy of vertebrates. 2nd Edition, H. Kimpton, London.

20./

20. Padykula, H.A. (1962). Fed. Proc., 21, 873.

21. Padykula, H.A., Strauss, E.W., Ladman, A.J. and Gardner, F.H. (1961). Gastroenterology, 40, 735.

22. Palade, G.E. (1953). J. Histochem. Cytochem., 1, 188.

23. Palade, G.E. (1955). J. biophys. biochem. Cytol., 1, 59.

24. Palade, G.E. (1955). J. biophys. biochem. Cytol., 1, 567.

25. Palade, G.E. and Siekevitz, S. (1956). J. biophys. biochem. Cytol., 2, 171.

26. (a) Palay, S.L. and Karlin, L.J. (1959). J. biophys. biochem. Cytol., 5, 363.

27. (b) Palay, S.L. and Karlin, L.J. (1959) J. biophys. biochem. Cytol., 5, 373.

28. Roodyn, D.B. (1962). Biochem. J., 85, 177.

29. Rouiller, C.H. (1960). Int. Rev. Cytol., 9, 227.

30. Robertson, J.D. (1959). Biochem. Soc. Symposium No. 16. University Press. Cambridge.

31. Ruska, C. (1960). Atlas of Electron Microscopy. Compiled by F. Scanga, Elsevier Publishing Co. 1964.

32. Thiery, J.P. (1960). In Ciba Foundation Symposium. Cellular aspects of immunity. J. & A. Churchill Ltd., London.

33. Verzar, F. and McDougall, E.J. (1936). Monogram. Absorption from the intestine. Langmons, Green & Co., London.

34. Watson, M.L. (1955). J. biophys. biochem. Cytol., 6, 147.

35. Weiss, J.M. (1955). J. biophys. biochem. Cytol., 1, 257.

36. Wells, H.S. and Johnson, R.G. (1934). Am. J. Physiol., 109, 387.

37. Wilson, T.H. (1962). Intestinal Absorption. W.B. Saunders Company, Philadelphia and London.

38. Wyburn, G.M. (1962). Surgical physiology of gastro-intestinal tract. R.C.S. (Edin) Symposium.

39. Wynn Williams, A. (1963). Gut, 4. 1.

40. Zetterquist, H. and Hendrix, T.R. (1960). Bull. John Hopk. Hosp., 106, 240.

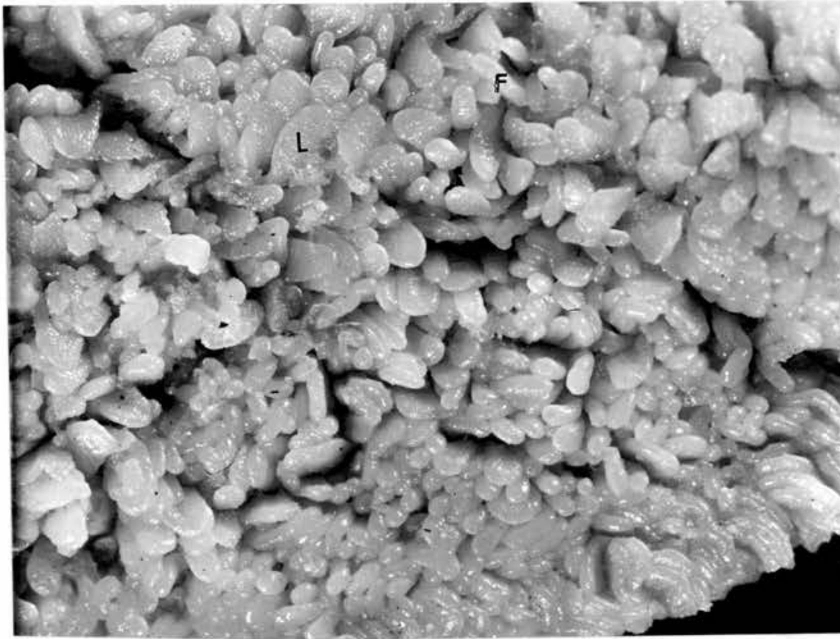


Fig. 2. Dissecting microscopic appearance of normal human jejunal mucosa.
F = finger shaped villus. L = leaf shaped villus. These finger and leaf shaped villi are seen in varying proportions. x 30

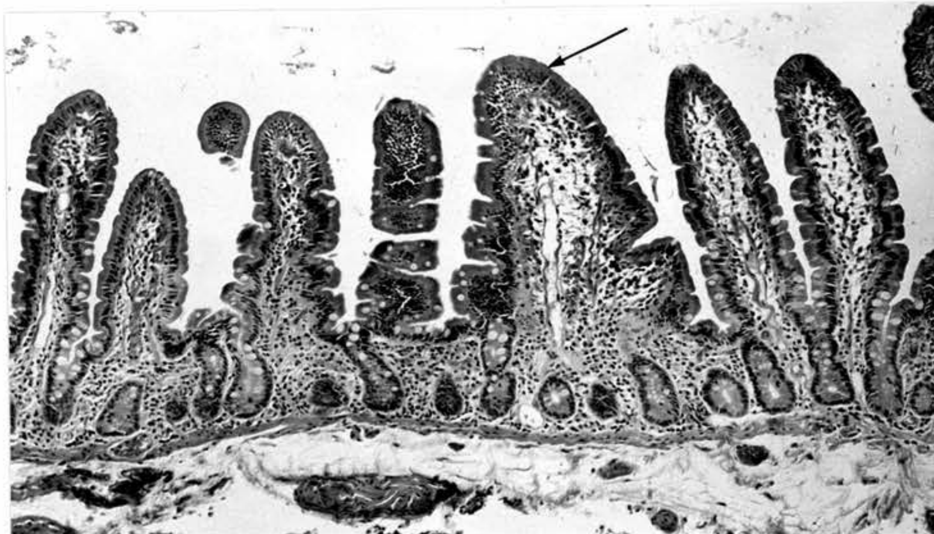


Fig. 3. Light microscopic appearance of normal human mucosa. Arrow shows a leaf shaped villus sectioned through its entire width. Villi on either side could be finger or leaf shaped. H & E x 80

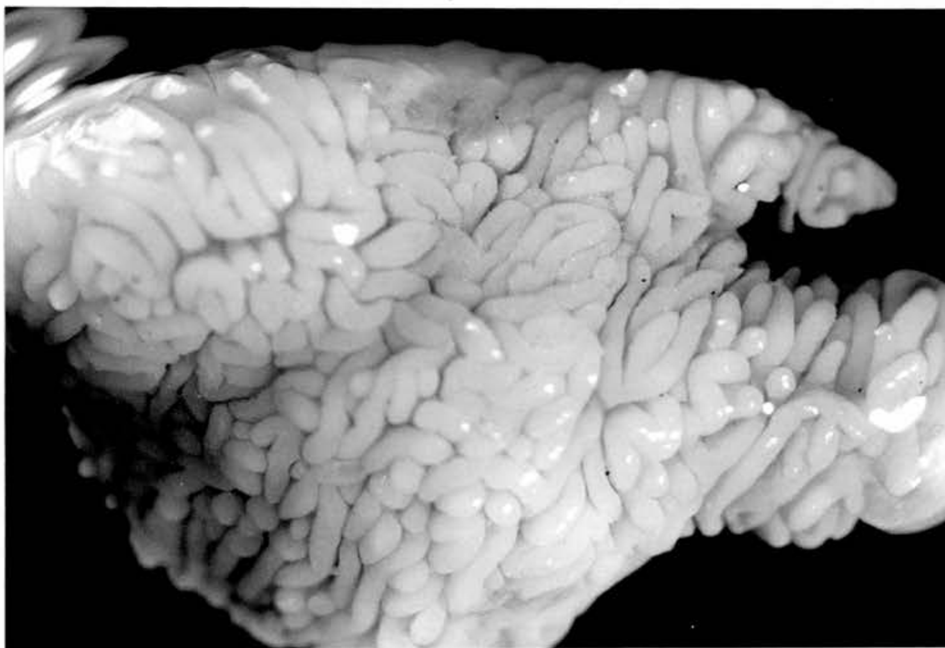


Fig. 4. Human jejunal mucosa showing predominantly ridge shaped and some finger shaped villi. x 30

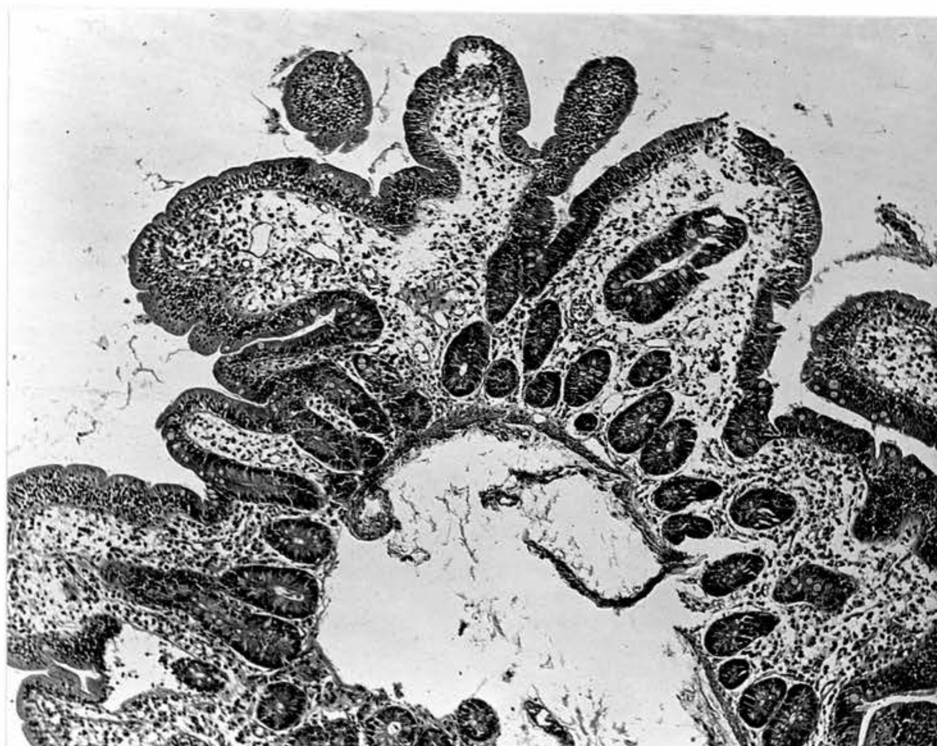


Fig. 5. Histologic section of the above mucosa. Note the diameter at the base of these ridge shaped villi is more than the diameter at the base of the leaf shaped villi. H & E x 80

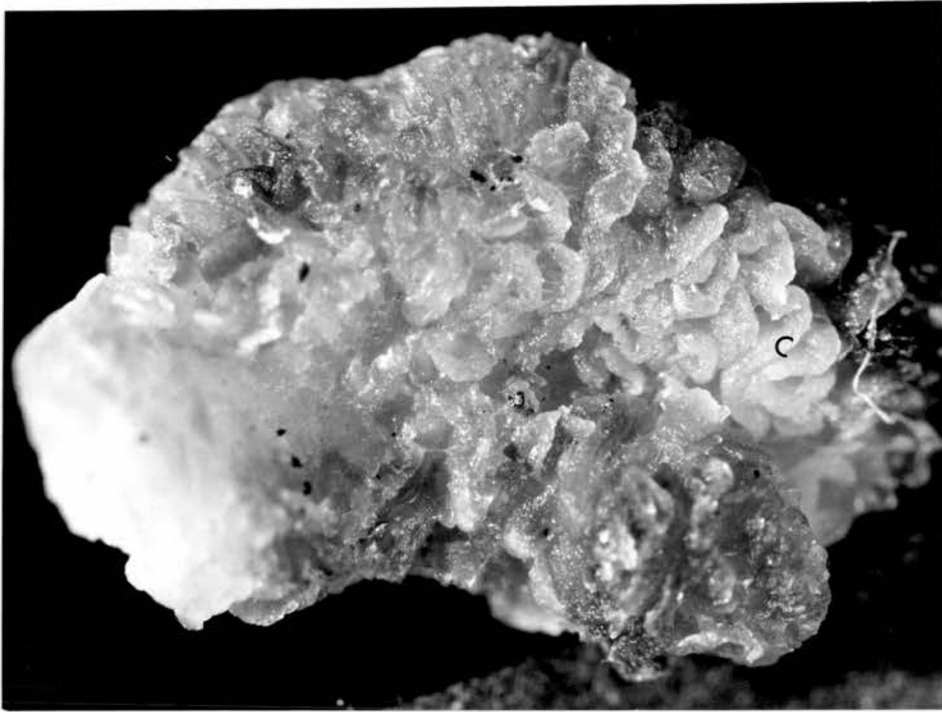


Fig. 6. Human jejunal mucosa. Note the convolutions (c). x 30

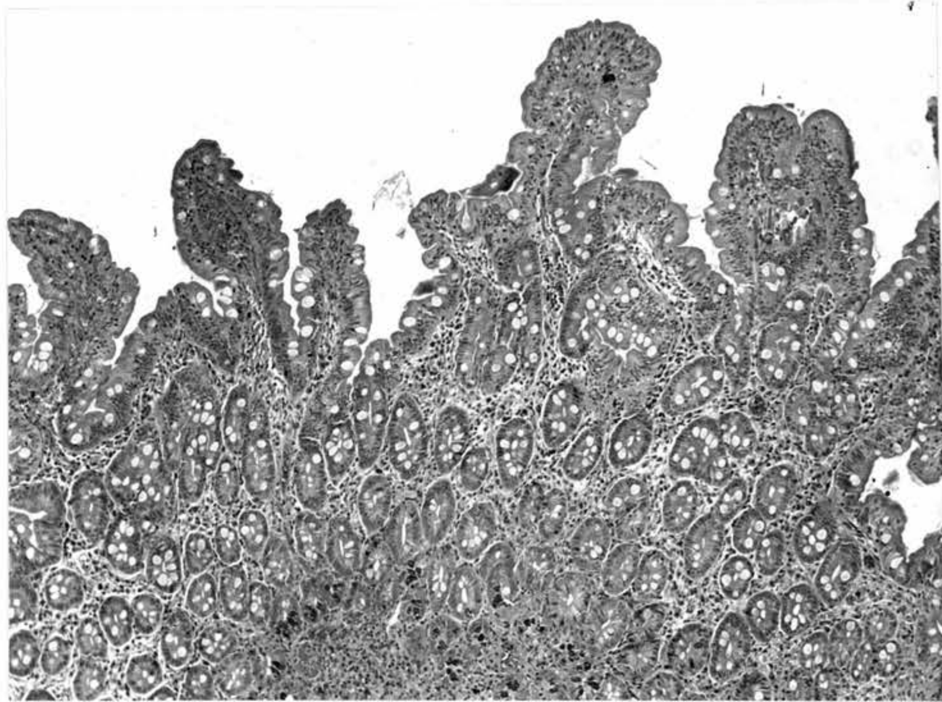


Fig. 7 Light microscopic appearance of the above mucosa. H & E x 80

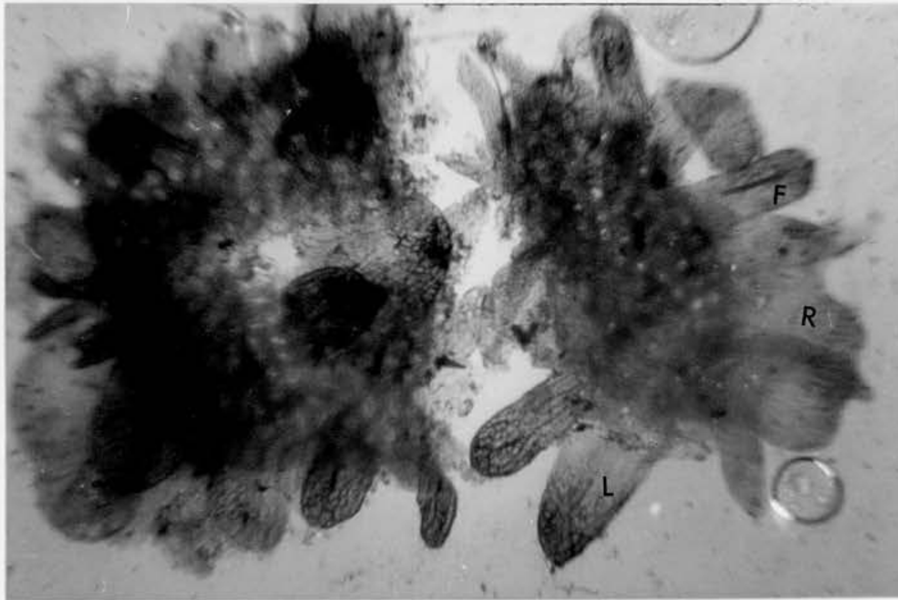


Fig. 8. Human jejunal mucosa. Note the three types of villi in one specimen. F = finger shaped. L = leaf shaped. R = ridge. In this photo leaf shaped villi appear to have fused to form ridge. x 30

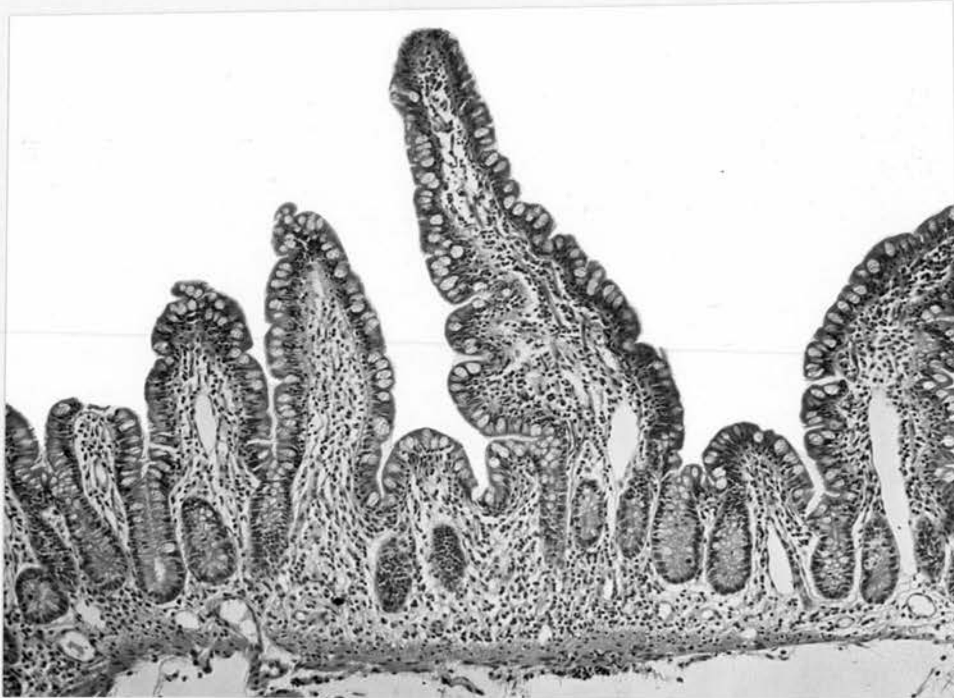


Fig. 9. Histologic section of normal mucosa. Note the deceptive variation in size of the villi according to the plane of section.
H & E x 80

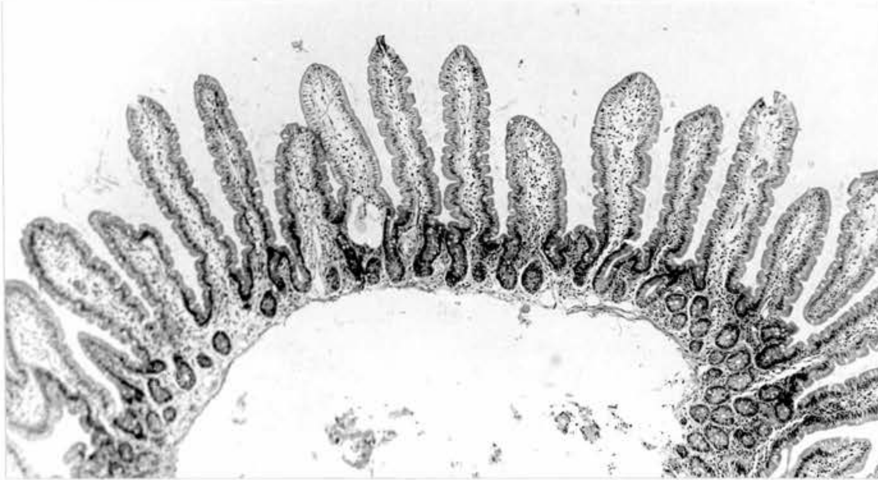


Fig. 10. Normal jejunal mucosa.
H & E x 40

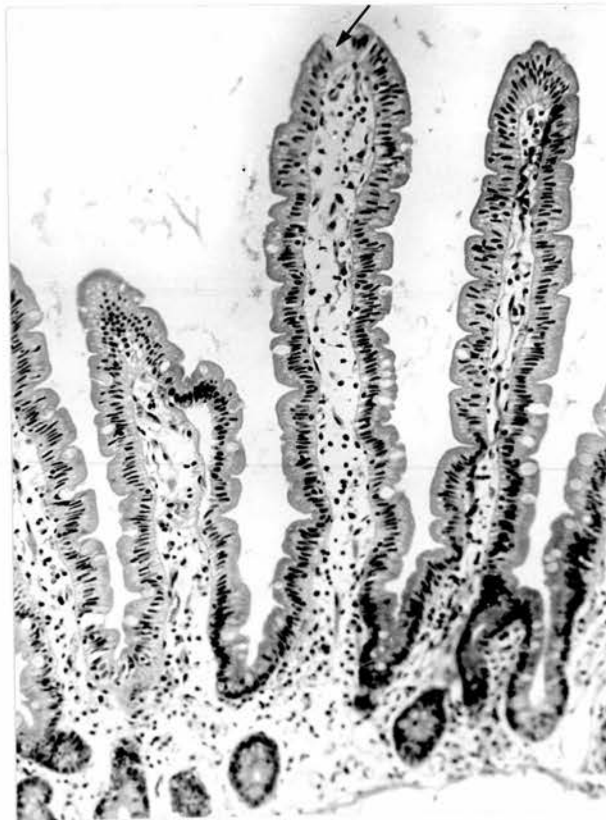


Fig. 11. Normal jejunal mucosa showing outline of the cell (arrow) at the lip of the villus undergoing dissolution.

H & E x 120

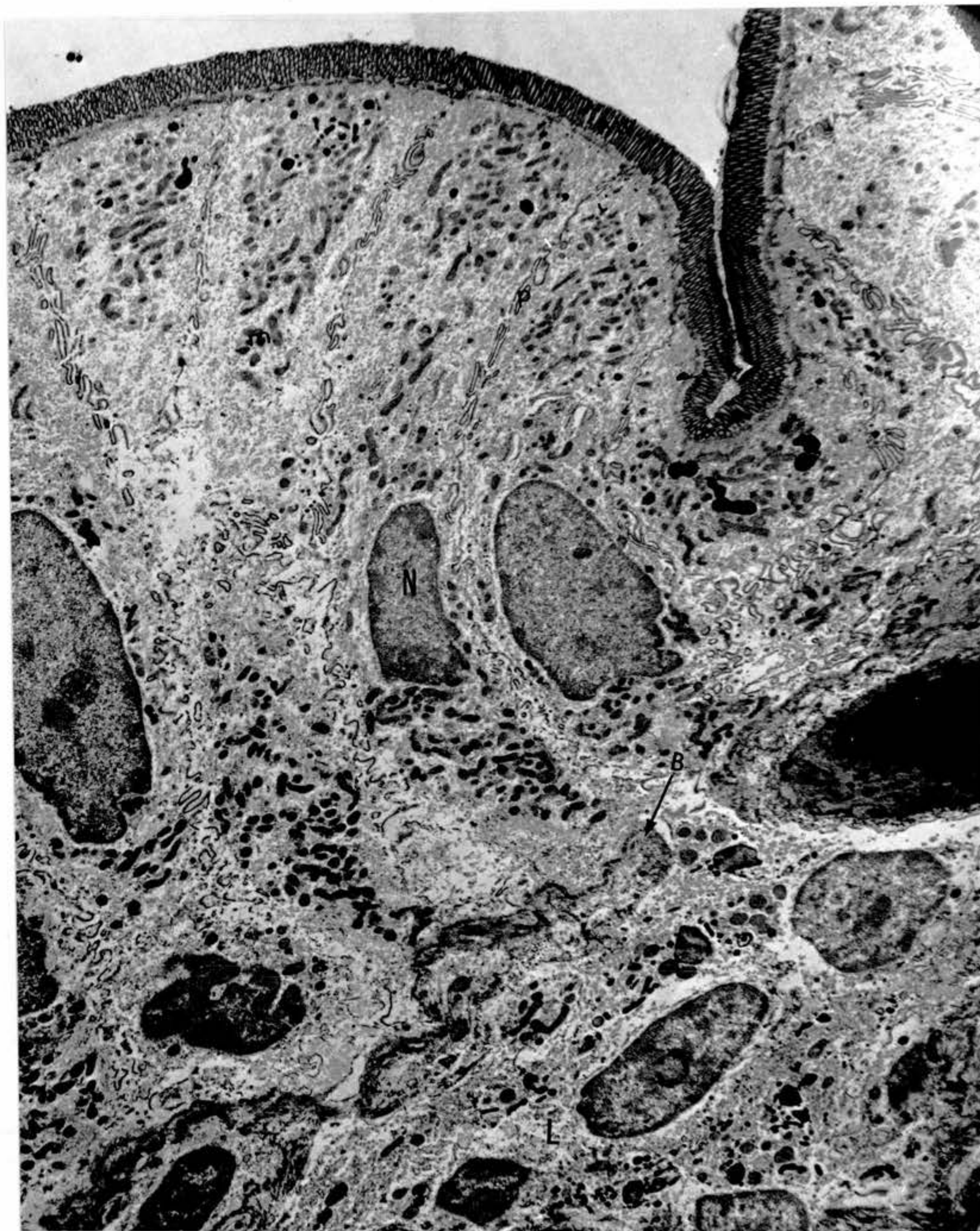


Fig. 12. E.M. of normal jejunal mucosa. MV = microvilli; m = mitochondria; p = plasma membrane forming lateral cell wall; N = nucleus of an absorbing epithelial cell; B = subepithelial basement membrane; L = lamina propria.

x 4,944

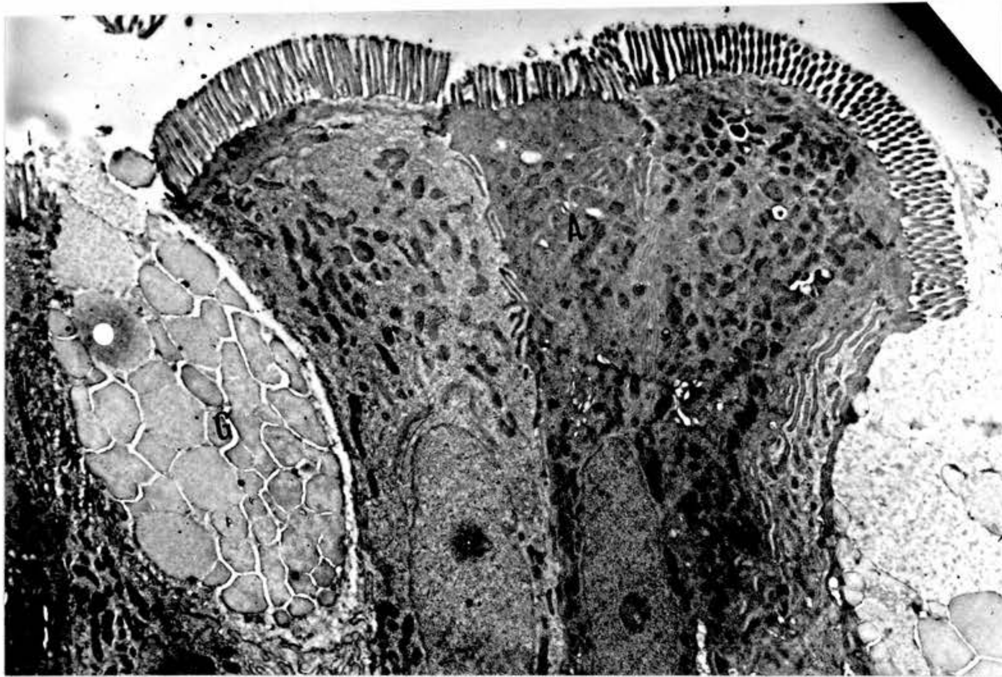


Fig. 13. E.M. of tip of human jejunal villus. A = ageing cell in the extrusion zone. Note its more electron dense cytoplasm and smaller microvilli; goblet cell (G). x 4,500

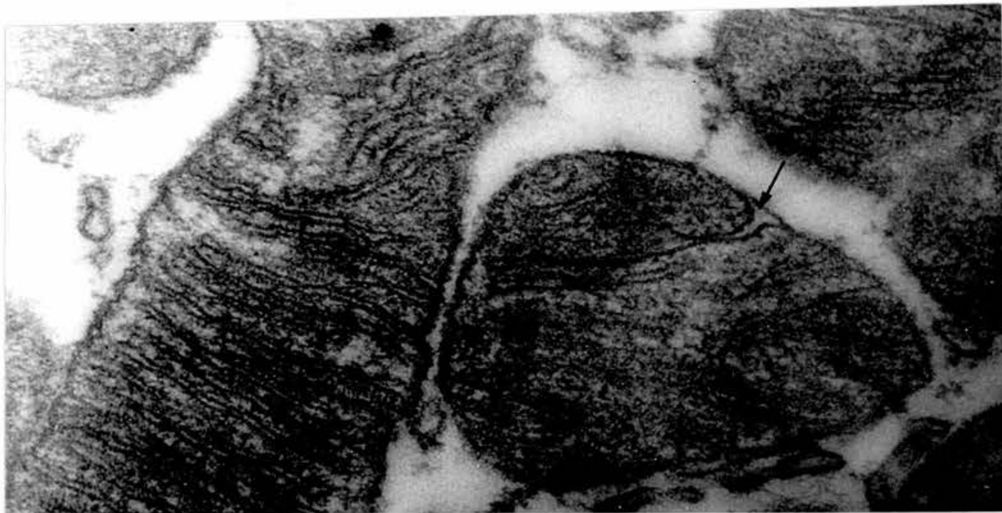


Fig. 14. Details of mitochondrial morphology. Arrow shows how cristae (c) of mitochondria are formed by reduplication of inner mitochondrial membrane and run transversely. x 120,000

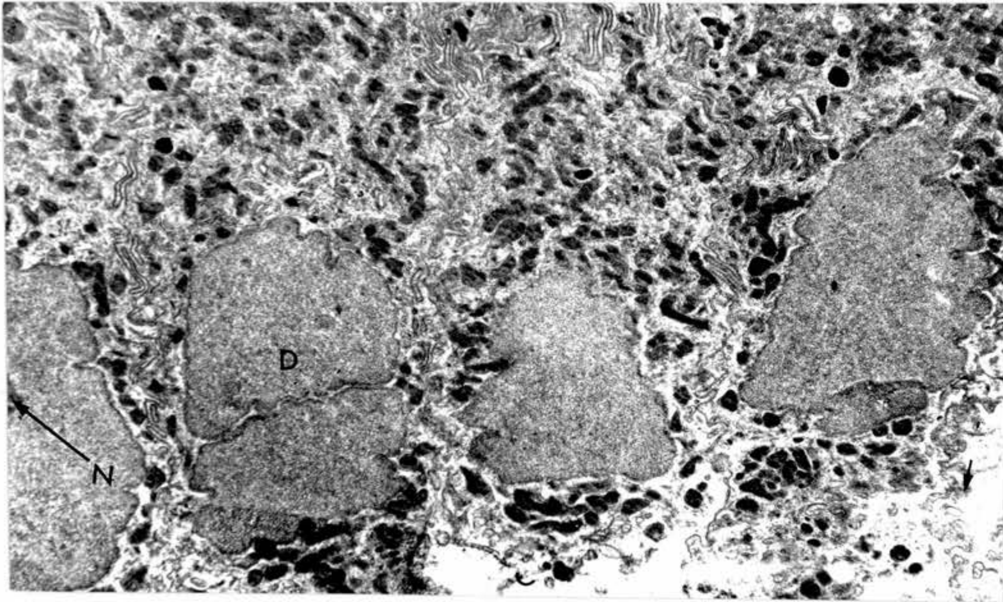


Fig. 15. E.M. of normal human jejunum showing nuclei and supranuclear cytoplasm of the epithelial cell. Nucleolus (N); dividing nucleus (D). x 6,000

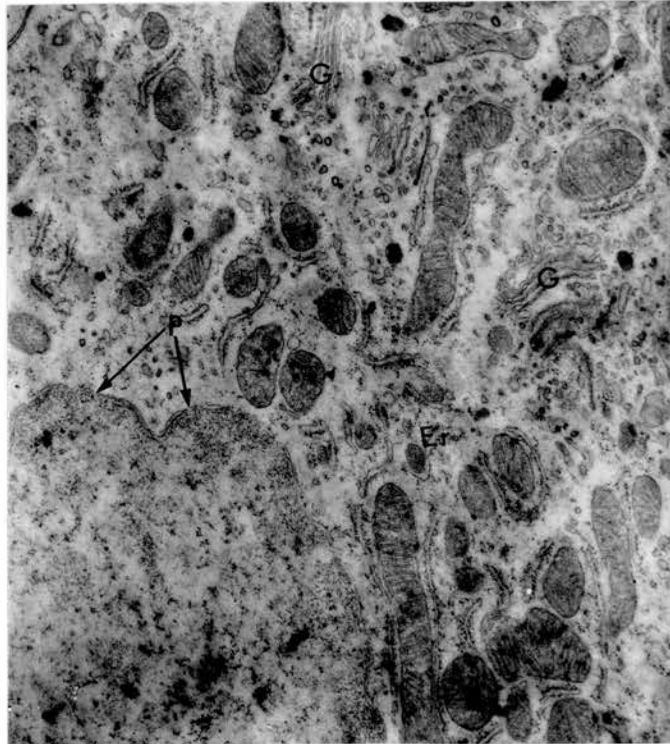


Fig. 16. E.M. of upper part of nucleus and supranuclear region showing mitochondria, golgi apparatus (G), endoplasmic reticulum (Er), and pores (P) in the double walled nuclear membrane. x 18,200

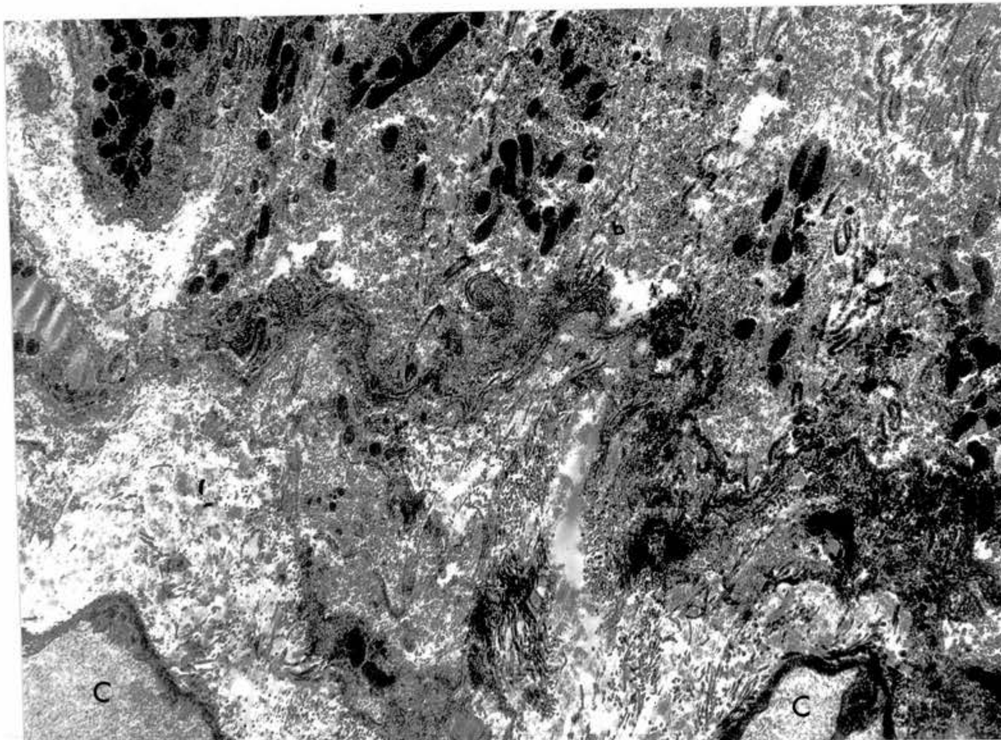


Fig. 17. E.M. of basal part of jejunal epithelial cell which rests on subepithelial basement membrane (B). This membrane separates the cell from capillaries (C) and other structures in lamina propria. L = loose areolar tissue which acts as packing material in lamina propria. x 7,200

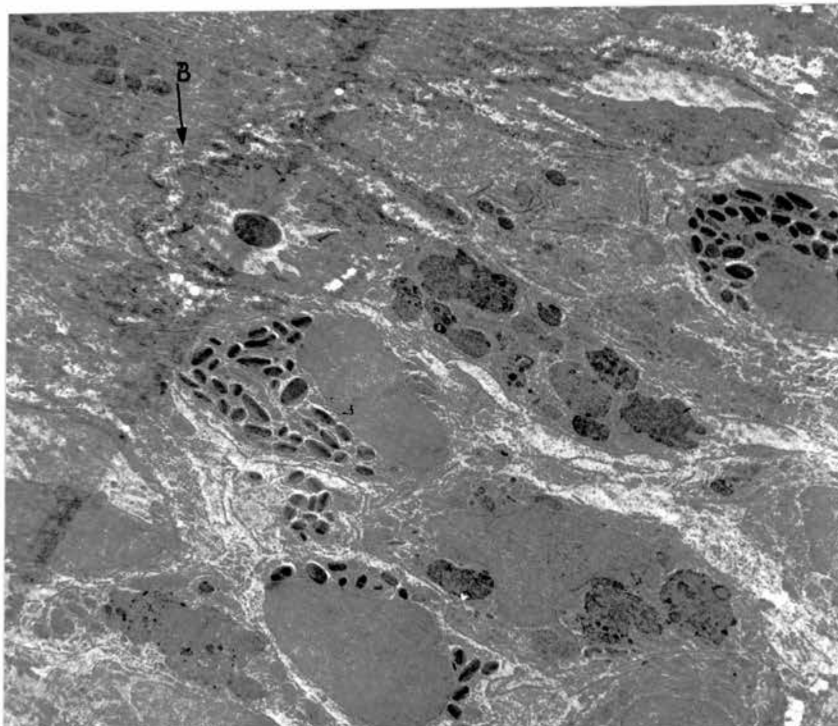


Fig. 18. E.M. of structures in lamina propria of rat small intestine. B = subepithelial basement membrane. Note predominance of eosinophils (E). Capillaries and monocytes are seen. x 2,700

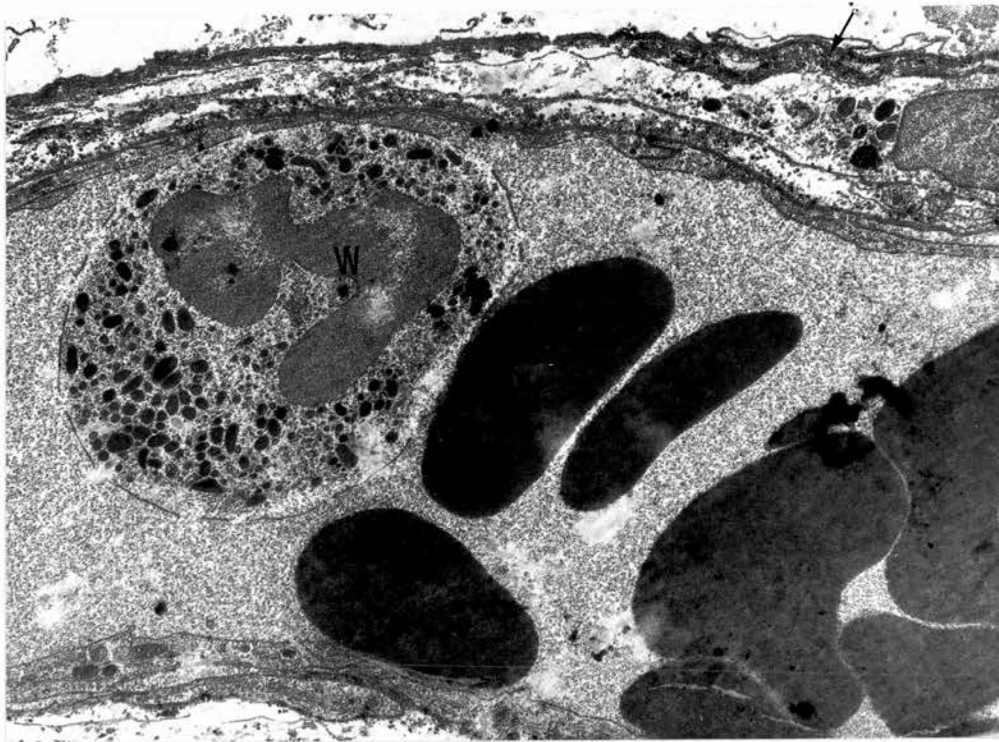


Fig. 19. E.M. of a capillary under the sub-epithelial basement membrane (arrow). Red cells and white cell (W) seen inside the lumen of the capillary. x 9,200

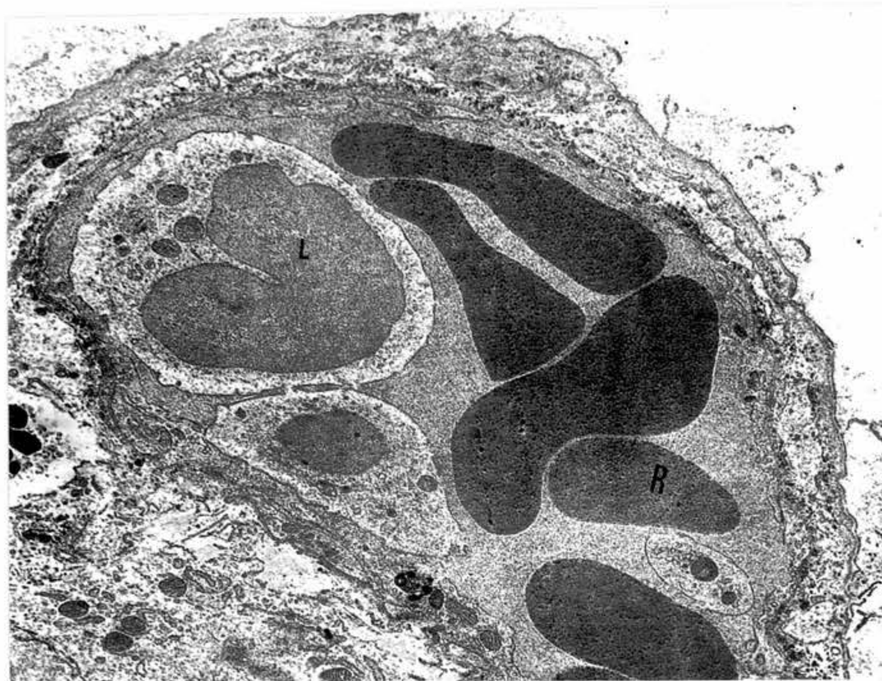


Fig. 20. E.M. of a capillary in the lamina propria, showing red cells (R) and a lymphocyte (L). x 6,400

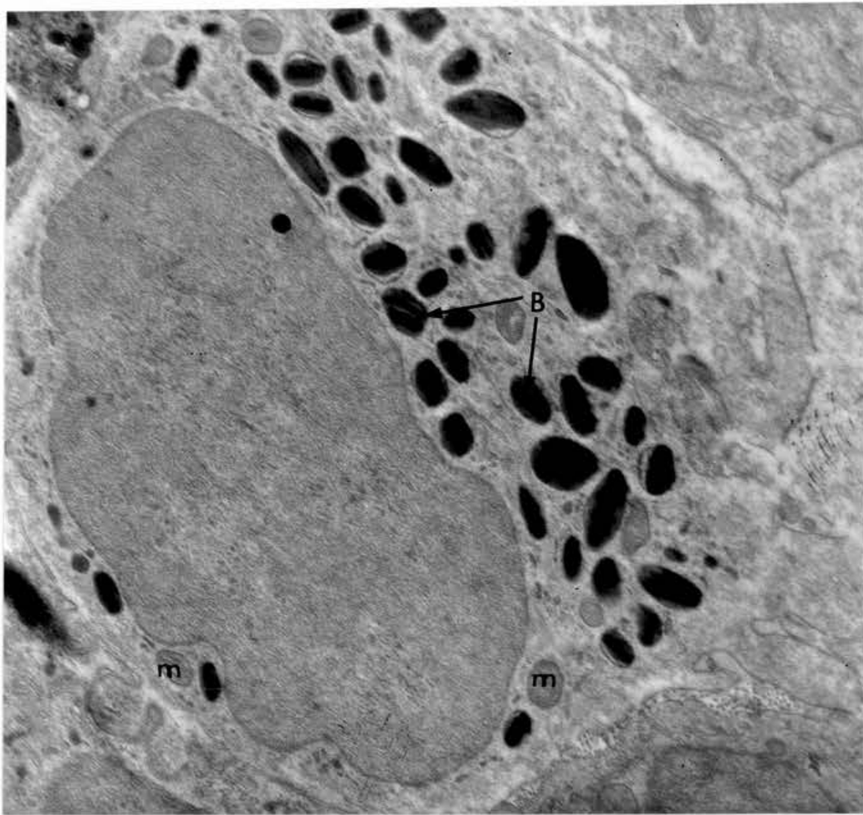


Fig. 21. An enlarged view of an eosinophil under the electron microscope. The granules of the eosinophils have a characteristic rectangular central bar (B).
m = mitochondria. x 14,000

PART II.

Small intestinal mucosa in abnormal states

SECTION A.

Experimental Pathology

CHAPTER 3.

CELL SHEDDING OF INTESTINAL EPITHELIUM: EARLY EVIDENCE OF AUTOLYSIS.

During the past thirty years various workers have tried to find a suitable explanation for the shedding of intestinal epithelia of experimental animals following death by humane killer. It is believed that ischaemia of the bowel, directly from occlusion of the mesenteric artery or secondary to severe blood loss, shock, fracture of bone or ischaemic muscular contraction of the intestine can cause shedding of the epithelium.

In the present study, a number of interesting observations have been made following simple removal by saline washing of the intestinal contents of experimental animals immediately after death. This paper comprises mainly an analysis of the effect of observations of various factors which maintain equilibrium between the vascular supply of the intestine and the actions of the enzymes on the intestinal epithelium.

It is an accepted fact that epithelial cells of the mucous membrane of the intestine become detached soon after death. Increased atmospheric temperature and humidity accelerate the autolytic process. But in the context of recent controversy on this subject,some of the recent observers have suggested that this is a process of disruption rather than dissolution (Cuthbertson, 1960; Fell, 1961). Alvarez (1948) suggested that there is a gradient in epithelial cell shedding compared with the metabolic gradient of the intestinal tract. Without perfusion of the

intestine the time of onset of post mortem cell shedding is as follows: duodenum 8 minutes, jejunum and ileum 10-12 minutes, caecum and colon 20 minutes. But perfusion of the intestine with 4% formol saline within 30 seconds of death can prevent the shedding of epithelial cells in both fasting and non-fasting rats and rabbits (Fell, 1961).

Verzar and McDougall (1936) mentioned that in an isolated intestinal loop (in vitro) anoxia leads to poor survival of the epithelium and therefore shedding of cells occurs. Anoxia of the epithelium could be due to a direct cause or secondary to prolonged intestinal contraction. This damage can be prevented by an improvement of the oxygen supply to these tissues as found by Fisher and Parson (1949). Cuthbertson (1960) was first to notice that fracture of the femur of a rabbit causes intestinal epithelial cell shedding, possibly due to reduced blood supply to the intestine. A similar picture has been reproduced in rats (Fell, 1961).

Various observers have considered epithelial cell shedding of intestine as an 'artefact' produced by damage to the epithelium from anoxia in the period that elapsed during slow or impeded fixation. There have been reports of exaggeration of this 'artefact' by feeding the animals a few hours before killing.

Materials and Methods.

The animals used in this experiment were all adult albino Swiss mice (8), Wistar rats (8), guinea pigs (2), rabbits (7), sheep (4). All these animals except 4 rats

had normal access to food and water. These 4 rats (two groups - two in each group) were kept without food (with access only to water) for 4 days before they were sacrificed. Scrupulous care was taken that these fasted rats should not have any access to their faeces (rats are notorious for coprophagia).

Anaesthesia and methodsof killing.

Anaesthesia was employed only where animals (rats and rabbits) were used for the study of the effects of differential vascular occlusion and also in order to study the effects on intestinal epithelium of vigorous and sustained muscular contraction following the artificial production of intestinal obstruction. The anaesthesia was induced and maintained by Nembutal per intravenous route (in rabbits) and intraperitoneally (in rats). The maximum dose given was 0.1 cc. per 100 g. Very rarely there was need of general ether to supplement the Nembutal anaesthesia.

All the other animals except the sheep were killed by a blow on the head. Sheep were killed by a shot in the frontal region with a captive bullet fired by a humane killer and then bled. The body cavity was opened up immediately afterwards. Death was timed by cessation of heart beats.

Preparation of segments.

Fluid intestinal contents of the proximal part were removed by making a cut in the wall. The contents of the distal part of the small intestine and the colon were

expressed by digital pressure. The length of the intestine was divided by ligation into loops of about 2-3 cm. long and were perfused with 10% formol saline at required time intervals. The shortest time taken (throughout this work) to perfuse any segment was 2 minutes from death. Previous work (Fell, 1961) suggests that perfusion fixation gives a better histological picture than immersion fixation. Therefore we limited our method to the perfusion fixation type only. During perfusion the segments took up the shape of a sausage and were left in 10% formol saline for 18-24 hours. Then from each loop a thin strip (about 2 cm. long and 1 cm. wide) was cut from the antimesenteric region. These strips of tissues were passed through the standard process of fixation, corrosive formol for a minimum period of 12 hours, dehydration by grades of alcohol, cleared with chloroform, impregnated with soft paraffin wax under vacuum pressor - 160 to - 180 mm. mercury for about one hour and then embedded in paraffin wax with the long axis of the tissue horizontal.

The sections were cut with a Cambridge microtome to 4μ thickness. Each section was stained with Haematoxylin and Eosin, Periodic Acid-Schiff and Masson Trichrome methods. Sometimes Picro-Mallory and Alcian green stains were used as an alternative method to the Masson Trichrome stain with a view to finding as good a staining method as Periodic Acid-Schiff which could obtain better tissue definition.

The intestinal epithelium was examined under the light

microscope for histological alteration of structure. Villi were examined for possible clues to the disintegration of the brush border, loss of epithelial cells and cellular infiltration into the lamina propria, and between epithelial cells. Fields were chosen where villi were exposed in full length.

Observations were made on four groups of animals kept under normal feeding conditions.

(i) From the first group, after the death of the animal (timed from cessation of heart beat) segments of different regions of the intestine were removed and perfused immediately and at varying intervals of 10', 20', 30', 40', 60'.

(ii) From the second group, immediately after death the pyloric region of the stomach was ligated and the rest of the intestine was washed with normal saline. Then at varying time intervals of 10', 20', 30', 40', 60' and 120', segments from different parts of the intestine were separated, the saline was allowed to drain off and the segments then perfused with 10% formol saline.

(iii) From both the above groups (that is, non-washed and washed segments of intestine) the tissues were left in an incubator at 37°C for varying time intervals (30' and 60') and then perfused with formol saline.

(iv) In the fourth group, after thoroughly washing the intestinal segments with normal saline, half of them were perfused with 0.5% Trypsin in normal saline and theother half perfused with equal parts of a-chymotrypsin in acetate

buffer at pH 5.6 and normal saline. At the end of a specified time (30' and 60') these solutions were allowed to drain off and the segments were perfused with 10% formol saline.

From animals (rats only) fasted for 4 days, unwashed segments of intestine were perfused immediately and at varying intervals of 10', 20', 30', 40', 60'. Saline washed segments were perfused at varying intervals, of 10', 20', 30', 40' and 60'; both unwashed and washed segments were left in the incubator at 37°C for 30' and 60' and then perfused with formol saline and examined under the light microscope.

Next our attention was directed towards the study of the effects of occlusion of inflowing (artery) and outflowing (veins and lymphatics) vessels of a loop of intestine. Laparotomy was performed on adult rabbits and rats under Nembutal anaesthesia. Artery, vein and lymphatics to the mid-jejunal segment of small intestine were isolated and occluded separately in different animals. The vessels were clamped with curl clips for 45' and then allowed 60' for recovery of circulation. At the end of the time interval, the tissues were sampled for examination under light microscope.

Finally, the effects of sustained intestinal contraction (to overcome induced obstruction to the lumen of the intestine) on the epithelium were studied. Laparotomy was performed on adult rabbits (2) under Nembutal anaesthesia supplemented with general ether. A

segment of jejunum was ligated with a thin tape (0.5 cm. wide) with meticulous care not to occlude any vessels of the mesentery. The incision was closed in layers and the animal was returned to the cage. The animals were sacrificed after 24 hours and the specimens were obtained from the intestine proximal to the ligated loop, from the loop itself and from the intestine distal to the ligated loop.

Observations.

1) In immediately fixed segments the cytology was preserved in all segments of the intestine.

2) Unwashed segments when perfused with formol saline at varying time intervals showed a gradient along the alimentary tract in the time of onset of shedding of epithelium, viz., earliest in the duodenum and next in the jejunum and ileum; most resistant was the epithelium of the large intestine. This was observed also by Fell (1961). Shedding of the epithelium was limited to the tips of the villi. Following delay in fixation for 10 minutes, shedding of the epithelium of the tips of the villi of the duodenum is more obvious in sheep, less marked in rabbits and guinea pigs and least marked in rats and mice (Figs. 22,23,24).

3) Following simple washing off of the intestinal contents immediately after death, remarkable preservation of the intestinal epithelium was observed (Figs. 25,26). Even after three hours there was no gross damage to the villi of the duodenum, although they looked stunted (Fig. 27). This procedure delayed further the onset of

damage to the epithelium of jejunum, ileum and colon. As the delay time was increased, the evacuated saline from the segments (before perfusion with formalin) was most thick in the duodenum; less so in jejunum and ileum and least in the colon. This phenomenon was more marked in sheep than in other animals.

4) After being kept in an incubator, the tissues were more fragile, this property being more marked in tissues from the small intestine than from the colon. After the specified time (30' and 60') more damage to the epithelium was noted in incubated segments than in non-incubated segments (both unwashed and saline washed groups).

5) Where ever intestinal contents were not completely removed (i.e. residual intestinal material noted by the light microscope) the villi of the adjacent area showed more dissolution of their tips (Fig. 28). The degree of this dissolution was less marked in washed segments, but enhanced after incubation.

6) After washing the segments (that is virtually removing the enzymes) treatment with trypsin or one of its degradation products (a-chymotrypsin) reproduced results similar to those of delayed fixation. (Fig. 29).

7) There was no significant difference in the appearance of the intestinal epithelium in rats 4 days fasting from that in rats with normal access to food. (Figs. 30,31,32).

8) The earliest histological appearance was more suggestive of dissolution of epithelium than of disruption, contrary to the observation of earlier workers. The

dissolution of cellular architecture observed at the tips of the intact villi suggest that this was an event preliminary to the actual shedding off of the epithelium. At first the refractility of the brush border disappears, then the brush border breaks up and becomes detached from the villi. This detachment first occurs from the intermediate area between the goblet cells at the subapical region of the villi. The definition of the cells at the tips of villi disappears next: the cytoplasm of the cells becomes confluent and vacuoles appear in this homogeneous mass. Finally the lamina propria herniates and the axial lumen communicates with the exterior.

9) The Periodic Acid-Schiff stain gave more information in the rats and mice than in the other animals where the mucin was too thin to take up the Masson Trichrome stain, although the bluish background of the Masson stain enabled us to bring to light very early changes of autolysis before the breaking-off of the tips of the villi.

10) When the artery to one segment of intestine was occluded, there was relatively less damage to the epithelium as compared with occlusion of both vein and lymphatic vessels. The damage to the epithelium caused by ischaemia for 45 minutes (by occlusion of the mesenteric artery and intramural vessels) was insignificant compared to the autolytic changes during an identical time interval.

Occlusion of the outflow vessels (veins and lymphatics) caused greater damage to the epithelium; there was more dilatation of the central lacteal, and more extravasation

of blood into the lamina propria. This damage was more marked when a small tributary was occluded than after occlusion of the main vein to the segment of intestine. Invasion of mononuclear cells into the epithelium of the mucosa of the villi was more marked after occlusion of vein and lymphatics than after occlusion of the artery. The changes here were not limited to the tips of the villi, contrary to observations in post mortem autolysis.

11) Following obstruction to the lumen of the intestine, the sustained, vigorous intestinal contraction of the segment of intestine proximal to the ligation caused damage to the epithelium. Here the picture was entirely different. The damage extended over the entire breadth and length of the villi as opposed to the changes due to delayed fixation which was limited to the tips of the villi and most marked in the region of the brush border.

Discussion.

Following the death of an animal the enzymes of the different systems of the body behave differently. The enzymes of respiration become inactive, whereas the action of hydrolytic enzymes persists at a more rapid rate and breaks down the tissue proteins to amino-acids and ammonium salts. These protein-splitting enzymes are called cathepsins: pH 6 is optimal for the hydrolytic action of the cathepsins. (Fruton and Simonds, 1958). Following death of the tissue, the pH of intestinal juice becomes slightly acidic, and this favours the autolytic action of the intracellular enzymes (cathepsins). Immediate fixation of tissue with formalin or alcohol denatures and

inactivates the cell proteins, including enzymes, and this prevents or arrests their autolytic action. Thus when early fixation of intestinal tissue is possible, the intracellular enzymes cannot damage the epithelium. (Very little is known about the physiological role of intracellular proteolytic enzymes of living cells).

Washing the intestinal lumen with saline reduces the enzymatic concentration of the intestinal content. This process alsoreduces contact between enzyme and substrate, dilutes the activators of enzyme (organic or inorganic ions) and diminishes the chemical combination between enzymes and their inhibitor substances. It also alters the pH optimum of enzyme action. In our experiment investigating the pH of intestinal juice by a crude method (pH paper) suggested that it becomes acidic one hour after death. Thus washing the intestinal lumen with saline delays the cell dissolution of intestinal epithelium by diminishing the potency of enzymatic action. This has been confirmed in all experimental animals - mice, rats, guinea pigs, rabbits and sheep. This observation (that is, preservation of intestinal epithelium following washing out of intestinal contents) casts doubt on the existing belief that ischaemia is the main cause of the shedding of intestinal epithelium.

Increased intestinal cell shedding has been reported after fracture of the femur in rabbits and rats (Cuthbertson, 1960). This can be argued to be due partly to increased concentration of proteolytic enzyme in blood following fracture of bone. Stern et al (1949) have reported

elevated peptidase activity after fracture of bone, which does not reach normal until the nitrogen balance becomes positive. Patients who have suffered a more severe and widespread injury to bone had a proportionate rise in peptidase activity. Moreover, following fracture of bone, increased corticotrophin in the blood could raise the blood peptidase level further. In the intestine of mammals, peptidases (Aminopeptidase, Prolidase, Tripeptidase and Dipeptidase) are the main proteolytic enzymes present in the lumen. These peptides and free amino-acids are absorbed into the blood from the intestinal tract. There is widespread distribution of peptidase in intestinal mucosa and in lymph, lymphocytes and polymorphonuclear cells invading the tissue (Annison, 1956). It may therefore be suggested that damage of the epithelial cells of the intestine associated with fracture of the femur was due to an increased concentration of intestinal proteolytic enzymes which cause damage to the epithelium, as a result of reduced blood supply to the gut during the phase of shock. The gastro-intestinal system is never digested by its own enzymes because of anti-enzymes produced as a result of repeated absorption of enzyme into the circulation. Ischaemia of the bowel reduces this barrier against enzyme action, by diminishing the release of enzyme inhibitor substance.

Mitosis is most active in the crypts of Lieberkühn from where the cells of the intestinal epithelium originate. These cells are gradually pushed upwards along the sides of the villi in an orderly fashion to become

the covering epithelium. The epithelium of the basal region of the villi consists of younger cells while older cells are seen as the epithelium is traced from base to apex of the villi, the dying cells being pushed to the tip. (Leblond and Stevens, 1948). Thus there is a slow but massive motion of the whole of the intestinal epithelium moving upwards to meet the inevitable process of ageing and disintegration. Autolytic enzymes seem to augment this process of disintegration following death. The younger epithelial cells towards the base of the villi can resist the autolytic enzymes better than the older cells at the tip, and thus the enzymatic effects are more marked at the tips than over the bases of the villi. The production of intestinal ischaemia adds further insult to those older cells at the tips of the villi and further lowers the resistance threshold to enzymatic action.

Dissolution of cells at the tip of the villi has been thought eroneously to be due to artefacts produced by physiological extrusion of cells or by anoxic damage in the period after death and before fixation. Exaggeration of these artefacts has been produced by feeding. (Fisher and Parson, 1949; Leblond and Stevens, 1948). The damaged tissue in thepresent study shows little similarity with cells that were extruded from the tips of the villi physiologically. Under physiological circumstances, after extrusion of epithelial cells at the villus tip, the basement membrane remains intact, whereas in the process of cell dissolution there was disruption of basement membrane as well. (Boyne et al, 1956). Changes due to

cell dissolution have been observed as an event preliminary to the actual shedding of epithelium. This observation was made possible because of the use of the Masson Trichrome stain. The bluish background of this stain brings these earlier changes to light, a distinct advantage over the yellow background of the Periodic Acid-Schiff stain used by earlier workers.

It is interesting to note that living animal tissues are more resistant than dead tissues to digestion by proteinase. Trypsin dissolves dead human tissue but is far less harmful to living tissue when it accumulates in certain diseases. In our experiment we have been able to reproduce the effects of delayed fixation by perfusing saline washed segments of intestine with a weak solution of trypsin or a-chymotrypsin with physiological saline before fixing the tissue with formalin.

The proteolytic activity of the ruminal content which increases by 5-10 fold immediately after feeding was recognised by Pearson and Smith (1943). This could be an explanation for the increased epithelial cell shedding in rabbits fed before death compared to the fasted controls (Fell, 1961). We have also noticed in our experiments that whenever intestinal contents were not completely removed from the lumen of the intestine, the villi of the adjacent epithelium showed dissolution of the tips. The destruction was proportional to the activity of the enzyme.

We are not convinced by earlier observations of Leblond and Stevens (1948) suggesting that there was very

little enzymatic activity of the intestinal juice of rats starved for four days. In our experiments, intestinal secretion was much less abundant in these rats which provided the cells extruded physiologically into the lumen of the intestine, but qualitatively there was no difference in the enzymatic activity of the intestinal juice of rats on normal diet and rats starved for four days, as judged by the almost identical picture of dissolution of tips of villi under delayed fixation. The enzymatic activity (i.e. effect on epithelium of these rats) was reduced by washing the intestinal contents and enhanced by increasing the atmospheric temperature before fixation with formol saline.

In the alimentary tract considerable addition of nitrogen to chyme occurs in the most proximal part of the small intestine. By using angiotomic methods on dogs, it was found that the proteolytic enzyme content of portal vein blood was three times greater than that of blood from the jugular vein (Boyne et al, 1956). It has been suggested that absorption of these substances from the gut is essentially a process of diffusion facilitated by a diffusion gradient; naturally, more diffusion occurs when the concentration of the solute in the intestinal content is greater with respect to the epithelial cell. Recent studies with isotopically labelled substances make it clear that abserption is not a simple physio-chemical process of diffusion. Movements of molecules and ions can take place between gut and blood against a concentration gradient, and this active process requires energy for its performance

(Bell et al, 1961). Soon after death, no more energy is provided to the tissue for the maintenance of process of diffusion, and thus the intestinal epithelium faces a static concentration of enzymes and their products of digestion.

These enzymes are carried by theportal circulation to the liver and other tissues to be used in the synthesis of tissue proteins. Analysis of the surface area of pigeon's intestine showed that the surface area of villi per square millimetre of duodenum is twice that of the jejunum (mean of 1st, 2nd and 3rd part) and six times that of the ileum. (Verzar and McDougall, 1936). If the draining vessels from the area of maximum absorption (i.e. veins and lymphatics) are occluded and hamper reabsorption of proteolytic enzymes, thus could cause increased injury tothe epithelium. It can be argued that cessation of activity of the draining vessels of the gut by death would predispose a unit area of duodenum to three times more concentration of proteolytic enzymes than in the jejunum or ileum. This accounts for the early onset of dissolution of cells of duodenal epithelium and this could be further exaggerated by feeding.

In neither vascular occlusion nor sustained vigorous intestinal contraction to overcome obstruction to the lumen, was the damage to the epithelium limited to the tips of the villi, which is a constant feature in the earliest stages of post mortem autolysis. It is not clear why certain villi showed more change than others in the same section; changes of autolysis were more frequently seen in tall villi

with a narrow base than in shorter villi with a broad base. The changes in the apical region of the tall villi were often associated with twists or kinks in their long axis.

The conclusions of this work might be summarised as follows:

1) Integrity of intestinal epithelium depends on a balance between the blood supply to the bowel and the enzymatic potentiality of the intestinal contents. Dimunition of blood supply to the gut makes the epithelium more vulnerable to the action of intestinal enzymes.

2) In the presence of total ischaemia of the bowel, if the intestinal enzyme action is diminished by washing the intestinal contents with saline after removing the segment of intestine from the body, the epithelium can be preserved for a longer period.

3) The enzymatic activity of the intestinal juice does not alter qualitatively after even 4 days fasting (with access to water only).

4) Differential vascular occlusion suggests that obstruction to the removal of intestinal metabolites causes far more damage to epithelium than isolated ischaemia to the bowel. I can therefore suggest that if the intestinal contents can be washed away by an effective method, the mucosa of an ischaemic loop of intestine can resist the insult longer than otherwise.

REFERENCES.

1. Alvarez, W.C. (1948). A n introduction to Gastroenterology, 4th Ed., chapter V. William Heinemann Medical Books Ltd., London.

2. Annison, R.F. (1956). Biochem. J., 64, 705.

3. Bell, George H., Davidson, J.N., Scarborough, H. (1961). Text Book of Physiology and Biochemistry. 5th Ed. E. & S. Livingstone, Edinburgh: London.

4. Boyne, A.W., Campbell, Rosa M., Cuthbertson, D.P. (1956). Brit. J. Nutr., 10, 325.

5. Fell, B.F. (1961). J. Path. Bact., 81, 251.

6. Fisher, R.B., Parson, D.S. (1949). J. Physiol., 110, 36.

7. Fruton, Joseph S., Simmonds, Sofia. (1958). General Biochemistry, 2nd Ed. John Wiley & Sons Inc., New York.

8. Leblond, C.P. and Stevens, C.E. (1948). Anat. Rec., 100, 357.

9. Leblond, C.P. and Stevens, C.E. (1947). Anat. Rec. Suppl., 97, 373.

10. Pearson, R.M., Smith, J.A.B. (1943). Biochem. J., 37, 153.

11. Stern, K., Cullen, A.M., Barber, V.T. (1949). J. clin. Invest., 28, 419.

12. Verzar, F. and McDougall, E.J. (1936). Absorption from the intestine. Longmans, Green & Co., London.

13. Cuthbertson, D.P. (1960). Biochemical response to injury, edited by H.B. Stoner, p. 216. Blackwell Scientific Publications, Oxford.

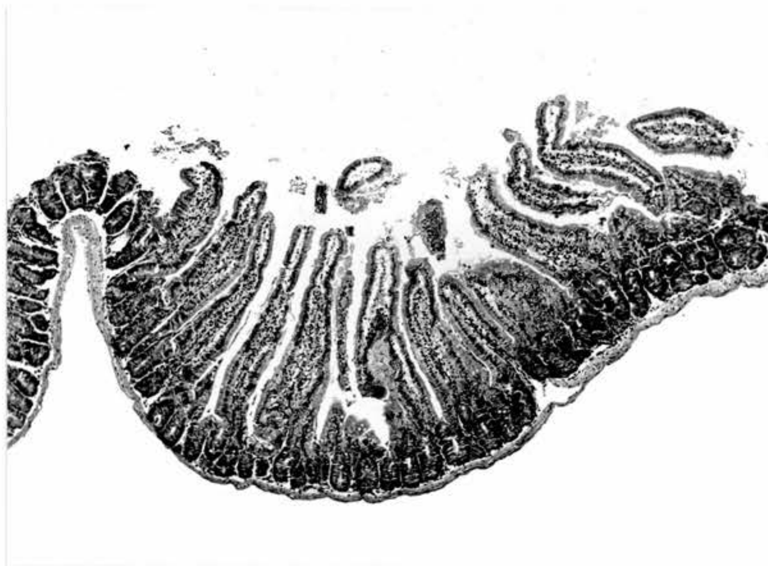


Fig. 22. Jejunum of mouse. Fixed by perfusion with formol saline 20 mins. after the death of the animal.
H & E x 60

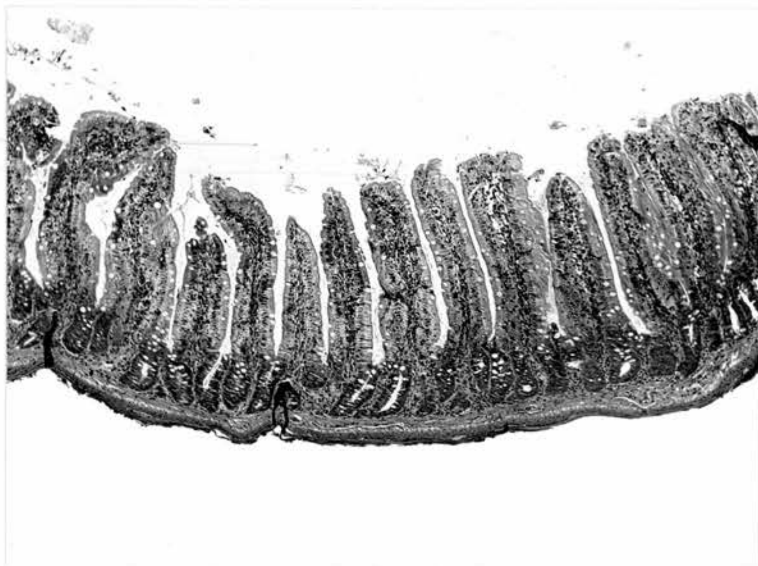


Fig. 23. Jejunum of rat. Fixed by perfusion 20 mins. after the death of the animal. H & E x 60

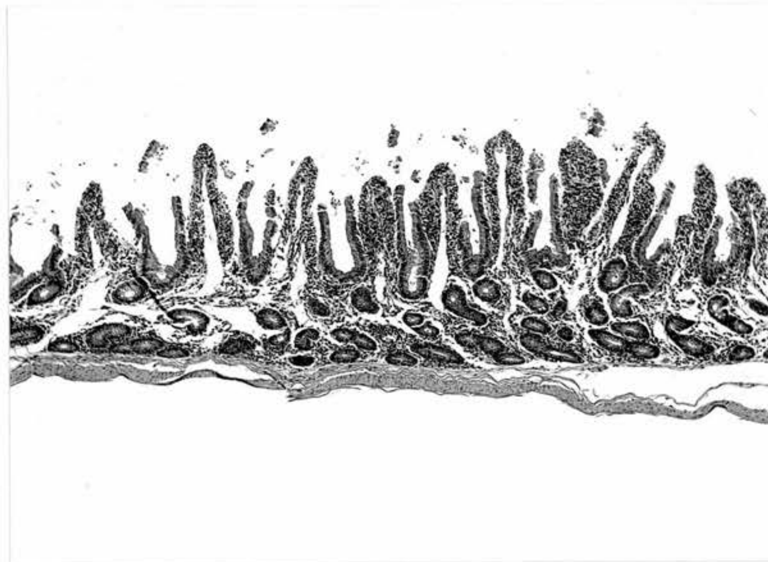


Fig. 24. Jejunum of sheep. Fixed by perfusion 15 mins. after the death of the animal. Note the autolytic changes more marked in sheep than in mouse or rat.
H & E x 60

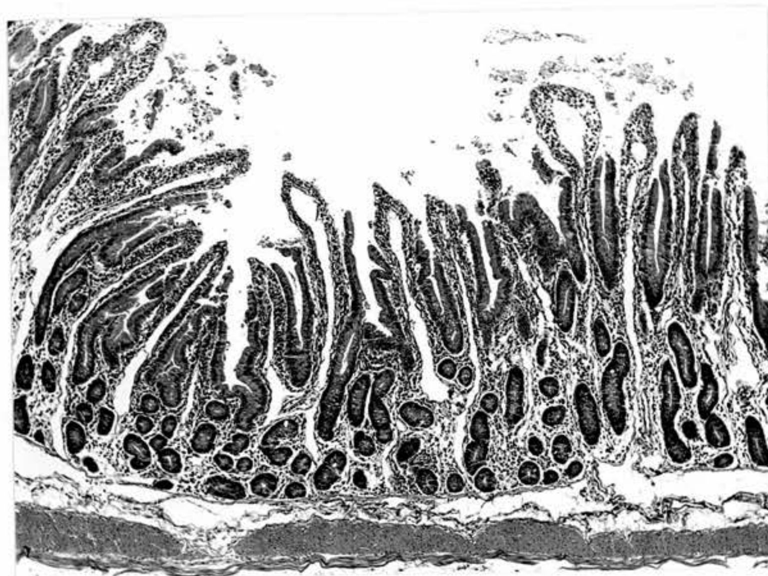


Fig. 25. Sheep duodenum. Fixed by perfusion 20 mins. after the death of the animal. Note marked destruction of the epithelium. H & E x 60

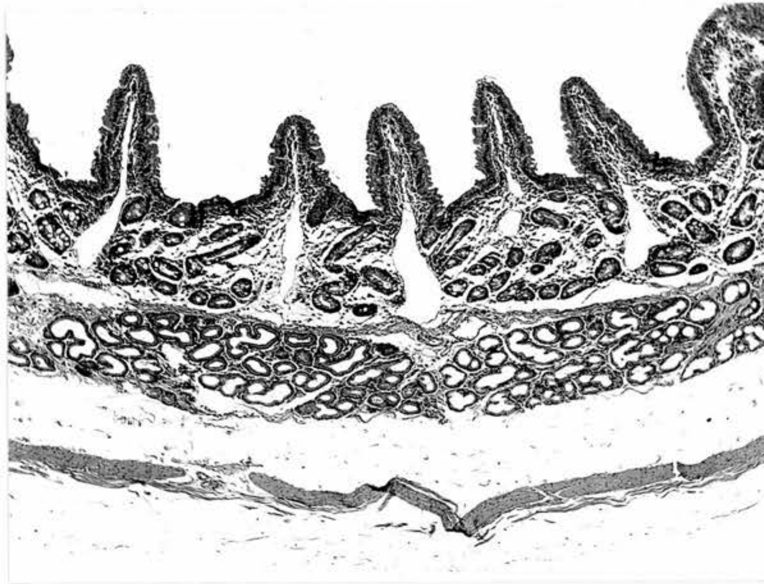


Fig. 26. Sheep duodenum washed with normal saline immediately after death and perfused with formol saline 120 mins. after death. Note the preservation of mucosa. H & E x 60

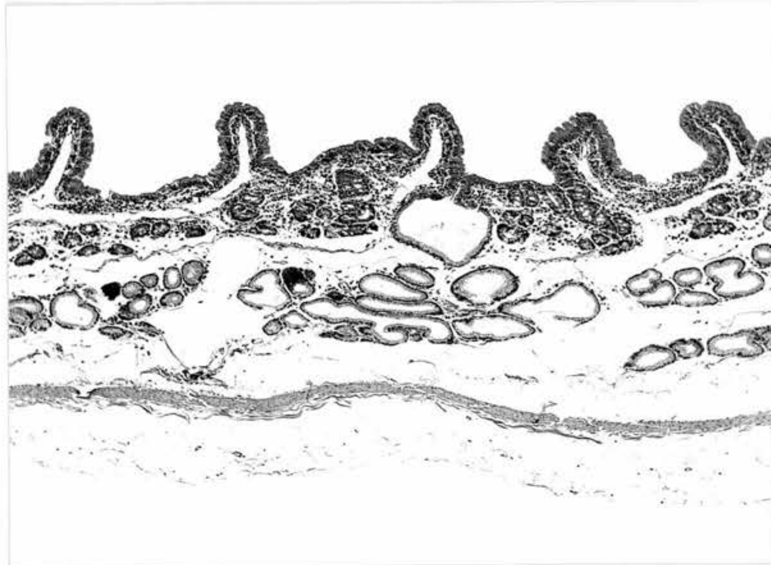


Fig. 27. Sheep duodenum treated as mentioned in figure 26 but perfusion fixation was delayed for 180 mins. after death. Note remarkable preservation of mucosa. H & E x 60

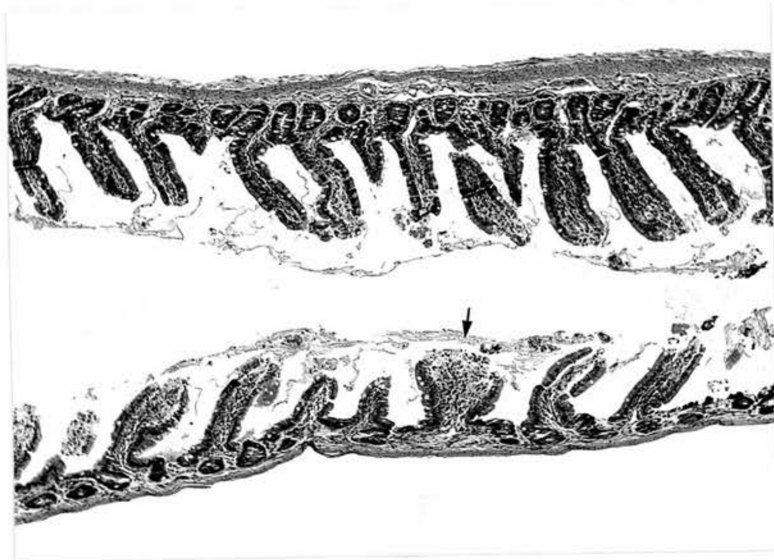


Fig. 28. Rat fasted 4 days. Jejunum fixed 30 mins. after death. Note wherever intestinal content (arrow) is left in contact with the mucosa there is destruction of the epithelium. H & E x 60

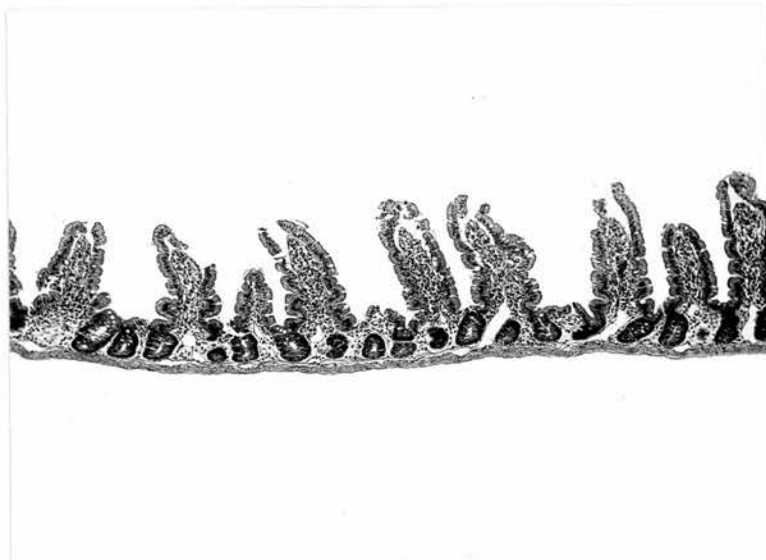


Fig. 29. Non-fasting rat jejunum. The segment was perfused with 5% Trypsin in normal saline and left at room temperature for 60 mins. before being perfused with formol saline. Note that the effect of Trypsin is identical with that of intestinal contents. H & E x 60

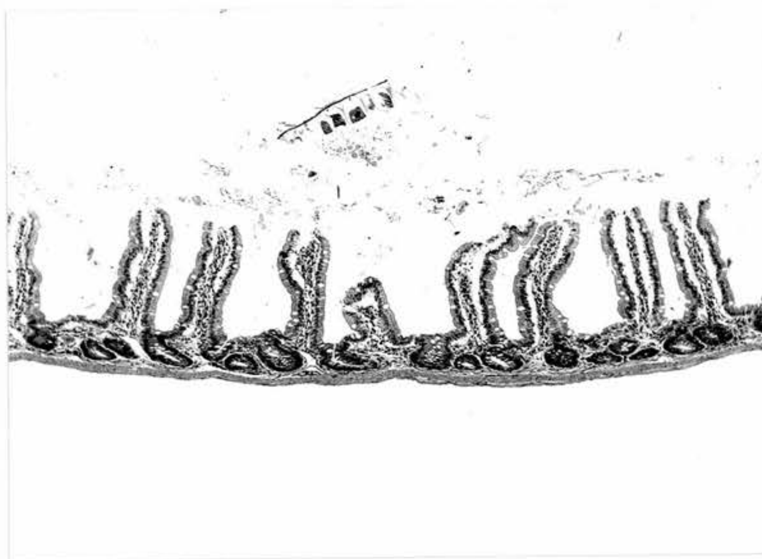


Fig. 30. Rat with normal access to food and water. Jejunum fixed by perfusion 60 mins. after death. H & E x 60

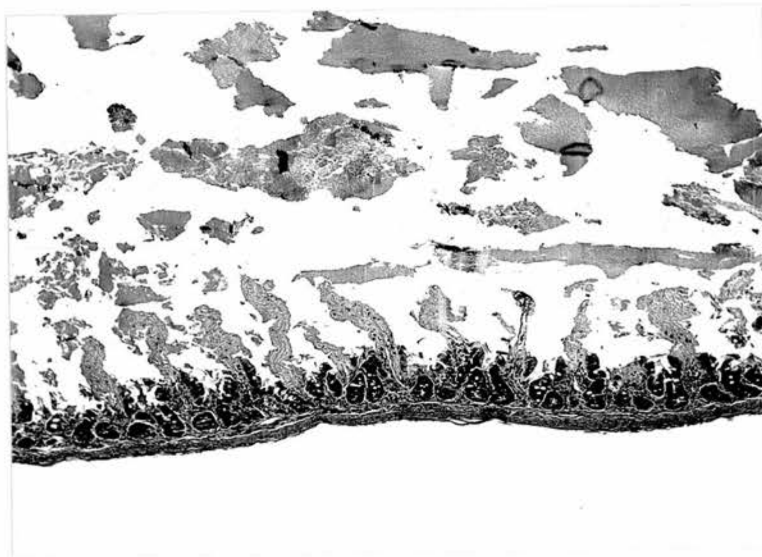


Fig. 31. Rat fasted for 4 days (allowed access to water only). Jejunum similarly fixed by perfusion 60 mins. after death. Note the autolytic effect of the intestinal content of fasting animal. H & E x 60

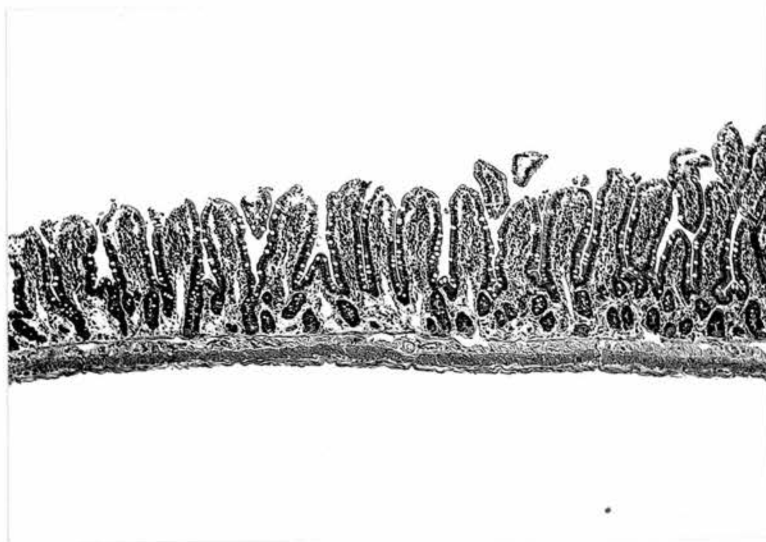


Fig. 32. Rat fasted for 4 days. Jejunum washed with saline immediately after death and perfused with formol saline 60 mins. afterwards. Note the beneficial effect of washing off the intestinal contents.
H & E x 60

CHAPTER 4.

ELECTRON MICROSCOPIC STUDY OF EARLY CHANGES DUE TO AUTOLYSIS IN THE INTESTINAL EPITHELIAL CELL.

The study of the structural alterations which accompany death of the cell is one of the fundamental studies of cellular pathology. These changes are more marked in the gastro-intestinal tract because its mucosa is vulnerable to the actions of digestive juices after death. The light microscopic study of this pathological phenomenon has received considerable attention in recent years, (Alvarez, 1948; Cuthbertson, 1960; Fell, 1961), but the study under the electron microscope has received only scanty attention. (Hartman et al., 1959). With modern techniques of obtaining intestinal mucosa by the peroral method (Crosby and Kugler, 1957) it is now possible to examine the small intestinal mucosa during life but there is often a delay in getting out the biopsy capsule or in dislodging the specimen from it. Thus there is a possibility of autolytic changes occurring before the specimen is put into the fixative. Such changes could be confused with those of diseases which affect the small intestinal mucosa. For this reason, the present chapter will deal with the electron microscopic study of the very early autolytic changes, within the absorptive cells of the small intestine; so that they may be recognised when observations are being made for the purpose of diagnosing disease processes.

Materials and Methods.

A strip of jejunum from a rabbit was dissected out when the animal was under anaesthesia. The segment was washed

with normal saline and left on a piece of gauze (soaked with normal saline) for 60 minutes at room temperature. From time to time a few drops of normal saline were put on the mucosa to keep it moist. At the end of this time specimens were obtained from the central part of this strip for light and electron microscopic examinations. (The details of the procedure are described in Part I, chapter 2). Araldite was used as the embedding material for electron microscopy.

Observations.

The light microscopic examination of this mucosa showed it to be normal.

Under the electron microscope, the microvilli which form the free surface of the intestinal epithelial cell were found to be constantly affected by the autolytic process. The upper half of the microvilli exhibited a curious fusiform dilatation. (Fig. 34). In places the tip of a microvilli was blown out into the shape of a bubble. (Fig. 35). Some microvilli were found to be affected over their entire length. Irregular spaces appeared in the filamentous core in the affected region of the microvillus and made it more electron translucent. The terminal web was well preserved. The normal homogeneous translucency of the cell was lost and there appeared irregular vacuoles in the cytoplasm. The ground substance was not conspicuous in most of the cells but in a few places it was found to be aggregated into irregular masses. The cisterns of the endoplasmic reticulum appeared irregular and swollen. The Golgi complex

appeared normal. (Figs. 36,37,38). The mitochondria appeared more electron opaque and their internal structure was found to be completely disorganised. The outer and inner nuclear membranes were found to be fused, thus obliterating the space between them. The nuclear substance which is normally uniformly granular showed a marginal aggregation of dense chromatin material (Fig. 39). The subepithelial basement membrane and the endothelium of the blood vessels were not affected at this stage of delayed fixation. (Fig. 40).

Discussion.

The curious affection of the upper halves of the microvilli is an important point to distinguish early autolytic changes from fusion of microvilli in primary malabsorptive disease (Part II, sec. B; Chapter 8). In this condition the tip of the microvilli are often curiously spared and the microvilli are mostly fused over the lower two thirds of their length. The terminal web is also affected early by this disease process but it is spared in the early stages of autolysis.

The cells of the different organs of the body vary so much in their structure function and their ability to withstand delayed fixation, that it is difficult to generalise criteria for good fixation. However, Pease (1960) has put forward some guiding points as criteria of good fixation. The nucleus and mitochondria of the cell are sensitive indicators which are affected early in the autolytic process. In an adequately fixed

specimen the nuclear substance is uniformly granular and is bounded by a double membrane. This space between the nuclear membranes is continuous with the cisterns of the endoplasmic reticulum. Due to delay in fixation, as pointed out by Pease (1960) the nuclear membranes fuse together obliterating the space between them and at the same time the homogeneous nuclear structure breaks up into aggregates of chromatin material.

Similarly the mitochondria swell up due to delay in fixation and vacuoles appear in the mitochondrial substance,which observation was also noted by Hartman and coworkers (1959).

The morphological beauty of the cytoplasm is dependent on effective fixation. Delay in fixation makes the cytoplasm look as if the ground substance had been finely precipitated. Hartman and coworkers (1960) noted small vesicles in the endoplasmic reticulum when fixation was delayed. These were also noted in the present work. These vacuoles in the cytoplasm should not be confused with shrinkage artefacts (which create artificial spaces) or spaces formed by tearing apart of the cell components (Pease, 1960). Artefacts can also be induced by polymerisation effects of the embedding material (methacrylate). In the present work these possibilities were kept in mind when observations were made.

Summary.

Early changes of autolysis in the intestinal epithelial cell were studied with the electron microscope.

An isolated piece of rabbit jejunum was left in a moist environment at room temperature and studied after 60 minutes. Notable changes were the fusiform dilatation of the upper half of the microvilli, the appearance of vacuoles in the cytoplasm, the increased electron opacity of the mitochondria and a distortion of their internal architecture. These features in the supranuclear cytoplasm are essentials to be remembered in differentiating cell autolysis from the changes due to primary malabsorptive and other diseases, which affect the epithelial cells of the upper intestine.

REFERENCES.

1. Alvarez, W.C. (1948). In An introduction to Gastro-enterology. 4th Ed., Wm. Heinemann, London.

2. Crosby, W.H., Kugler, H.W. (1957). Amer. J. digest. Dis., 2, 236.

3. Cuthbertson, D.P. (1960). In Biochemical response to injury, edited by H.B. Stoner, Blackwell Scientific Publications, Oxford.

4. Fell, B.F. (1961). J. Path. Bact., 81, 251.

5. Hartman, R.E., Smith, R.B.W., Hartman, R.S., Butterworth, C.E.Jr., and Molesworth, J.M. (1959). J. biophys. biochem. Cytol., 5, 171.

6. Pease, D.C. (1960). Histochemical techniques for Electron Microscopy. Academic Press, New York, London.

Fig. 33. E.M. of apical cytoplasm of rabbit jejunum. The microvilli are of regular shape. x 28,800

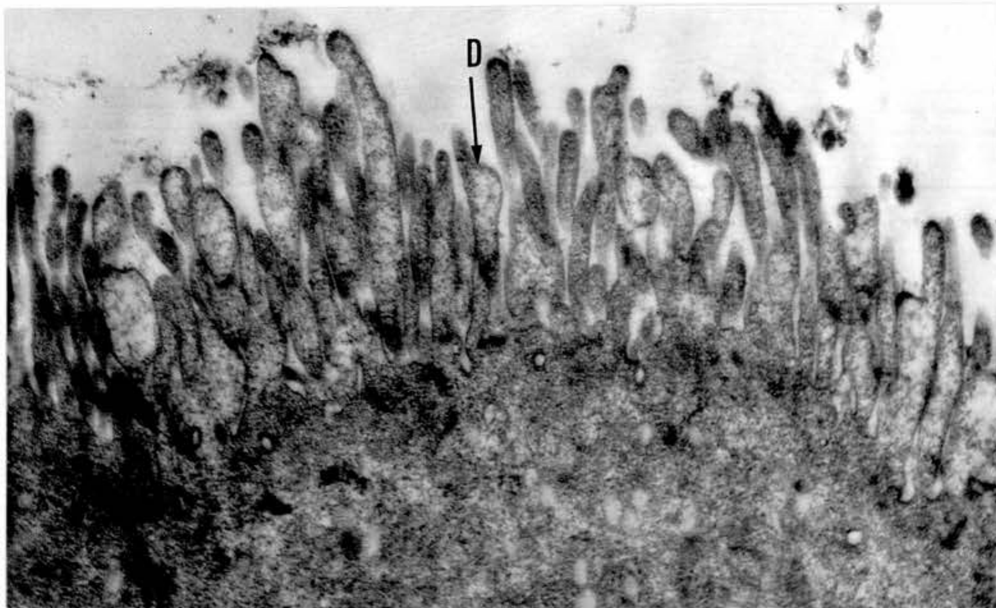


Fig. 34. Electron micrograph of the epithelium from the upper half of a villus from rabbit jejunum washed with saline and left unfixed for 60 mins. Note fusiform dilatation (D) of microvilli. x 40,000

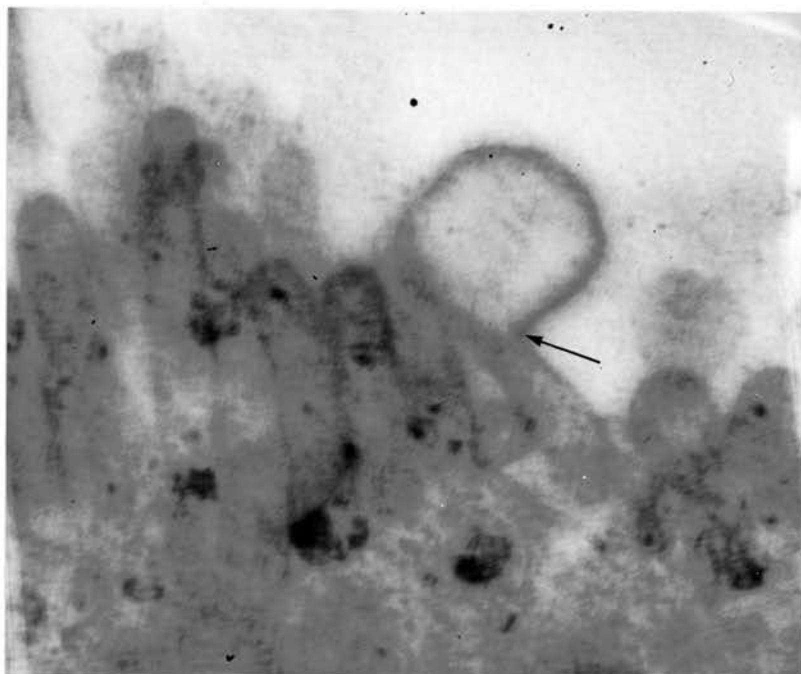


Fig. 35. Electron micrograph. Note saccular dilatation of the tip of a microvillus (arrow) due to delay in fixation. Dark particles in the cytoplasm are manganese precipitated from the staining solution. x 82,000

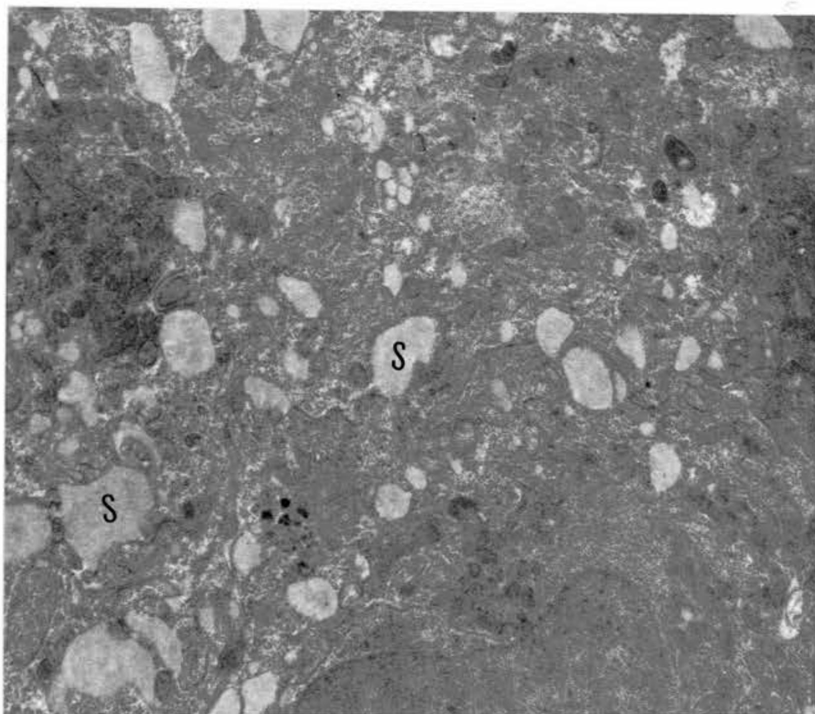


Fig. 36. E.M. of epithelial cell showing irregular spaces (S) in the cytoplasm. Mitochondria are less distinct. x 13,600

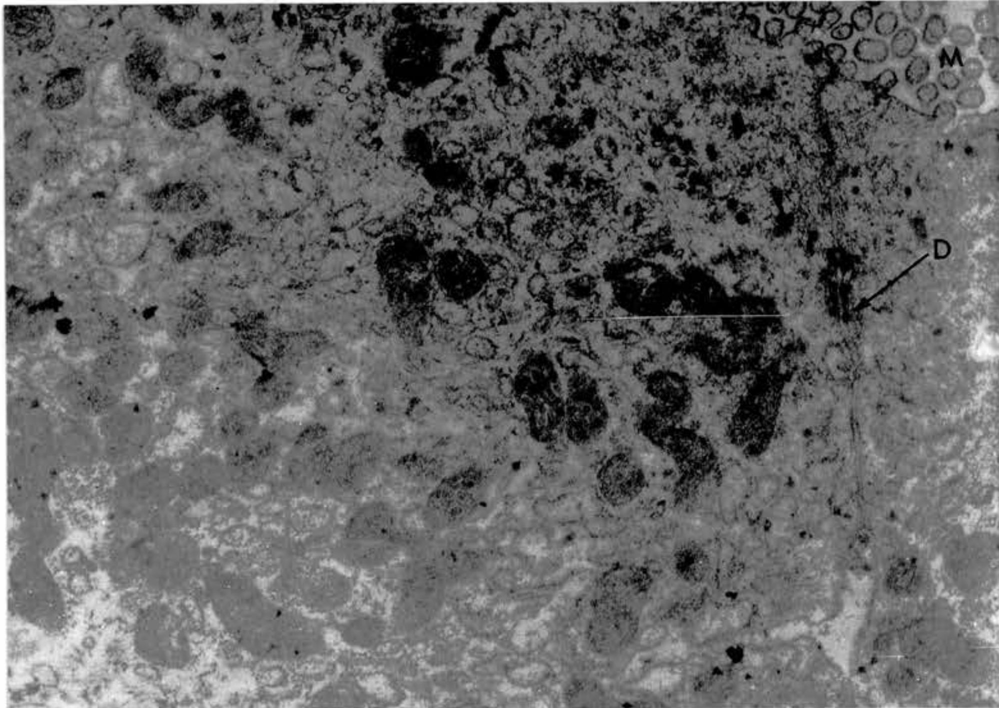


Fig. 37. E.M. of supranuclear cytoplasm of an absorbing epithelial cell. M = transverse section of microvilli; D = desmosome binding the adjacent lateral cell walls. Mitochondria are irregular and more electron opaque. x 35,200

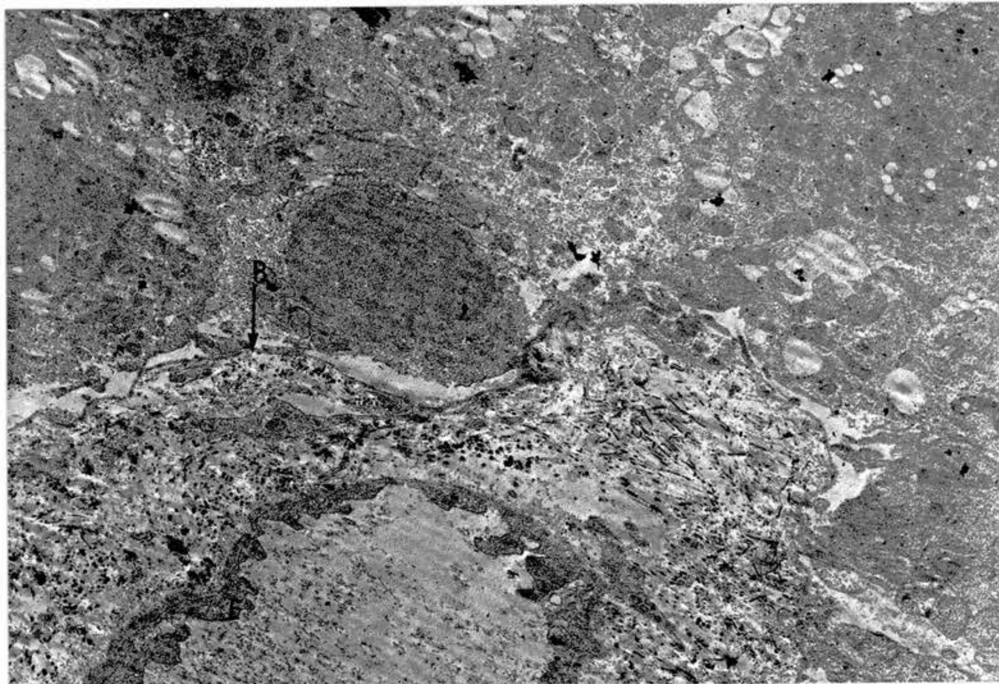


Fig. 38. Basal part of epithelial cell showing vacuoles in the cytoplasm. B = subepithelial basement membrane; E = endothelium of a capillary in the lamina propria. x 10,200

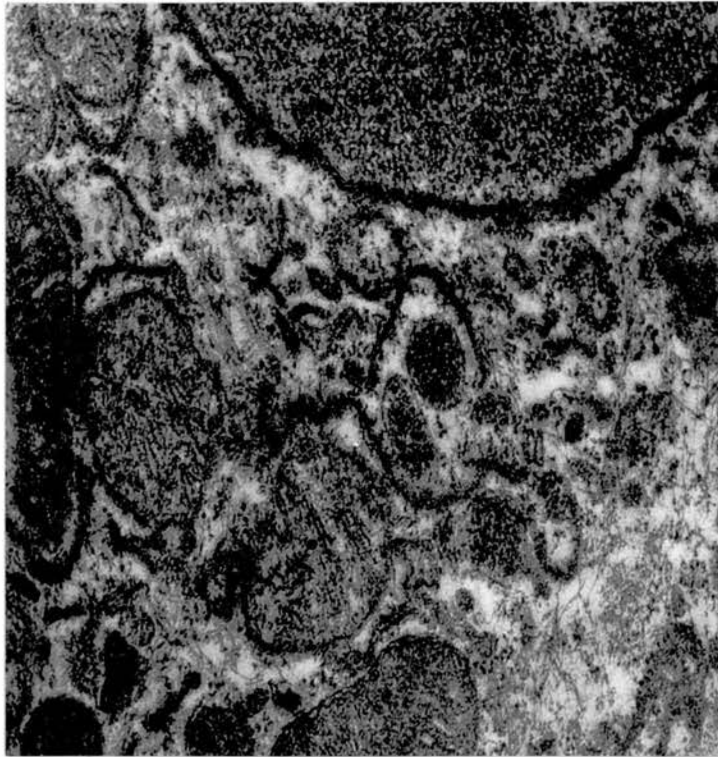


Fig. 39. E.M. showing the lower region of the nucleus and infranuclear mitochondria. The cristae of these mitochondria are better preserved than mitochondria of supranuclear region (shown in fig. 37). x 49,600

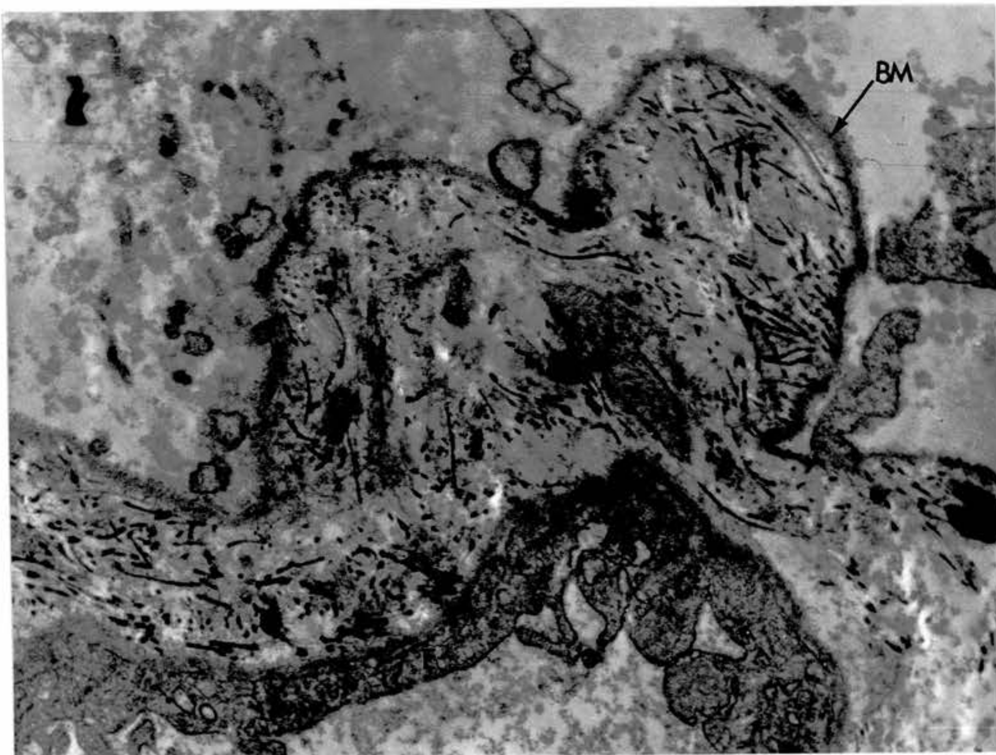


Fig. 40. E.M. showing well preserved subepithelial basement membrane (BM) and endothelium of capillary (E) at this stage of delayed fixation. x 22,000

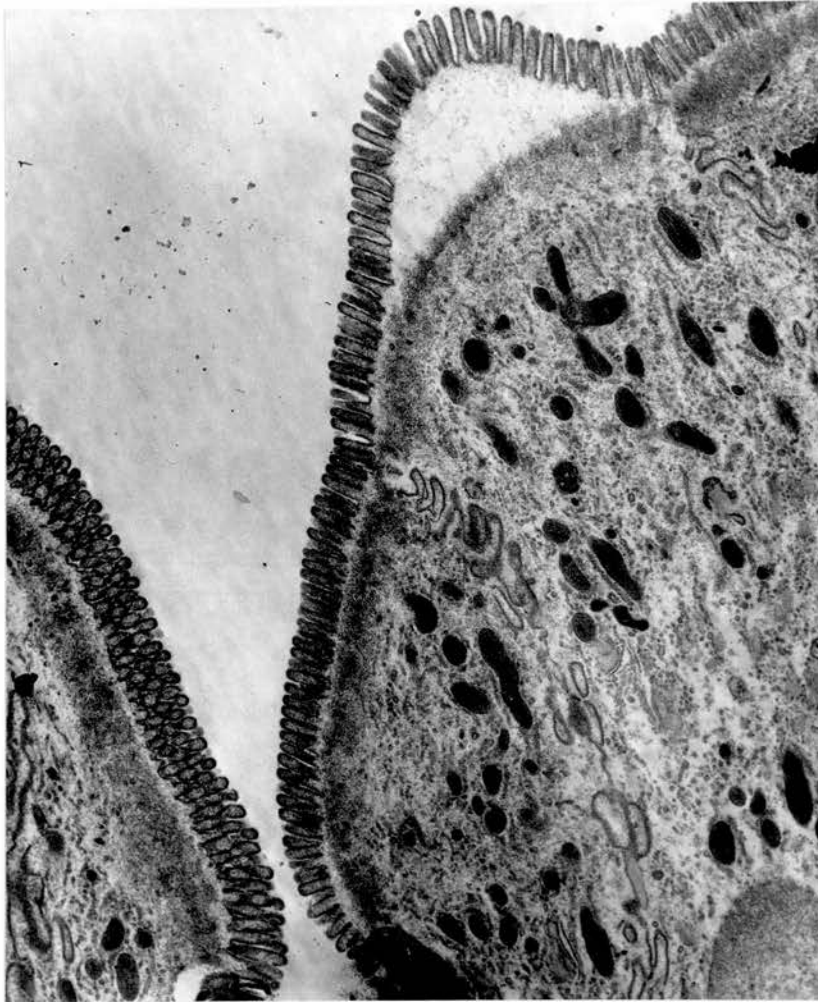


Fig. 41. E.M. showing effect of inadequate dehydration on the epithelial cell. The microvilli are detached from the rest of the cell.
x 6,400

CHAPTER 5.

EFFECT OF PROTEIN DEFICIENT STATE ON THE MUCOSA OF PROXIMAL SMALL INTESTINE OF RAT.

Protein is one of the most valuable nutrients in the diet. It is mainly absorbed from the proximal part of the small intestine. Strangely enough only very scattered reports have appeared describing alterations in the mucosa of the small intestine in the protein malnutrition state. We must now consider the problems of how much protein deficiency affects the cells of the small intestine.

Although much attention has been given to the essential pathological changes in Kwashiorkor, they are far from complete so far as intestinal pathology is concerned; very little study of the intestinal mucosa has been carried out under the dissecting and electron microscopes. This pathological state is largely limited to those parts of the world where facilities for such advanced techniques are scanty. In addition, the per oral biopsy technique is more dangerous in protein malnutrition, because of the subtotal atrophy of the small intestine which occurs in this state.

To get a complete intestinal picture of the protein malnutrition state one has to depend on experimental animals. During the last forty years there have been attempts by various workers in this field to reproduce human disease in the experimental animal. Radnakrishna Rao (1942) produced a lesion in monkeys identical with the human type by feeding them a rice diet similar to that of poor rice eaters inIndia, and studied the histopathological

changes in the gastro-intestinal tract. He noticed atrophic changes in the lower third of the small intestine. Degenerative changes were noted in all the layers of the gut, including the intramural nerve plexus. According to Radnakrishna Rao in the experiment carried out by McCarrison (1921) on monkeys, pigeons and guinea pigs feeding them a grossly ill-balanced diet which induced lesions of an acute and fulminating type. None of the animals lived beyond three months. In man, however, the protein deficient state induces a subacute or chronic type of lesion quite different from the disease produced in monkeys by McCarrison. Ramalingaswami (1964) induced this deficiency state in rhesus monkeys in preference to albino rats because in the latter progress was slow. The lesion in monkeys was very similar to those of Kwashiorkor. There was reduction in height of the villi with sluggish migration of epithelial cells. Electron microscopic examination revealed blunting of the microvilli, sparse mitochondria and ribosomes and less dense protein matrix of the cytoplasm.

The aim of the present work is to compare the appearances of the proximal part of small intestinal mucosa of the rat in normal and protein-deficient states. The mucosa was examined under the dissecting, light and electron microscopes, and the examination confirms a generalised atrophy of the intestine at all these magnifications.

Materials and Methods.

Adult male Wistar-strain albino rats were used for the experiment. The animals weighed between 260-320 grams

(average 295 grams), were put in groups of six into metabolic cages. No precaution was taken against coprophagy because the amount of faeces recovered daily seemed to be too small to affect the investigation. The rats were weighed and examined weekly. Those showing signs of infection were excluded from the investigation.

The rats were divided into two groups. Half of them were fed on an adequate diet of synthetic protein and the other half were given a synthetic protein free diet (Table 1). Each rat received 20 grams of appropriate diet per day. After about three weeks the protein deficient group consumed only 15 grams per rat per day, while those on the protein adequate diet continued to consume 20 grams per rat per day. The protein deficient animals lost weight continuously (about 75 grams per rat in eight weeks) while rats on a protein adequate diet gained weight steadily (55 grams per rat in eight weeks).

After eight weeks, rats from both groups were anaesthetized and laparotomy was performed. Immediately a small strip of mucosa about 2 mm. square was removed from the anti-mesenteric surface of the duodeno-jejunal region of the small intestine for electron microscopic examination. An adjoining area (towards the jejunum) about 5 cms. long was ligated and perfused with 10 per cent formol saline, and this intestinal segment and the pancreas were then removed. The intestinal mucosa (anti-mesenteric segment) was first examined under the dissecting microscope and then processed for light microscopy. The pancreas was processed only for light microscopic examination.

The strip of mucosa for electron microscopic examination was put immediately into 1% isotonic osmium tetroxide solution, and it was then removed on to a piece of cork. The edges of the specimen were trimmed and it was then chopped into small pieces by means of a sharp (new) razor blade. These were again put into the osmium tetroxide solution and left for 1-1½ hours. The tissue was passed through ascending grades of alcohol for dehydration and embedded both in araldite and in methacrylate. From these tissues ultra-thin sections were cut with the aid of glass knives on the Huxley microtome and examined in an A.E.I., E.M.6 electron microscope.

Table 1.

Composition of the Synthetic Diets
(gram per 100 gram of diet)

	Protein-adequate	Protein-Free
Dextrose	48.8	70.8
Casein	22.0	-
Corn Oil	4.0	4.0
Agar	5.0	5.0
* Salt mixture	4.0	4.0
ø Multi-vitamin mixture	2.2	2.2
Water	14.0	14.0

* USP XV 883, 1955.
ø N.B., Co., 1962.

Results.

On opening the abdominal cavity of the rat one finds that it is entirely filled with coils of intestine. More than three-quarters of these coils are small intestine, the proximal part of which is distinct from its distal half, being lighter in colour and having greater vascularity.

The soft consistency of the intestinal contents gives the proximal part a uniform smoothness, whereas the darker lower half of the small intestine is beaded, due to its scybalous content.

In the protein deficient rat the proximal half of the small intestine looked pale and lustreless compared with the normal. There was no distension of the small intestine.

Dissecting Microscopy.

The mucosa of the control rats had no finger-shaped villi over the proximal quarter of the small intestine. The villi were present in parallel ridges which zig-zag (Fig. 42), a finding which Baker and co-workers described in 1963. In places these ridges presented a convoluted appearance instead of straight surface. These ridge-shaped villi have such broad bases that they appear as if they are pressing against each other leaving very little intervillous space. In protein deficient rats the ridges were not prominent; they were very small over some areas and were even absent altogether (Fig. 43). Their height was reduced, the zig-zag pattern was lost, and the gaps between successive rows appeared to be widened.

Light Microscopy.

The villi emerged as outgrowths of the mucous membrane. They were on average 380-480μ in length and 90-110μ in width. (Fig. 44). The epithelium which covered these villi and the glands of Lieberkuhn was made up of columnar epithelial cells, the majority of which were absorptive in nature and were about 30μ in height. A very thin section

of the villi (0.5 - 1.0μ thickness) cut from methacrylate or araldite blocks, when stained with 1% Toluidine Blue and examined under the light microscope (x 320 magnification) revealed further details in the structure of the villi which were not clearly seen in paraffin sections stained with Haematoxylin and Eosin. The free surface of the cells presented a striated appearance. The supranuclear cytoplasm and the cytoplasm below the nuclei contained fine rod shaped bodies which were the mitochondria of the cell. The vacuolated cytoplasm just above the nucleus comprised the Golgi complex. The epithelial cells rest on a dense membrane called the subepithelial basement membrane. It is a mucoprotein film and is P.A.S. positive. This membrane is in turn supported by the reticulin fibres of the lamina propria. It has been found by Leblond and Stevens (1948) that these columnar epithelial cells covering the mucosa originate in the glands of Lieberkuhn and escalate over the subepithelial basement membrane along the sides of the villi. At the tip of the villi there is an extrusion zone from where the older cells are shed and replaced by younger cells.

The mucus secreting goblet cells are distributed among the absorbing epithelial cells. They have the shape of a wine-glass - hence the name. The goblet cells form mucus and expel it over the surface of the adjacent absorbing cells, giving them covering which is protective against the noxious substances in the intestinal lumen.

The lamina propria forms the core of the villi. Its

reticular fibres hold the central lacteal, blood vessels and nerve fibres. In addition to these structures there are lymphocytes, plasma cells and eosinophils found in the core of the villi. Their origins and functions have not been ascertained.

The intestinal glands are known as glands of Lieberkuhn They open to the surface by a crypt which communicates through a pore with the surface of the mucosa at the base of the villi. They are lined by columnar epithelial cells and their epithelium is continuous with that of the villi. The Paneth cells which manufacture intestinal enzymes are situated at the bases of these glands,and these cells are acidophilic.

The muscularis mucosae separates the mucosa of the intestine from the submucosa which holds Meissner's nerve plexus, and blood and lymph vessels. It is not very prominent in the upper part of the small intestine of the rat, but in this region the muscle coat is prominent. It comprises an inner circular and outer longitudinal muscle layer, between which Auerbach's plexus is situated. The external or serosal covering of the intestine is contributed by the peritoneum and connective tissue.

In protein malnutrition rats the intestinal mucosa showed no ulceration. There was a generalised atrophy of the mucosal structures and the muscle coat. The height of the mucosa was reduced to about 300μ (normal 540μ): marked reduction, almost 50%, was noted in the height of the villi, whereas there was comparatively little

reduction in their width (Fig. 45). The striated border of the epithelial cells was well preserved. Their height, although apparently reduced, was in fact within the lower limits of normality. The size and distribution of the goblet cells and the staining character of the mucin was unchanged. The glands were smaller in size and the glandular epithelium was reduced in height to 50-60μ compared with the normal of about 90-120μ. The cellularity of the lamina propria was slightly reduced. It is difficult to know how much this reduction in the cellular infiltration was secondary to the reduction in size of the stroma of the villi. Nowhere was there evidence of inflammation of the mucosa. There was no evidence of increased mitotic activity compared by reduced villous height as seen in coeliac spure. The muscularis mucosae was reduced in thickness and was discontinuous in places. There is no oedema of the submucosa. Another striking change was that the subserosal muscle was reduced to about half its normal thickness (normal $35 \pm 5\mu$), - ref. Table II. The nuclei of the muscle fibres were rather round, dark and crowded together. The last feature suggests reduction in size of individual muscle fibres. In places the striations of the muscle are seen less clearly.

Table II.

	Height of villi	Thickness of subserosal muscle	Height of mucosa
Normal rat	430 ± 50	35 ± 5	540 ± 70
Protein-deficient rat	190 ± 40	18 ± 4	300 ± 40

Measurements in μ.
Values considered abnormal if they are outside the range of mean - standard deviation.

Electron Microscopy.

Improved methods of cutting ultra-thin sections and their examination under the electron microscope has brought to light the structural details of the mucosal surface of the intestinal epithelium of the rat (Granger and Baker, 1950; Dalton et al, 1951; Palay and Karlin, 1959). The striated free surface of the epithelial cells is found to possess tubular or finger-like processes running at right angles to the surface of the cell. These minute structures resemble the shape of the villi and hence are called microvilli. Granger and Baker (1950) in a masterly piece of work calculated that these microvilli in the rat are on average 0.62μ in length and 0.08μ in breadth (Fig. 46). Their tips are rounded: the core of the microvillus appears to be an extension of the cellular protoplasm. They noticed also that these microvilli are packed closely in long rows. The even spacing of the rows brings into view their arrangement as regular hexagons noticed by Palay and Karlin (1959). Granger and Baker (1950) found that an intestinal epithelial cell holds about

3000 microvilli and they increase the absorbing surface of the intestine by a factor of 30. Sometimes minute vesicles are seen in the intermicrovillous spaces which open into the lumen of the intestine. It is suggested that pinocytosis takes place at these sites (Palay and Karlin, 1959).

The cytoplasm below the attachment of the microvilli has a smooth velvety appearance and is called the ectoplasm. Below the ectoplasm the fibrillar element in this terminal part of the cell spreads like a web (the terminal web), binding the ectoplasm and the microvilli with the subjacent endoplasm by its microfibrils.

The cytoplasm of the cell can be divided into three regions, supranuclear, nuclear and infranuclear (or basal). The central part of the cell has a vast network of cytoplasmic membrane, and because of its position in the centre of the cell it is called endoplasmic reticulum. It comprises a series of channels and vesicles. The vesicles are of two types: some of them are smooth walled and found diffusely in the superficial part of the endoplasmic reticulum, while the surface of the others is studded with numerous dense particles or ribosomes (Palade and Porter, 1954). The cytoplasm holds in its matrix various organelles, namely mitochondria, microsomes, the Golgi complex, centrosomes, fat droplets, myofibrils and various other inclusion bodies. These are worth mentioning at this stage, since they share in cytoplasmic alterations in the protein malnutrition state.

The mitochondria are very specialised cell organelles, which are distinguished from other cytoplasmic inclusions by well defined chambers and cristae. The electron microscope has brought into light the double membranous capsule of the mitochondrion which divides it into two chambers. The outer chamber lies between the two membranes and the inner chamber is bounded by the inner membrane. The inner limiting membrane folds on itself to form cristae (Palade, 1953). Occasionally dense granules are seen in the mitochondrial matrix and these appear to be arranged along the long axis of the cell in the supranuclear cytoplasm, thereas in the infranuclear region they appear to be crowded together. This impression is more apparent than real because the abundant space in the long axis of the cell in the supranuclear region and the limited space below the nucleus tends to alter the disposition of the mitochondria.

Another interesting feature noted in the cytoplasm is the presence of numerous small vesicles which are often found to be broken. Fine (RNA) granules are seen studded over the wall of some of them, but others seem to be devoid of these granules and comprise the microsomal fraction of the cytoplasm. Amidst all these fine structures, there is a tiny electron opaque cytoplasmic organelle, found very close to the Golgi region of the cell. It always maintains its individual entity, and because of its central position in the cell it is called the 'centrosome'. The electron opaque materials which give

shape to this organelle are grouped around the centre of the cell. Its function is not yet known but it is often suggested that it organises the fibrillar material of the cell.

The Golgi apparatus occupies the cytoplasm just above the nucleus. It is often referred to as the Golgi complex because it includes a complex arrangement of a number of small and large vesicles and smooth walled sacs which are closely packed in a lamellated fashion (Fig. 47). Besides these there are many fibrillar structures in the cytoplasm surrounded by microfibrils, some of which are found in close association with the terminal bar and the terminal web.

The nucleus of the cell is a homogeneous mass which appears uniformly granular under the electron microscope. It is surrounded by a double membrane separated by a perinuclear space. The nuclear membrane is interrupted in places which appear as pores (Gall, 1959). There is controversy regarding the structural detail of these pores, but aggeement concerning their functional importance. Watson (1959) suggested that through these pores selective transfer of substances takes place between the nucleus and cytoplasm.

The broad basal part of the cell rests on the sub-epithelial basement membrane. This electron dense membrane is about 500Å in thickness and consists of hyaline amorphous material probably secreted by the epithelial cells of the intestine.

The cell is enveloped by a plasma membrane. Just below

the microvilli the plasma membrane presents a wedge shaped thickening called the terminal bar, and this gives extra support to the physiologically active absorbing surface of the cell. The membranes of adjacent cells fold on each other and are bound together by desmosomes. This allows greater surface contact between the adjacent cells.

In addition to reticular fibres the core of the intestinal villus accommodates various blood cells (namely, lymphocytes, eosinophils, plasma cells and macrophages), blood capillaries and unmyelinated nerves. The lymphocytes have very little cytoplasm around their nucleus, which is round or ovoid with a little indentation on one side. The nucleus is more granular than the nuclei of any other blood cells. The eosinophils are characterised by granules which have a rectangular or square shaped. The plasma cells present an excess of rough-surfaced vesicles similar to the endoplasmic reticulum. The lymphatic vessels differ from blood capillaries in having a very thick wall which is devoid of fenestrations, and lacks a definite basement membrane. Unmyelinated nerve fibres are found underneath the subepithelial basement membrane and are again seen in association with the blood vessels.

One of the most striking findings in the protein deficient rat under the electron microscope was the change in the mucosal absorptive surface. The mucosa contained fewer microvilli, which were shortened, their length varying between 0.2 - 0.3μ (Fig. 49). Over most areas they retained a regular shape, and an even contour. Their hexagonal array was preserved over these areas. There

were some areas where the microvilli had an irregular shape and an uneven contour, and showed a tendency to fuse with one another (Figs. 50 and 51). Over some other areas they were probably lost altogether.

The velvety appearance of the cytoplasm below the microvilli was lost in the protein-malnutrition state and looked similar to the remaining cytoplasm of the cell. The fibrillar network over the terminal part of the cell (terminal web) was less prominent than normal. The cytoplasm looked hazy and less even. Electron microscopy further unveiled minute vacuoles in the cytoplasm which were not evident under the light microscope. The presence of innumerable vacuoles of various sizes and shapes gave a distorted appearance to the ergastoplasm and accounts for the altered appearance of the cytoplasm. The cell matrix looked less electron dense. The cytoplasmic granules were coarser and achieve prominence because they are separated by vacuoles. There was also an accumulation of fat in the cytoplasm of the epithelial cells and in the intercellular spaces. (Figs. 52 and 53).

The mitochondria were found to have become spherical and appeared somewhat swollen. Their outer and inner walls showed a tendency to fuse. The cristae appeared wavy and short and mitochondrial morphology was distorted. In addition to these mitochondrial changes there appeared electron opaque bodies of various sizes and shapes in the cytoplasm. These cytoplasmic bodies had a single wall, and some had a central vacuole while others contained remnants of the mitochondrial cristae. These features

suggest that these cytoplasmic bodies are degradation products of the cell organelles (Novikoff and Essner, 1962).

At this stage the nuclei of the epithelial cells of the intestine of the rat in protein malnutrition did not show any notable change. The empty spaces normally found between the basal part of the adjoining cells were reduced a great deal and occupied by some material of low electron density. This may be glycogen, as noted in the liver cells in Kwashiorkor patients (Theron and Liebenberg, 1963). Curiously enough one notices many electron opaque particles (lipid) making their way between adjacent cells in the protein deficient state. This was not seen in control animals. The subepithelial basement membrane and the blood vessels showed no change.

Discussion.

Baker et al (1963) asserted that the duodenum of the new born rat presents typical finger shaped villi. At 10 days the villi look somewhat leaf-like, but in older animals they assume a ridged appearance. This they suggested was due to toxic substances in the food,whereas the gaps between the ridge-shaped villi in the control rats in the present experiment were small, they were appreciably widened in the protein deficient rats. This we assume to be due to the generalised atrophy of the mucosa.

The intestinal proteolytic enzymes act on and hydrolyse dietary proteins. The products of protein digestion, especially peptides, (Newey and Smyth, 1960) are absorbed mainly from the duodenum and upper jejunum, by diffusion

and also by active transport. In the mucosal cell the peptides are hydrolysed into amino-acids which are then released into the portal blood. After passing through the liver the products of protein assimilation are poured into the general circulation and are reconstituted as tissue proteins. In cell cytoplasm proteins are not stored as inclusion bodies: they are present as RNA granules in the endoplasmic reticulum and as the lipoprotein complex of the cementing substance. Under the condition of protein malnutrition this reserve cellular protein is utilised. Histochemical studies suggest that the morphological changes observed in the cells in this condition are due to a disturbance of intracellular proteins and intracellular hydrogen ion concentration. (Anderson, 1961). The cells look swollen and lose their translucent appearance. Lack of protein affects the physical and chemical state of the cell, and may alter the osmotic state of intracellular substance. The permeability of the cell membrane is increased, causing it to imbibe water and bringing about structural distortion.

It is a well known fact that a protein and caloric deficient state leads to improper manufacture of tissue protein and slows down cellular activity. But we do not know the exact role of protein in maintaining the dynamic state, nor do we know at what level the basic functions of the cells are affected in the deficiency state. Much attention has been focused on the changes in liver cells in this condition (Radnakrishna Rao, 1942; De Silva, 1955; Theron and Liebenberg, 1963; Ramalingaswamy, 1964).

The cell membrane becomes prominent and the mitochondria show evidence of degeneration. A very similar but less severe picture is noted in the mucosal cells of the upper part of the small intestine.

Reports concerning the sites of affection of the small intestine in a protein malnutrition state are of great interest. Radnakrishna Rao (1942) noticed degenerative changes in the lower third of the small intestine of monkeys fed on a rice diet. He commented that the affection of the entire small intestinal mucosa exhibited by McCarrison's monkeys was related to a severity of artificial malnutrition rarely met with in humans. Ramlingaswami (1964) commented that although protein deficient monkeys showed predominant damage of the mucosa of the ileum, the photomicrographs showed that there was a striking atrophy of the proximal jejunal mucosa as well. In our protein malnourished rats the mucosal atrophy was noticed in the proximal half of small intestine and the changes were more marked in the duodenum than in the distal jejunum. We, however, limited our observations to the sites from which protein is absorbed in the small intestine.

Deficient protein in the diet appears to induce generalised atrophy of the mucosa. Although the height of the villi were reduced to about half of the normal size there was very little change in their breadth, and their general configuration remains more or less unchanged. In contrast to coeliac disease or primary malabsorption disease

of the intestine there was no increase in the mitotic activity of the epithelial cells of the intestine. There was also reduction in the size of the glands and reduction in the thickness of the glandular layer of the intestine. Ramliganswami (1964) reported a study of the turnover of the epithelial cells of the intestine by labelling in vivo with tritiated thymidine. From a comparative study of the migration of the epithelial cells of the intestine in normal and in protein deficient monkeys he came to the conclusion that in unit time normal epithelial cells cover two and a half times the distance covered by the intestinal epithelial cells in the protein deficient state. From these observations it appears as if in the protein malnutrition state the mucosa is in a state of induced hibernation.

Severe protein restriction causes inadequate synthesis of hormones of protein composition like thyroxine and anterior pituitary hormone. Similarly the enzyme content of the liver may be expected to fall. (Cantarrow and Schepartz, 1962). Serious protein insufficiency causes diminution of the supply of amino-acids which are essential for the maintenance of the liver cells, and these show an accumulation of fat and a reduced ability to stand stress. This change is reversible with the addition of protein to the diet. (Davidson and Passmore, 1963). Gastro-intestinal enzymes are also diminished in extreme cases of protein depletion, and this has been blamed for gastro-intestinal symptoms. De Silva (1955), while describing the pathology of protein malnutrition

(Kwashiorkor) mentioned that the primary change is loss of zymogen granules from the intestinal glands. For the same reason the acinar cells of the pancreas atrophy as well. Thus there is selective atrophy of certain exocrine glands and the manufacture of enzymes is reduced. In protein deficient rats in this experiment the phase of protein deprivation was not severe enough to bring about marked reduction in zymogen granules in the proximal half of the small intestine.

Another noticeable feature in the intestine is a reduction in muscle mass. Although both submucosal and subserosal muscles are affected, the subserosal muscle appears to be affected to a greater extent. Radnakrishna Rao (1942) noticed atrophy of the muscular coat of the small intestine of monkeys fed on a rice diet. He commented that although there was reduction in the volume of the intestinal muscle, so much so that the intestine looked translucent when held up to the light, there was no change in the individual muscle fibres and their nuclei. In the protein deficient rats described here, in addition to a reduction in the muscle mass of the intestine there was definite crowding of the nuclei suggesting a reduction in the size of individual muscle fibres.

The striking feature of the mucosa under the electron microscope was the change in the microvilli. Although they were shorter they were mostly regular in shape and had an even contour. Another feature noted in this condition is fusion of microvilli. I think this is a very early manifestation of microvillus affection in primary

malabsorptive disease of the intestine as well (Chapter 8) but I cannot give an explanation from my observations, as to why such fusion should occur. I have noticed that methacrylate seems to preserve the lipoprotein membrane covering the microvilli of protein deficient tissues better than araldite. The latter causes unevenness of the contour of the microvilli and induces an artificial irregularity in their outline.

In general the granular part of the endoplasmic reticulum is depleted in starvation and at the same time the a-granular part, which plays a role in glycogen metabolism, is increased. This might account for the accumulation of electron dense material above the subepithelial basement membrane. Distortion of mito-chondrial morphology was another salient feature of protein malnutrition. This damage to the structural morphology of themitochondria is reflected in functional disturbances in the cell. Harman (1958) believes that the structural transformation of mitochondria is closely associated with depletion of cellular enzymes which in extreme cases causes or is associated with destruction of cellular structure. From various observations it is concluded that the mitochondrial membrane is selectively permeable to certain substances (Oberling, 1959). Florey (1962) suggested that in the malnutrition state the mitochondrial membrane is damaged and its permeability is altered. The mitochondria and lyzosomes are rich in proteolytic enzymes (Cathepsins). These proteolytic enzymes are now supposed to gain the upper hand and cause further damage

to the cell.

Degeneration of the mitochondria affects cellular respiration in particular, and depresses the metabolism of the cell, which fails to cope with the removal of imbibed water and sodium. The concept of water transport across the cell wall is now better understood (Robinson, 1960); the water transport system depends equally upon the osmotic concentration of the extracellular fluid and the metabolism of the cell. Mitochondria maintain the cell respiration and in addition produce energy for active transport across the walls in order to maintain water balance. In the protein-malnutrition state damage to the mitochondria depresses the metabolism of the cell. Decreased cellular oxygen tension leads to the ingress of sodium and water to the cell, where they displace potassium.

Unfortunately our studies are insufficient to give us any clue as to how much these morphological changes are reversible. Kaltenbach and Hartmann (1955) thought that the mitochondrial state in cloudy swelling is not reversible. Novikoff and Essner (1962) suggested that cytolysomes which are noted in protein-deficient tissues are commonly found in specimens of liver and kidney when the cells are reversibly injured or regenerating. It would be interesting to discover what particular protein fraction has to be lacking for atrophy of the intestinal mucosa to develop and what extent of cellular damage is reversible, by supplementing the protein fraction. Such information may be of use in clinical practice.

Summary.

1. The proximal part of the small intestine obtained from normal rats and rats on a protein-deficient diet was studied with the dissecting, light and electron microscopes. There is a suggestion of subtotal atrophy of the small intestine in the protein malnutrition state.

2. Dissecting microscopic examination showed that the villi of the upper part of small intestine of the normal rat are ridge-shaped. In the protein-deficient state, although the gross appearance of the villi remained unchanged, they are smaller, thin and separated, causing an increase in the intervillus space.

3. Examination under the light microscope similarly showed preservation of the gross structure of the mucosa. However the height of the villi and the thickness of the submucosal muscles are grossly reduced. At the same time a moderate reduction of the glandular layer and absence of increased mitotic activity amongst the epithelial cells clearly distinguished the mucosal changes from those seen in coeliac-sprue.

4. Changes in the microvilli are another striking feature. These are shorter and fewer than normal, and show a tendency to fuse with one another. The mitochondria are swollen, and present complete disorganisation of their internal structure. The rhibosomes of the endoplasmic reticulum are rather less prominent than normal.

It would be interesting to note how far these structural changes are reversible by the restoration of protein

REFERENCES.

1. Anderson, W.A.D. (1961). Pathology 4th Ed. Henry Kimpton, London.

2. Baker, S.J., Mathan, V.I., Cherian, V. (1963). Lancet, i, 860.

3. Cantarrow, A., Shepartz, B. (1962). Biochemistry. W.B. Saunders Company Ltd., Philadelphia, London.

4. Dalton, A.J. (1951). Am. J. Anat., 89, 109.

5. Dalton, A.J., Kahler, H., Lloyd, B. (1951). Anat. Rec. 67, 111.

6. Davidson, Sir Stanley, Passmore, R. (1963). Human Nutrition and Dietetics. E. & S. Livingstone, Ltd., Edinburgh and London.

7. De Silva, C.C. (1955). Ceylon med. J., 3, 55.

8. Florey, Sir Howard. (1962). General Pathology, 3rd Ed., Lloyd-Luke Medical Books Ltd., London.

9. Gall, J.G. (1959). J. biophys. biochem. Cytol., 6, 115.

10. Granger, B., Baker, R.F. (1950). Anat. Rec., 107, 423.

11. Ham, A.W., Leeson, T.S. (1961). Histology, 4th Ed. Pitman Medical Publishing Co. Ltd., London.

12. Harman, J.W. (1958). J. Clin. Path., 11, 495.

13. Kaltenbach, J.C. and Hartman, J.W. (1955). Exp.Cell Res., 8, 435.

14. Leblond, C.P. and Stevens, C.E. (1948). Anat. Rec., 100, 357.

15. McCarrison. (1921). Quoted by Radnakrishna Rae, M.V. Indian J. Med. Res., 30, 273.

16. Newey, H. and Smyth, D.H. (1960). J. Physiol., 152, 367.

17. Novikoff, A.B., Essner, E. (1962). Fed. Proc., 21, 1130.

18. Oberling, C. (1959). Intern. Rev. Cytol., 8, 1.

19. Palade, G.E. (1953). J. Histochem. Cytochem., 1, 188.

20. Palade, G.E., Porter, K.R. (1954). J. exp. Med., 100, 641.

21./

21. Palay, S.L., Karlin, L.J. (1959). J. biophysic. biochem. Cytol., 5, 363.

22. Tamayo, R. (1961). Mechanism of disease. W.B. Saunders Co., Philadelphia and London.

23. Platt, B.S. (1962). Proc. roy. Soc., B, 156, 337.

24. Radnakrishna Rao, M.V. (1942). Indian J. Med. Res., 30, 273.

25. Ramalingaswami, V. (1964). Nature, 201, 546.

26. Robinson, J.R.(1960). Physiol. Rev., 40, 112.

27. Theron, J.J. and Liebenberg, N. (1963). J. Path. Bact., 86, 109.

28. Watson, M.L. (1959). J. biophysic. biochem. Cytol., 6, 147.

Fig. 42. Dissecting microscopic appearance of mucosa of proximal small intestine of normal rat. x 30

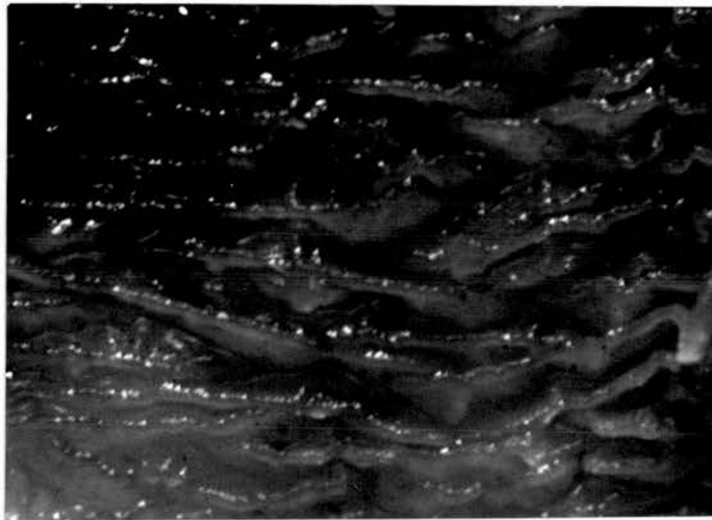


Fig. 43. Dissecting microscopic appearance of mucosa of proximal small intestine of protein malnutrition rat. Note shrinkage of the villi thus widening the intervillus space. x 30

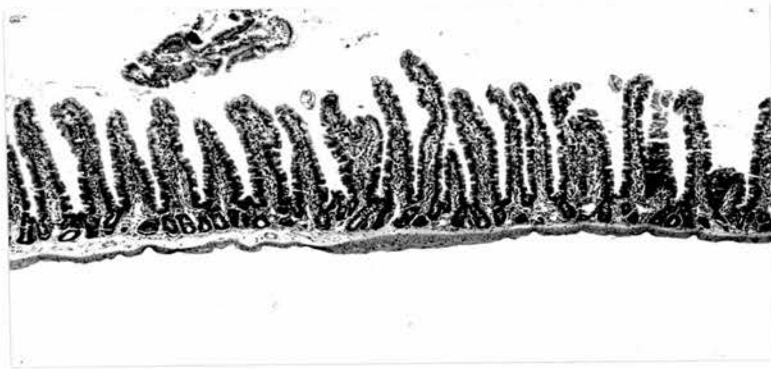


Fig. 44. A section of normal proximal small intestinal mucosa of rat. x 40

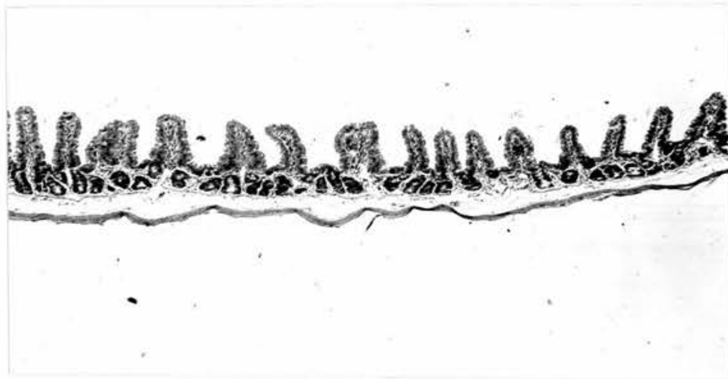


Fig. 45. A section of proximal small intestinal mucosa of protein malnutrition rat. Note generalised atrophy of mucosa. The heights of villi, glandular layer and muscularis mucosae are reduced. Reduction of the heights of villi are most marked. x 40

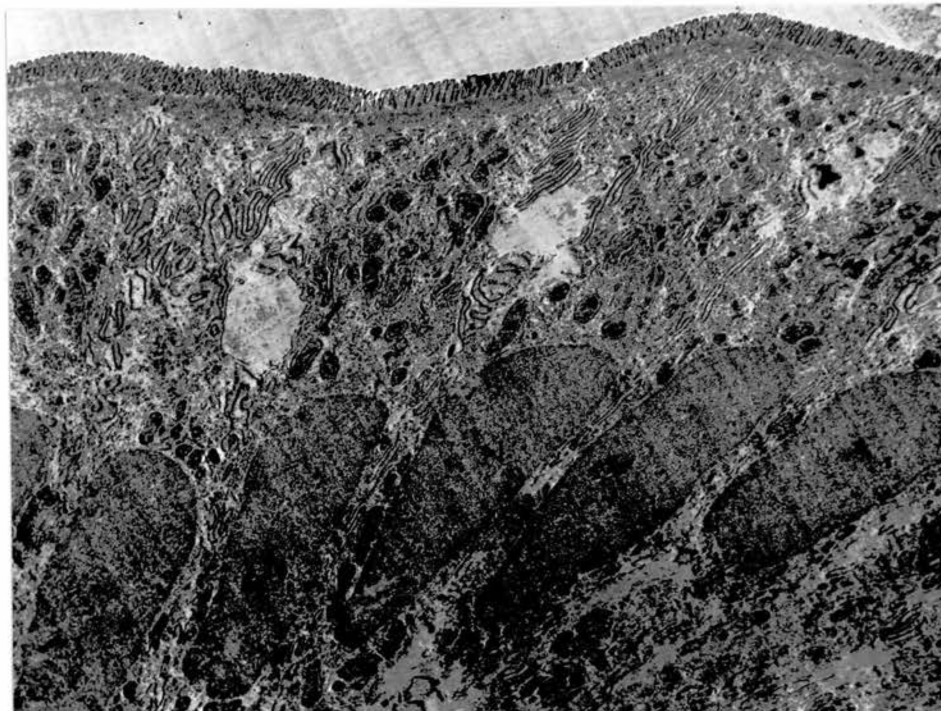


Fig. 46. Electron micrograph of normal epithelial cell of small intestine of rat. N = nucleus; m = mitochondria; mv = microvilli. x 2,850

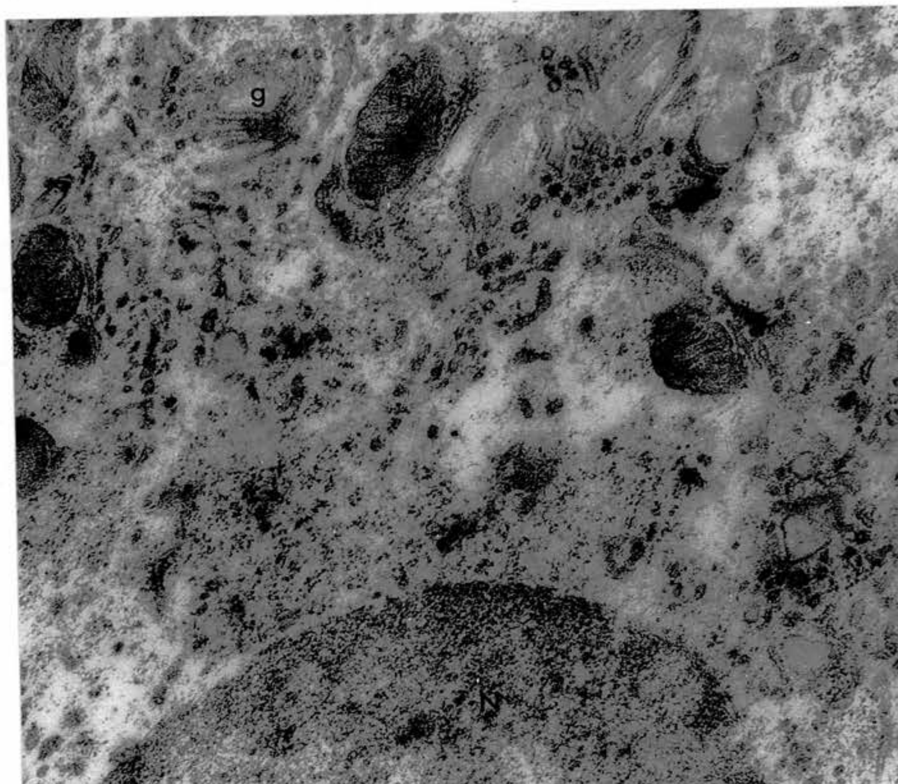


Fig. 47. E.M. showing upper part of nucleus (N) and supranuclear cytoplasm of normal epithelial cell. m = mitochondrion; g = golgi complex. x 40,000

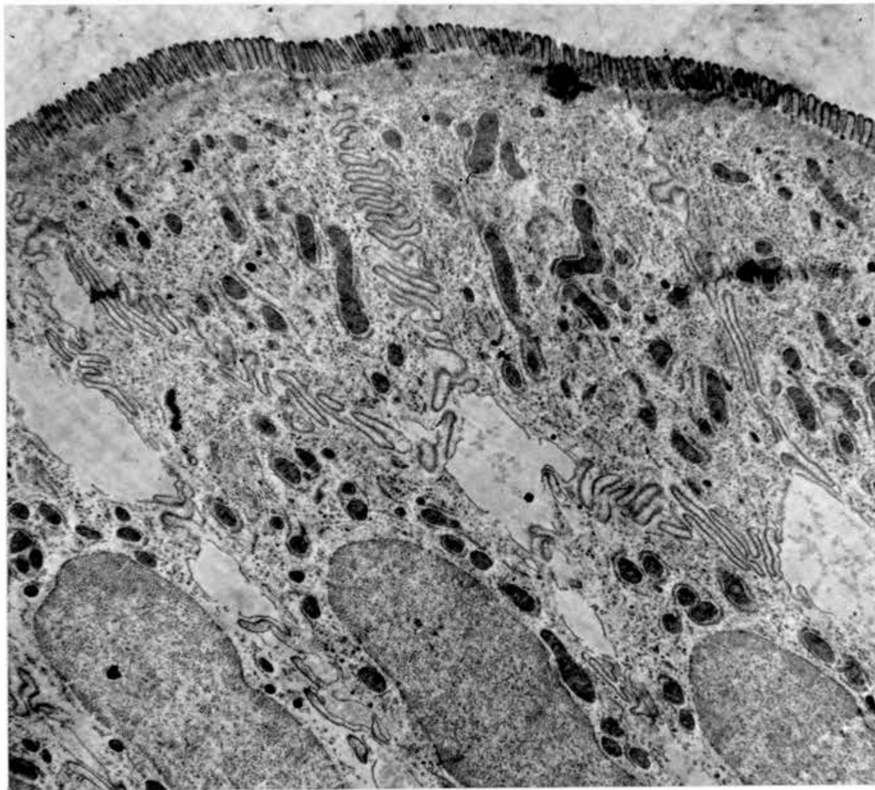


Fig. 48. Microvilli (M) of the absorbing cells of normal upper small intestinal mucosa of rat. x 4,050

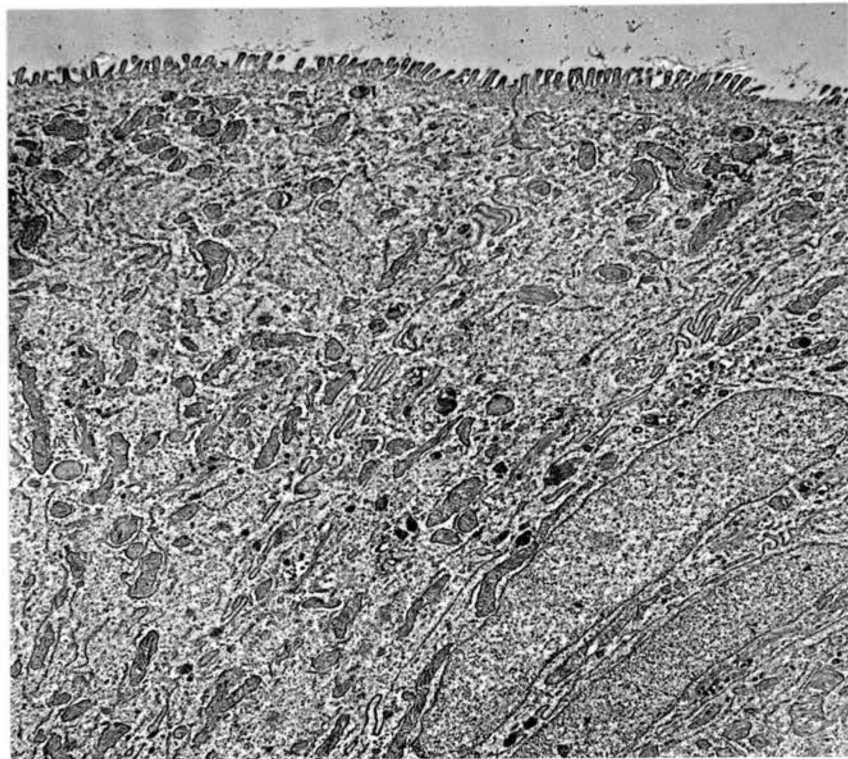


Fig. 49. Epithelial cell of upper small intestine of protein deficient rat for comparison. Note the reduction in size of the microvilli (M). There is also increased accumulation of fat in these cells. x 4,050

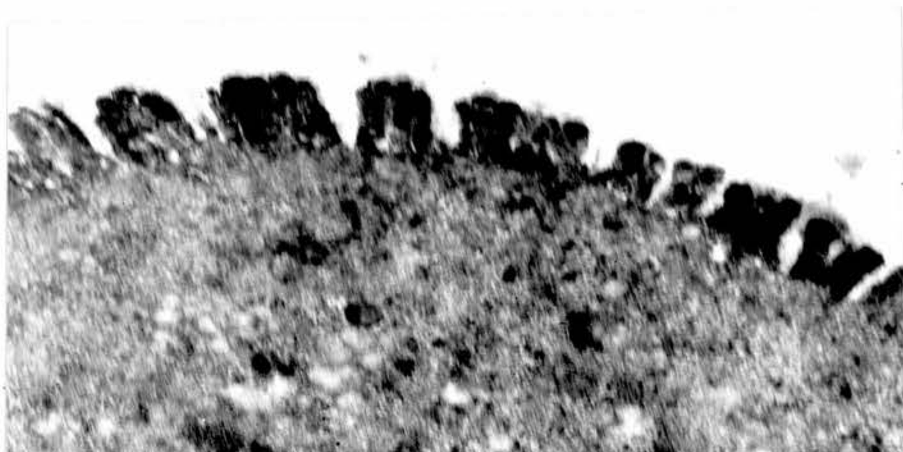


Fig. 50. Microvilli of epithelial cell of small intestine of protein deficient rat. Note aggregation of microvilli in groups. x 40,000

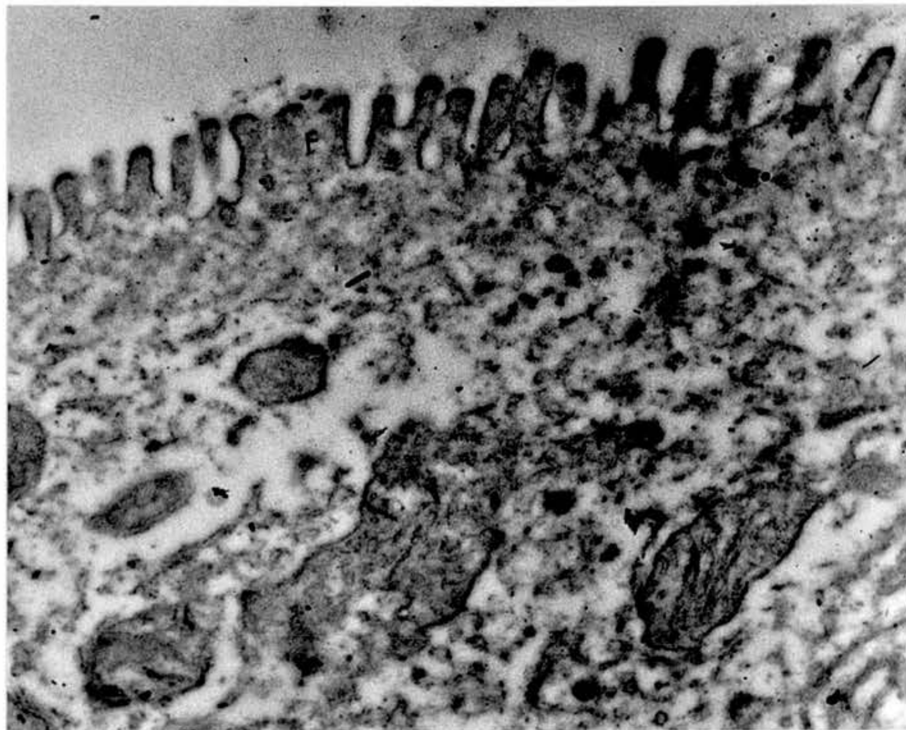


Fig. 51. Note fusion (F) of the microvilli of protein deficient rat. The microvilli are also shorter in height ($0.25 - 0.3\mu$). x 32,000

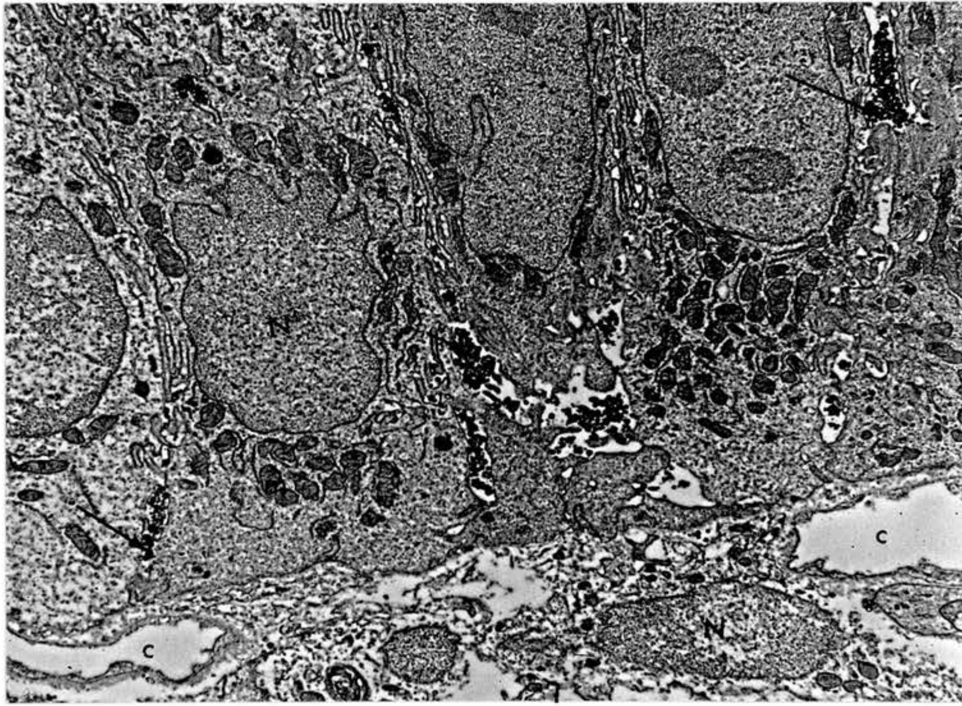


Fig. 52. Electron micrograph of bases of epithelial cells of upper small intestine of protein malnutrition rat. The fat droplets (arrow) mostly lie between the epithelial cells. They are also seen in the cytoplasm of the cells. N = nucleus; m = infranuclear mitochondria; c = capillary; N = nucleus of a fibroblast in the lamina propria. x 5,750

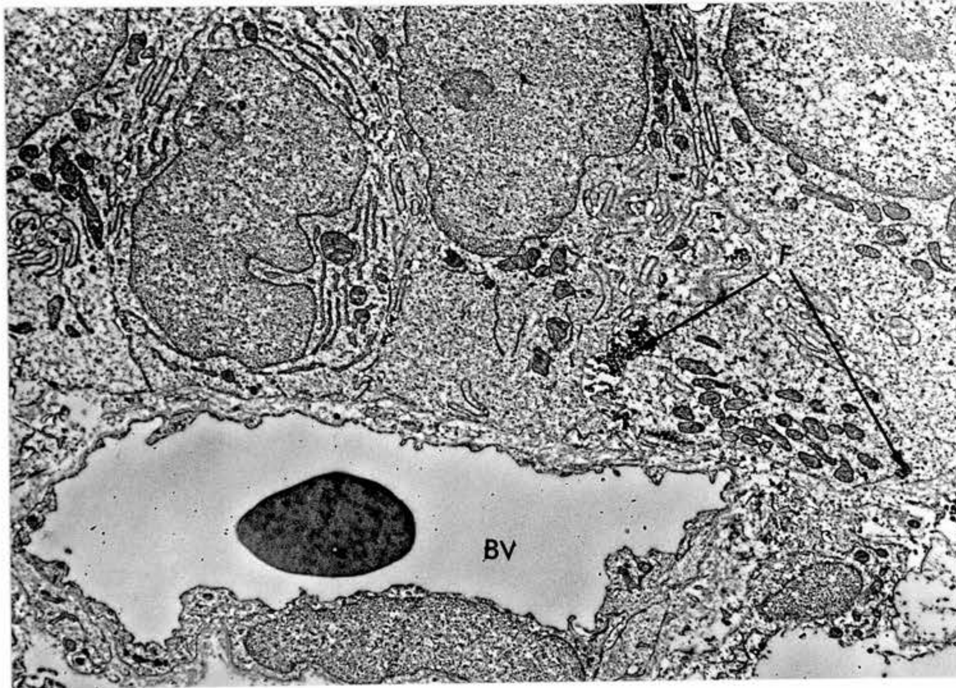


Fig. 53. Basal part of epithelial cells of protein deficient rat showing excess accumulation of fat particles which are shown to be crossing (F) the sub-epithelial basement membrane. BV = blood vessel in the lamina propria. x 4,050

CHAPTER 6.

MUCOSAL CHANGES OF SMALL INTESTINE IN EXPERIMENTAL INFECTION WITH V. CHOLERAE.

In recent years with the availability of a peroral biopsy method it is now possible to obtain specimens of small intestinal mucosa from persons at different stages of infection with Vibrio cholerae. It is now realised that the mucosa of the small intestine is preserved throughout the entire course of the disease (Gangarosa et al, 1960; Sprinz, 1962; Datta, 1964). The older view which states that in this disease there is sloughing and desquamation of the small intestinal mucosa is no longer held and it is believed that these are post-mortem changes. Our knowledge is still fragmentary as regards the pathogenesis of the disease. There is still a search for an explanation to account for the massive loss of fluid and electrolytes from the intestine.

In the present work the mucosa of the intestine of rabbit was studied under the dissecting, light and electron microscopes during the early hours following introduction of the V. cholerae into a ligated intestinal loop. An attempt has been made to correlate the early morphological changes observed under the electron microscope with the massive loss of fluid that occurs in this disease.

Materials and methods.

The rabbit intestinal loop technique was performed with the minor modifications of the method described by De and Chatterjee (1953). Rabbits 8-12 months old,

weighing 2-2½ kilograms were deprived of solid food overnight. Pentobarbitone sodium B.P. (30 mg/kg., I.V.) was used for anaesthesia and was supplemented by open ether when necessary. The strain of V. cholerae used in this experiment was (Ogawa) # 12 r, which was isolated from a patient in India. It was preserved by freeze drying until required. The inoculum was 0.5 ml. of an overnight broth culture of the vibrio, which had been regenerated on nutrient agar and it contained about 3×10^8 viable cells.

The abdomen of the rabbit was opened by a 4 cm. long paramedian incision. The ileo-caecal junction was identified. Loops were made by ligating both ends of 10 cm. segments of ileum taking meticulous care not to obstruct the blood supply. The first loop was made about 40 cm. from the ileo-caecal junction and the subsequent loops were made at 10 cm. intervals proceeding towards the oral end. The segments of intestine in between the loops served as untreated controls. Each rabbit had 1-2 loops which were inoculated with culture and one loop inoculated with physiological saline or broth. After replacing the intestines the abdomen was closed up in two layers and the animal allowed to recover. After the required intervals, 3 hr., 6 hr., 12 hr., and 18 hr. the rabbit was anaesthetised again using ether alone if the interval was less than 9 hours. A fresh incision was made to reopen the abdominal cavity and the loops were identified by their positions.

The infected loops were always distended. At first

some fluid was removed from the loop by a syringe, With the aid of a fine pair of scissors a strip of mucosa was removed from the antimesenteric region of the loop and was put on a piece of filter paper to remove the intestinal fluid. The strip of mucosa was then put into 2 ml. of chilled osmium tetroxide fixative for about 30 seconds. It was then removed on to a piece of cork with the aid of forceps.1 mm. square of mucosa was isolated from the central region of this strip by means of a new razor and chopped into small pieces. These were again put into 2 cc. of fresh osmium tetroxide fixative and processed for electron microscopic examination. An adjacent area of the loop was ligated and perfused with 10% formol saline. After 18-24 hours a segment of intestine from the antimesenteric region was removed, first examined under the dissecting microscope and then processed for light microscopic examination.

The control loops were always empty. The mucosae for the microscopic examinations were removed as mentioned above. After collecting the specimens the animals were killed by deepening the anaesthesia.

Vibrio cholerae embedded in agar. The bacterial suspension was centrifuged and the supernatant fluid was drained off. Normal saline was added to the suspension which was mixed and centrifuged. The supernatant saline was drained off. Chilled osmium tetroxide was added to the suspension, stirred with a wooden stick and left in a refrigerator for 4 hours. At the end of this time the

mixture was centrifuged and the osmium tetroxide was poured off. The bacterial suspension was given 2-3 washings in the buffer (2% potassium dichromate and 1.7% sodium chloride solutions in equal parts) at room temperature. ½ cc. of 2% agar at 60°C was added to the bacterial suspension, mixed with a wooden stick and poured onto a watch glass. After the agar solidified it was divided into small blocks of 1 mm. square. These agar blocks were dehydrated with ascending grades of alcohol,

10%	-	2 changes	
50%	-	2 changes	) 15 minutes
70%	-	2 changes	) per change
90%	-	2 changes	)
100%	-	4 changes	

and embedded in araldite, after two changes of epoxypropane.

<u>Observations.</u>

1. <u>Ultra structure of Vibrio cholerae</u>. The haematoxylin and eosine stained sections of an infected loop under the low power of the light microscope often presented basophilic substances close to the striated surface of the villi. These substances under the high power of the microscope (x 1000) showed masses of curved rod-shaped structures resembling <u>Vibrio cholerae</u>. When an ultrathin section of the infected epithelium was viewed under the electron microscope the structural details of these rod shaped structures came to view (Figs. 54 and 55). They were found to be identical with sections of <u>V. cholerae</u> embedded in agar.

Sections of the comma shaped vibrio under the electron microscope presented a granular cytoplasm which is limited by two membranes and a terminal flagellum. The outer of the two membranous structures is the cell wall which has a uniform thickness of about 80Å. In a very thin section the cell wall appeared double: two dark zones separated by a lighter zone. The inner membrane is called the plasma membrane. Like the cell wall it is a double walled structure and is almost equal in thickness to the cell wall. In most places the two membranes run parallel with each other. The space between them is occupied by a substance which is very slightly electron opaque. Strands were noted bridging the gap between the two membranes causing the outer cell membrane to be drawn towards the plasma membrane, and thus the cell wall appeared wavy. (Fig. 56).

The granular appearance of the cytoplasm is presumably due to close packing of the ribonucleoprotein particles as suggested by the work of Huxley and Zubay, 1960. In places these granules appear to aggregate into small masses. The cytoplasm surrounds uniformly the central more electron-translucent area, called the nucleus. The nuclear region is filled with a network of fine fibrillar material. Glauert, (1962) suggested that during the cell division this fibrillar material is re-arranged in a parallel and more regular fashion.

V. cholerae has a polar flagellum well seen when a shadowed specimen of the vibrio is examined under the

electron microscope. An ultra thin section of the flagellum presents electron opaque material (which is 100Å thick) forming the core, and is enveloped by a sheath which is less electron opaque. The total diameter of the flagellum is about 250Å. The sheath of the flagellum appears as if it were continuous with the cell wall of the vibrio. (Fig. 57). The ultra structure of *Vibrio cholerae* are identical with *Vibrio metchnikovic* described by Glauert and co-workers (1963).

2. External appearance of the loop. In contrast to the control loop which remained empty, the intestinal loop into which *V. cholerae* was injected was distended with fluid. At 3-hours there was only 1 or 2 ml. of clear fluid in the loop. At 6 hours the fluid amounted to about 5-6 mls. At 9 hours blood vessels were prominent over the serosal surface of the loop. At 12 hours the distension of the loop was maximum and patches of haemorrhage appeared over the serosal surface. These haemorrhagic areas increased with further lapse of time. At 18 hours it looked as if the loop was developing gangrene. (Figs. 58 and 59).

At 9 hours the accumulated fluid inside the loop was noted to be under pressure. The fluid was at first greenish brown in colour. With lapse of time the colour of the fluid changed in sequence to yellowish green, reddish yellow and finally reddish brown in colour. At the same time the pH of this fluid which was originally 7.5-8 became 9 at a time when the red colour appeared in the fluid.

The fluid did not contain any inflammatory cells till haemorrhage occurred.

3. Appearance under the dissecting microscope. The mucosa of the ileum of rabbit is covered with short finger shaped and leaf shaped villi. The earliest noticeable change was at 6 hours, when the villi overlying the larger mucosal blood vessels appeared congested. (Fig. 60). At 12 hours the villi over the entire mucosa looked uniformly congested and a purple line appeared over the midline of the entire length of the villus (Fig. 61). With further lapse of time the villi looked darker as if the mucosa was about to slough off. (Fig. 62). A transilluminated view of the mucosa showed rupture of the bigger vessels and clots of blood were noted at these sites. (Fig. 63). This would account for the change in the colour of the accumulated fluid from greenish yellow to red.

4. Light microscopic appearance. At 3 hours after infection the mucosal picture under the light microscope was normal, except the submucosal oedema which separated the subepithelial basement membrane from the structures in the lamina propria (Fig. 64). At 6 hours the villi were of normal dimensions but the glandular layer of the mucosa appeared flattened and less prominent. The epithelial cells and their striated luminal border appeared normal. The goblet cells, for the first time, looked smaller and empty. The subepithelial basement membrane was further lifted by the submucosal oedema and was broken

in places. There was a slight increase in the number of mononuclear cells in the stroma of the villi (Fig. 65), a feature identical to that noted in the acute diffuse enteritis of Asiatic cholera (Gangarosa et al, 1960; Fresh et al, 1964). Dilated spaces appeared in the stroma of the villi. With further lapse of time these spaces increased in size and blood corpuscles were noted in it. These features progressed until extravasated blood masked the entire mucosal picture at 18 hours (Fig. 66).

5. Electron microscopic changes. The studies under the electron microscope were confined to the mucosa taken up to 6 hours following infection with *V. cholerae*. At this stage there was no demonstrable change in the epithelial cells under the light microscope. At six hours the microvilli covering the epithelial cells were of uniform size and evenly distributed. Under the high resolution of the electron microscope each microvillus is found to have an enveloping lipoprotein membrane. This membrane comprises two dark zones and an intermediate lighter zone. (Fig. 68). At 6 hours after infection the lipoprotein membrane presented localised areas of saccular dilatation. The inner and outer dark zones of the lipoprotein membrane appeared to join on either side of these vesicles. In places these dilatations were found to be broken and the core of the microvillus communicates with the intestinal lumen. (Figs. 69 and 70). The terminal web appeared to be normal at this stage. The cytoplasm of the cell appeared more electron opaque. The mitochondria appeared irregular in shape, more electron opaque and there was a distortion

of the internal details. (Figs. 71 and 72). There were neither clear spaces in the cytoplasm nor was there any gross enlargement of the size of the mitochondria to suggest excessive reabsorption of intestinal fluid. (Wynn Williams, 1963). Under the subepithelial basement membrane there were spaces which appeared to be filled with substances of low electron density. (Fig. 73). These spaces separated the basement membrane from the structures in the lamina propria. The endothelial cells of the capillaries appeared separated instead of overlapping each other, and thus the gap between them was widened. At these sites it seemed as if the lumen of the blood vessel was separated from the perivascular region by only the basement membrane of the blood vessels. (Figs. 74 and 75).

Discussion.

It was originally believed that following infection with V. cholerae the epithelium of the small intestine desquamates. These sites of the desquamation behave like burnt areas from where a massive amount of fluid is lost into the intestinal lumen and the denuded mucosa was thought to contribute to the characteristic rice-water stool. This view was further supported by observations on the experimental infection of V. cholerae into the ligated intestinal loop of rabbit (De and Chattijee, 1953). This popular view has fallen into disrepute during the recent years. Gangarosa and coworkers (1960) obtained specimens of small intestinal mucosa from proven cases of Asiatic cholera during the different stages of the disease

and did not find desquamation of the epithelium. They suggested that the mucosal changes noted by the earlier workers could be due to post mortem autolysis or due to terminal vascular collapse or both. This view was supported by Spirnz (1962). Datta (1964) reproduced the human disease in infant rabbits by feeding them with sonicated cholera vibrios and noticed that the small intestinal mucosa remained intact during the entire course of the infection. The late mucosal changes noted after infection and the ligated intestinal loop of rabbit could be due to pressure necrosis exerted by the massive exudate which accumulates in the confined space. In the present study, at 6 hours, following infection, there was a moderate accumulation of exudate into the loop but the mucosa was found to be intact on microscopic examination. There was no shedding of the microvilli covering the epithelial cells.

The mechanism by which V. cholerae affects the intestine is a subject of debate. It is established that the organisms do not penetrate the intact mucous membrane. They stick to the striated surface of the villi and probably produce enzymes, metabolites and toxic substances which are absorbed through the intact intestinal mucosa and cause inflammation at various sites. This leads to the sudden outpour of a large amount of fluid and salts into the lumen of the intestine (Freter and coworkers, 1961). There is still a search for an explanation on the basis of morphological alteration to account for the excessive loss of fluid.

It is suggested that increased capillary permeability is one of the early changes in the intestine following infection with V. cholerae (Formal, 1961; Spirnz, 1962). In the present study it was noted that as early as 3 hours following infection there was demonstrable submucosal oedema detectable with the light microscope. Examination under the electron microscope showed that subepithelial basement membrane was widely separated from the subjacent structures by tissue spaces containing material of low electron density. At 6 hours following infection there was a demonstrable gap between the endothelial cells of the blood vessels of the lamina propria. According to Manjo and Palade (1961) this "partial dislocation of the endothelial sheath" allows the fluid to escape more readily from the blood vessels. They also suggested that the outpour of water is far greater than can be accounted for by pinocytotic transport mechanism across the vascular wall.

It was the aim of the present work to suggest possible explanations for the source of the enormous quantity of fluid which is poured into the lumen of the intestine following infection with V. cholerae. In the normal intestinal mucosa there is a balance of pressure between the intravascular and extravascular fluids, maintained by the selective permeability of the endothelial cell wall. The effect of certain toxins, such as the V. cholerae toxin, may be to inhibit this selective permeability with the result that fluid pours out of the vessels into the extravascular spaces until the hydrostatic

pressure of the surrounding tissues equals that of the intravascular space. The saccular dilatations and openings in the lipoprotein membranes of the microvilli, that were found in the present study, are probably the routes by which the fluid leaves the epithelial cells to enter the lumen of the intestine. This evacuation of intracellular fluid prevents the rise of extravascular tension in the lamina propria. Therefore the outpouring of fluid from the vessels becomes a continuous process.

There is also impaired absorption of water from the small intestine. The epithelial cells of the terminal part of the ileum, which is the most active site for absorption of water (Scholar and Code. 1954) are the ones mainly affected in infection with V. cholerae. Although they appeared normal under the light microscope, the cells were more electron opaque. The mitochondria of these cells looked irregular and there was a loss of internal detail. These observations suggest that there is a derangement of the metabolic activity of the cell - leading to impaired absorption of water. (Ref. previous chapter).

Thus failure to absorb water in addition to the excessive fluid loss from the mucosal surface of the intestine leads to the hypovolaemea and haemo-concentration characteritic of cholera gravis.

Summary.

1. The ultra structure of Vibrio cholerae appears to be identical with those of Vibrio metchnikovii.

2. At 6 hours following the introduction of V. cholerae

into the ligated loop of rabbit ileum, when the exudation into the loop was only moderate, the mucosal changes under the light microscope were very much identical with those of enteritis noted in human disease.

3. At this stage the electron microscopic examination revealed a breaking up of the lipoprotein membrane covering the microvilli. The mitochondria of the epithelial cells appeared swollen. These as well as the cytoplasm of the cell looked more electron opaque. There were demonstrable gaps between the endothelial cells of the blood vessels of the lamina propria from where the fluid is presumed to leak into the perivascular space.

4. The sources of excessive fluid loss from the lumen of the small intestine is discussed.

REFERENCES.

1. De, S.N. and Chatterje, D.N. (1953). J. Path. Bact., 66, 559.

2. Dutta, N.K. (1964). W.H.O. Cholera Information, 1, 7.

3. Formal, S.B., Kundel, Di, Schneider, H., Kunev, N., Spirnz, H. (1961). Brit. J. exp. Path., 42, 504.

4. Fresh, J.W., Versage, P.M., Reyes, V. (1964). Arch. Path., 77, 529.

5. Freter, R., Smith, H.L. Jr., Sweeney, F.J. Jr. (1961). J. infect. Dis., 109, 35.

6. Glauert, A.M. (1962). Brit, med. Bull., 18, 275.

7. Glauert, A.M., Kerridge, D., Horne, R.W. (1963). J. cell. Biol., 18, 327.

8. Gangarosa, E.J., Beisel, W.R., Chanyo Benyajati, Spirnz, H., Prapont, Piyaratn. (1960). Amer. J. Trop. Med., 9, 125.

9. Huxley, H.E. and Zubay, G. (1960). J. molec. Biol., 2, 10.

10. Manjo, G., and Palade, G.E. (1961). J. biophys. biochem. Cytol., 11, 571.

11. Spirnz, H. (1962). Fed. Proc., 21, 57.

12. Scholer, J.F., and Code, C.E. (1954). Gastroenterology, 27, 565.

13. Wynn Williams, A. (1963). Gut, 4, 1.

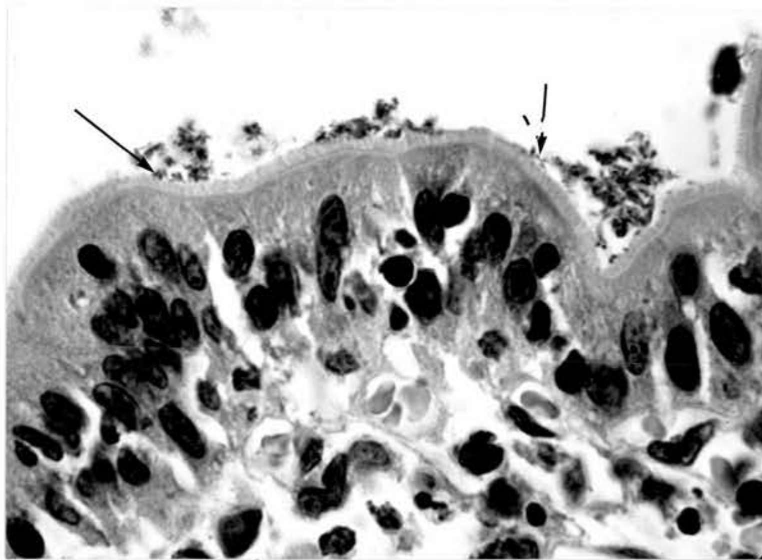


Fig. 54. Mucosal surface of rabbit ileum infected with V. cholera. Note particulate material close to the striated surface of the villi (arrows), which under E.M. showed V. cholerae (v.c.) (Below).
H & E x 1,000

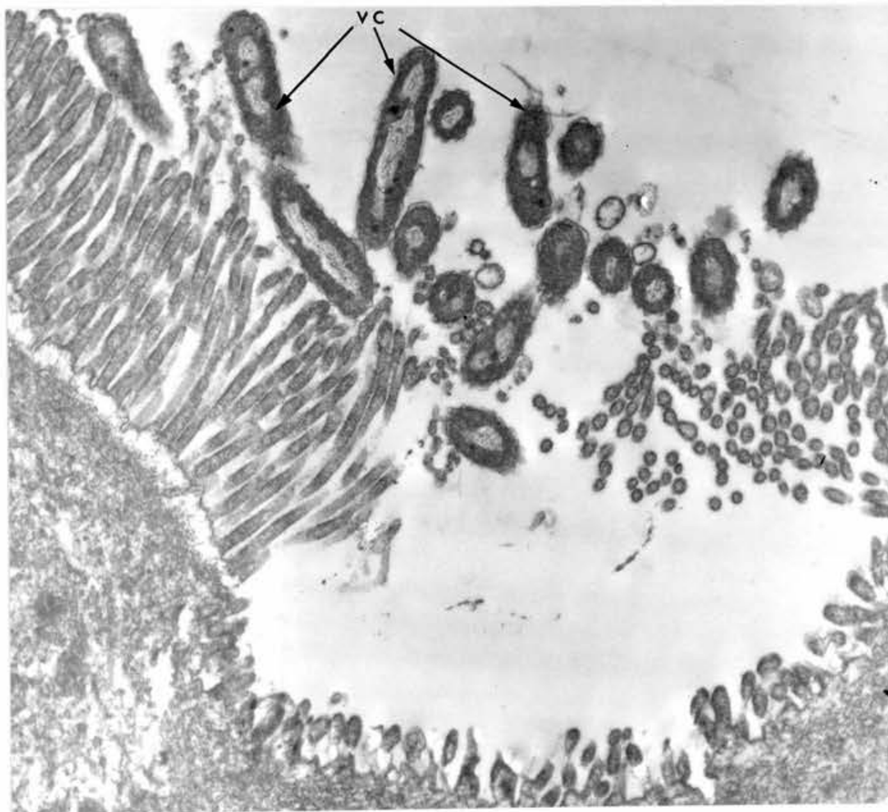


Fig. 55. Electron micrograph x 14,000

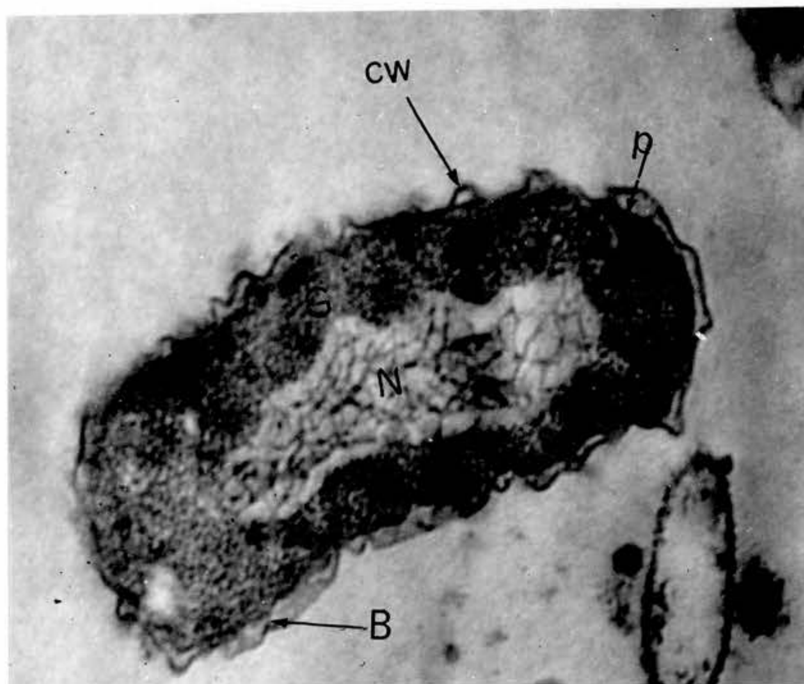


Fig. 56. Electron micrograph of thin sections of V. cholerae. CW = cell wall; p = plasma membrane; B = strand binding cell wall and plasma membrane; G = cytoplasm; N = nucleus. x 125,000

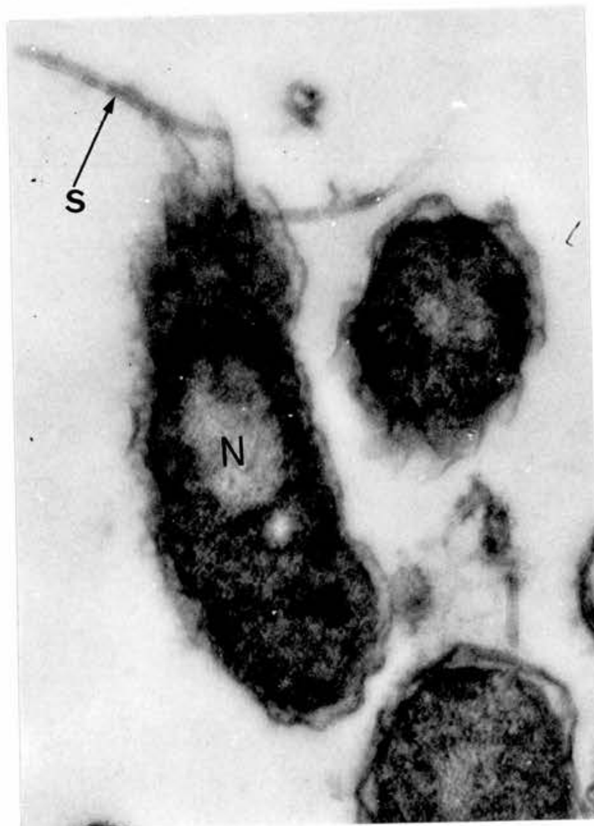


Fig. 57. E.M. of thin section of V. cholerae. S = sheath of the terminal flagellum. N = nuclear material. x 70,000

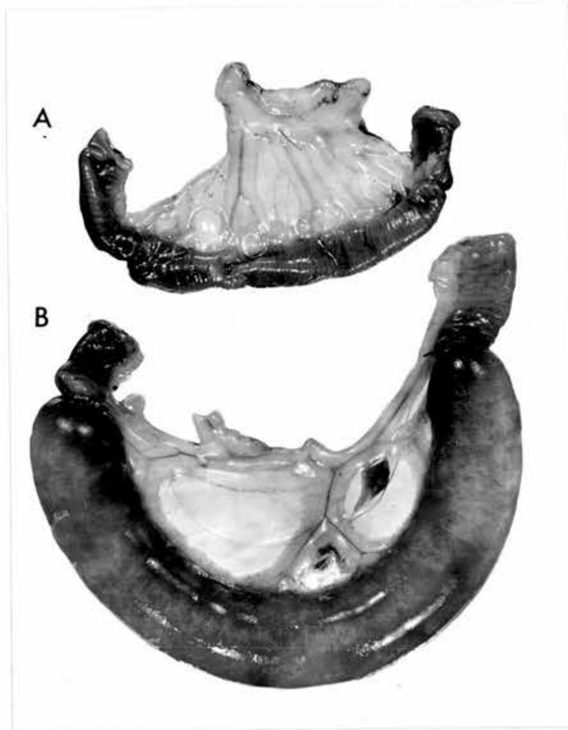


Fig. 58. Appearance of the loop of rabbit ileum after injection with V. cholerae.
A = control loop. B = positive loop at 18 hours. Note gross distension of this positive loop and patchy areas of dark colouration of the loop.

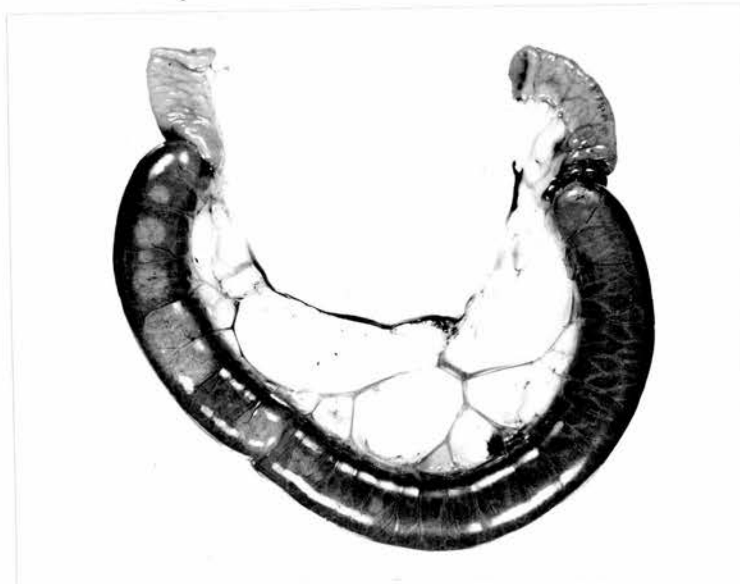


Fig. 59. Appearance of a positive loop at 6 hours.

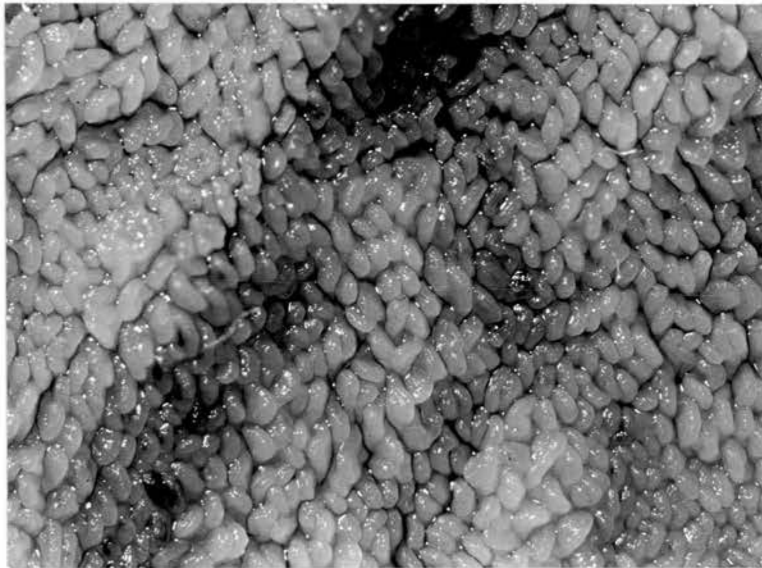


Fig. 60. Appearance of the mucosa of ileum under dissecting microscope 6 hours after injection. The villi overlying mucosal vessels look congested. x 14

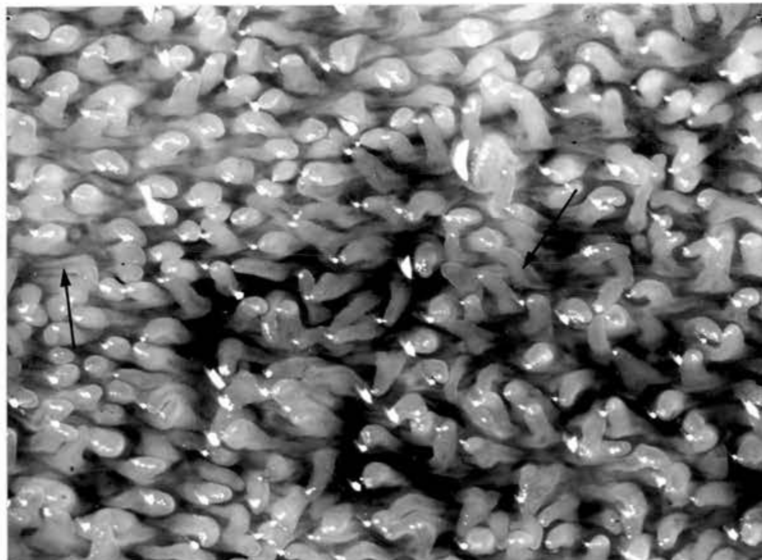


Fig. 61. Mucosal appearance of a positive loop at 12 hours. Note distension of blood vessels in the centre of the villi (arrow). Villus architecture is normal. x 14

Fig. 62. Mucosal appearance of a positive loop at 18 hours showing gross haemorrhage into the mucosa. x 14

Fig. 63. Trans-illuminated view of the above showing (arrow) haemorrhage from the larger mucosal vessels and blood clots in the mucosa. x 14

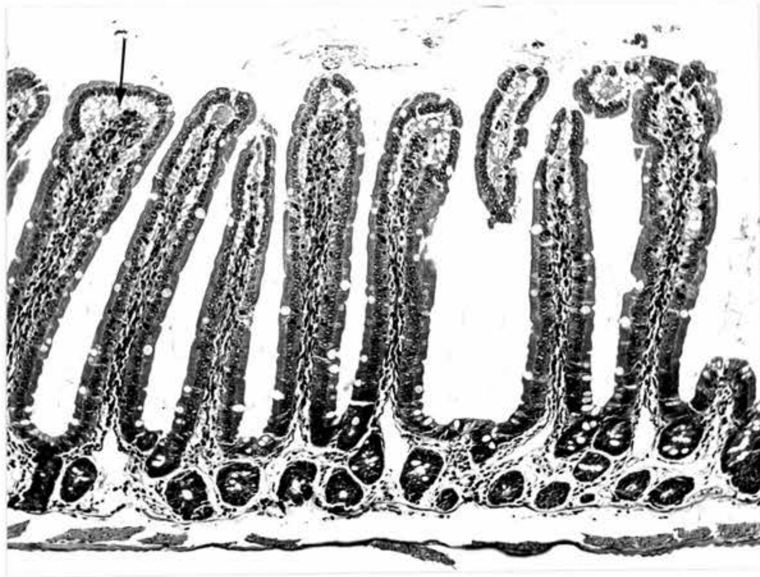


Fig. 64. Histological appearance of rabbit ileum 3 hours after infection. Normal apart from submucosal oedema (arrow). H & E x 110

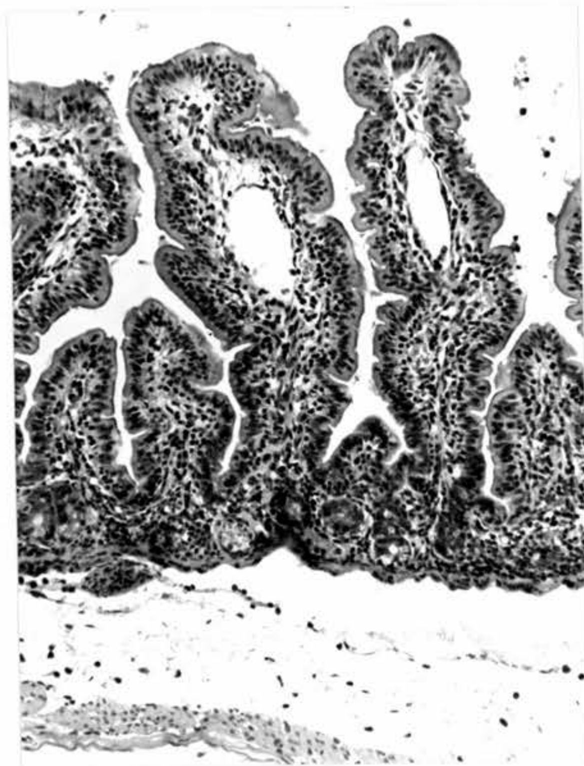


Fig. 65. Specimen from rabbit ileum 6 hours after infection. The stroma of the villi shows appearance of empty spaces and slight increase in cellular infiltration. H & E x 110

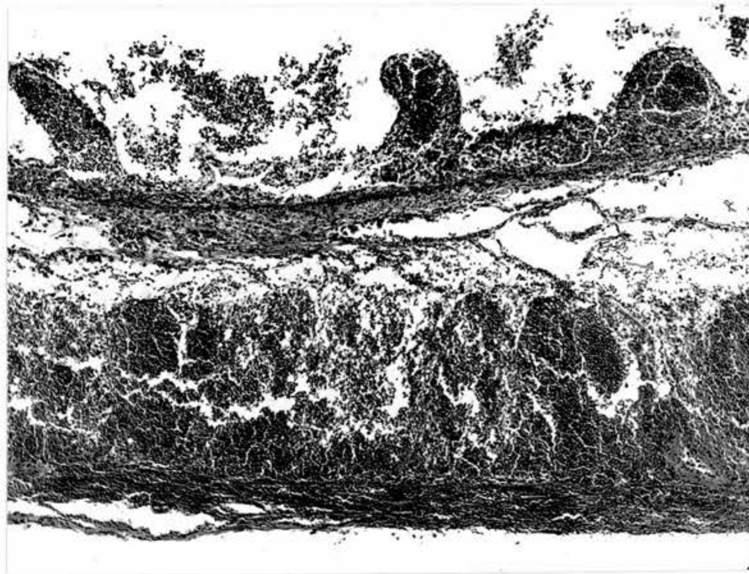
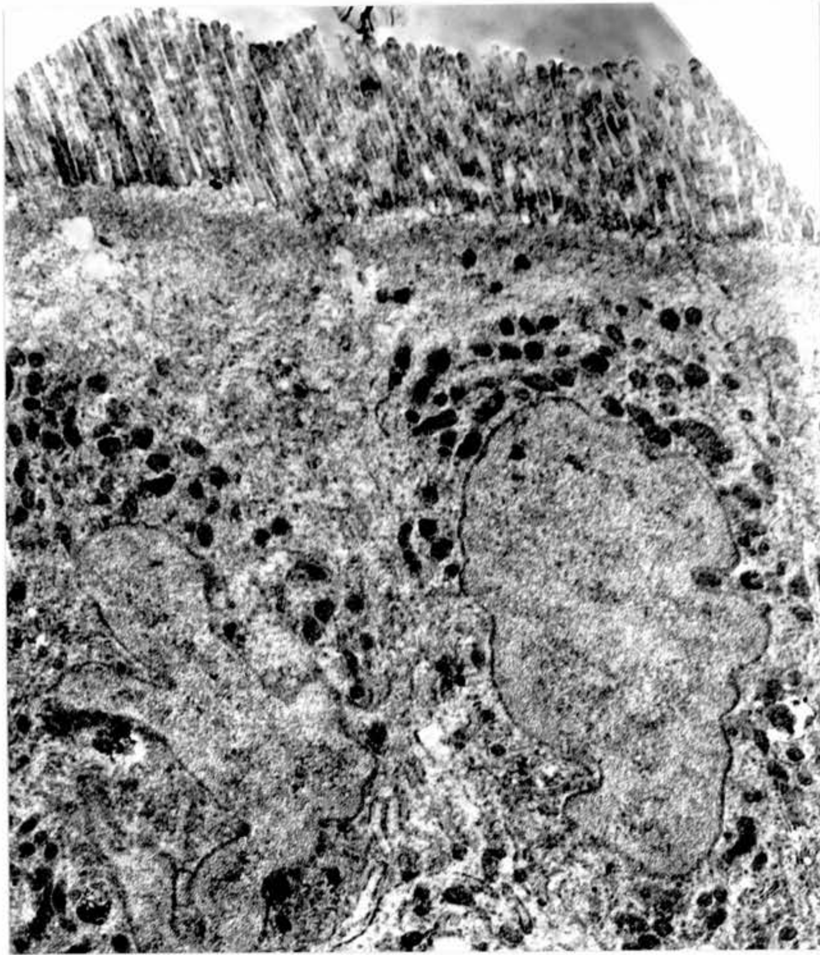


Fig. 66. Mucosal condition of a ligated loop of ileum 18 hours after infection. Note gross haemorrhage and distortion of mucosal architecture. H & E x 110



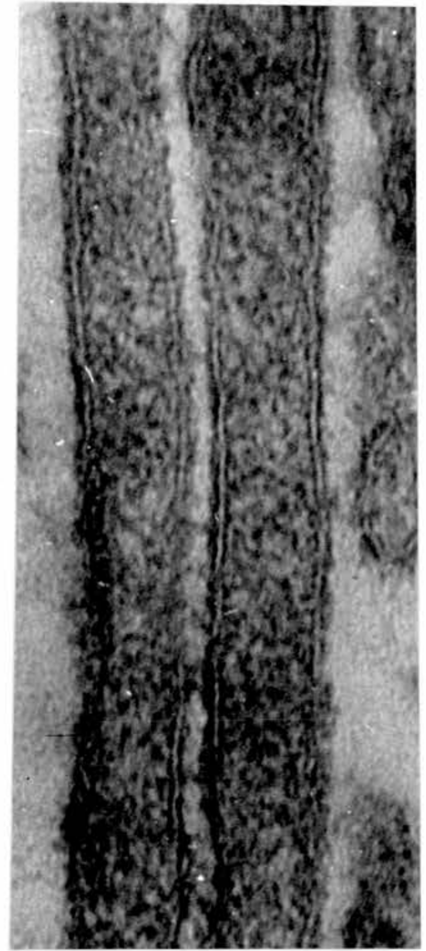


Fig. 67. x 13,800

Fig. 68. x 240,000

Fig. 67. E.M. of epithelial cell of rabbit ileum (control loop).

Fig. 68. E.M. demonstrating plasma membrane bounding microvillus. The membrane comprises of a pair of electron dense zones separated by a lighter zone.

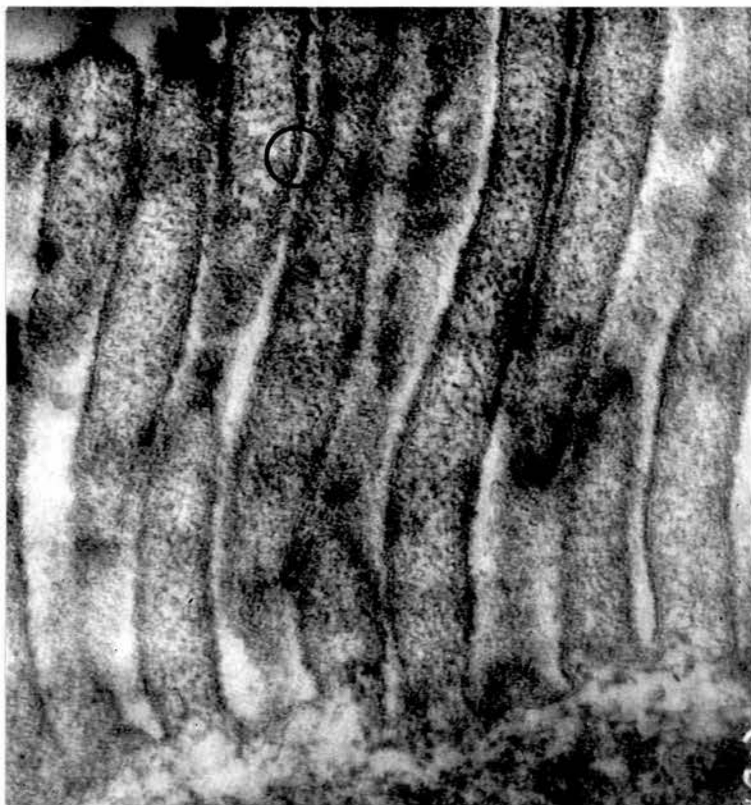


Fig. 69. E.M. of microvilli of an epithelial cell of rabbit ileum 6 hours after infection. Note small saccular dilatations in the walls of the microvilli (indicated by circle). x 120,000

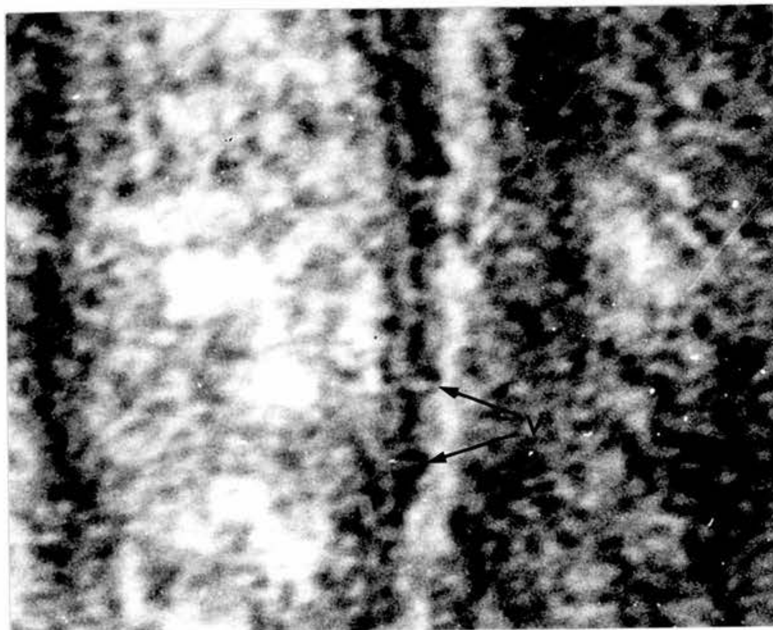


Fig. 70. Circle marked area of the above picture magnified to show the saccular dilatations (V). In places these dilated areas have ruptured communicating the core of the microvillus to the exterior. x 600,000

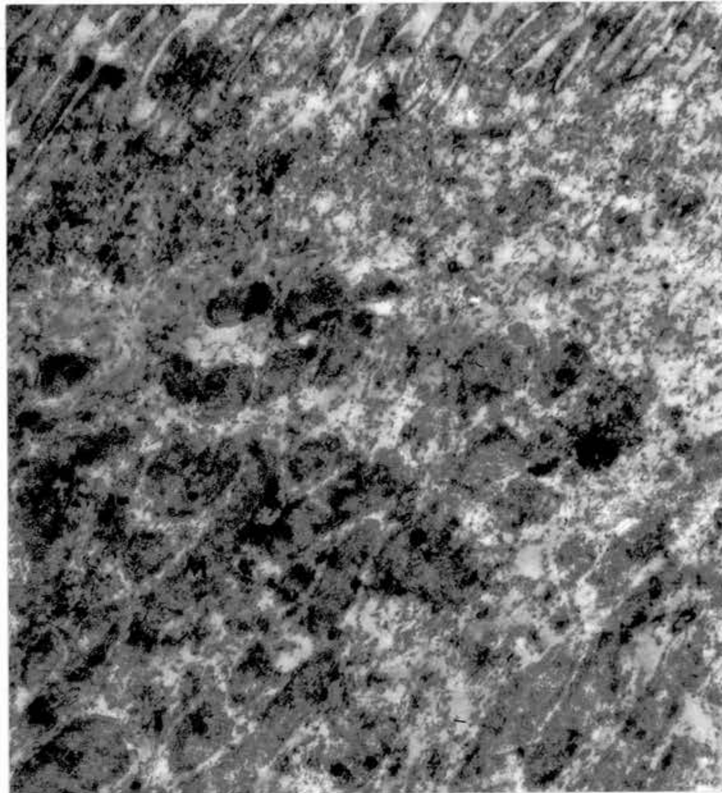


Fig. 71. E.M. of epithelial cell of rabbit ileum 6 hours after infection with V. cholerae. Note the mitochondria (M) have an irregular shape. They are swollen and their internal details are blurred. x 51,000

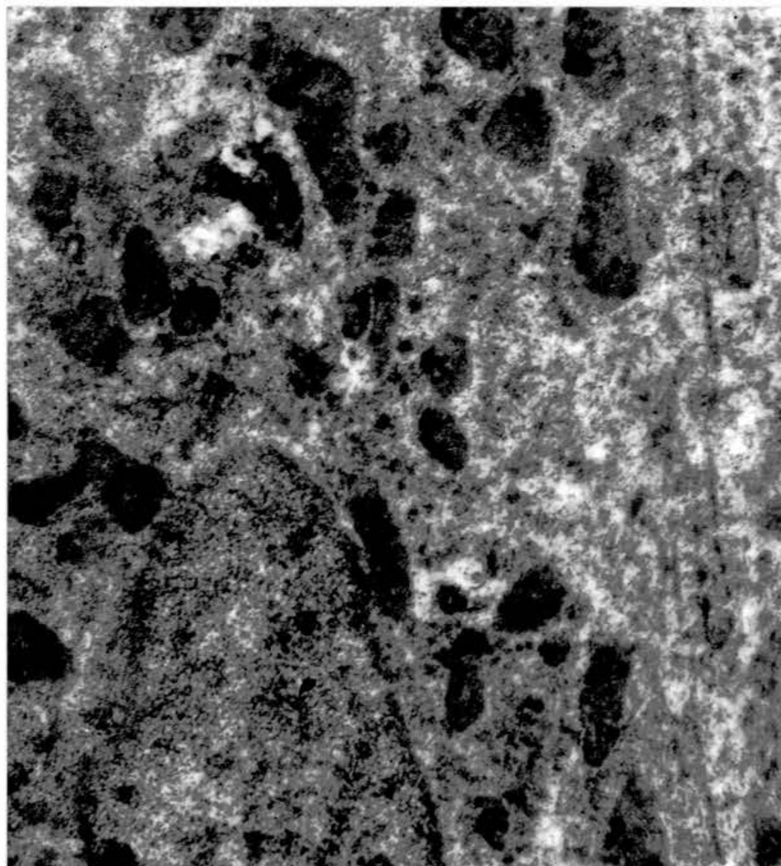


Fig. 72. E.M. Epithelial cell. x 40,000

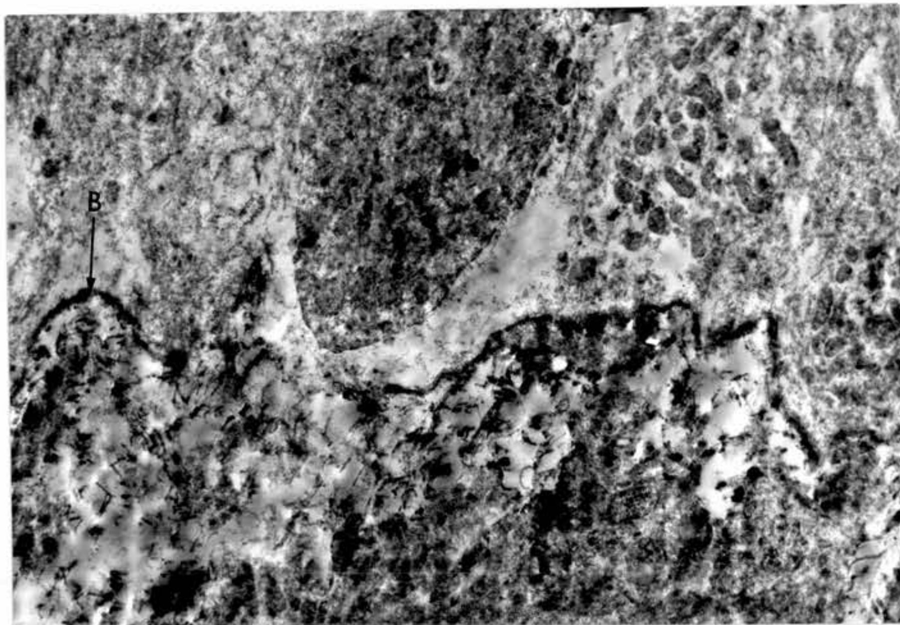


Fig. 73. E.M. of infected mucosa at 6 hours. Note increased separation of subepithelial basement membrane (B) from the structures in lamina propria (probably due to accumulation of fluid). x 16,000

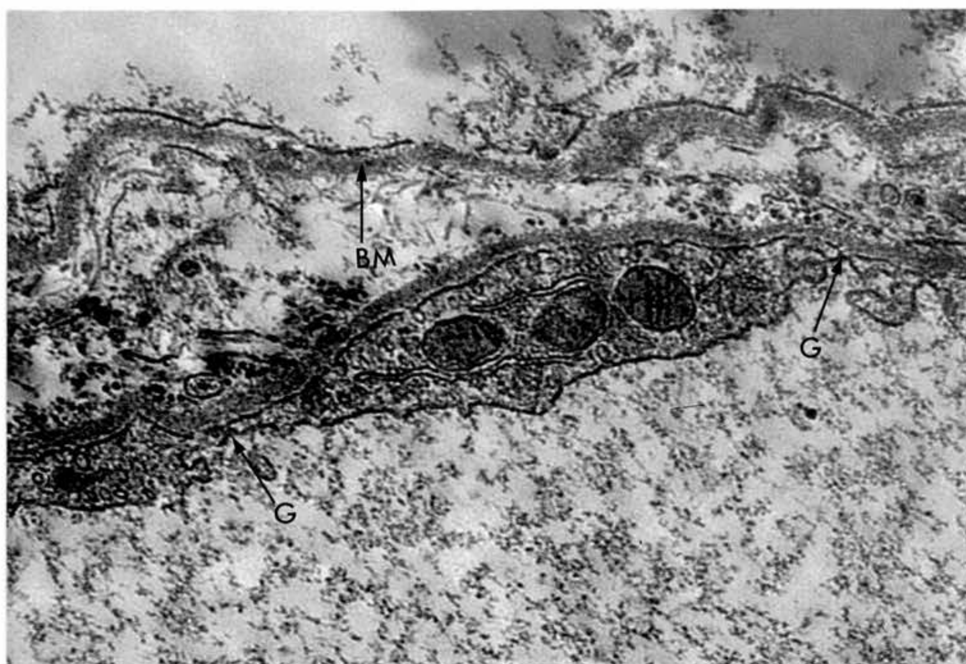


Fig. 74. E.M. of a small vessel in the lamina propria of a villus, infected for 6 hours. Note the gaps (G) between the endothelial cells of the vessel facilitating escape of water into the perivascular space. BM = subepithelial basement membrane; x 24,000

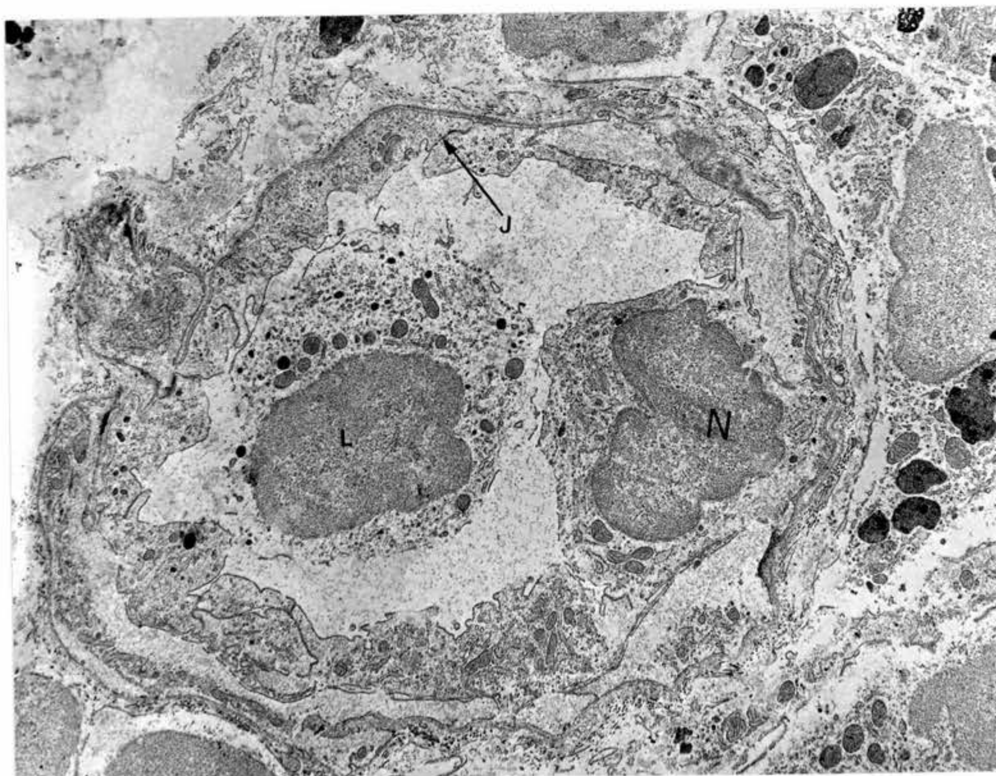


Fig. 75. E.M. of a normal small vessel. Note the endothelial cells overlap on each other (J). N = nucleus of an endothelial cell; L = lymphocyte within the lumen of the vessel. x 5,600

SECTION B.

Jejunal Mucosa in Human Disease causing Steatorrhoea.

CHAPTER 7.

JEJUNAL MUCOSA IN PRIMARY MALABSORPTIVE DISEASE

Introduction.

Mucosal affection of the proximal part of the small intestine is a usual feature of primary malabsorptive disease. This was first brought to light by Paulley in 1954. The pathogenesis is still in a speculative stage and the disease is therefore given various names (viz. adult coeliac disease, coeliac sprue, nontropical sprue, gluten induced enteropathy, idiopathic steatorrhoea). The most appropriate name is yet to come. Rubin and coworkers (1962) exposed the apparently normal mucosa of the small intestine of two symptom-free cases of coeliac sprue to wheat flour. The symptoms returned along with the alteration of mucosal morphology. Girdwood (1962) reported that although in some instances there is no definite evidence of sensitivity to gluten, these patients respond well when gluten is excluded from the diet for long periods. Defects in the enzyme system of the small intestine, which maintain the dynamic property of absorption, have been held responsible for the disease but enzymatic defects may be a secondary rather than a primary feature of the condition. (Bolt, et al, 1960; Spiro et al, 1964). An autoimmune mechanism, probably related to gluten, has been suggested by Ashworth and Cheers (1962). Despite all these efforts there is no uniformity of opinion as regards the aetiology and pathogenesis of this disease; nor is there any agreement that a constant association exists between the clinical features and the morphological

changes of small intestinal mucosa in this disease (Rubin and coworkers, 1960; Girdwood and coworkers, 1965, unpublished).

In this chapter I intend to compare the value of dissecting, light and electron microscopy in the diagnosis of primary malabsorptive disease. I will also make an attempt to show how the qualitative affection of the mucosa is as much an important factor as the quantitative affection in this disease.

Materials and methods.

Mucosal specimens from the upper small intestine were obtained from two groups of patients. The control group comprises 17 patients who had no intestinal disease. Some of them had previous gastro-jejunostomy for a duodenal ulcer, four suffered from chronic rheumatoid arthritis and one case was free from gastro-intestinal symptoms. All the specimens were examined under the dissecting and light microscopes. Only six of these specimens were examined under the electron microscope as well. The diseased group comprises 63 patients admitted to various wards of the Royal Infirmary of Edinburgh during the past five years. Most of these cases presented as cases of steatorrhoea, or megaloblastic anaemia and were diagnosed clinically and on biochemical grounds as cases of primary malabsorptive disease. (A more detailed account of the clinical data will be shortly published by Girdwood and coworkers). 35 of these cases had their biopsy taken prior to this present study. All the specimens were

examined under the dissecting and light microscopes, and 14 of these were examined under the electron microscope as well. The biopsies were obtained with the peroral biopsy capsule (Crosby and Kugler, 1957) from the upper part of the small intestine being located under fluoroscopic observation. Every attempt was made to minimise the time between obtaining the biopsy and putting it into the fixative. The delay was always within 2-3 minutes. Each specimen was placed on a piece of cork and divided into two halves. One half was cut into small pieces of ½ - 1 mm. diameter and placed in chilled buffered 1% osmium tetroxide solution (Palade's fixative) and left at room temperature for 1-1½ hours. After dehydration with graded alcohols the fragments were embedded in methacrylate or araldite. Sections were cut with glass knives and examined under AEI E.M.6. The other half of the specimen was put into 10% formol saline and examined under the dissecting microscope before it was processed for conventional light microscopy. A thick layer of mucus is often seen in primary malabsorptive disease and this binds the villi together and hides the mucosa. These specimens, whenever necessary, were treated with a mild mucolytic agent for 10-15 minutes (half saline and half glycerine and spun in a 'Blood-cell suspension mixer, Matburn Limited'). Such treatment usually cleared the mucous layer and exposed the mucosa for a clearer view under the dissecting microscope. The sections of tissue for light microscopic examination were stained with haematoxylin and eosin. Additional staining with P.A.S.

and reticulin stains were used whenever necessary.

In re-examining the older specimens there was no difficulty in obtaining sections for light or electron microscopic examination. But for dissecting microscopic examination, the tissues in paraffin blocks had to be trimmed off, put into xylol and then passed through descending grades of alcohol into 10% alcohol. After reviewing the specimens under the dissecting microscope the tissues were dehydrated and embedded in paraffin. Such a procedure, though laborious, is quite simple for re-examination of the old tissues.

Observations.

(A) Dissecting microscopic changes. Under the dissecting microscope, the mucosa of the small intestine is found to be covered with villi. These villi vary considerably in different individuals. They may be finger-shaped, leaf-shaped, ridge-shaped or convoluted. The control group presented finger and leaf shaped villi, the predominant type being finger shaped. The diameter at the base of these finger shaped villi was about a fourth or a fifth of the height of the villi. (Fig. 1). This is the most common type of villus met with in carnivorous animals and it has the ability to contract in its long axis (Verzar and McDougall, 1936). The leaf shaped villi which are flattened from side to side and have a broad base which is about onehalf to a third of the height of the villi, were usually present in less than a quarter of the total villous population of our normal subjects. Very rarely

they were found in excess of the finger shaped villi. Occasionally the leaves coalesce to form distinct ridges. Such a feature is commonly seen over the mucosa of the duodenum (Holmes, Hourihane and Booth, 1961). Ridges and convolutions which have been found to contribute to 20% and 24% respectively of the control subjects in the tropics (reported by Baker and coworkers, 1962) were absent amongst the control subjects of the present study. Occasionally leaf shaped villi held closely together by a thick mucous layer, or ridges at the edge of a biopsy give a false impression of convolutions. Baker and coworkers (1962) overcame this difficulty by slightly stretching the specimen and pinning it out on a cork. But the same result was obtained here by treating the specimen with a mild mucolytic agent as described above. In the ridge shaped villi the diameter at the base is roughly twice the height of the villi. This feature is clearly demonstrated in the photomicrograph where a ridge shaped villus is cut along the entire transverse diameter. In the case of convolutions the diameter at the base is 5-6 times the height of the villi. But when a convolution is sectioned for examination under the light microscope, because of its shape, a section cannot pass through its entire length. The villi therefore present as small separate villi.

In primary malabsorptive disease the villi lose their individual identity. In a moderate degree of mucosal affection occasionally the villi were ridge shaped or in convolutions, but mostly they were found to be held

together in small islands which looked knobbly under the dissecting microscope. This appearance is popularly called "mosaic pattern". (Fig. 83a). In an apparently earlier stage of the disease the individual convolutions were seen in these islands (convolutions in mosaic pattern) but in a later stage the convolutions merged with one another leaving behind grooves which widely separated the islands one from another. In an extreme case these knobs disappeared and the geographical contour was replaced by a featureless flat mucosa. (Fig. 84).

(B) Light microscopic changes. The villi of the small intestinal mucosa are lined by columnar epithelial cells. These cells are formed in the crypts of Lieberkuhn. The epithelial cells over the base of the villi are basophilic in their staining characteristics. They escalate along the sides of the villi and reach the extrusion zone at the tip from where they are shed into the lumen of the intestine. (Fig. 11). As they reach the tip they become more specialised and become the active site of absorption. Over the apical third of the villus, the epithelial cells differ in their morphological characteristics from the cells at the base. The cytoplasm of these matured cells looks acidophilic with basophilic strands in it. They are taller, and their Golgi region is more conspicuous (Palade, 1955). The goblet cells over the apical third of the villus are found vacuolated suggesting that due to increased activity they discharge more mucus than they can form (Florey, 1962). The lamina propria contains chronic inflammatory cells mostly

lymphocytes. An occasional plasma cell or an eosinophil cell is also seen. In between the villi the mucosa dips down and forms crypts. Paneth cells are seen at the bases of these crypts. The cells in the crypts, in addition to mucus, secrete many enzymes.

In our cases with primary malabsorptive disease both the villi and the glandular layer of the mucosa were affected. In a moderate degree of mucosal affection the villi showed marked irregularity in size and shape. They were club-shaped and showed a tendency to fuse with one another. The brush border was well preserved. The columnar epithelial cells were of normal height and had abundant cytoplasm. The nuclei of these columnar cells had a regular shape and maintained normal position. Goblet cells were inconstant in their number. A similar degree of mucosal affection in tropical sprue is accompanied by an increase in the number of goblet cells (Butterworth and Perez-Santiago, 1958). The stroma of the villi showed a moderate degree of infiltration with inflammatory cells, mostly lymphocytes, histiocytes and plasma cells and occasionally polymorphs were found. Occasionally there was a slight increase in the fibrillar element of the stroma The crypts of Lieberkuhn appeared deeper than normal. The Lieberkuhn glands showed some degree of hyperplasia. Mitotic figures were slightly increased in number. Paneth cells were normal. Peculiarly the mucosal lesion fades into the normal with no clear dividing line and it was sometimes difficult to distinguish it from the minor differences of normal villi (Deller et al, 1962). Such a

mucosal picture is popularly known as partial atrophy of the villi (Doniach and Shiner, 1957). (Fig. 83b).

In a severe degree of mucosal affection the mucosal surface looked almost completely flat. The villi were shorter still and they were replaced by mucosal folds over most areas. These shorter villi showed a peculiar tendency to fuse in the subapical region. The brush border looked irregular and was absent in places. The columnar epithelial cells were sometimes reduced to about half of the normal size. Their nuclei looked round in shape and were basally situated. The supranuclear cytoplasm sometimes showed vacuolation. The epithelial cells were infiltrated with chronic inflammatory cells which made the epithelial cells irregular and gave the impression of overcrowding of the nuclei. Thus the mucosa appears to be lined by pseudo-stratified columnar epithelium. The surface goblet cells appeared to be reduced in number. The sparsity of goblet cells in the surface epithelium was in contrast to their normal number in partial atrophy of villi. The stroma of the villi showed an increase in fibrous element and showed more infiltration with chronic inflammatory cells. There was a curious increase in the number of plasma cells. A homogeneous acidophilic material seemed to encroach upon the subepithelial basement membrane, and induced the separation of the epithelial cell from the stroma of the villi. The mitotic figures were very much increased in number and sometimes even the cells covering the surface of the mucosal folds showed mitotic activity. The crypts

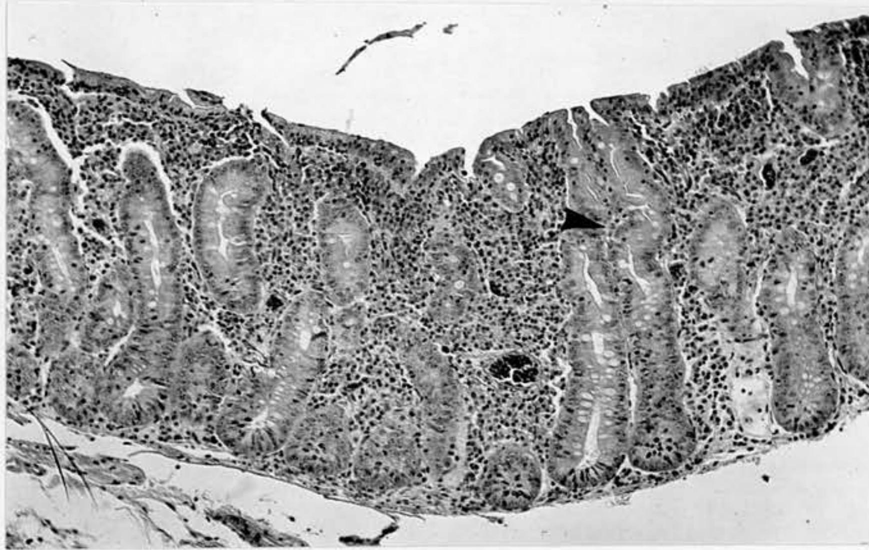


Fig. 76. Histological appearance of flat jejunal mucosa. Indicator points at racemose character of the hypertrophied glands. H & E x 80

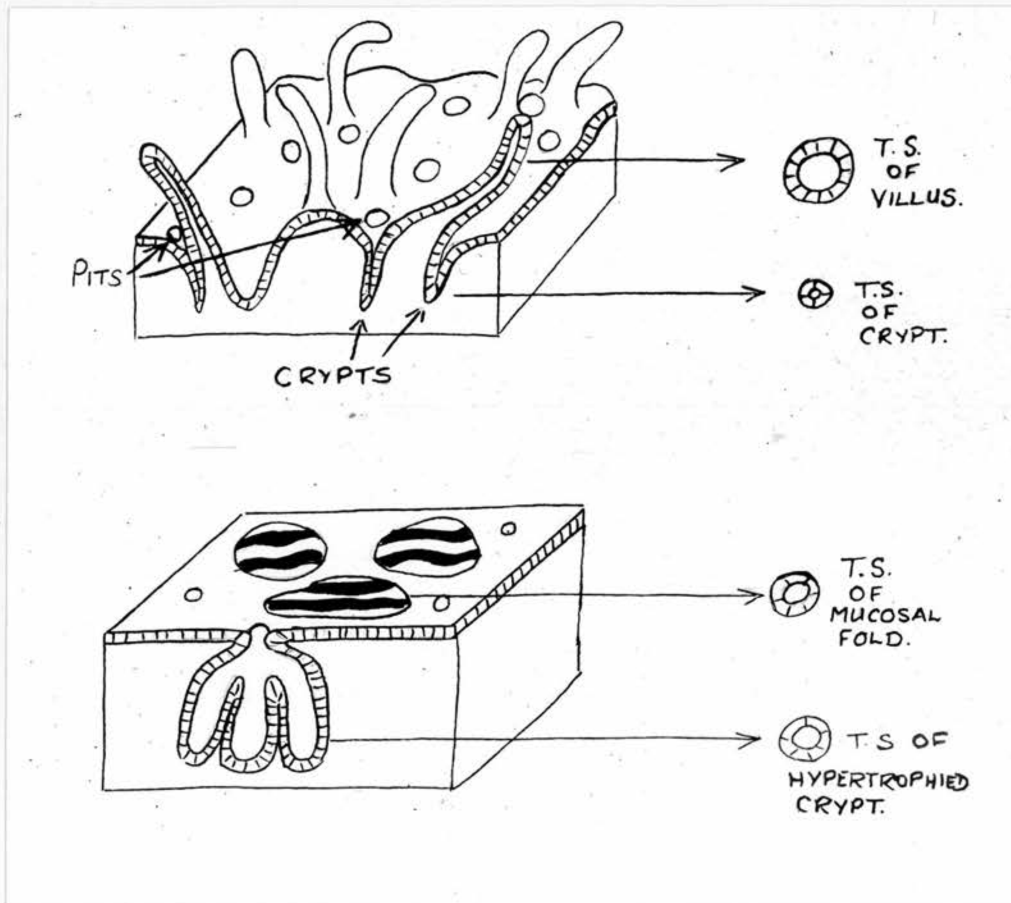


Fig. 77. Diagramatic representation of small intestinal mucosa in health (top) and in primary mal-absorptive disease (bottom). Note reduction in number of adult cell population and increase in number of crypt cell population in the latter condition.

of Lieberkuhn from where regeneration of epithelial cells takes place, were elongated, dilated and tortuous. The glands were often racemose in character, that is, two or three glands opening into one crypt. In primary malabsorptive disease the basic regeneration potential of the intestinal mucosa remains unaltered (Ashworth and Cheers, 1962) but due to a maturation arrest of epithelial cells these cells disappear from the surface at a greater rate than normal (Padykula et al, 1961). This might initiate a vicious circle and the gland tend to regenerate more cells to cope with the loss. (Figs. 76 and 77). Occasionally, Paneth cells did not appear very prominant in this type of mucosa. Such a type of mucosa, although often showing complete absence of villi is popularly called subtotal villous atrophy (Doniach and Shiner, 1957). (Fig. 85).

The conventional classification of the degree of mucosal affection is based on the measurement of villous height only. Villi between 300µ - 150µ are considered to indicate partial villous atrophy and where the villi are below this range, subtotal villous atrophy (Doniach and Shiner, 1957). Blunting of the villi may be seen as an artefact when the plane of section instead of being perpendicular to the mucosal surface is cut slightly askew (Rubin et al, 1960). Moreover partial villous atrophy (according to the above criteria) is difficult to distinguish from the minor abnormalities of the villi that occur in the normal individual (Dellar et al, 1962). To avoid these fallacies in this series the heights of both

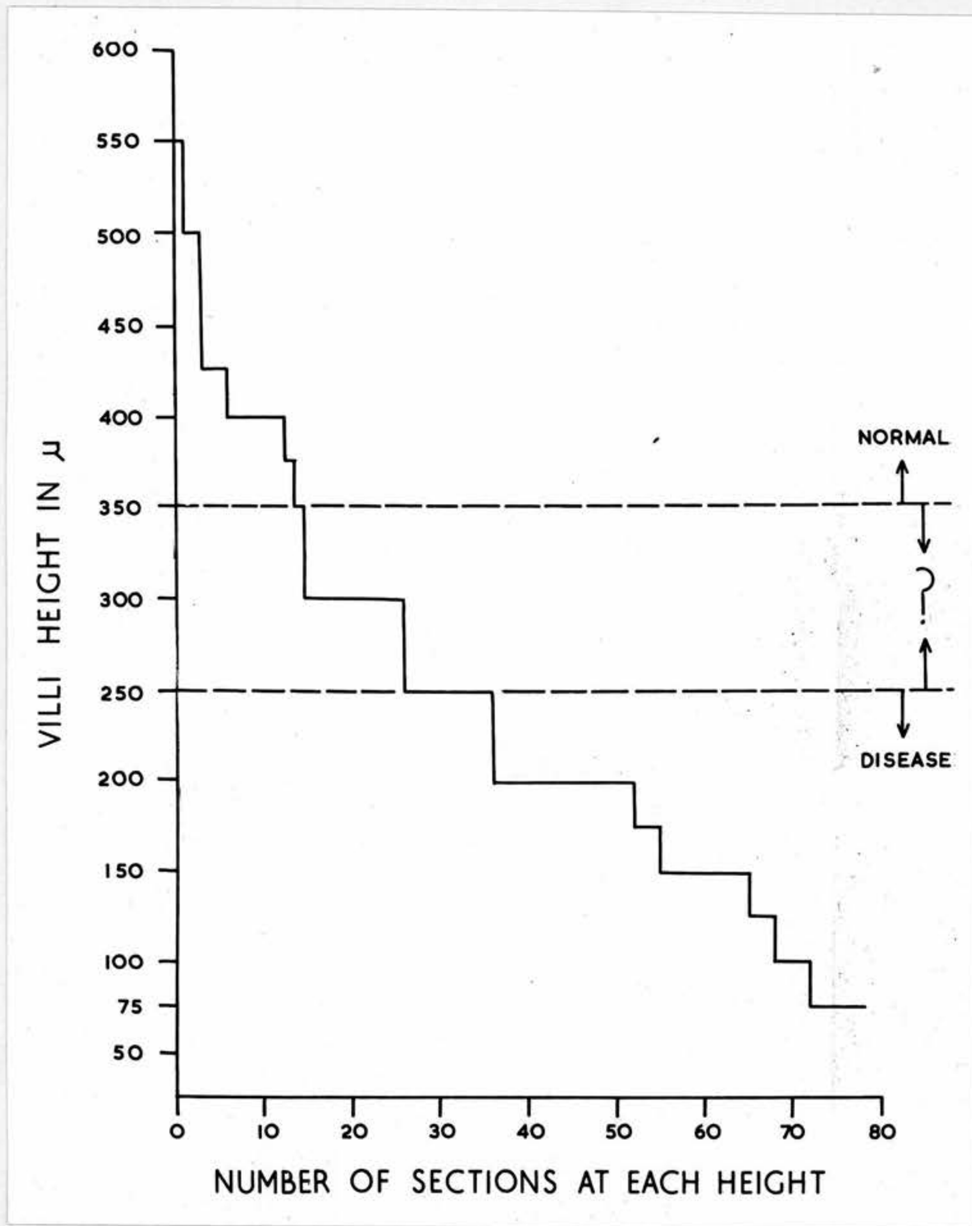


Fig. 78. Comparison of the heights of the villi in normal and in primary malabsorptive disease ("?" indicates doubtful range).

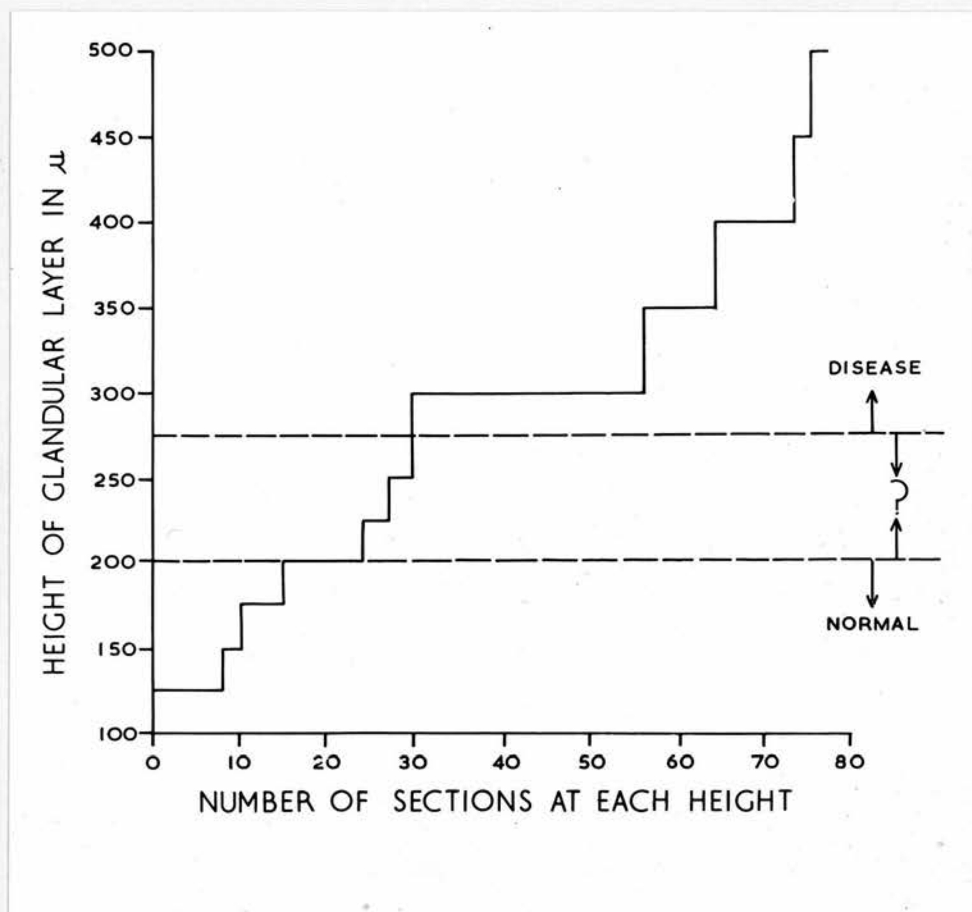


Fig. 79. Comparison of the heights of the glandular layer in normal and in primary malabsorptive disease. ("?" indicates doubtful range).

the villi and the glandular layer were taken into consideration. Villous and glandular heights of both normal and abnormal cases from our series were plotted (Figs. 78, 79, and 80). From the graphs it was assessed that the range of normal villous height was between 550-350µ and the range of abnormal villous height was between 250-75µ. (Fig. 78). In a similar evaluation of gland height it was found that the normal height of the glandular layer was up to 200µ. Beyond 275µ they were considered abnormal. (Fig. 79). Some cases showed villi and,/or gland heights to be between the abnormal and normal ranges, that is, in a doubtful range. In a properly orientated specimen, if either villi height or gland height is normal and the other is in the doubtful range then electron microscopic studies should be done or another biopsy should be obtained as in the following two examples.

<u>Example 1.</u>

	Height of villi in µ	Height of glandular layer in µ
Mrs. M.	300 ?	200 ?
	200 ab.	180 N
	200 ab.	200 ?
	450 N	250 ?
	400 N	250 ?
	400 N	250 ?
	320 ?	280 ab.

<u>Example 2.</u>

A.R.	250 ? ab.	100 N
	250 ? ab.	100 N
	300 ?	150 N
	300 ?	150 N

N = normal. ab. = abnormal. ? = doubtful.

Example 1 shows that the majority of gland heights lie in the doubtful range. The villi height showed normal,

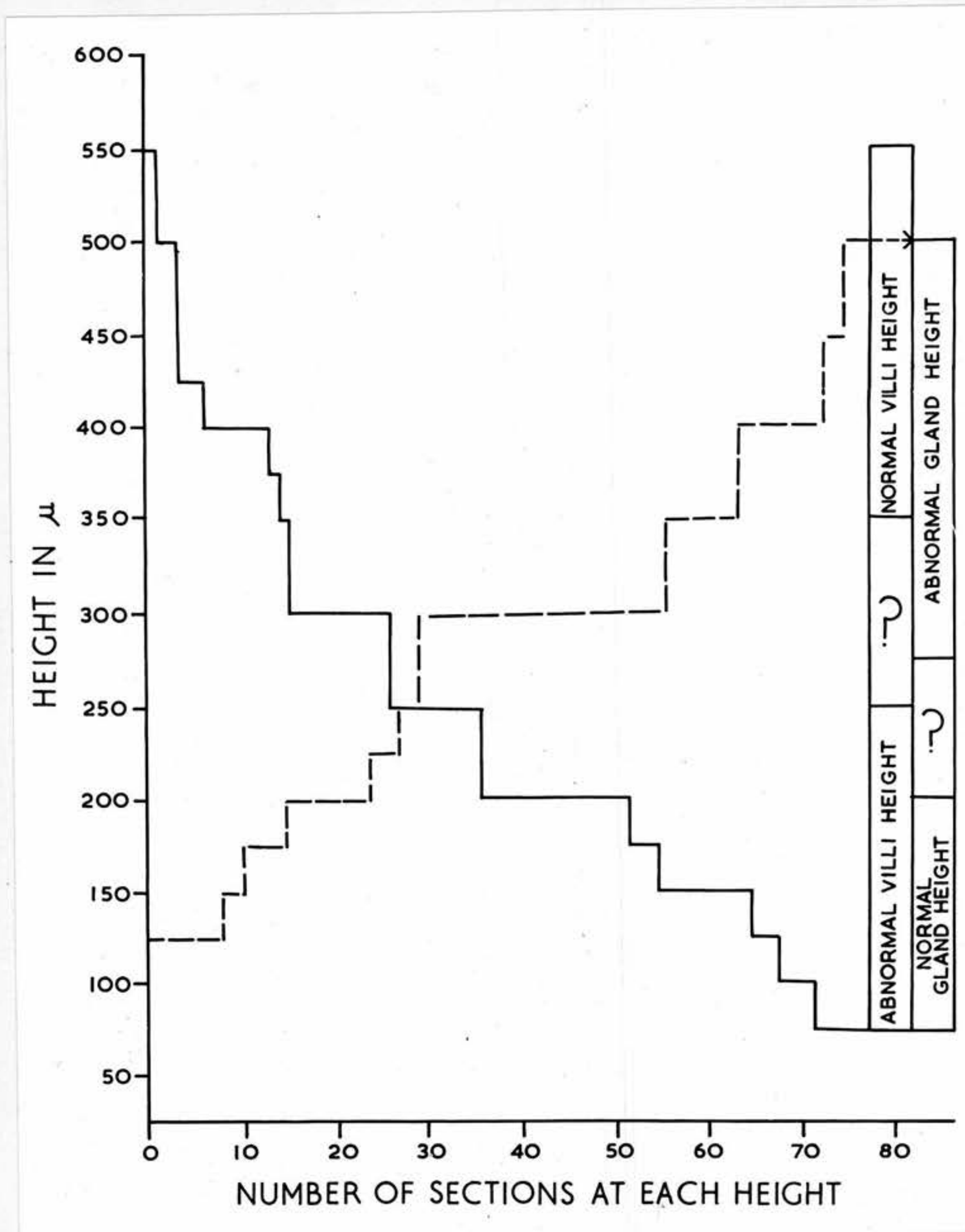


Fig. 80. Opposite behaviour between the heights of villi and glandular layer of intestinal mucosa in primary malabsorptive disease. The mucosa of three cases from the 'doubtful range' were found to be abnormal under the electron microscope.

abnormal, and doubtful values. In example 2 the glands were of normal height and the villi height were found to be doubtful or abnormal. These mucosae were examined under the electron microscope and abnormalities were noted. (Figs. 88 and 89). By the careful use of these two graphs (Fig. 80) the amount of material requiring electron microscopic examination can be cut down. Micrometric studies take an average 2 days and therefore this can be used in conjunction with biochemical tests to confirm the diagnosis of primary malabsorptive disease quickly.

(C) Electron microscopic changes. Under the electron microscopic examination, the absorptive surface of the epithelial cells over the apical third of normal villi present tall microvilli which are closely packed. (Fig. 91). These increase the absorptive surface that comes in contact with the intraluminal nutrients. The microvilli over the base of the villi are shorter, broader and fewer in number. (Brown, 1962). In primary malabsorptive disease affection of the microvilli is oneoof the cardinal morphological characteristics. These microvilli get shorter and irregular in shape and in their density. (Fig. 92). Often a thick layer of mucus was seen to cover the microvilli. This mucous layer in addition to preventing the nutrients from coming in contact with the absorptive surface, bind the microvilli together and diminishes their absorptive area(described in detail in the next Chapter). Over the terminal part of the cell, just below the attachment of the microvilli, the fibrillar element is

uniformly spread as a web. This was another site constantly affected early in the disease process and its involvement might precede that of the microvilli. In a severe degree of mucosal affection the terminal web was less distinct and might even be missing. The reticular arrangement of the terminal web may govern the formation of the pinocytotic vesicle, because, along with affection of the terminal web substance, the pinocytotic vesicles are found to be smaller and less capable of accepting lipid particles (Ashworth and Cheers, 1962). The mitochondria of the supranuclear cytoplasm were affected earlier than those of the infranuclear region. They looked swollen and round. The outer limiting membrane showed a tendency to fuse with the inner membrane. The internal structure was completely disorganised and the swollen matrix appeared in places to encroach over the cristae. (Figs. 93 and 94). Occasionally, one noticed rudimentary cristae inside the mitochondria and the rest of the substance was occupied by an electron opaque material. In addition to the altered mitochondrial morphology large cytoplasmic bodies were noticed in the supranuclear region. (Fig. 95). Section through one of these bodies showed a lamellated appearance (Fig. 96). Similar oval dense bodies (probably lysosomes) in the supranuclear cytoplasm have been reported by Ashworth and Cheers,(1962). Novikoff and Essner (1962) mentioned that these cytoplasmic bodies are seen in reversibly injured cells or regenerating tissues of liver and kidney.

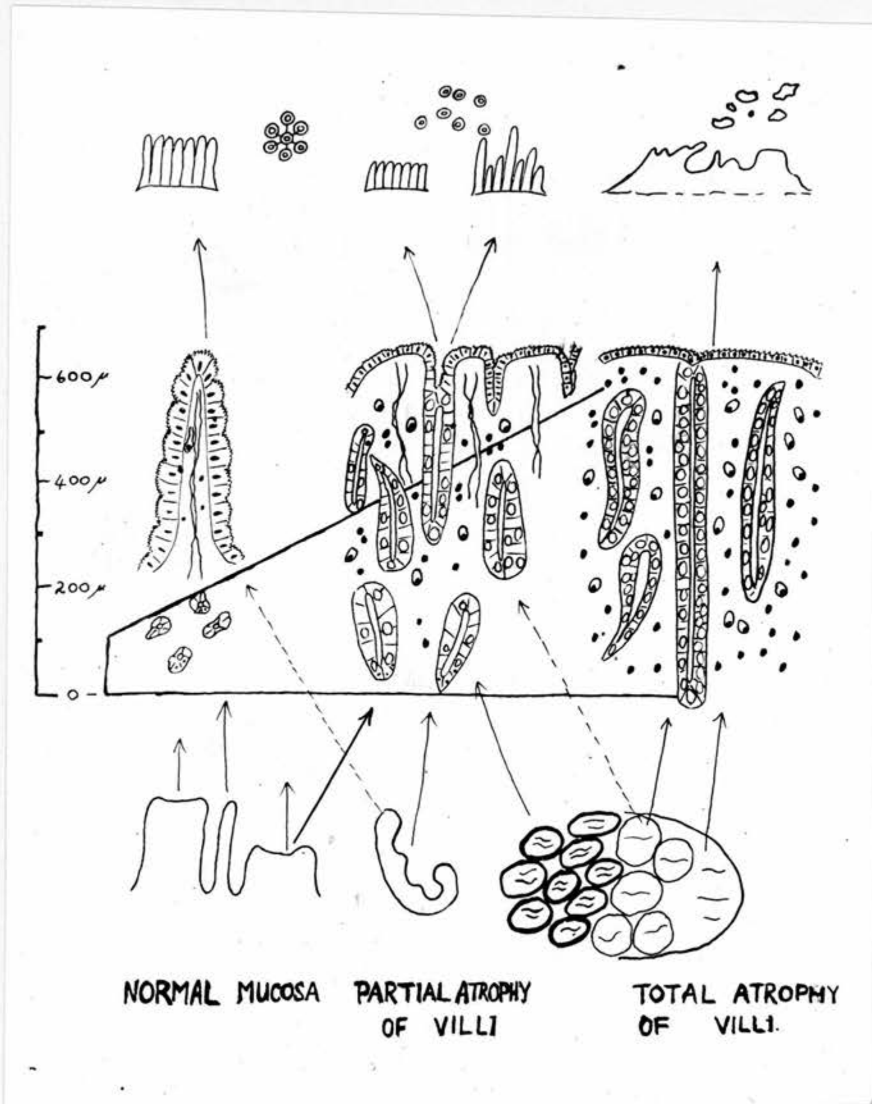


Fig. 81. Schematic diagram illustrating correlation between the appearances of mucosa in primary malabsorptive disease under the dissecting, light and electron microscopes.

The nuclei of the epithelial cells appeared smaller. As regards nuclear change, electron microscopy did not add to that which can be observed under the light microscope. But the electron microscopic examination revealed that in an early case, when microvilli and the mitochondria of the supranuclear cytoplasm were affected the nuclei were found to be normal. This suggested that a local injurious substance is the initiating factor and it is not the cell which is at fault to start with. Mononuclear cells were seen to invade between the basal parts of the epithelial cells; a feature already noted under the light microscopic examination. In a minimal degree of mucosal affection the subepithelial basement membrane was found to be unaffected and had maintained its uniformity of thickness. (Figs. 97 and 98). But when the mucosa was grossly affected it was found to be broken, discontinuous and might be absent altogether in places. Slightly electron opaque material was seen to be deposited below the basement membrane separating it from the subjacent structures. A similar observation was reported by Curran and Creamer (1963).

Discussion.

The superiority of dissecting microscopy as a tool for routine diagnosis.

In the present work the most striking fact which came to light was that in about 95% of the cases reviewed the mucosal abnormality in primary malabsorptive disease was noted under the dissecting microscope, this being confirmed by light and electron microscopy. (Fig. 81). In an

extreme degree of mucosal affection the picture under the dissecting microscope was a featureless flat mucosa or a flat surface which had a knobbly appearance. The light microscopic appearance of such a mucosa showed that there were no true villi over such mucosal surfaces but they were instead replaced by mucosal folds. At this level of mucosal affection there was a curious coincidental increase in mitotic activity of the epithelial cells and an increase in the plasma cells of the lamina propria. The glandular layer was markedly hypertrophied and the Paneth cells appeared to be less prominent. Electron microscopic examination showed that the microvilli have variable lengths, density and might be absent over some areas. Cross sections of the microvilli showed that in addition to the loss of hexagonal array the circumference of each microvillus had altered. The terminal web was grossly affected. When the mucosa was less severely affected the individual villi could be identified as a convolution or convolutions held together in mosaic pattern. The light microscopic appearance of this mucosa revealed abnormally shaped villi which were shorter and broader (club-shaped). There was a moderate increase in infiltration with mononuclear cells, predominantly lymphocytes, into the lamina propria. The glandular layer was moderately hypertrophied. Electron microscopic examination showed that the microvilli were regular in shape and even contoured. They were either variable in length (some tall, some short) or uniformly shorter (about a third of normal size). Transverse sections of the microvilli showed that although

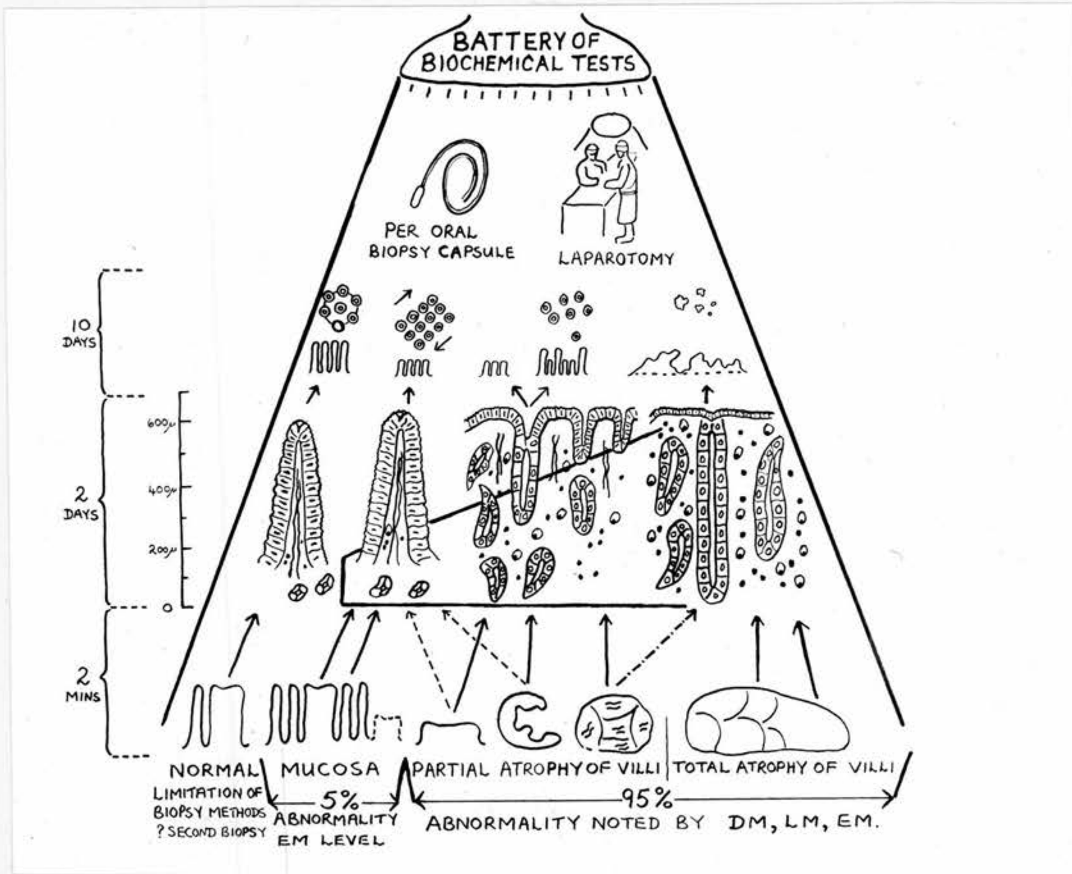


Fig. 82. Diagramatic representation of the available methods of investigation into primary malabsorptive disease: the efficacy of the different types of microscopy in the diagnosis of the disease.

the microvilli have maintained uniformity of dimensions their hexagonal array was lost. At this stage the terminal web was only moderately affected.

In this series there were three cases (5 per cent) of primary malabsorptive disease as established by biochemical investigation which exhibited a normal appearance, under the dissecting and light microscope. The only abnormalities noted under the electron microscopic examination were uniformly small sized microvilli and an increase in translucency of the terminal web substance. To arrive at these conclusions, electron microscopy took on an average ten days per case. In these cases dissecting microscopy took 2 minutes and the full process for light microscopic examination took 2 days. (Fig. 82).

The time has now come to make a comparative study of the relative efficacies of the microscopic methods available to us for diagnosing and following up cases of primary malabsorptive disease. From the present comparative study it is quite evident that the dissecting microscope in addition to being a simpler and more easily used diagnostic tool, can be easily accomodated in the clinical sideroom of the ward and can give us as much information as the light and electron microscopes in 95% of cases. (In the remaining 5% of cases where the electron microscope has shown its superiority, it has nevertheless contributed little to treatment which was in any case based on biochemical evidence). The dissecting microscope can view a field of mucosa which is about 100 times or more than the field examined under the light microscope.

It presents a three dimensional impression of a diseased mucosa (viz. mosaic pattern) which impression can not be obtained from light microscopy. Even in a normal mucosa a two dimensional view (from a histologic section) of a finger or leaf or even a ridge shaped villus looks identical. These differences in villous pattern cannot be differentiated in histologic sections (McCarthy and coworkers, 1964). In the light microscopy an improperly orientated specimen or a tangential cut could give a normal villus an abnormal shape (Rubin, 1961). Such interpretive difficulties could be avoided by the dissecting microscope. Coming back to the diseased mucosa, a preliminary screening under the dissecting microscope can easily separate a grossly abnormal mucosa from an apparently normal one. The latter only, should then be processed for electron microscopic examination.

The question now arising, is whether electron microscopy has got any place in the diagnosis of primary malabsorptive disease? Only a few cases will present where dissecting microscopy and light microscopy are not of much help and the biochemical findings are equivocal. For such cases, it is now left to ultramicroscopic studies to prove their efficacy in detecting the early lesions. In the presence of positive biochemical evidence, failure to detect mucosal abnormality under the electron microscope does not, however, exclude the possibility that the biopsy could have been obtained from a normal area of a diseased intestine.

Altered mucosal pattern: a non-specific change.

Several diseases each with a different aetiology can present similar morphological pictures. The mucosal changes seen in primary malabsorptive disease are also found in a variety of other pathological conditions, viz.- tropical sprue (Butterworth and Perez-Santiago, 1958); coeliac disease (Zetterquist and Hendrix, 1960); ulcerative colitis (Salem et al, 1964); Crohn's disease (Shiner and Drury, 1962); Lymphosarcoma involving small intestine (Sleisenger et al, 1953); anaplastic carcinoma and reticulum cell sarcoma (Shearman et al, 1962); adenocarcinoma (Premysl Eric et al, 1963); post gastrectomy malabsorption syndrome (Ashworth and Cheers, 1962); Whipple's disease and some steatorrhoeas of unknown aetiology (Chapter 10); following acute bacterial enteritis (King and Joske, 1960); following acute bacillary dysentery and Asiatic cholera (Gangarosa et al, 1960; Spirnz et al, 1962); in acute epidemic hepatitis (Astaldi et al, 1964); in intestinal infestation with Giardia lamblia and strongyloidosis (Da Silva et al, 1964); hookworm disease (Sheehy et al, 1962; Salem and Truelove, 1964); following neomycin therapy (Jacobson and associates, 1960); following 4-aminopteroyl glutamic acid (Wynn Williams, 1961); and following mesenteric artery occlusion (Cameron and Khanna, 1959). In none of these caseswas there a report of concommitant hypertrophy of the glandular layer and increase in the mitotic activity of the epithelial cells which are commonly noted in primary malabsorptive disease. These

features might be present in the mucosa affected by the malignant tumours of the small intestine - where the possibilities of predisposition of such mucosae to malignant changes cannot be excluded.

Quantitative versus qualitative studies.

Mucosal affection of the small intestine in primary malabsorptive disease is very patchy. Girdwood and co-workers (1961) noted normal mucosae in about a fifth of cases. They argued that by the peroral biopsy technique a sample could be obtained from a relatively less affected area. Therefore a single normal biopsy does not exclude the possibility of this disease. McCarthy and coworkers (1964) examined specimens of mucosa obtained from various levels of the small intestine in a patient with non tropical sprue. They noticed severe mucosal affection in the proximal part of the small intestine. The mucosa was found to be mildly affected over the intermediate part and almost normal over the lower ileum. Even in a single biopsy specimen under light microscopic examination varying grades of mucosal affection are present. (Deller et al, 1962). One often notices a transition from rudimentary villi to an area of mucosa devoid of villi. Shiner and coworkers (1962) have gone a stage further to show the patchy nature of mucosal affection. Under electron microscopic observation they noted that a cell with normal microvilli was found adjacent to a cell with grossly abnormal microvilli. From the present work it was noted that in addition to a normal and an abnormal cell lying side by side the abnormal and normal

cells could alternate over a wide area, or there could be a group of normal cells next to a group of abnormal cells. (Figs. 99, 100,101 and 102).

The mucosa of the small intestine is specially constructed to increase the absorbing surface to cope with the demand. In recent years there is a growing tendency to measure the total absorbing surface of the small intestine. Attempts have been made by various authors to estimate the quantitative loss of absorptive surface in the malabsorptive diseases affecting the small intestine. Butterworth and Perez-Santiago, (1958) came to the conclusion that in tropical sprue the affected mucosal surface of an untreated subject is about a forth of the normal subject. Ashworth and coworkers (1961) demonstrated that in non tropical sprue microvilli are reduced in number to about one half to one third of the normal. Rubin and coworkers (1958) suggested that in both coeliac disease and idiopathic sprue the reduction of absorptive surface of the small intestinal epithelium might account for malabsorption.

Contrary to the above observation Flint (1912) and Althausen et al (1950) showed that normal small intestinal mucosa has a great compensatory capacity. Following massive resection of the small intestine there is a functional increase in the absorptive capacity . The absorptive area of the small intestine could be increased by even 400 per cent (Flint, 1912). Althausen et al (1950) showed that in man when only 15% of the small bowel is retained, functional balance is achieved, without

much difficulty. In primary malabsorptive disease the involved mucosa is mostly limited to the upper half of the small intestine. The mucosa of the ileum does not usually show any microscopic abnormality. Had there only been a quantitative involvement of the mucosa the biochemical balance could be easily obtained. Since there is a failure to obtain a biochemical balance it seems that the mucosal cells are also affected qualitatively. Moreover microscopic methods are inadequate to assess correctly the quantitative mucosal loss in this disease. The dissecting microscope is greatly limited in giving an accurate estimation of the overall mucosal affection, and the two dimensional view under the light microscope is still less adequate for such information. An affected epithelial cell under electron microscopic examination presents microvilli which are very dissimilar in their dimensions. The electron microscope has also revealed that the cell surface is found to have a dynamic structure of some depth. Therefore any attempt to assess the mucosal loss in this disease by measuring the microvilli will give inaccurate information. The time has now come to lay stress on the qualitative affection of the cell in this disease. The absence of pinocytotic vesicles in the apical part of the diseased cell, the inability of the cell to perform pinocytosis along with the affection of the terminal web region of the cell suggests that the molecular distortion of lipoprotein membrane and of the terminal web must be playing a major role in guiding the pathology of the diseased cell. These

morphological alterations according to Spiro and coworkers (1964) are followed by the enzymatic defect. Thus the mucosa, in this disease is covered with a peculiar surface epithelium which has morphological and biochemical deficiencies.

Role of gluten: allergic hypothesis.

It is now a well known fact that gluten is in some way related to the pathogenesis of malabsorptive disease, though the exact mechanism by which it brings about changes in the mucosa of the small intestine is not yet known. Skin tests to gliadin and other compounds when performed in patients with coeliac disease are found to be negative (Sheldon, 1955; Kramer and Weijers, 1955). This could suggest that an antibody to gluten might be localised to the site of absorption. Frazer and coworkers (1959) autoclaved an enzymatic metabolite of gluten and made it free from any antigenic properties. When such a substance was given, it still produced harmful effects on the mucosa. Hawkins (1961) suggested that such an experiment does not exclude the possibility of a local immunochemical process apart from allergy. He has also described how often the disease fails to respond to a gluten-free diet and to explain this puts forward the argument that gluten might not have been excluded completely from the diet or omitted for long enough; diagnosis of such cases might not have been correct or there might yet be some unknown factor in operation. If tropical sprue, primary malabsorptive disease and coeliac disease are one disease entity why does the gluten have no adverse

effect in tropical sprue? Curiously enough a gluten-free diet may have a beneficial effect in other conditions namely Crohn's disease and postgastrectomy steatorrhoea with hypoproteinaemia (Forshaw, 1958).

Zetterquist and Hendrix (1960) demonstrated regeneration of microvilli and disappearance of malabsorption in two patients with idiopathic steatorrhoea four months after exclusion of gluten from the diet. Rubin and coworkers (1960) although they reported in their preliminary study, a failure of the mucosa to improve following exclusion of gluten from the diet, in subsequent studies (Rubin, 1961) was able to repeat the above findings of Zetterquist and Hendrix. Rubin and coworkers (1962) in a classical experiment showed that wheat, barley and rye can produce identical damage to intestinal mucosa in coeliac sprue. These substances could be equally harmful to even the distal part of the small intestine. Since the duodenum and the upper jejunum are constantly exposed to a higher concentration of these toxic substances and as these are the areas from where these substances are absorbed it is not surprising that for this reason the diseased mucosa is mostly confined to the upper part of the small intestine and the ileum escapes these insults. Ashworth and Cheers (1962) from their observation of six cases of non tropical sprue noticed that while the untreated cases continued to have a grossly abnormal mucosa (flat mucosa devoid of villi) exclusion of gluten from the diet brought about clinical remission. Light microscopic examination showed that there had been

regeneration of the intestinal villi and electron microscopic examination showed that the microvilli had also returned to normal size and density. In addition to the above morphological evidence of gluten sensitivity in primary malabsorptive disease there is a high titre of circulating antibody to a soluble fraction of gluten (Taylor and coworkers, 1961). In support of the allergy hypothesis Taylor and coworkers (1964) demonstrated that a state of induced hypersensitivity was observed when gluten and similar protein were given to persons suffering from coeliac disease or idiopathic steatorrhoea. In these persons there was also a high titre of circulating antibodies to these dietary antigens. In addition an excess of mucous production in the small intestine was a sign of an allergic reaction. The beneficial effect of corticosteroids in the disease supports the allergic hypothesis. In the present series of cases the plasma cell infiltration of the mucosa found under light microscopy was confirmed by the finding under the electron microscope of large cells rich in endoplasmic reticulum and having large electron opaque substances identical to Russell bodies, thus supporting the above hypothesis.

Enzymatic hypothesis.

Those who support the enzymatic defect theory argue that basically the epithelial cells of the small intestine, in this disease, have defective enzymatic systems. Therefore they cannot deal with gluten properly. Thus an intermediate metabolite forms which enters the general

circulation and exerts harmful effects on different systems of the body. (Sheldon, 1955). Weijers and coworkers (1959) fed gliadin to healthy children and children with coeliac sprue. They found that the level of glutamine peptide in the blood rose higher in coeliac children. Ashworth and Cheers (1962) in their study of the pathologic physiology in this disease suggested that the defective enzyme system hampers the dynamic process of absorption. Histochemical examination of the intestinal mucosa in primary malabsorptive disease show that the enzyme systems concerned with the release of energy are at fault. But Spiro and coworkers (1964) in their study of functional histochemistry came to the conclusion that in the malabsorptive syndromes this might be a secondary factor rather than a primary factor because the enzymatic change follows the morphological mucosal changes.

Summary.

1. From the above observations it is quite evident that in the majority of cases there is a good correlation between the different microscopic observations of the altered mucosal morphology in primary malabsorptive disease. In 95% of cases the dissecting microscopic picture is confirmed under the light and electron microscopes. Thus dissecting microscope is a better tool for routine diagnosis and follow up of patients.

2. In only 5% of cases is the biochemical abnormality confirmed by a morphological abnormality detected only under the electron microscope. In these cases electron

microscopic examination took on the average per specimen 10 days to give sufficient information to arrive at the diagnosis.

3. An identical light microscopic picture is met with in a variety of other conditions. Micrometric estimation of the heights of both villi and glandular layer gives a better impression of the diseased mucosa, than the measurement of the height of the villi alone. By this procedure the limitations of the light microscopic technique, such as bad orientation of specimen, or an oblique section, can be avoided.

4. By plotting the height of the villi and height of the glandular layer, in primary malabsorptive disease, a doubtful zone is now determined. Such specimens exhibiting doubtful measurements should be further investigated under the electron microscope. Thus the number of specimens subjected to this laborious and time-consuming procedure could be reduced.

REFERENCES.

1. Aulthausen, T.L., Doig, R.K., Khan, Ugeyama, Weidens, S. (1950). Gastroenterology, 16, 126.

2. Ashworth, C.T., Cheers, W.C. Jr. (1962). Fed. Proc., 21, 880.

3. Ashworth, C.T., Sanders, E., Pearce, M.B. (1961). Arch. Path., 71, 13.

4. Astaldi, G., Grandini, U., Poggi, C., Strasselli, E. (1964). Amer. J. dig. Dis., 9, 237.

5. Baker, S.J., Mercy, Ignatius, Mathan, V.I., Vaish, S.K., Chacko, C.C. (1962). Intestinal Biopsy. Ciba Foundation study group, No. 14. J. & A. Churchill Ltd., London.

6. Bolt, R.J., Pollard, H.M., McCool, S. (1960). Am. J. clin. Pathol., 34, 43.

7. Brown, A.L. (1962). J. Cell.Biol., 12, 623.

8. Butterworth, C.E. Jr., Perez-Santiago, E. (1958). Ann. intern. Med., 48, 8.

9. Cameron, G.R. and Khanna, S.D. (1959). J. Path. Bact., 77, 505.

10. Crosby, W.H., and Kugler, H.W. (1957). Am. J. dig. Dis., 2, 236.

11. Curran, R.C. and Creamer, B. (1963). J. Path. Bact., 86, 1.

12. Da Silva, J.R., Continho, S.G., Dias, L.B. , Defigueiredo, N. (1964). Am. J. dig. Dis., 9, 355.

13. Deller, D.J., Richards, W.C.D., Wilts, L.S. (1962). Quart. J. Med., 31, 89.

14. Doniach, I., and Shiner, M. (1957). Gastroenterology, 33, 71.

15. Flint, J.M. (1912). Johns Hopk. Hosp. Bull., 23, 127.

16. Florey, Sir Howard. (1962). General Pathology, 3rd Ed. Lloyd-Luke Ltd., London.

17. Forsahw, J.W.B. (1958). Brit. med. J., 2, 1020.

18./

18. Frazer, A.C., Fletcher, R.F., Ross, C.A.C., Shaw, B., Sammons, H.G., Schneider, R. (1959). Lancet, 2, 252.

19. Gangorosa, E.J., Beisel, W.R., Benyajati, C., Spirnz, H., Piyaratn, P. (1960). Am. J. trop. Med., 9, 125.

20. Girdwood, R.H. (1962). John Matheson Shaw Lecture. The Royal College of Physicians of Edinburgh.

21. Girdwood, R.H., Delamore, I.W., Wynn Williams, A. (1961). Brit. med. J., 1, 319.

22. Holmes, R., Hourihane, O'B. D., Booth, C.C. (1961). Postgrad. med. J., 37, 717.

23. Jacobson, E.D., Prior, J.T., Falcon, W.W. (1960). J. Lab. clin. Med., 56, 245.

24. Kamer, J.H., Van de Weigers, H.A. (1955). Acta. Paediat., 44, 465.

25. King, M.J., and Joske, R.A. (1960). Brit. Med. J., 1, 1324.

26. McCarthy, C.F., Borland, J.L., Kurtz, S.M., Ruffin, J.M. (1964). Am. J. Path., 44, 385.

27. Novikoff, A.B., and Essner, E. (1962). Fed. Proc., 22, 1130.

28. Padykula, H.A. (1962). Fed. Proc., 21, 873.

29. Padykula, H.A., Strauss, E.W., Ledman, A.J., Gardner, F.H. (1961). Gastroenterology, 40, 735.

30. Palade, G.E. (1955). J. biophys. biochem. Cytol., 1, 59.

31. Paulley, J.W. (1954). Brit. Med. J., 2, 1318.

32. Premysl, Fric., Blahoslav, Bednar, Bohuslan, Neiderle, Jiri, Lepsik. (1963). Gastroenterology, 44, 330.

33. Rubin, C.E. (1961). Ann. Rev. Med., 12, 39.

34. Rubin, C.E., Brandborg, L.L., Phelps, P.C., Taylor, H.C., Murray, C.V., Stemler, R., Howry, C., Volwiler, W. (1960). Gastroenterology, 38, 517.

35. Rubin, C.E., Flick, A.L., Macdonald, W.C., Parkins, R.A., Parmentier, C.M., Phelps, P., Sribhibhadh, S., Trier, J.S. (1962). Intestinal Biopsy, Ciba Foundation study group No. 14. J & A Churchill Ltd. London.

36. Rubin, C.E., Phelps, P.C., Taylor, H.C. Jr., Wash, S. (1958). J. clin. Invest., 37, 927.

37. Salem, S.N., Truelove, S.C. (1964). Brit. med. J., 1, 1074.

38. Salem, S.N., Truelove, S.C., Richards, W.C.D. (1964). Brit. med. J., 1, 394.

39. Sheehy, T.W., Meroney, W.H., Cox, R.S., Soler, J.E. (1962). Gastroenterology, 42, 148.

40. Sheldon, W. (1955). Lancet, 2, 1097.

41. Shearman, D.J.C., Girdwood, R.H., Wynn Williams, A., Delamore, I.W. (1962). Gut, 3, 16.

42. Shiner, M., Drury, R.A.B. (1962). Amer J. dig. Dis., N.S., 7, 744.

43. Shiner, M., Lacy, D., Hudson, R.H. (1962). Intestinal Biopsy, Ciba Foundation study group, No. 14, J. & A. Churchill Ltd., London.

44. Sleisenger, M.H., Almy, T.P., Barr, D.P. (1953). Amer. J. Med., 15, 666.

45. Spirnz, H., Sribhibhadh, R., Gangarosa, E.J., Benyata, C., Kundel, D., Halstead, S. (1962). Amer J. clin. Path., 38, 43.

46. Spiro, H.M., Filipe, M.I., Stewart, J.S., Booth, C.C., Pearse, A.G.E. (1964). Gut, 5, 145.

47. Taylor, K.B., Thomson, D.L., Truelove, S.C., Wright, R. (1961). Brit. med. J., ii, 1727.

48. Taylor, K.B., Truelove, S.C., Wright, R. (1964). Gastroenterology, 46, 99.

49. Verzar, F. and McDougall, E.J. (1936). Absorption from the intestine. Longman's Green & Co., London.

50. Weijers, H.A., Kamer, J.H., Vande, (1959). World Congress of Gastroenterology, Washington. Williams and Wilkins, Baltimore.

51. Wynn Williams, A. (1961). Gut, 2, 346.

52. Zetterquist, H., and Hendrix, T.R. (1960). Johns Hopk. Hosp. Bull., 106, 240.

53. Hawkins, C.F. (1961). Postgrad. Med. J., 37, 761.

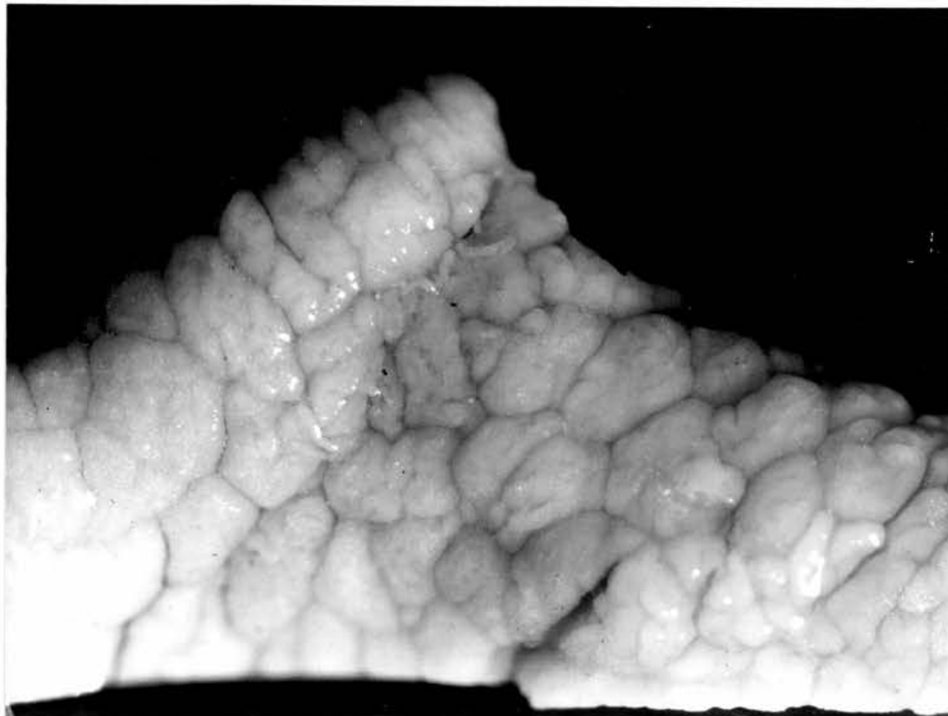


Fig. 83a. Jejunal mucosa from a patient with primary malabsorptive disease showing 'mosaic pattern'. x 30

Fig. 83b. Histological section showing the abnormal villi in a mucosa which presented a mosaic pattern under dissecting microscope similar to above figure. H & E x 95.

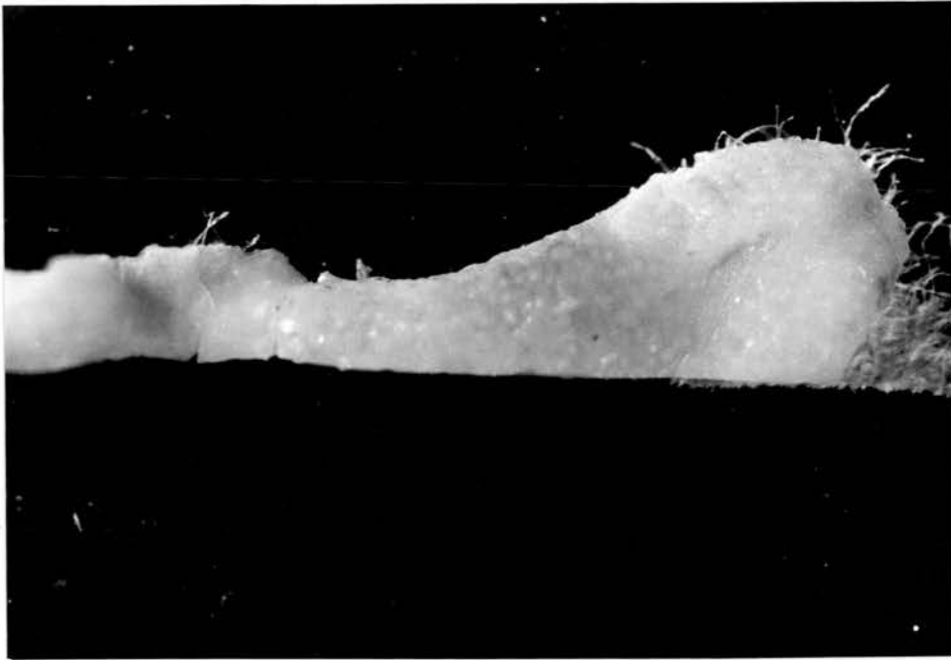


Fig. 84. Flat jejunal mucosa from a patient with primary malabsorptive disease. x 30

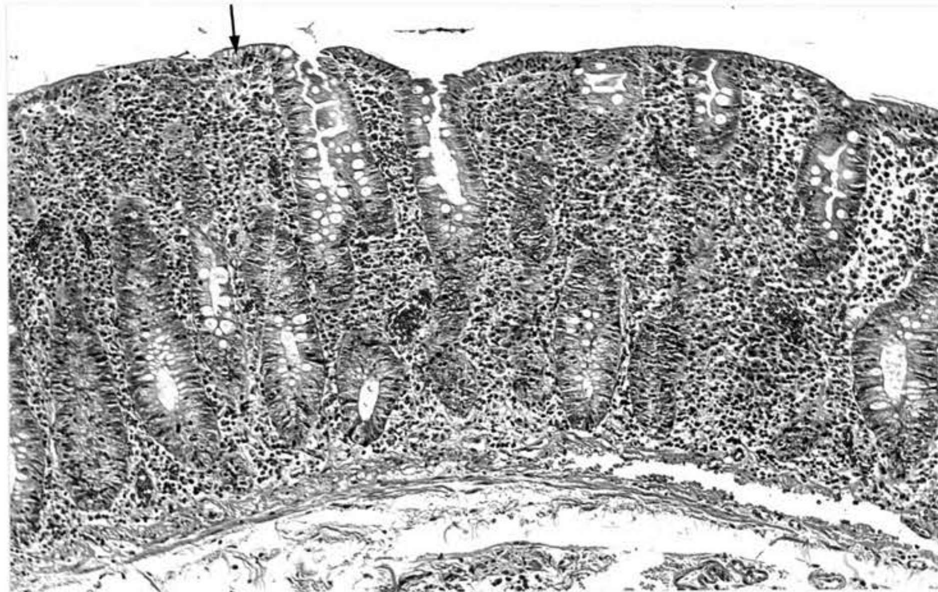


Fig. 85. Histological section of the biopsy shown in figure 84 showing mostly mucosal **folds**. Supranuclear cytoplasm of the epithelial cells show vacuolation (arrow). There is a marked cellular infiltration of the mucosa. H & E x 80

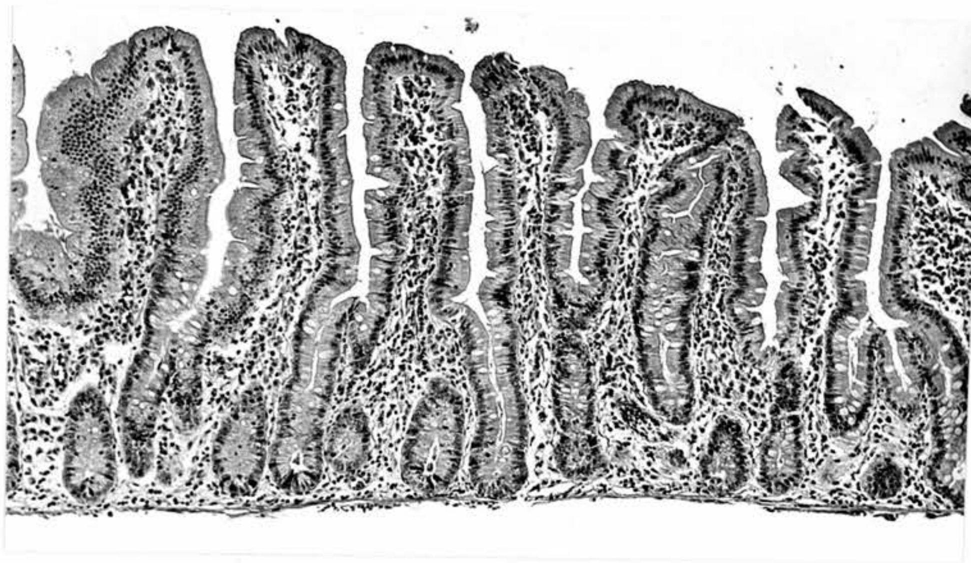


Fig. 86. H & E x 80

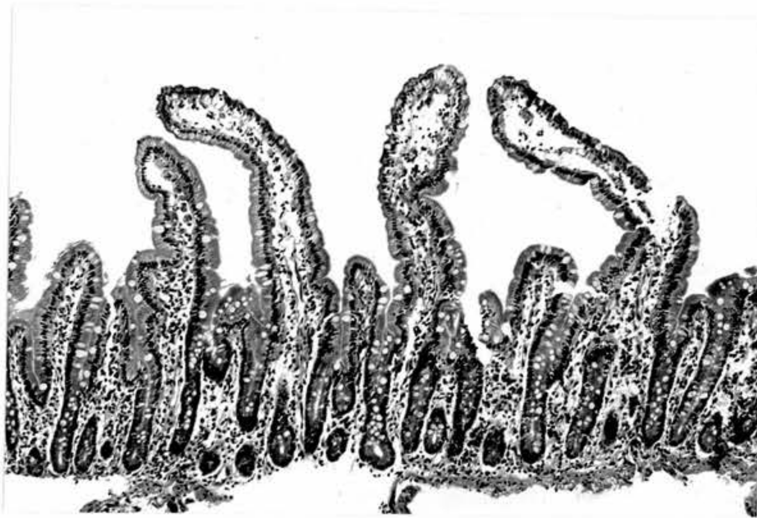


Fig. 87. H & E x 80

Histological appearances of jejunal mucosa in two cases of primary malabsorptive disease (presented above). Although most of the villi are of normal shape and there is no increase in cellular infiltration into the stroma of the villi, the abnormal appearance of the glands merit their investigation under the electron microscope.

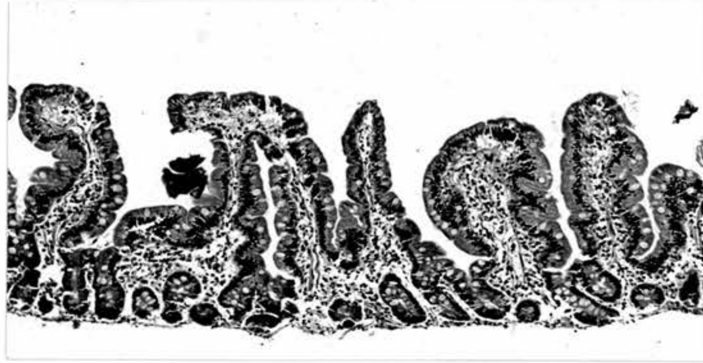


Fig. 88. Histological appearance of jejunal mucosa of a case biochemically diagnosed as primary malabsorptive disease. Reduction in the height of the villi is not accompanied by hypertrophy of the glandular layer. There is no increase in the cellular infiltration into the stroma of the villi. H & E x 80

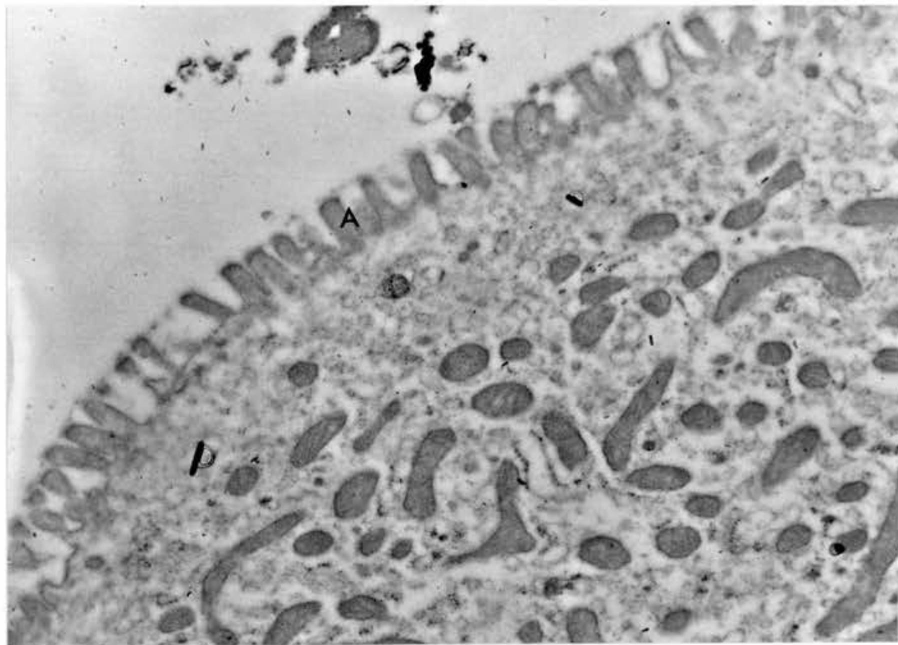
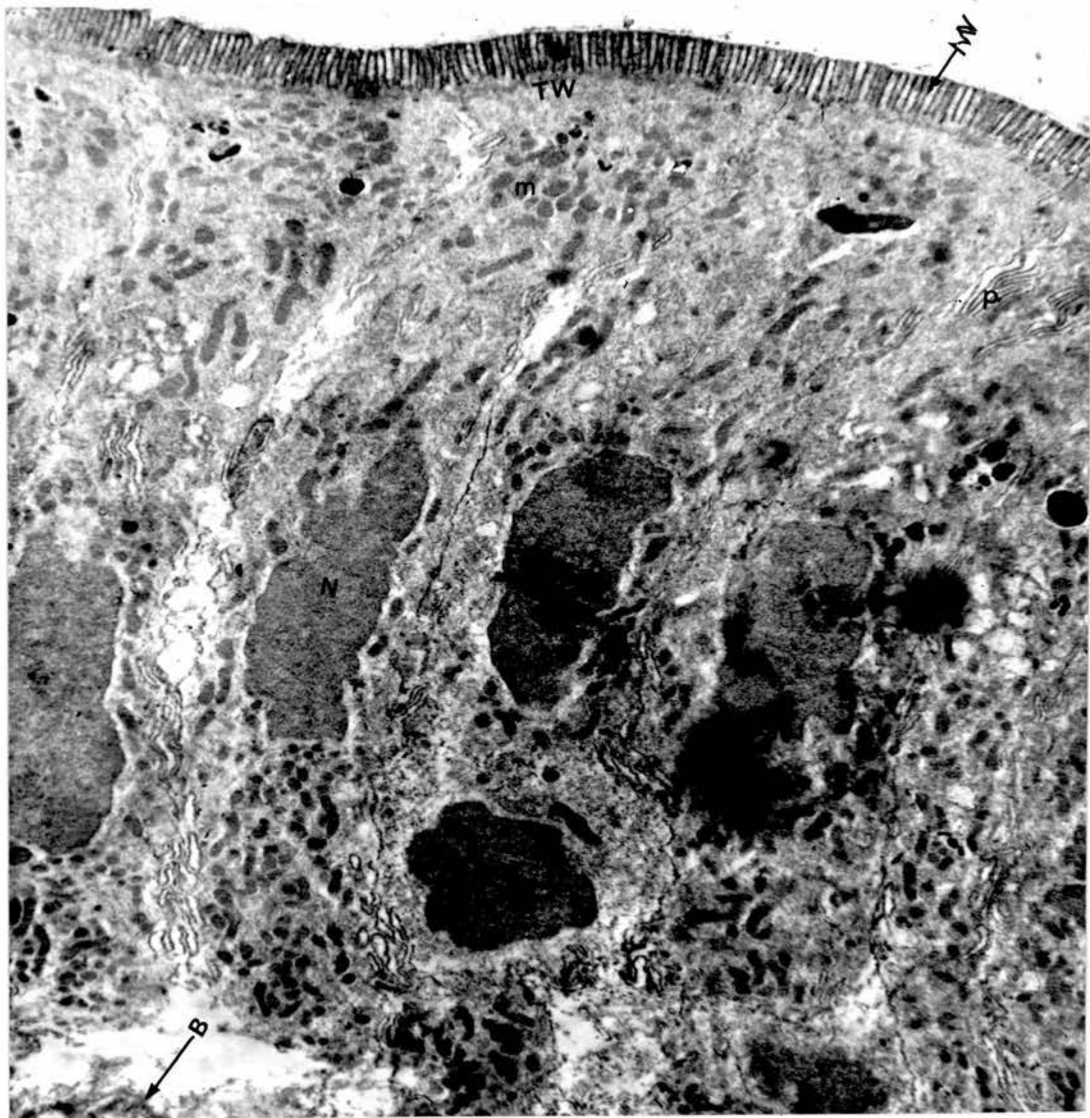


Fig. 89. Electron micrograph of the above mucosa showing abnormal microvilli (A) which are shorter and fewer in number x 16,000



<u>Fig. 90.</u> Electron micrograph (E.M.) of columnar absorbing cell of human jejunum. MV = microvilli; TW = terminal web; m = mitochondria; p = plasma membrane covering the lateral surface of the cell; N = nucleus; B = subepithelial basement membrane.
x 6,000

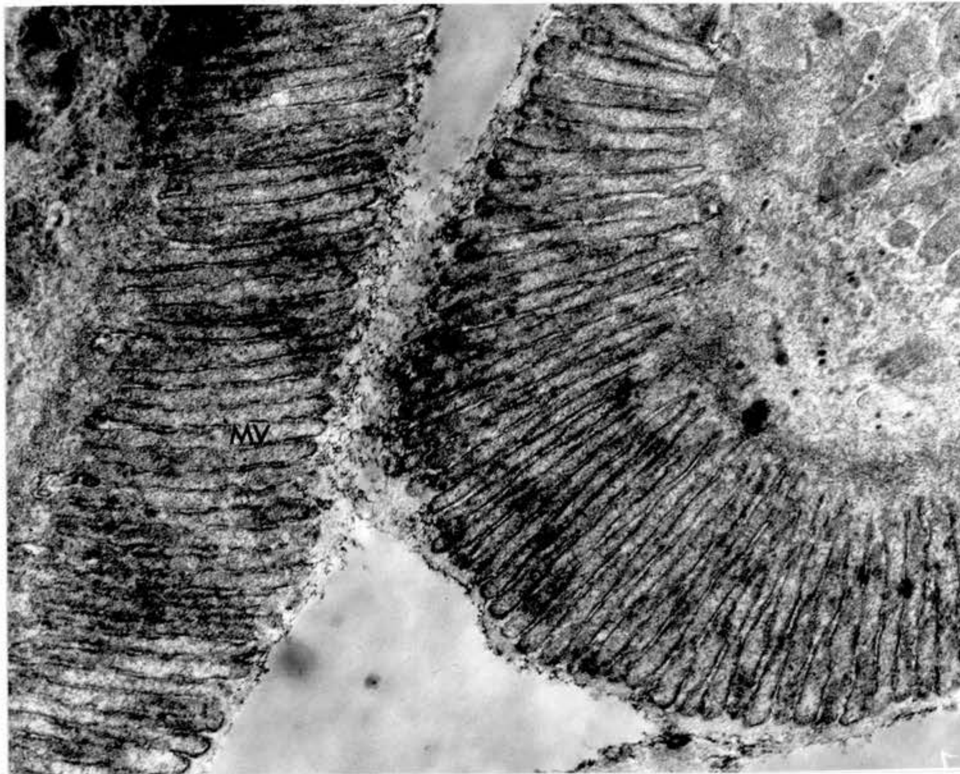


Fig. 91. E.M. of microvilli (MV) of normal epithelial cell of jejunum. x 25,000

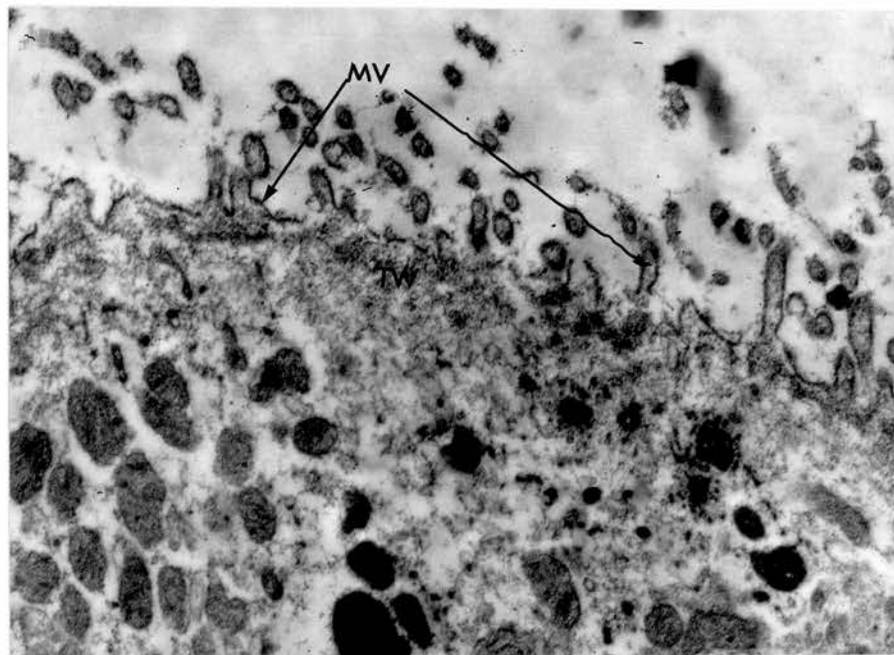


Fig. 92. E.M. showing advanced mucosal change in primary malabsorptive disease; note complete absence of normal microvilli. Microvilli (MV) vary widely in size and shape, terminal web (TW) is less prominent and there are osmiophilic bodies (O) in supranuclear cytoplasm. x 27,200

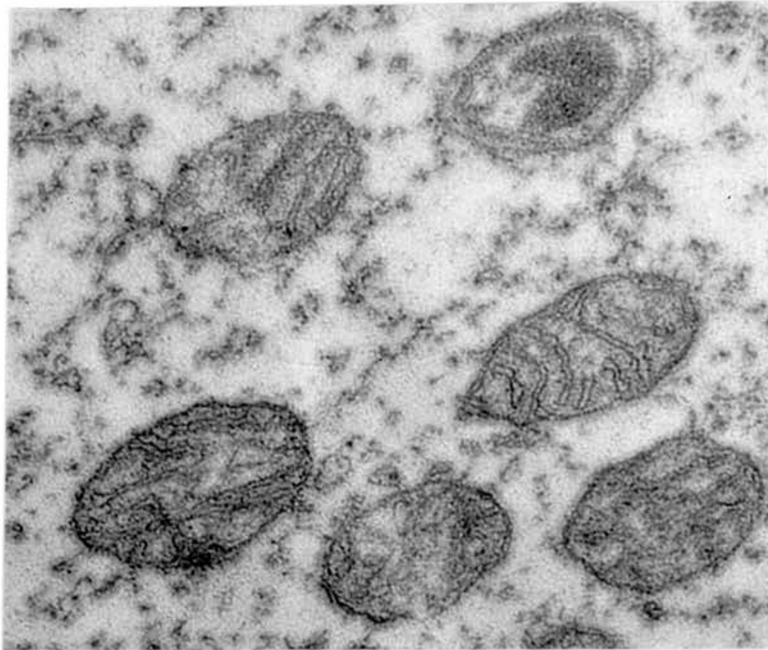


Fig. 93. Mitochondrial changes in primary malabsorptive disease. The mitochondria appear swollen. x 60,000

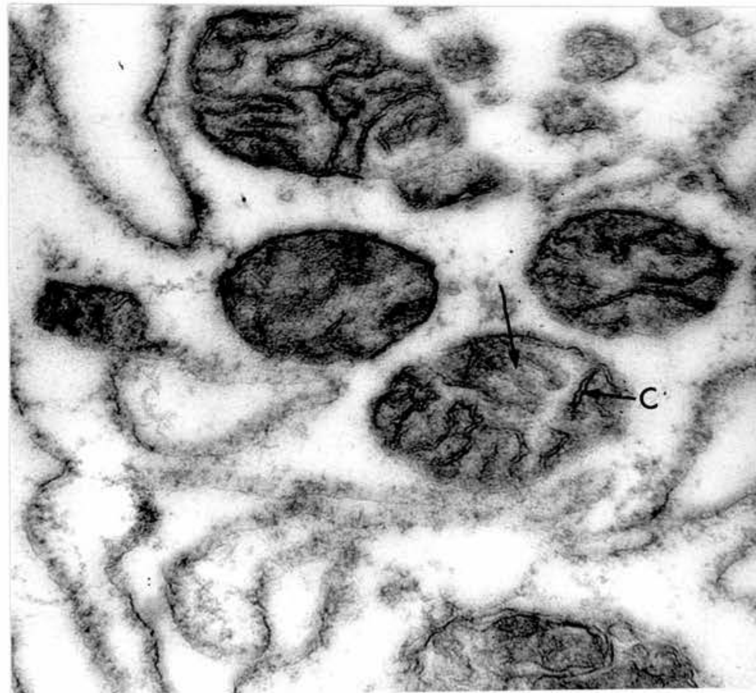


Fig. 94. Note disorganisation of mitochondrial morphology. Swollen mitochondrial matrix (arrow) encroaches upon the cristae (C) of the mitochondria. x 60,000

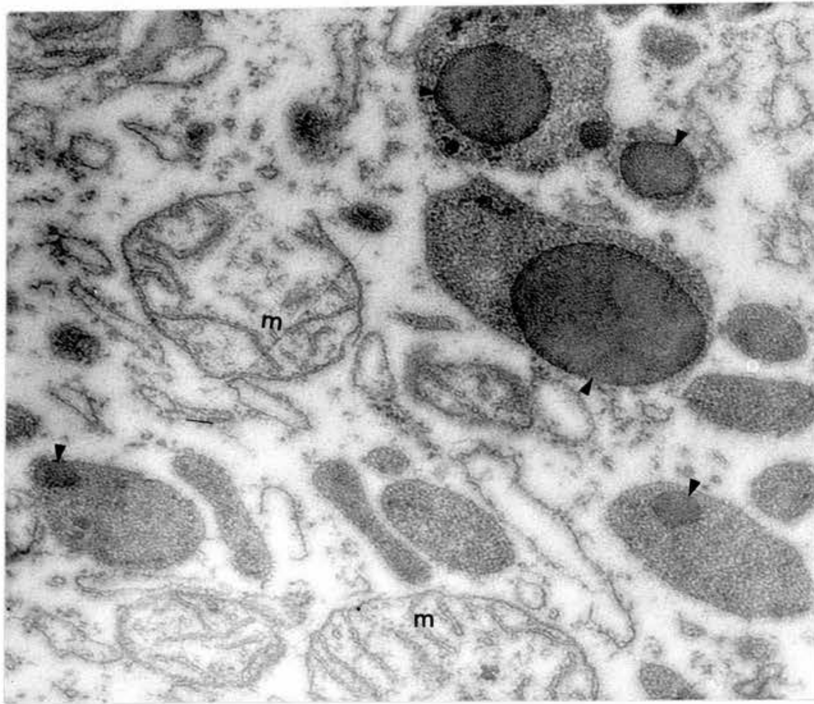


Fig. 95. E.M. of supranuclear cytoplasm in a case with primary malabsorptive disease. Pointers indicate osmiophilic granules in the cytoplasm; mitochondria (m) appear swollen. x 56,000

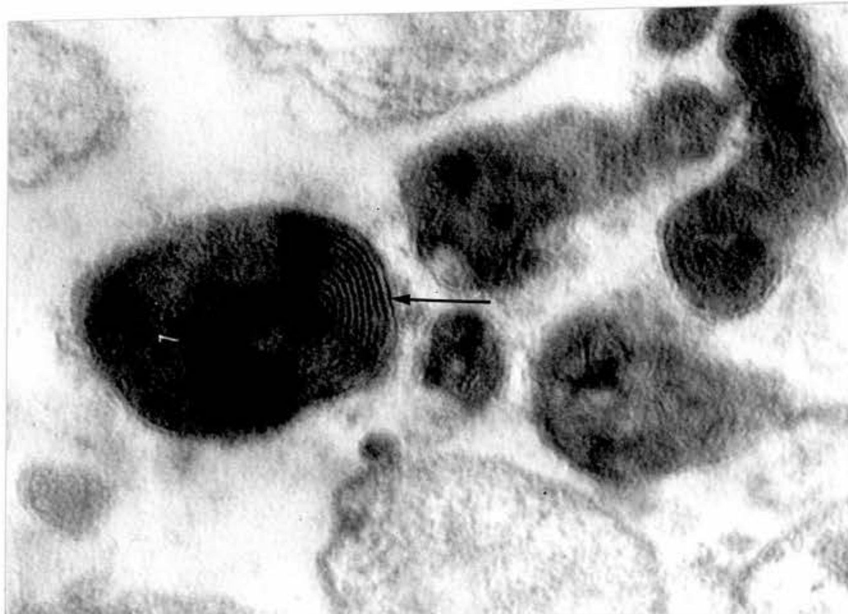


Fig. 96. E.M. of an osmiophilic body in the supranuclear cytoplasm showing 'myeline figure'(arrow). Lysosomes also show similar morphological feature. x 105,600

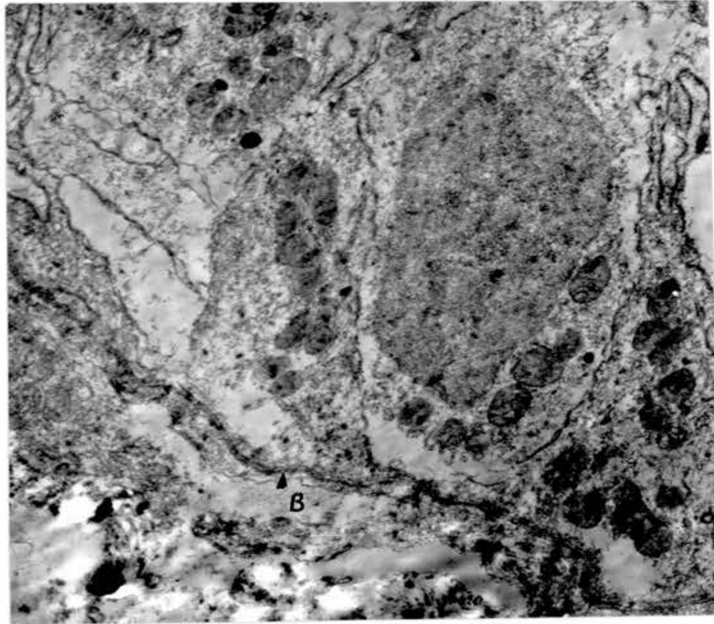


Fig. 97. E.M. of basal part of epithelial cell in primary malabsorptive disease. Note that the subepithelial basement membrane (B) does not show any alteration in its thickness from the normal. x 14,000

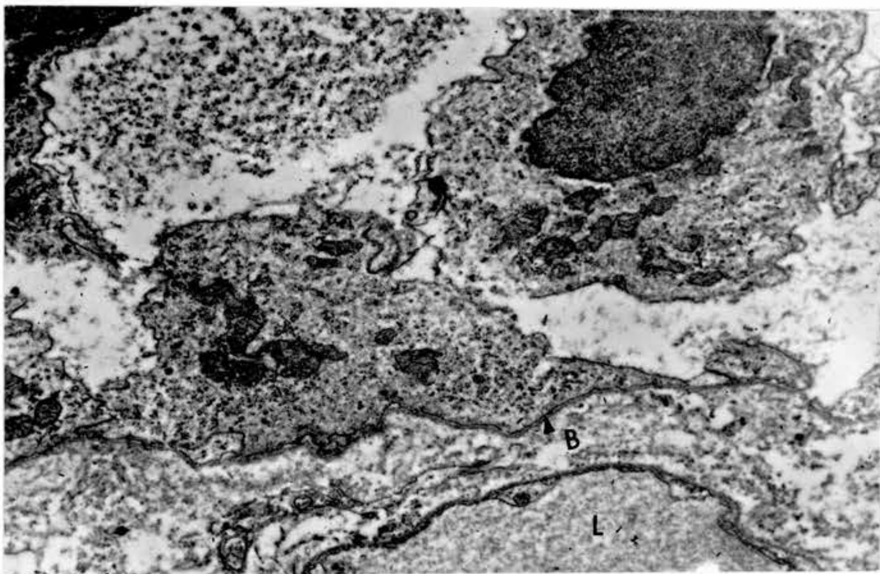


Fig. 98. Note deposition of homogeneous electron opaque material underneath the subepithelial basement membrane (B). L = lumen of a capillary in the lamina propria. x 9,600

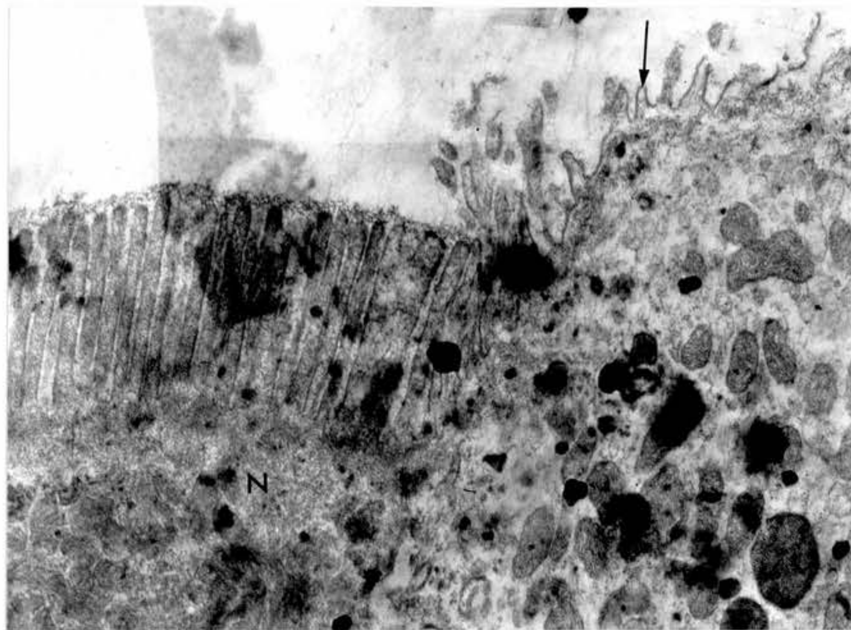


Fig. 99. E.M. of jejunal epithelial cells. A normal cell (N) is seen adjacent to another cell with abnormal microvilli (arrow) and abnormal mitochondria. x 22,400

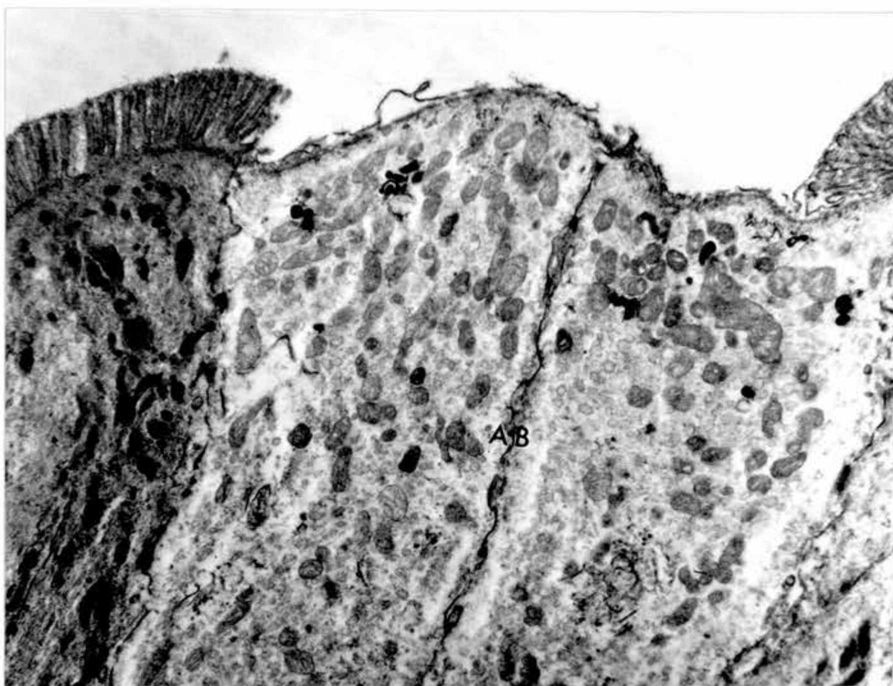


Fig. 100. Two abnormal cells (A & B) in between two cells with normal microvilli. x 8,000

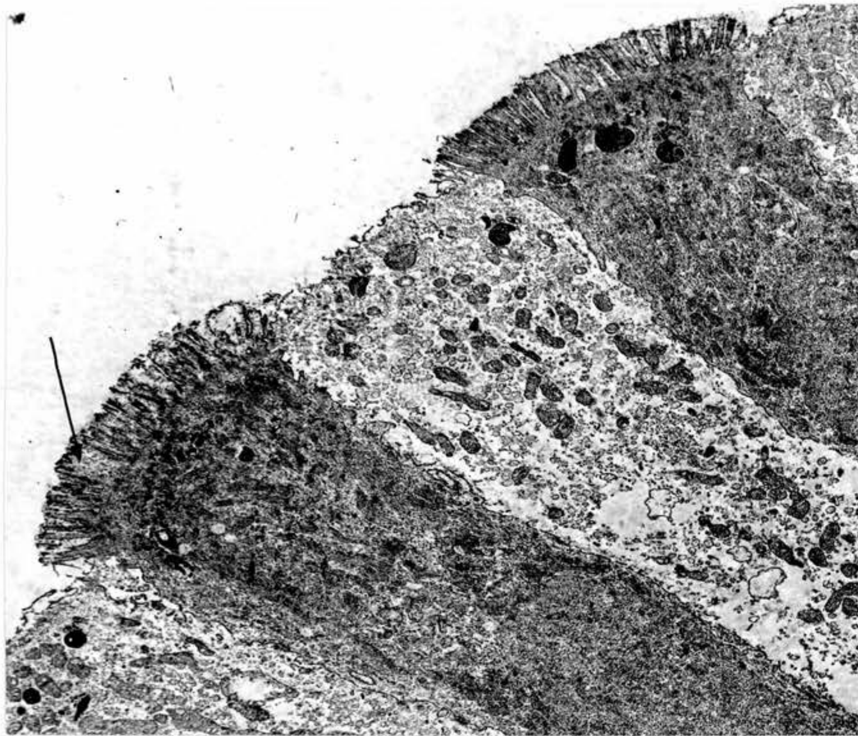


Fig. 101. E.M. of jejunal mucosa in a case with primary malabsorptive disease. Abnormal cells alternate with cells which show early changes like fusion of microvilli (arrow). x 4,200

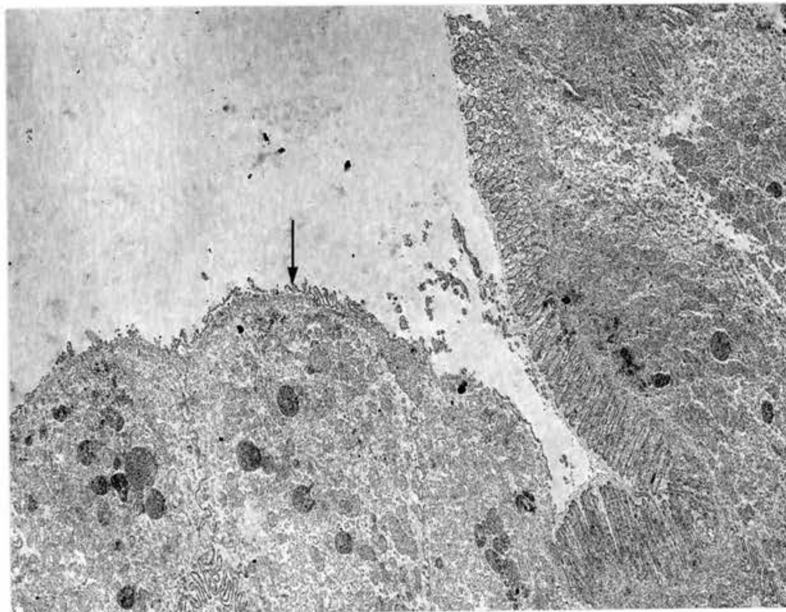


Fig. 102. A group of cells with normal microvilli adjacent to a group of cells with abnormally few microvilli (arrow). x 7,600

CHAPTER 8.

PATHOGENESIS OF MICROVILLOUS ATROPHY IN PRIMARY MALABSORPTIVE DISEASE

The anatomy and functional significance of the free surface of the epithelial cells of the small intestine has been a topic of interest for over 120 years. It was first described as a clear and structureless area. Funke (1856) noted striations in this homogeneous layer and suggested that they resembled cilia. Granger and Baker (1950) using the electron microscope were the first to confirm that the processes of the brush border are cylindrical processes originating from the cytoplasm of the free surface of the epithelial cells. Their shape resembled the villi of the intestine: hence the term microvilli. Further examinations showed that the microvilli of the human intestine are of relatively uniform dimensions; the height varies from 0.8μ to 1.3μ and the maximum width is 0.12μ (Hartman and coworkers, 1960). Their long axes are parallel and they are thickly packed in hexagonal array, each microvillus being equidistant from the six surrounding it (Palay and Karlin, 1959). The plasma membrane of each microvillus appears as a pair of dark lines separated by a paler zone of uniform thickness. The close packing of the microvilli and their strong binding to the terminal web results in a stable structure which is able to deal effectively with the semisolid food material of the intestine. The intermicrovillous space dips into the terminal web and forms an open vesicle where pinocytosis takes place.

In primary malabsorptive disease, good correlation is noted between the appearances of the villi under the light microscope and the heights of the microvilli of the covering epithelial cells: (previous chapter). An untreated or relapsing case presents a featureless flat upper small intestinal mucosa under the light microscope. When such a mucosa is examined under the electron microscope it shows abnormally shaped microvilli which are small in size and reduced in number. Although many observers have noted the above irregularities in the microvilli (Ashworth et al, 1961; Padykula et al, 1961; Shearman et al, 1962; Shiner et al, 1962; Rubin et al, 1962; Curran and Creamer, 1963) none have suggested any possible explanation for their variation in size and shape and density of spacing.

In the present chapter,I would venture to throw some light on this problem from my study of the functional epithelium of the upper small intestine at a very early stage in primary malabsorptive disease.

Materials and Methods.

Jejunal biopsies were obtained from 14 cases presenting biochemical evidence ofprimary malabsorptive disease (idiopathic steatorrhoea). The specimens were obtained by the peroral small intestine biopsy capsule (Crosby and Kugler, 1957) the details of which have been described in the previous chapter. The specimens were examined under the dissecting, light and electron microscopes.

Observations.

Of the 14 cases, 11 showed the typical mucosal changes of primary malabsorptive disease under the dissecting and light microscopes (described in Chapter 8). The remaining 3 cases showed changes under the electron microscope only, presenting an apparently normal appearance, under the dissecting and light microscopes. The electron microscopic changes were confined to the microvilli, the rest of the epithelial cell being normal in all respects, i.e. the terminal web was normal and there was a normal number of pinocytotic vesicles (Fig. 104).

Apart from being shorter than normal (less than 0.6μ in height) some of the microvilli were fused, a characteristic which was noted in two other of the 14 cases. There appeared to be two stages of fusion; simple aggregation in which the microvilli retained their enveloping plasma membranes (Fig. 105) and complete fusion where there was a breakdown of the plasma membrane of two or more microvilli (Figs. 106, 107 and 108). The actual size of fusion varied and was seen to affect either the whole length of the microvilli or their middle thirds or the greater part of their length leaving the tips free (Fig. 109). In this way, two, three, four or more microvilli were seen to be fused with one another (Figs. 110 and 111). At the point of contact the plasma membranes seem to have dissolved away giving rise to a continuity of the core of the microvilli. The contents of the microvilli which had fused together

were replaced in many cases by a material less electron dense than normal. In extreme conditions the change in the cytoplasmic appearance extended from the microvilli into the terminal web. All these changes disturbed the normal relationship of the microvilli to the upper part of the epithelial cell.

As one of the above five cases showing fusion of microvilli had rheumatoid arthritis, four other rheumatoid arthritis cases were examined. None of these showed fusion of the microvilli, thus excluding the possibility that this disease was the cause of the microvillous fusion in the one case included in the present study.

Discussion.

As fusion of the microvilli was found in only five cases, three of which were otherwise morphologically normal, it is presumed to be a feature of the very early stage of primary malabsorptive disease and to precede the shortening, irregularity in shape and unequal distribution of the microvilli seen in the other cases. The fusion of the microvilli may be a feature manifested when the disease is present at a subclinical stage. It is possible that whatever noxious factor is responsible produces patchy fusion of the microvilli, and when this factor is removed fusion remains in the parts of the microvilli near the terminal web, only the more distal regions remaining free. Thus the microvilli on the surface of the epithelial cell appear to be shortened and distorted. With repeated insult to the

mucosa the microvilli take up a bizarre shape. Thus the customary electron microscopic picture (Fig. 113) seen in this disease is presumed to be the end result of the above process of fusion of the microvilli.

It is of interest to speculate on what toxic material could cause such fusion of the microvilli. It has been shown that gluten-casein complex may excite adhesive inflammation (gluten-casein is a protein preparation employed in intestinal surgery for this purpose). Hence the intraluminal formation of such a complex might be operative in causing fusion of microvilli.

Mucus may play an important role in binding the microvilli together before they fuse with one another. Although there is no good correlation between the severity of mucosal atrophy and changes in the sise and number of goblet cells, it is often noted under the dissecting microscope that the mucus covering the intestinal surface is rather thicker in long-standing and relapsing cases of primary malabsorptive disease than in a case with short history. Under the electron microscope a thick layer of mucus is often seen covering the surface of a mucosa which is moderately affected. In a more advanced stage of mucosal involvement the microvilli are shorter and fewer, and are often noted to be free from mucus, possibly because of lack of adequate microvilli to adhere to it, so that it is more easily washed away from the cell surface by the process of fixation for electron microscopy. A thicker layer

of mucus may limit the free movement of microvilli. This could be a factor that brings the mobile parts of the microvilli in apposition to one another.

Flexibility in the construction of the microvilli facilitates the passage of food over them. The food particles take up a mucus coating which smooths their surface and minimises the frictional resistance as they move over the microvilli. However small food particles may be, or how well they may be coated with lubricating mucus, their pressure is always felt by the microvilli. Fig. 103 shows that at the point of contact of food material with the brush border, the microvilli are bent and have therefore been cut transversely, whereas the microvilli on either side of this point are cut longitudinally. These food particles move as innumerable small masses over the mucosa of the small intestine (Verzar and McDougall, 1936). These "food balls" could be very large compared to the size of the microvilli on which they roll. (Fig. 112). When the microvilli are fused together they lose their individual flexibility and cannot bend effectively to minimise the bombardment from these food particles. As a result they may be injured or even dislodged, possibly another factor contributing to the bizarre arrangement of microvilli of a long-standing case.

Another point to be clarified is whether the changes described in the microvilli could be due to autolysis from delayed fixation. This would seem unlikely since all precautions were taken to minimise the time that

elapsed between obtaining the biopsy and putting it into fixative. Delay in fixation causes a fusiform dilatation of the upper third of the microvilli but not fusion with one another (Fig. 34, Chapter, 4.) The typical changes that occur in the cytoplasm, the mitochondria and nucleus of a cell which is inadequately fixed were not present in any of the examined material. Such changes were not noted in the tissues with inadequate dehydration (Fig. 114) where the entire mass of microvilli become separated from the apical part of the cell.

Summary.

1. Five of the fourteen cases diagnosed as primary malabsorptive disease has presented fusion of the microvilli under the electron microscope.

2. Fusion of microvilli are seen more frequently in three cases whose mucosae are normal under the dissecting and light microscopes. These mucosae are therefore thought to be presenting the very early changes due to this disease. It is therefore suggested that fusion of microvilli may precede their classical picture in primary malabsorptive disease.

3. Various degrees of this fusion are presented and possible explanations for the cause of fusion are discussed.

REFERENCES.

1. Ashworth, C.T., Saunders, E. and Pearce, M.B. (1961). Arch. Path., 71, 713.

2. Crosby, W.H. and Kugler, H.W. (1957). Amer. J. digest. Dis., 2, 236.

3. Curran, R.C. and Creamer, B. (1963). J. Path. Bact., 86, 1.

4. Funke, O. (1856). Zeitschr. Zool., 7, 315.

5. Granger, B. and Baker, R.F. (1950). Anat. Rec., 107, 423.

6. Hartman, R.S., Butterworth, C.E. Jr., Hartman, R.E., Crosby, W.H., Shirai, A. (1960). Gastroenterology, 38, 506.

7. Padykula, H.A., Strauss, E.W., Ladman, A.J., Gardner, F.H. (1961). Gastroenterology, 40, 735.

8. Palay, S.L. and Karlin, L.J. (1959). J. biophys. biochem. Cytol., 5, 363.

9. Rubin, C.E., Brandborg, L.L., Flick, A.L., MacDonald, W.C., Parkins, R.A., Parmentier, C.M., Phelps, P., Sribhibhadh, S.I., Trier, J.S. (1962). Intestinal Biopsy. Ciba Foundation study group, No. 14, J. & A. Churchill Ltd., London.

10. Shearman, D.J.C., Girdwood, R.H., Wynn Williams, A., Delamore, I.W. (1962). Gut, 3, 16.

11. Shiner, M., Lacy, D., Hudson, R.H. (1962). Intestinal Biopsy. Ciba Foundation study group, No. 14. J. & A. Churchill Ltd., London.

12. Verzar, F. and MacDougall, E.J. (1936). Absorption from the intestine. Longman's Green & Co. London.

13. Dornald's illustrated medical dictionary, 23rd Ed. 1957, W.B. Saunders Company, Philadelphia and London.

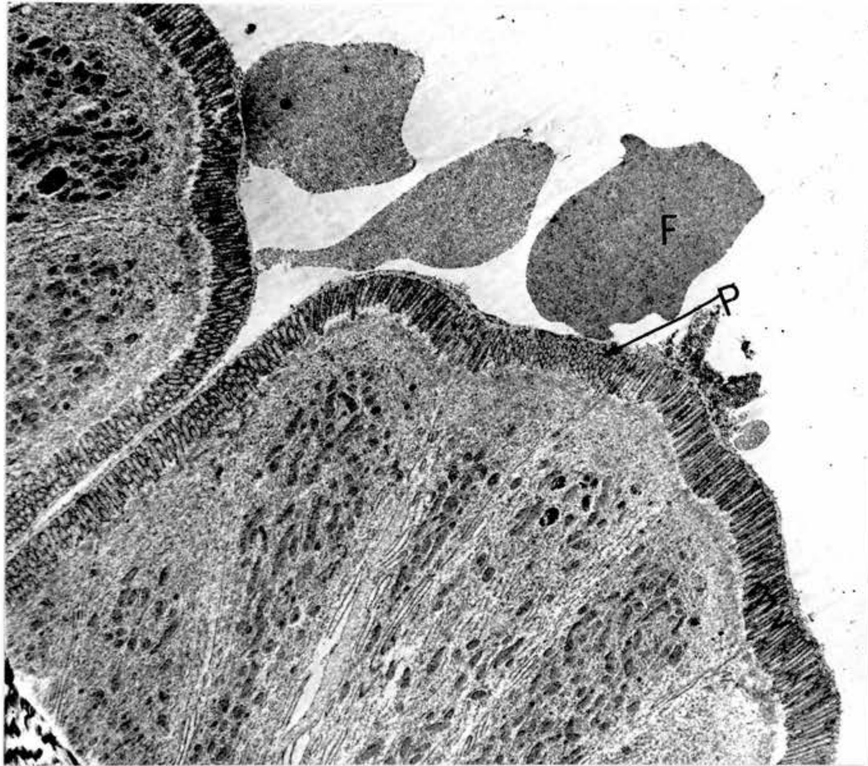


Fig. 103. Electron micrograph of a normal epithelial cell from proximal jejunum. Note the effect (P) of the pressure exerted by the intestinal content (F) on the microvilli (mv). At the point of contact the microvilli are bent, therefore have been cut transversely. x 6,800

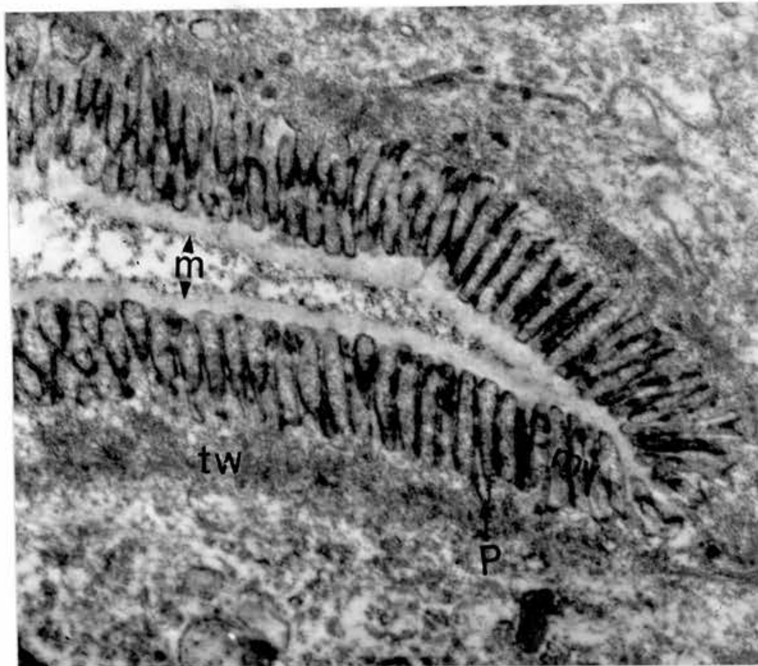


Fig. 104. Electron micrograph of a jejunal biopsy from a patient with primary malabsorptive disease. Note the early mucosal changes evident only in shortening of microvilli (mv). A thick layer of mucus (m) covers the surface of the microvilli. The pinocytotic vesicles (P) are normal in number and the terminal web (tw) is well preserved. x 22,400

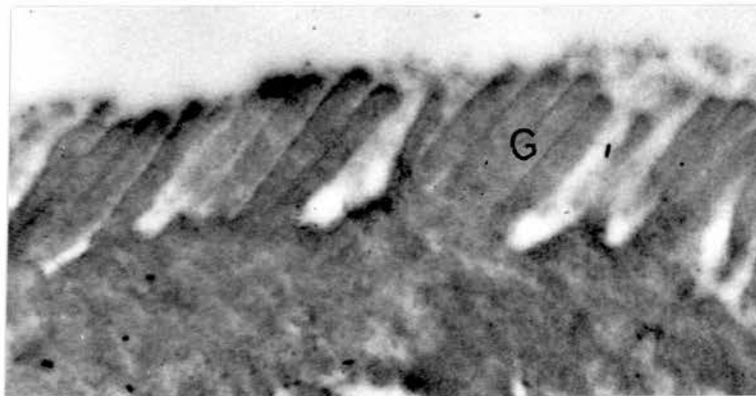


Fig. 105. Jejunal biopsy from a mild case of primary malabsorptive disease. Note grouping (G) of the microvilli. x 40,000

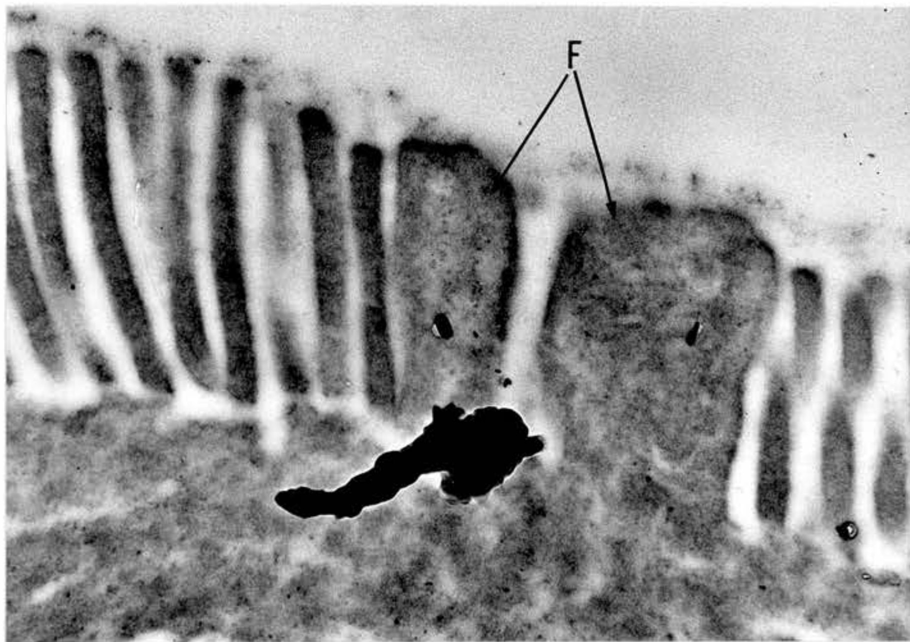


Fig. 106. E.M. showing complete fusion of microvilli. x 35,200

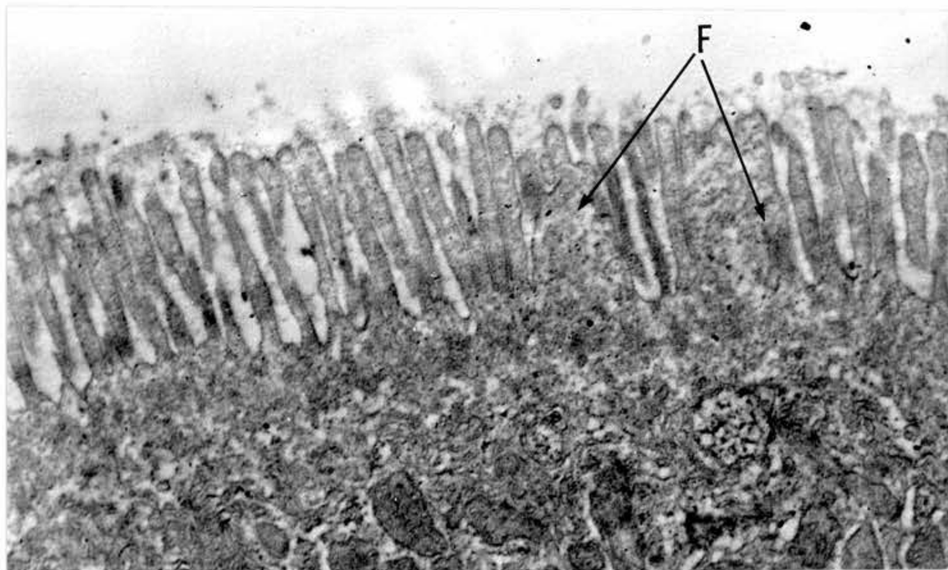


Fig. 107. Electron micrograph showing fusion (F) of the microvilli of an early case of primary malabsorptive disease. x 28,800

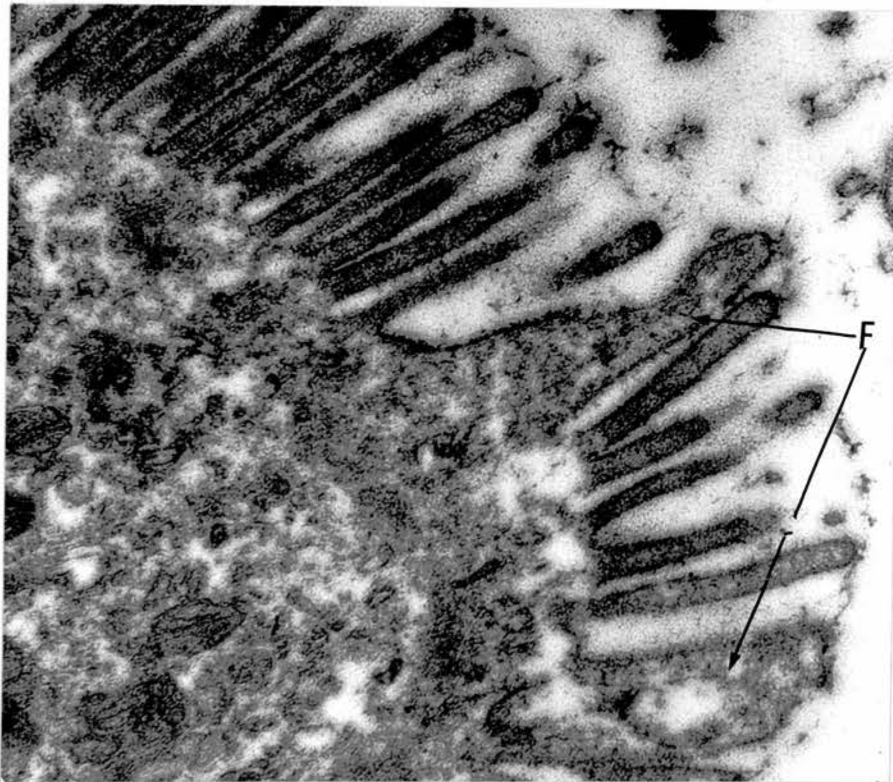


Fig. 108. Electron micrograph showing complete fusion (F) of the microvilli. x 51,000

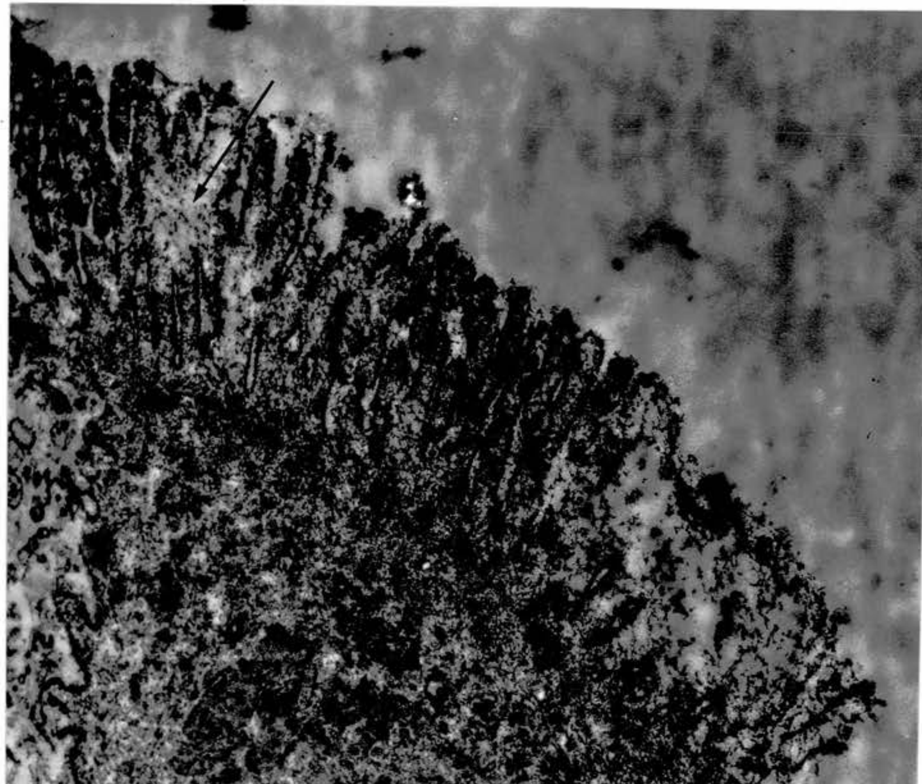


Fig. 109. Fusion of microvilli at their mid region (arrow). The apical and basal part of these microvilli are seen clearly. The fused parts of the microvilli is replaced by a homogeneous mass (f). x 35,200

Fig. 110. Electron micrograph showing more fusion amongst the microvilli. x 30,000

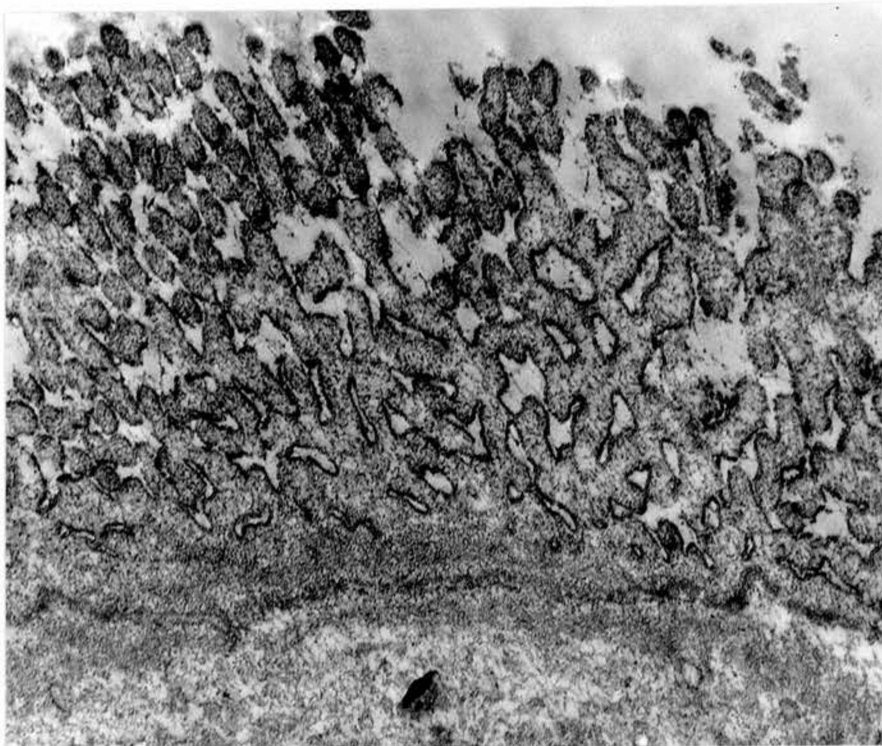


Fig. 111. Many microvilli fused together and stand out as arborisations of a tree. x 40,000

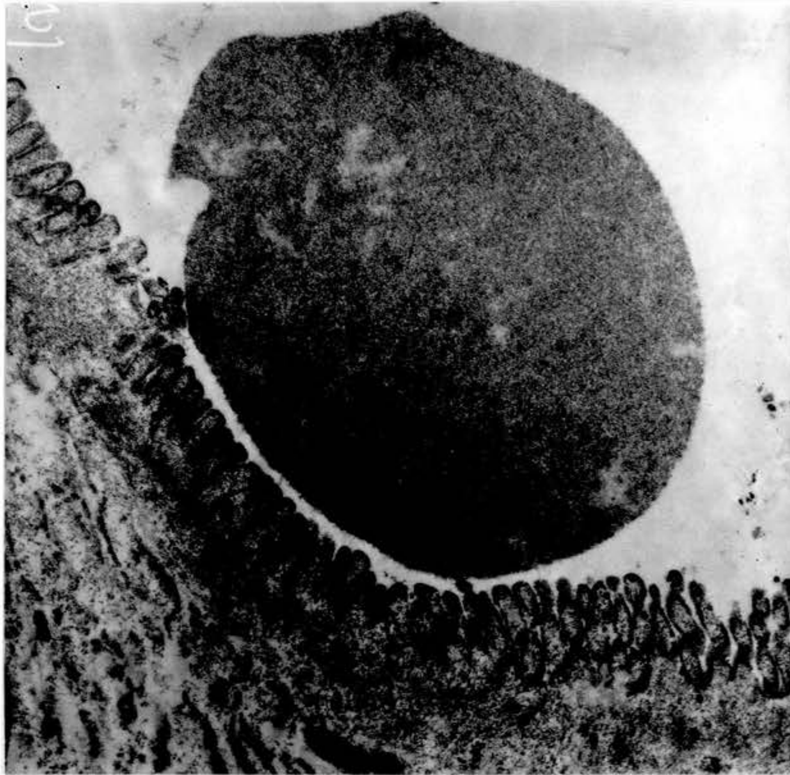


Fig. 112. Food particle (FP) on microvilli (mv) of epithelial cell. x 15,600

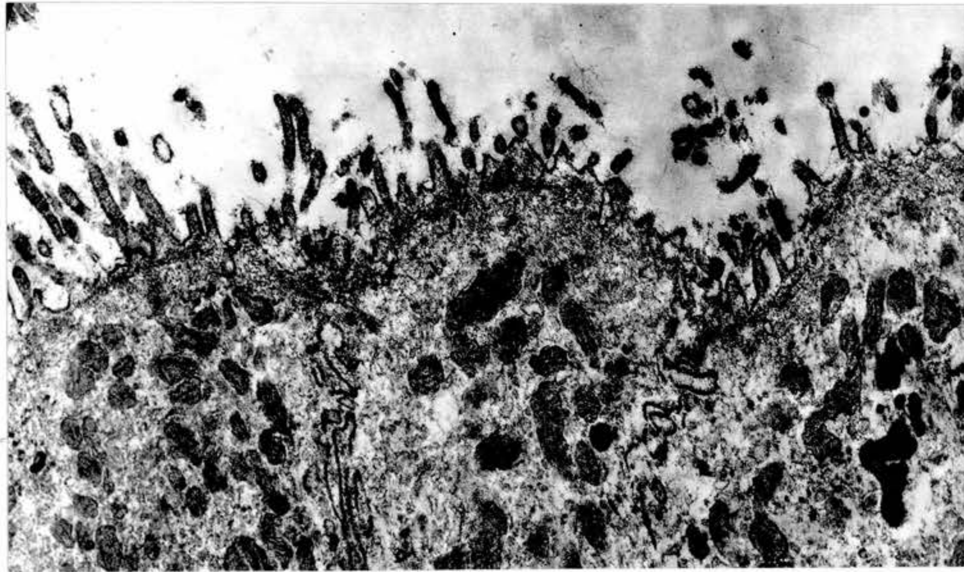


Fig. 113. E.M. showing variety of size and shape of microvilli in epithelial cells from a patient with primary malabsorptive disease. x 18,000

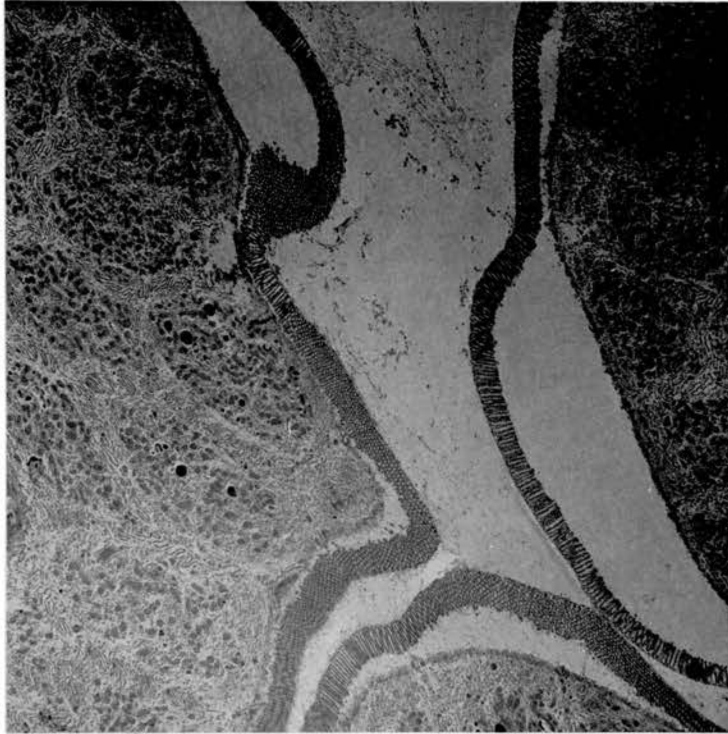


Fig. 114. Effect of inadequate dehydration on the jejunal mucosa. x 1,950

CHAPTER 9.

ULTRA STRUCTURE OF THE LUMINAL SURFACE OF THE ABSORBING CELL IN PRIMARY MALABSORPTIVE DISEASE: PATHOGENESIS OF STEATORRHOEA.

Introduction.

The luminal surface of the epithelial cell of the duodenum and jejunum has received much attention during the past ten years since the original observations of Paulley in 1954. He suggested that an abnormal mucosal pattern of the proximal part of the small intestine is a constant feature in idiopathic steatorrhoea. Since this time, various workers have reported mucosal changes in the sprue-syndrome under both the light and electron microscopes (Hartman et al, 1960; Rubin et al, 1962; Ashworth et al, 1961, 1962; Girdwood et al, 1961; Padykula, et al, 1961; Shearman et al, 1962; Curran and Creamer, 1963). The mucosa of duodenum and jejunum has great functional significance. It is the site of active nutrient absorption and therefore a lesion of the mucosa of this region leads to a multiple deficiency syndrome. In this chapter I shall make an attempt to throw some light on the dynamic interpretation of altered mucosal morphology in this multiple deficiency state, which I would like to designate as Primary Malabsorptive Disease (although non tropical sprue, idiopathic steatorrhoea, adult coeliac disease and coeliac sprue are synonymous terms).

Review of Literature.

Granger and Baker (1950), with the aid of the

electron microscope, found that the luminal border of the intestinal epithelium of the rat has minute finger like projections, and called them microvilli. Transverse section through these microvilli showed that they have a very small diameter and are arranged in close horizontal or possibly spiral rows. Palay and Karlin (1959) made a more extensive study of the epithelial cell of the intestinal villi of the rat and found that the microvilli when cut in the long axis have an average length of 1μ and a width of 0.07μ. When the plane of section was transverse to the long axis of the microvilli, their detailed arrangement came to light. In each small area there is one microvillus in the centre surrounded by six others arranged in the shape of a regular hexagon. Hartman et al (1960) found that in man the height of the microvilli ranged from 0.8 to 1.3μ and their maximum width was 0.12μ. Brown (1962) made a comparative study of the microvilli over the crest of the villus, in the intervillous space and in the crypts of the villi of human jejunal epithelium. He found that microvilli over the villous crest are roughly twice the height of microvilli in the crypt.

Hartman et al (1960) found that in non tropical sprue the microvilli of the jejunal epithelium are deficient in size and number. Ashworth et al (1961), in addition to the comments made above regarding the microvilli, remarked that in this disease process the terminal web, a dense zone of fibrillar element just beneath the microvilli, is constantly affected and the

pinocytotic vesicles are markedly reduced in number. In a subsequent study Ashworth et al (1962) found that when lipid emulsions were given to non tropical sprue cases, although the lipid droplets were visible over the luminal surface, none were seen inside the epithelial cell. Rubin (1961) found that mucosal affection in this disease, although very diffuse, is mainly limited to the jejunum; ileal mucosa could be affected to varying degrees.

Cellular dynamics in quantitative reduction of mucosal surface.

In recent years, with increased knowledge of the morphological details of the cell, there is a growing tendency to interpret the dynamic property of cells in health and in disease states. Mucosal cells of the small intestine are unparalleled in their function of absorbing water, electrolytes and nutrients from the food. Therefore there is great need for maintaining the maximum surface area to cope with this demand. Granger and Baker (1950) found that there are 3,000 microvilli per cell or 200,000,000 microvilli per square millimetre of mucosal surface of the rat intestine. These finger like projections from the luminal border of the intestine increase the absorptive surface of intestine by 30 times. Ashworth and coworkers (1961) made an identical quantitative study of the absorptive surface of jejunal mucosa in man and found that their figures were almost the same as the above estimations of rat intestine. Brown (1962) found from his studies

of jejunal mucosa in man that there is progressive increase in the surface area of individual microvilli from the crypt to the crest of the villi. On the crest, the microvilli are taller, thinner and more numerous, but in the crypt the microvilli are shorter and wider. Although there is a decrease in the volume of individual microvilli over thecrest of the villi, the available absorptive surface is greatly increased. Butterworth and Perez-Santiago (1958) found that the mucosal lesions of the small intestine in tropical sprue are probably identical with those in non tropical sprue. They further calculated that within an identical area of the bowel, the mucosal surface of a normal person is about four times the mucosal surface in an untreated case of the above disease. Ashworth and coworkers (1961) found in two cases of non tropical sprue that in this diseased state the microvilli are reduced to about one-half or one-third of those seen on healthy mucosa.

Plasma membrane.

Much attention has been focused on the plasma membrane, which covers the microvilli and the cell, in recent years. Our present conception of the details of the fatty layer on the cell surface originates from the observations of Gorter and Grandel (1925). They noticed that the lipid layer is arranged as a bimolecular leaflet. At 37°C. (body temperature) cephalin and lecithin form an expanded film, whereas cholesterol and sphingomyelin form a condensed film. Danielli and Harvey (1934) found that the low tension observed at

the surface of living cells is due to the absorption of protein on a lipid layer. From this the conclusion was drawn that the bimolecular lipid layer has protein absorbed on its inner and outer surfaces. Although it looks like a single layer, time-lapse cine-films of living cells in tissue culture and the high resolution of the electron microscope have revealed a similar molecular configuration of the plasma membrane. It presents as two opaque lines which include an intermediate zone of low capacity (Weiss, 1955) and supports the Danielli-Harvey model of 'protein/lipid:lipid/protein'. The molecular configuration of the cell membrane can be studied stage by stage in its process of construction (Stoeckenius, 1962). When lipid extract from tissues is brought in contact with water, fixed with osmium and then sectioned, its rough profile is identical with those of plasma membrane. It has two darker outer layers separated by a central lighter layer and osmium is seen over the surface of the lipid·molecules. By adding an appropriate protein (globulin) to the solution, it was found that the protein was absorbed to the surface of the lipid layer and increased the thickness of the outer dark bands. The cell membrane is mainly comprised of two substances, namely protein and lipid. Over each half of the lipid layer protein forms an artificial membrane which exerts stabilising effect on the polar head of the lipids and is electron dense. The paraffin chains of the lipid molecules are electron thin and

therefore form a central lighter zone. Palay and Karlin (1959) found that the plasma membrane covering the microvilli of the adult rat has a pair of dark lines, each 40Å thick, and they are separated by a structureless zone of 25Å width. The findings are identical with Zetterquist's (1956) drawing of plasma membrane covering the microvilli of the absorbing cells of mouse intestine. When the tissue is exposed to hypertonic sucrose, the lighter zone of the cell membrane is reduced and brings the electron opaque lines closer to each other. (Robertson, 1959).

Mucus factor.

The excessive production of mucus with or without alteration in the size of number of the goblet cells is another feature constantly met with in primary malabsorptive disease. Frazer, French and Thompson (1949) suggested that unabsorbed free fatty acids are a potential source of mucosal irritation which causes excessive mucus secretion and probably contributes to the deficiency pattern picture of barium meal examination of small intestine. Baker and coworkers (1962) suggested that the thick layer of intestinal mucus is one of the causes of failure to obtain a jejunal biopsy in tropical sprue cases: however, the thick layer of mucus which covers the microvilli in primary malabsorptive disease, binds them together, thereby reducing the effective surface of microvilli. This factor has received very little attention by electron microscopists, although most of their published electron photomicro-

graphs of affected mucosa show a good deal of mucus. This appears to be of great significance.

Materials and Methods.

Described in Chapter 7.

Observations.

The absorptive cells of the intestinal villus under the electron microscope have finger like projections of minute size called microvilli. These microvilli have uniform dimensions, rounded apices and are present over the mucosal surface of the epithelial cell orientated vertically. Their height, over the villous crest, (to which part the present study is confined) ranges from 0.85 to 1.20μ and the width from 0.08 to 0.10μ (Fig. 119). The core of each microvillus is occupied by a prolongation of cytoplasmic substance from the terminal web, and this cytoplasmic substance is transformed into a dense fibrillar network. Most of the fibrillar structures are stretched along the long axis of the microvilli and merge below the ectoplasm of the apical part of the cell. This co-ordinates the microvilli with the apical part of the cell and constitutes a static structure. Some filaments are orientated in other axes as well. Each microvillus is enveloped by a plasma membrane. Under high resolution electron microscopy, the plasma membrane comprises two electron dense zones each 20Å thickness, which are separated by an electron thin zone of 30-35Å width. (Figs. 121 and 122). The outer dark zone is often found to be a little wider than the inner one.

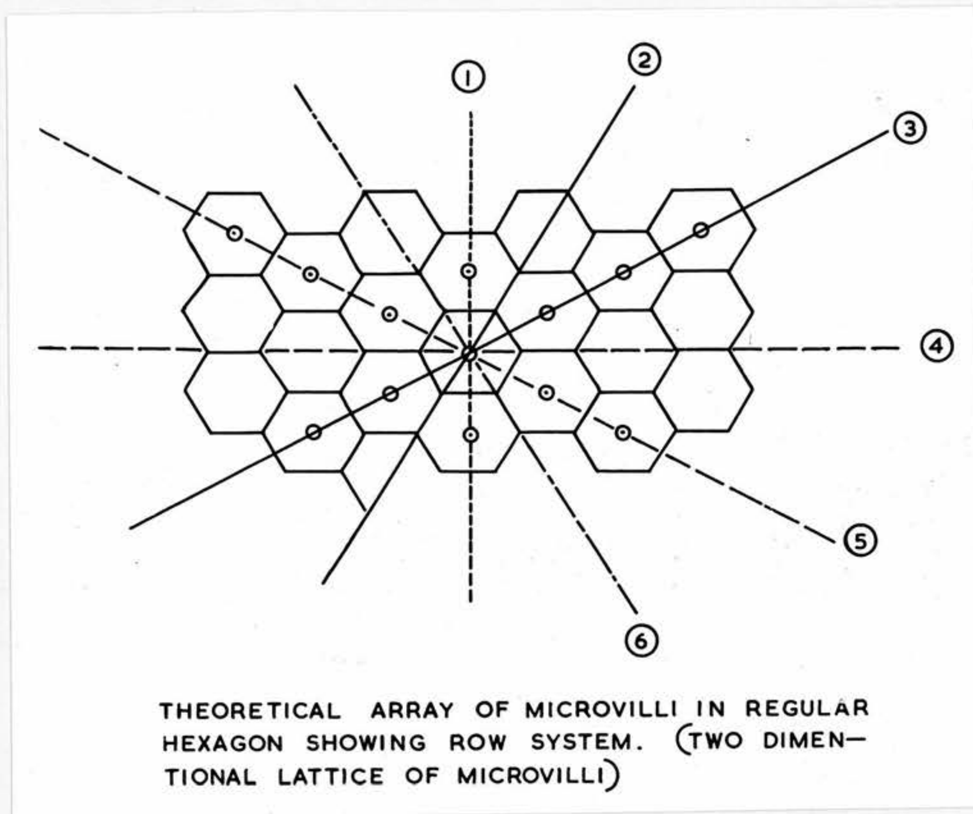


Fig. 115. A diagramatic presentation of E.M. 124 and 125.

Occasionally this apical plasma membrane dips down from the base of two adjacent microvilli for a short distance into the apical cytoplasm forming a gutter shaped open vesicle. These are the sites from which active pinocytosis takes place. (Palay and Karlin, 1959).

Origin of microvillus unit.

Analysis of the plane of section which has passed transversely through the microvilli has brought to light the pattern of packing of the microvilli. They are actually arranged in long rows, in the shape of arcs. When these long rows are present equi-distant from one another, their arrangement in hegular hexagons comes into view (Palay and Karlin, 1959). (Figs. 120 and 122). Arrangement of microvilli in long rows was noticed by Granger and Baker (1950), but the hexagonal array seemed more acceptable. In fact, both are correct in their observations. If the position of the microvilli are plotted on graph paper so that the microvilli are equidistant, the lines joining them together will form regular hexagons. As a result, the microvilli are found arranged in six symmetrical row systems. (Fig. 115). When an ultra thin section passing through adjacent epithelial cells is viewed under the electron microscope the actual arrangement of the microvilli is well seen. In such a picture of the junctional zone of the cells, the microvilli in addition to the hexagonal array, display a multiple row system. (Figs. 123, 124 and 125). The ratio of the distance between the rows and the distance

between adjacent microvilli is $\frac{\sqrt{3}}{2}$ or (0.875):1. This picture is now identical with the pattern of packing of microvilli seen in rats and in man.

In primary malabsorptive disease the ectoplasm of the core of the microvilli, the apical part of the cell and the terminal web as a unit shows various grades of involvement which follows closely the affection of the microvilli. When the microvilli are only slightly affected, the ectoplasm (subcuticular region) is less electron dense under the electron microscope. This suggests that this region is slightly oedematous. When the microvilli are grossly abnormal there is gross structural distortion of the apical part of the cell and the terminal web is found to be far less distinct. Another striking feature that has come to notice is that in a very early case changes in the ectoplasm often precede the changes of the microvilli.

Integrity of the ectoplasm and terminal web maintain the crystalline array of the microvilli. A very slight degree of molecular distortion (? due to oedema) of this apical region of the cell disturbs the hexagonal array by widening the gap between the successive rows of the microvilli. (Figs. 126 and 127). It was noticed that there was alteration in the arrangement of the microvilli in two of the three cases where biochemical evidences suggested that the diagnosis was primary malabsorptive disease (M.Y.; A.R.; Mrs. M.), but the biopsy specimen was found to be normal under the dissecting and light microscopes. The mucosal

abnormality was detected only under the electron microscope; the microvilli were uniformly shorter and less dense. Transection of the microvilli showed that the hexagonal array was lost but the microvilli were arranged in long rows of arcs. The only other abnormality was that the apical part of the cell was slightly less electron opaque. Whenever light microscopy showed partial atrophy of the villi, electron microscopic examination revealed irregularity in the height of the microvilli and a further degree of distortion of the arrangement of the microvilli. At this stage, although the dimensions of the microvilli have remained constant, they are arranged neither in the shape of a hexagon nor in long rows. (Figs. 128 and 129). The terminal web of such a cell is more indistinct.
In extreme cases of mucosal affection, where the light microscope shows a flat appearance, i.e. the villi are replaced by mucosal folds, the microvilli are found to be grossly abnormal. Their number is reduced; they have an irregular shape, an uneven contour; and variable length. Some are reduced to about one-third or one-fourth of the normal size; over some probably more affected areas only remnants of microvilli are seen and in extreme instances they are almost absent and replaced by a markedly undulating plasma membrane. At this stage, the cross section of the microvilli shows that in addition to complete distortion of their arrangement, they vary in their dimensions. (Fig.130). The apical part of the cell presents extreme oedema, so much so

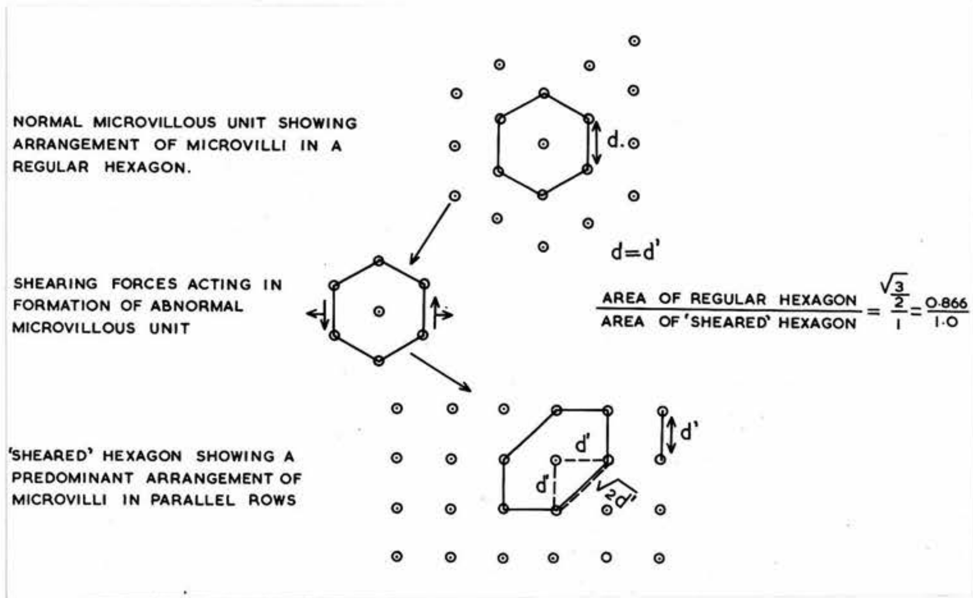
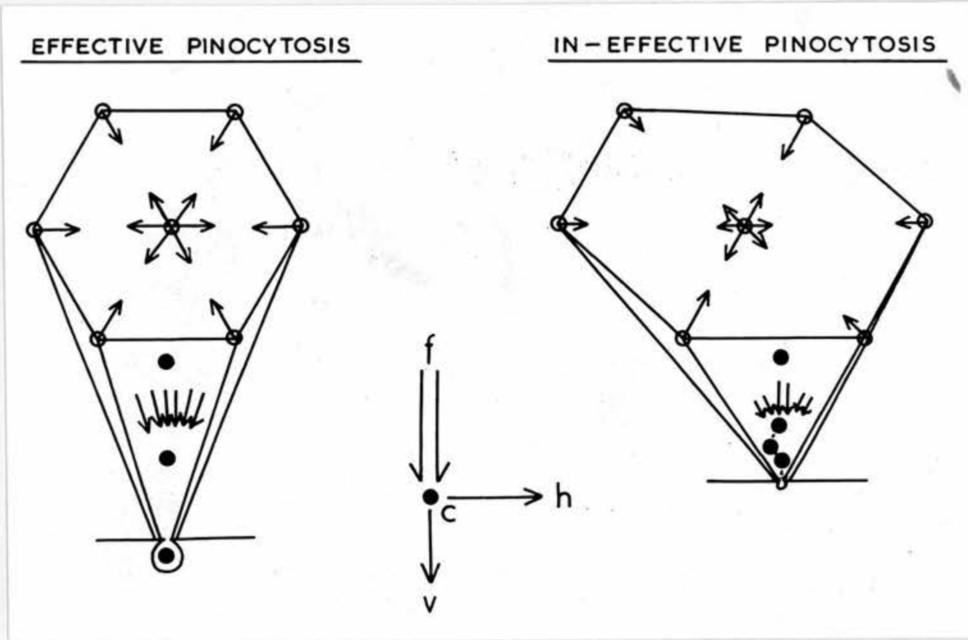


Fig. 116. Schematic diagram illustrating qualitative diminution of absorptive surface of upper part of small intestine even at a very early stage in primary malabsorptive disease. d and d' = distance between two microvilli in each row.

that the terminal web is not seen at all. (Fig. 131). This feature should not be confused with improper fixation (Fig. 34) or inadequate dehydration (Fig. 114), because there were no concomitant changes in the cytoplasm nor was there detachment of the microvilli.

Qualitative Affection of Mucosa.

There has been much discussion regarding quantitative reduction of the mucosal surface in both tropical and non tropical sprue. (Butterworth and Perez-Santiago, 1958; Ashworth et al, 1961; Padykula et al, 1961). In primary malabsorptive disease there is also a qualitative diminution of the absorptive surface of the cell. Normally the microvilli are arranged in a regular hexagon. If 'd' is the distance between two microvilli, then the area occupied by a microvillus hexagon unit is $d^2 \times 2.58$. Even early in the disease process, the molecular distortion of the apical cytoplasm of the cell exerts a differential pull to the regular hexagon and converts it into "a parallel array hexagon". The arrangement in long rows is now more distinct. The space between the successive rows has widened, giving an altered shape to the microvillus unit. (This phenomenon can be demonstrated by placing ink dots on a piece of thin rubber. By applying shearing forceps on one half of opposite sides of the regular hexagon, this altered shape will be evident). The area of this newly formed microvillus unit is $3d^2$. If d=d' (i.e. the distance between two microvilli in one row remains unaltered) it is quite



<u>Fig. 117.</u> Demonstration of the effect of alteration in the arrangement of microvilli on the pinocytotic activity. f = pinocytotic force acting on the chylomicron (c). h = horizontal component of the effective force. v = vertical component of the effective force.

evident that the altered microvillus unit will occupy a larger area. In other words it contains less mucosa within the unit area and is thus qualitatively poor. (Fig. 116).

Forces behind pinocytotic activity.

The altered shape of the microvillus unit has great functional significance also. The symmetrical arrangement of the microvilli might aid in the pinocytotic activity of the plasma membrane. A regular hexagon can be divided into six equilateral triangles. It can be argued that in addition to the gravitational effect, an electrical force appears to act on a chylomicron to push it into the pinocytotic vesicle. Such forces can be resolved into horizontal and vertical components. In a symmetrical figure all corners contribute equal horizontal forces which act in such directions that the resultant horizontal force is zero. Therefore only the vertical components remain acting on the particles (Chylomicrons) and therefore these move downwards to the apex of pinocytotic vesicle. (Fig. 12). The microvilli of healthy mucosa can therefore push chylomicrons into the pinocytotic vesicles which are present in the apical part of the cytoplasm. But in a non-symmetrical figure the horizontal force components will not, in general, cancel. Thus the resultant motion will be affected by asymmetrical horizontal forces exerted by the corners. As a result of this there will be dissipation of the downward motion. (Fig. 117). The bigger the figure, the weaker is the downward force.

Coulomb's law of two electrical charges is $f \alpha \frac{1}{r^2}$ when f = force, r = distance between two objects. If the distance increases, the force decreases. Apart from the change in the force exerted, the molecular distortion of the apical part of the cytoplasm of the cell in disease fails to respond normally to the action of the force exerted on it. (Just as when the neck of a balloon is twisted, it cannot be inflated). That is why the pinocytotic vesicles are found to be smaller and empty in primary malabsorptive disease even after feeding emulsions of lipid. (Ashworth and Cheers, 1962).

<u>Distortion of molecular arrangement of lipo-protein membrane.</u>

It is believed that pinocytosis accounts for only a fraction of lipid absorbed from the small intestine. Most of the lipid, is diffused through the lipo-protein membrane from a "micellar phase". (Hoffmann and Borgström, 1962). The lipids in this phase are usually not seen by the electron microscope. After crossing the membrane of the epithelial cell and during its transit through the cytoplasm, the fatty acids are converted into triglycerides which can be seen by the electron microscope. Under these circumstances in cases of malabsorption we should look for evidence of molecular distortion of the plasma membrane, preventing transport of lipids into the epithelial cell.

There is excess of lipo-protein membrane over the lateral part of the cell where it is folded. Therefore by unfolding it can accomodate increasing cytoplasmic

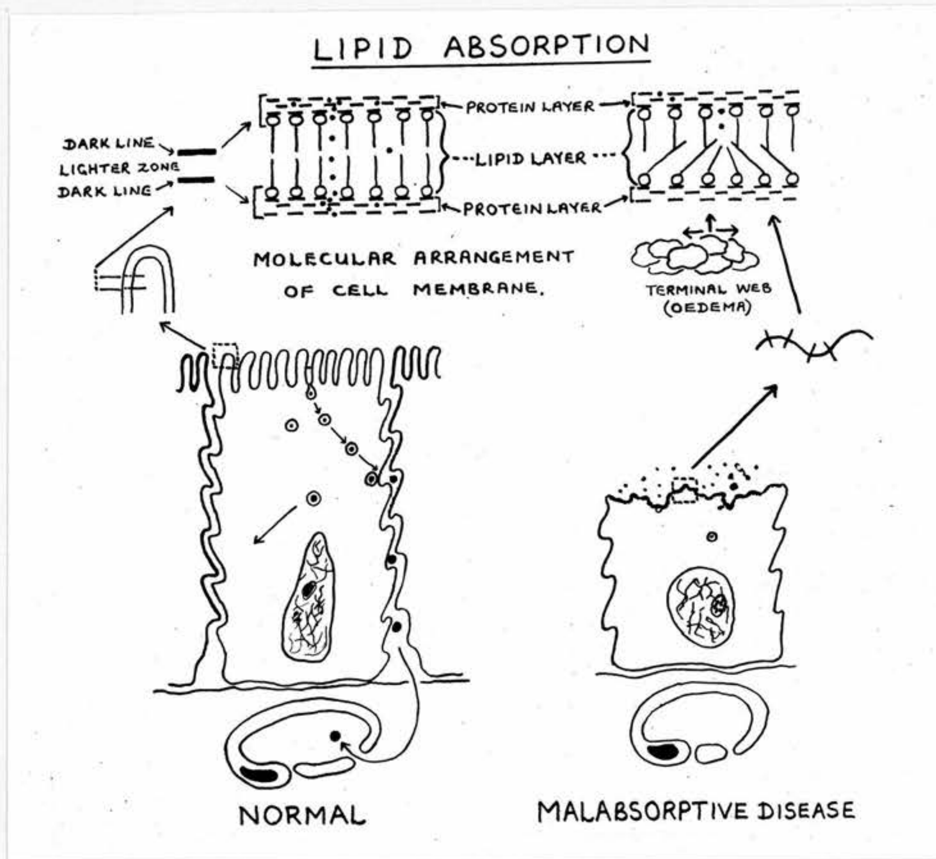


Fig. 118. Note the effect of oedema in the region of terminal web and subcuticular region of the epithelial cell in primary malabsorptive disease causing distortion of themolecular arrangement of the lipoprotein membrane enveloping the microvilli.

volume while absorbing water or when the cell is brought into contact with hypotonic intestinal content. (Rusca, 1960; Wynn Williams, 1963). Unfortunately there is no excess of lipo-protein membrane covering the microvilli. Therefore swelling of the substance of the core of the microvilli and ectoplasm alters the molecular arrangement of the lipo-protein membrane which covers the absorptive surface of the cell. Normally the paraffin chains of one set of lipid molecules (of the bimolecular lipid leaflet of the lipo-protein membrane) meet end to end with the paraffin chain of the other set of molecules. The swelling of the cytoplasm distorts this arrangement, as a result of which the paraffin chains of the lipid molecules cross each other and meet each other at an angle. This could prevent the effective diffusion of lipids into the cell. (Fig. 118). The distortion of molecular arrangement dan be demonstrated by drawing sets of parallel lines on a non-inflated balloon. By creating differential inflation, the parallel lines over the inflated area are spread out and meet with the lines over the non-inflated area at an angle.

Mucus factor.

Excessive mucus production from the intestinal epithelium is another constant feature met with in primary malabsorptive disease. (Fig. 104). Mucus is opaque to electron beams. The thickness of the mucus layer varies inversely with the density of the microvilli: in other words, in a very early stage, where microvillous

affection is minimal, a thick layer of mucus is seen to form a film over the surface of the microvilli and to bind them together. In some cases the microvilli show a tendency to fuse with one another (previous chapter). In an advanced stage of mucosal affection, when only a few microvilli remain, the mucus layer has no static structure to adhere to, and therefore is washed away leaving the microvilli bare.

Discussion.

In primary malabsorptive disease, the microvilli present a remarkable picture of various grades of affection. There have been reports of lack of correlation between the mucosal picture and the severity of the illness; the mucosal affection of intestine in this disease condition can be very patchy and a biopsy could be obtained from a relatively unaffected area. (Girdwood et al, 1961; Shiner et al, 1962). Rubin and coworkers (1962) reported that the clinical condition of the patient correlated better with the length of the bowel affected than the severity of the lesion of the proximal part of the small intestine. In the present series it was found that in this disease, cells which have normal microvilli were adjacent to another cell with grossly abnormal microvilli. Similar findings have been reported by Shiner and coworkers (1962), but a regular occurrence of a histological lesion of the jejunal mucosa has been constantly reported by most of the workers in this field of research.

It can be argued that the arrangement of the microvilli in a regular hexagon is probably the body's best economy. By this arrangement, the maximum absorptive surface is held in contact with the nutrients in the food within the minimum space. In nature one notices that a bee-hive consists of innumberable hexagonal cells inside which a maximum quantity of honey can be collected within a very limited space. In the plant kingdom, chlorophyll, which is such an essential substance is formed mainly by the combination of hexagonal rings. The arrangement of the florets in the flower head of the daisy type compositae are arranged in spiral arcs which in small regions are in hexagonal array. Any deviation from this structural (aromatic) arrangement would probably make the dynamics of the construction poor qualitatively. In primary malabsorptive disease, in addition to quantitative reduction of the absorptive mucosa of the small intestine, the cells are poor qualitatively. From my observations, it would seem that the qualitative affection of the mucosa precedes the quantitative change.

From the above observations it seems quite evident that the molecular arrangement of ectoplasm and terminal web preserves the crystalline array of the microvilli. Ashworth and coworkers (1961) noticed absence of the terminal web in the intestinal epithelial cells of two cases who suffered from non tropical sprue. Shiner and coworkers (1962) noticed that the central core of substance is lacking from the abnormal microvilli and that

at the same time the terminal web was less well demarcated. But there has been no mention of affection of the terminal web substance when the microvilli are only minimally affected. From the present observations it would be argued that molecular distortion of the terminal web affects the crystalline array of the microvilli and makes the cell poor qualitatively. Probably the oedema of the apical cytoplasm alters the molecular configuration of the plasma membrane which envelopes the apical absorptive surface of the cell.

The small intestine is the main site from which fats and lipids are absorbed. As soon as the fat (chyme) enters the small intestine it comes in contact with the lipolytic enzyme of the pancreatic juice. Bile salts are added to it and intestinal peristalsis enhances the process of emulsification of lipids. Triglycerides in the lumen of the intestine are now broken down into fine droplets, and intestinal lipase act on them. About one half to one third of the lipid is split up and hydrolysed into fatty acids and glycerol: the remainder of the glyceride is partially hydrolysed to mono and diglycerides. The exact composition of the intestinal glycerides is not clear, but it is known that monoglycerides comprise the major part of the result of pancreatic lipolysis (Hoffmann and Borgström, 1962). These observations do not support the theory of absorption of lipid from the intestine as an emulsion and suggest that fatty acids and glycerides are absorbed by a process of active transport, across

the cell membrane into the epithelial cells of the intestine (Wilson, 1962).

Protein, which forms an artificial membrane over the polar head of the lipid molecules, does not offer resistance to free diffusion of the nutrients from the food into the intestinal epithelial cell. (Widdas, 1963). Therefore the double layer of lipid molecules probably contributes to the permeability barrier. It can be argued that the distortion of the molecular arrangement of the lipo-protein membrane in primary malabsorptive disease might be the primary cause which hinders the passage of the lipid from the "micellar phase" beyond the outer protein layer of the membrane. In addition to the lipid other nutrients might be similarly prevented from entering the epithelial cells and thereby account for the multiple deficiency syndromes.

The same mucosal affection alters the pinocytotic activity of the intestinal epithelial cell. The lipid particles enter the cytoplasm of the cell by means of membrane invasion. Although nobody has so far demonstrated how these vesicles are formed, it is believed that the mechanism of engulfment by pinocytosis does exist. In primary malabsorptive disease the distortion of the molecular arrangement of the apical part of the cell hinders the active process of pinocytosis. Although one notices minute pinocytotic vesicles in the affected mucosa, they cannot engulf

lipid, and remain empty. This observation suggests that there is distortion of some electro-mechanical force which normally aids the process of pinocytosis.

In addition to these structural alterations in primary malabsorptive disease, there must be some additional change in the enzymatic transference mechanism of the mucosal wall of the intestine. It is now an established fact that there is no selective area for the absorption of lipid from the small intestine in the rat: probably the distal half of the small intestine is capable of absorbing as much lipid as the proximal half (Bennett, 1964). Removal of 40% of the small intestine (either jejunum or ileum) does not reduce the absorptive capacity for tricaprylin. Is the intestinal absorptive capacity and site of fat absorption in the rat the same as in man? If so, the problem now arises that in primary malabsorptive disease, where the ileal mucosa remains normal, there is deficient absorption of fat. The probable explanation to this riddle is that there is an abnormal change which takes place in the products of lipid digestion, in this disease, which make it unabsorbable.

A molecule in the interior of a liquid is attracted in all directions by the other molecules of the liquid. But at the surface there is a nett inward attractive force which acts on the molecule, tending to draw it inwards from the liquid surface. This may be the explanation of how water-soluble food substances

in the intestinal juice come in contact with the intestinal epithelial cell. Moreover, when a molecule is in a liquid, the pressure on the concave side of the liquid meniscus is greater than the pressure on the convex side. This constitutes an inward pull. But when one liquid nutrient in the intestine is spread on another liquid (mucus) in which it does not dissolve or with which it mixes only partially, the inward pull of nutrients in the intestinal juice is decreased and this is accompanied by a decrease of free energy. That is why in the presence of a thick mucous layer (as in primary malabsorptive disease), besides the mechanical factor exerted by this layer of mucus, the surface energy is altered, thus preventing the particles from coming in contact with surface of mucosa. Very little quantitative information is yet available regarding 'inter facial surface energy'. (Glasstone, 1960).

Under the electron microscope, the cell surface is found to have a dynamic structure of some depth. Time-lapse cine film has revealed that the surface of the living cell extends deep into the cell substance. Moreover in primary malabsorptive disease microvilli vary much in their height and density: therefore any attempt to measure the qualitative diminution of the absorbing surface of the cell by measuring the size of the cell (Lancet, 1962) or taking the microvilli into account will give an erroneous result. In my opinion the qualitative affection of the cell and the alteration of cell dynamics would be better explained in terms of

affection of the plasma membrane.

<u>Summary.</u>

1. The small intestinal microvilli are arranged in long rows in the form of arcs. When these rows are closely and regularly packed, the arrangement of the microvillus unit as a regular hexagon comes to light.

2. Affection of the apical cytoplasm is a very early sign of mucosal change in primary malabsorptive disease. This may precede the changes in the microvilli.

3. The molecular arrangement of the apical cytoplasm and terminal web have a close association with the hexagonal array of the microvilli. In primary malabsorptive disease oedema of the apical cytoplasm disturbs the packing of the microvilli and make the absorptive surface poor qualitatively.

4. Both the diffusion hypothesis and the pinocytotic hypothesis of lipid absorption are discussed in relation to the altered dynamics of the cell in this disease state. Alteration of the molecular configuration of the lipo-protein membrane covering the apical region of the cell is one of the primary factors which brings about the multiple deficiency syndrome.

5. Finally, a thick layer of mucus,which covers the microvilli in this disease introduces an additional barrier between the nutrients, and the mucosa of the intestine.

REFERENCES.

1. Ashworth, C.T. and Cheers, W.C. Jr. (1962). Fed. Proc., 21, 880.

2. Ashworth, C.T., Saunders, E., Pearce, M.B. (1961). Arch. Path., 71, 13.

3. Baker, S.J., Ignatius, M., Mathan, V.I., Vaish, S.K., Chacko, C.C. (1962). Intestinal biopsy. Ciba Foundation study group, No. 14. J. & A. Churchill Ltd., London.

4. Bennett, S. (1964). Quart. J. exp. Physiol., 49, 210.

5. Brown, A.L. (1962). J. Cell. Biol., 12, 623.

6. Butterworth, C.E. and Perez-Santiago, E. (1958). Ann. intern. Med., 48, 8.

7. Curran, R.C. and Creamer, B. (1963). J. Path. Bact., 86, 1.

8. Danielli, J.F. and Harvey, E.N. (1935). J. cell. comp. Physiol., 5, 483.

9. Davson, H. (1962). Circulation, 26, 1022.

10. Frazer, A.C., French, J.M., Thompson, M.D. (1949). Brit. J. Radiol., 22, 123.

11. Girdwood, R.H., Delamore, I.W., Wynn Williams, A. (1961). Brit. med. J., i, 319.

12. Glasstone, S. (1960). Text book of Physical Chemistry, 2nd Ed. Macmillan & Co. Ltd., London.

13. Gorter, E. and Grendel, F. (1925). J. exp. Med., 41, 439.

14. Granger, B. and Baker, R.F. (1950). Anat. Rec., 107, 423.

15. Hartman, R.S., Butterworth, C.E. Jr., Hartman, R.E., Crosby, W.H., Shirai, A. (1960). Gastroenterology, 38, 506.

16. Hoffmann, A.F. and Borgström, B. (1962). Fed. Proc., 21, 43.

17. Lancet, (1962). Annotation. The cell surface. ii, 597.

18. Padykula, H.A., Strauss, E.W., Ladman, A.J., Gardner, F.H. (1961). Gastroenterology, 40, 735.

19. Palay, S.L. and Karlin, L.J. (1959). J. biophys. biochem. Cytol., 5, 363.

20. Paulley, J.W. (1954). Brit. med. J., 2,1318.

21. Robertson, J.D. (1959). Biochemical Society Symposium, Vol. 16, 3. The University Press, Cambridge.

22. Rubin, C.E. (1961). Ann. Rev. Med., 12, 39.

23. Rubin, C.E., Brandborg, L.L., Phelps, P.C., Taylor, H.C. (1960). Gastroenterology, 38, 28.

24. Rubin, C.E., Brandborg, L.L., Flick, A.L., Macdonald, W.C., Parkins, R.A., Parmentier, C.M., Phelps, P., Sribhibhadh, S., Trier, J.S. (1962). Intestinal biopsy. Ciba Foundation study group, No. 14. J. & A. Churchill Ltd., London.

25. Ruska, C. (1960). Atlas of Electron Microscopy. Compiled by F. Scange. Elsevier Publishing Co., 1964.

26. Shearman, J.C., Girdwood, R.H., Wynn Williams, A., Delamore, I.W. (1962). Gut, 3, 16.

27. Shiner, M., Lacy, D., Hudson, R.H. (1962). Intestinal Biopsy, Ciba Foundation study group, No. 14. J. & A. Churchill Ltd., London.

28. Stoeckenius, W. (1962). Circulation (Part 2), 1066.

29. Weiss, J.M. (1955). J. exp. Med., 102, 775.

30. Widdas, W.F. (1963). Recent Advances in Physiology, 8th Ed., Edited by R. Greese. J. & A. Churchill Ltd., London.

31. Wilson, T.H. (1962). Intestinal Absorption, W.B. Saunders & Co., Philadelphia.

32. Wynn Williams, A. (1963). Gut, 4, 1.

33.

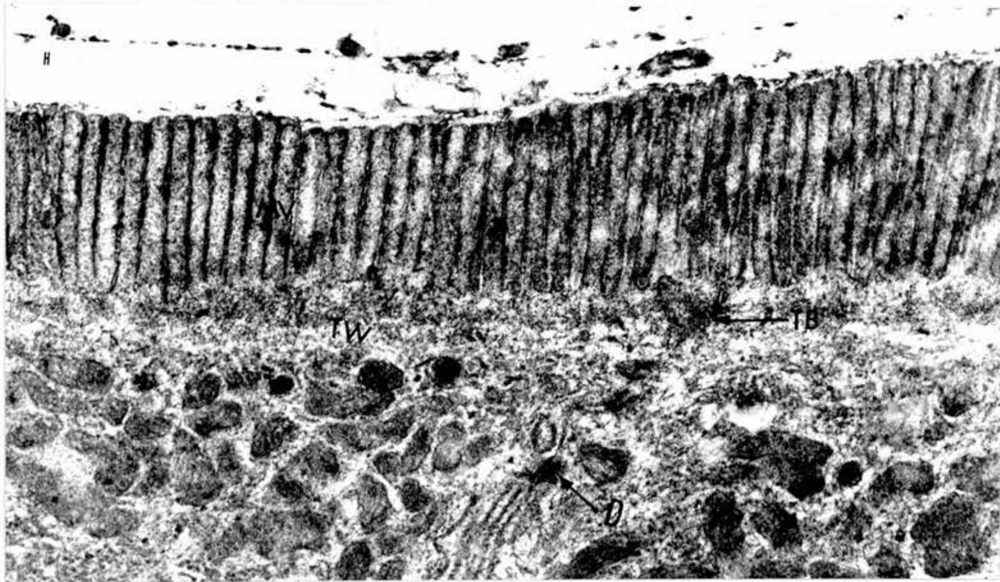


Fig. 119. Electron micrograph of upper part of normal villous epithelium. MV = microvilli; TW = terminal web; TB = terminal bar; D = desmosomes binding the lateral walls of adjacent cells. x 24,700

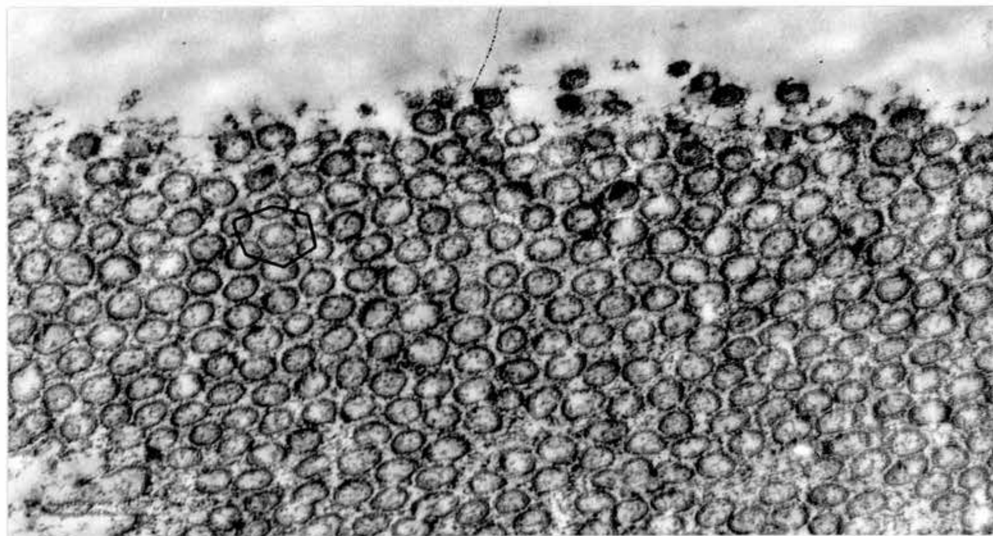


Fig. 120. Transverse section through the microvilli. The multiple row system of the arrangement of the microvilli is not clearly seen because the plane of section is probably nearer the tip of the microvilli. The hexagonal array is only seen in places. x 48,000

Fig. 121. Longitudinal section of microvilli. x 52,000

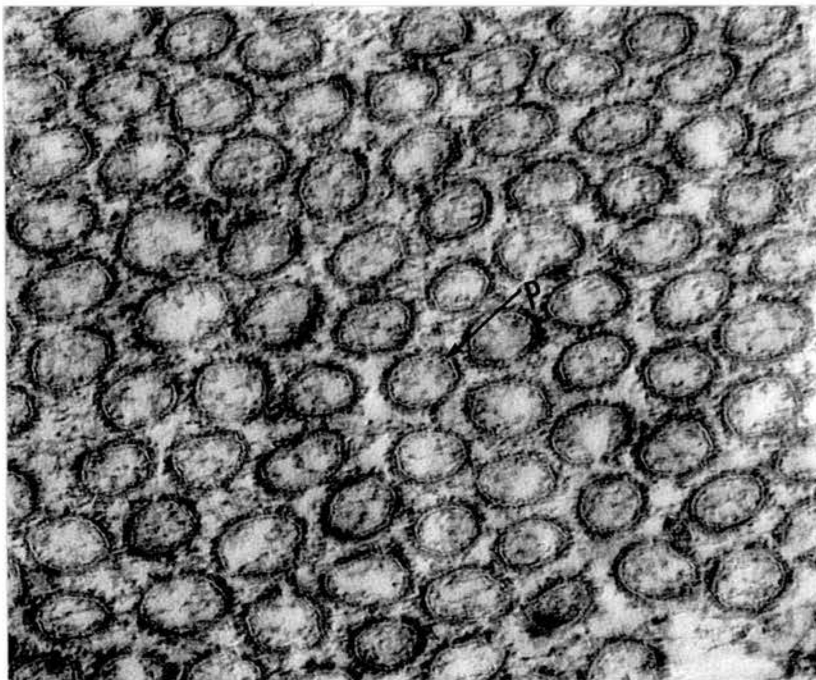


Fig. 122. Transverse section of microvilli. x 92,800. E.M. showing the plasma membrane enveloping the microvilli.(P) It comprises a double electron dense layer separated by a less dense layer.

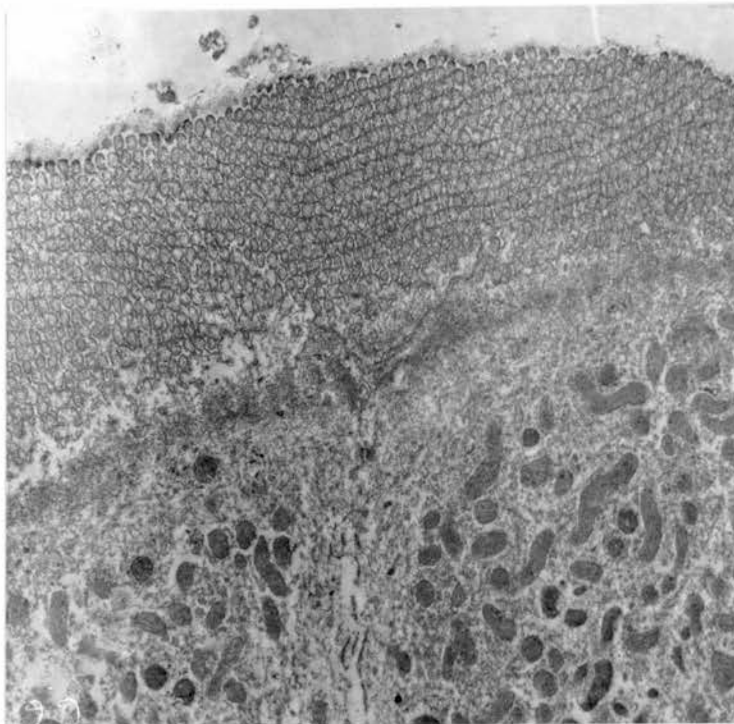


Fig. 123. E.M. of the junctional zone of epithelial cells. The microvilli clearly display row system. x 12,000

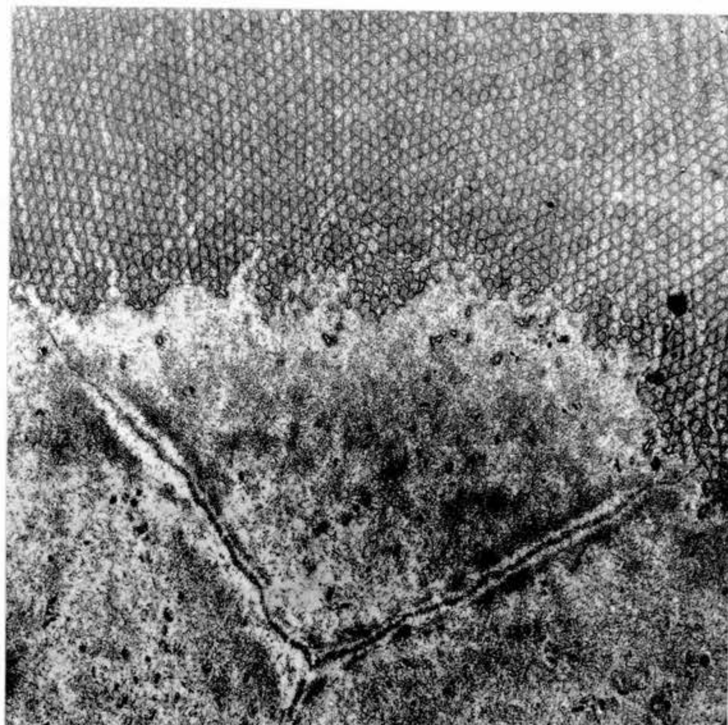


Fig. 124. Section passing through a greater area of the junctional zone bringing into picture the multiple row system in the arrangement of the microvilli. x 7,200

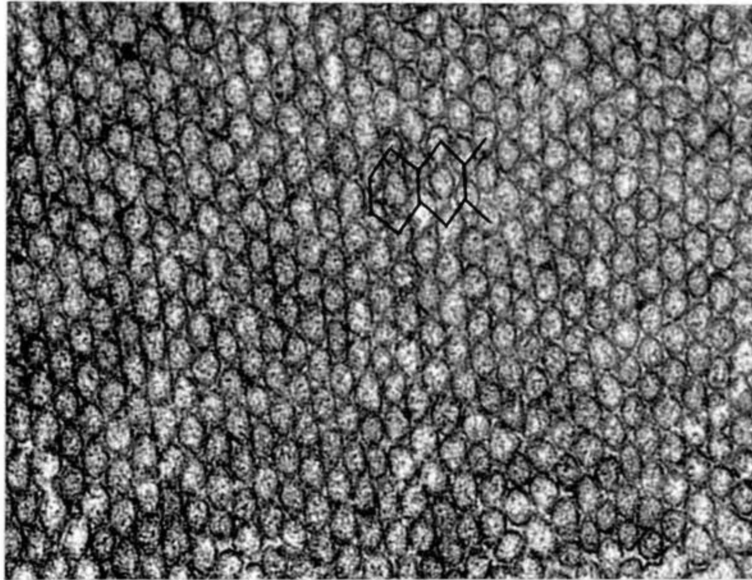


Fig. 125. E.M. showing both hexagonal array and multiple row system in the arrangement of the microvilli. x 28,000

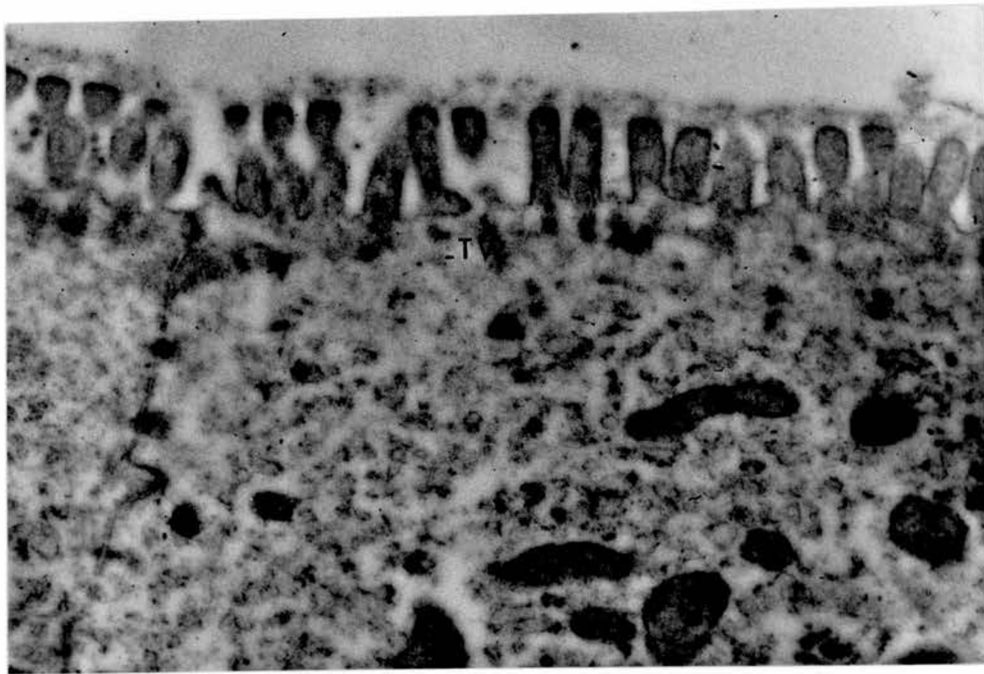


Fig. 126. Electron micrograph of jejunal mucosa of primary malabsorptive disease showing only minimal affection. The microvilli are shorter and fewer. The terminal web (TW) is less electron opaque than normal. x 27,200

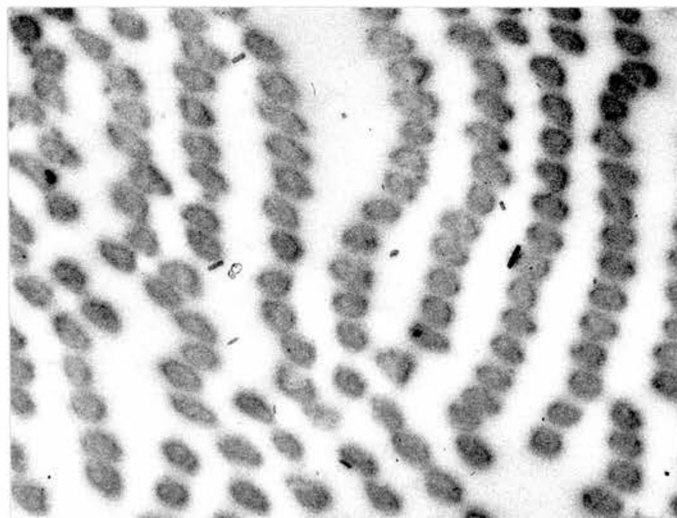


Fig. 127. Transverse section through the microvilli shown in Fig. 126) showing their arrangement in parallel rows, but the microvilli are not equidistant from each other. x 48,000

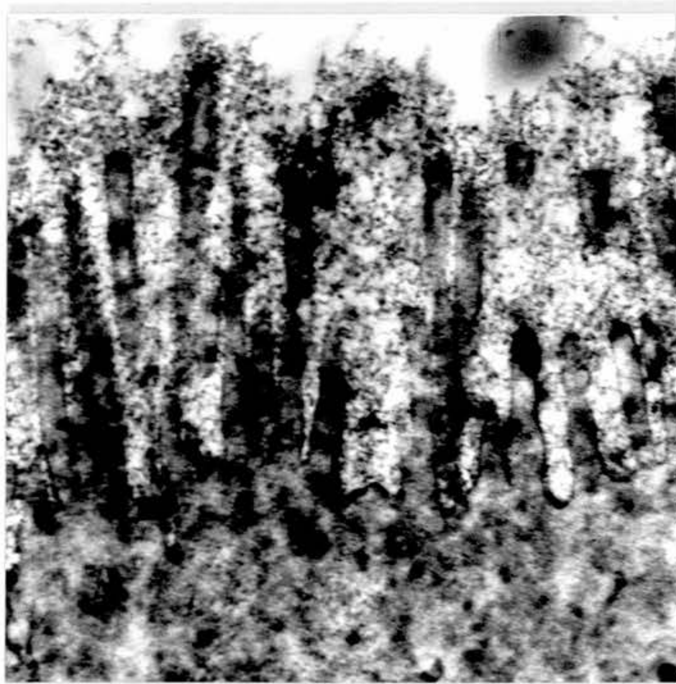


Fig. 128. The microvilli are of unequal size. x 48,000

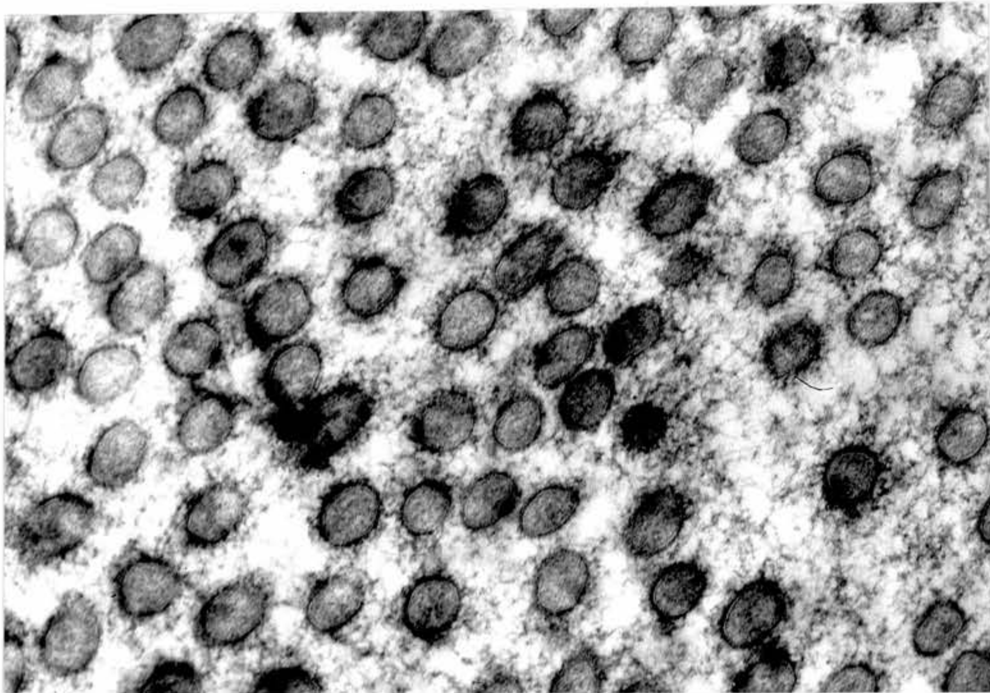


Fig. 129. Electron micrograph of transverse section through the microvilli shown above. There is loss of row system of their arrangement, and hexagonal array. x 112,000

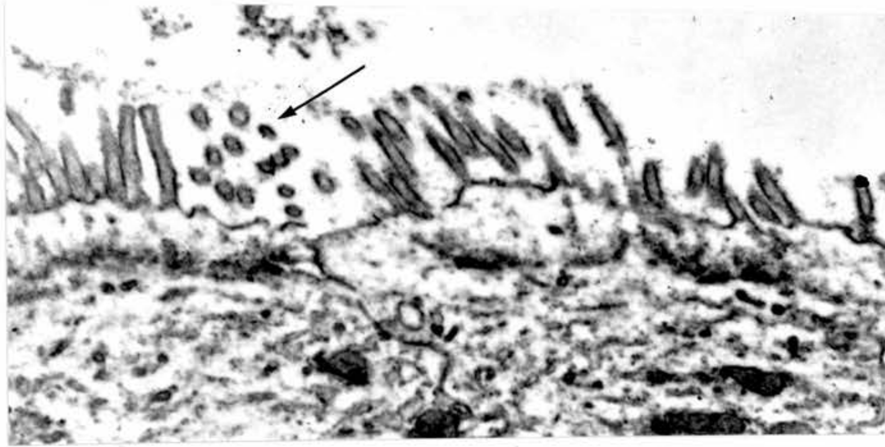


Fig. 130. E.M. showing more advanced degree of affection of jejunal epithelium due to primary malabsorptive disease. Abnormal microvilli are seen on the surface of these cells and there is marked oedema, in the subcuticular region of the cell. Their shape, diameter and the hexagonal array (arrow) is completely distorted. x 28,000

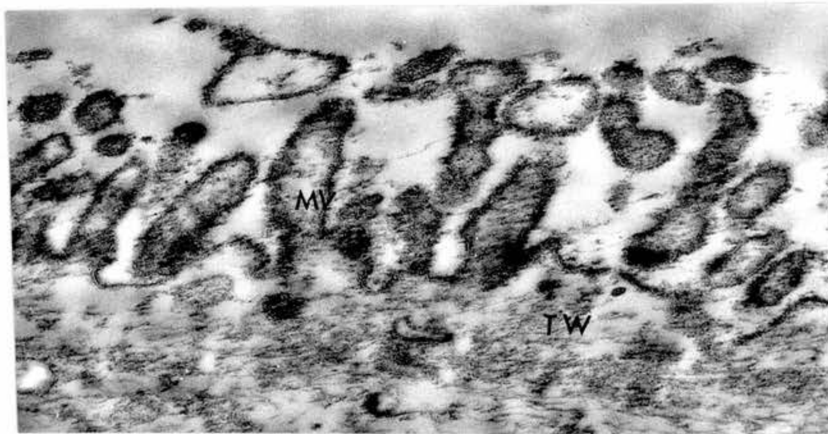


Fig. 131. Electron micrograph showing moderate degree of affection of epithelial cell due to primary malabsorptive disease. The terminal web (TW) is less distinct than Fig. 126 . Microvilli (MV) are absent over some areas. x 56,000

CHAPTER 10.

JEJUNAL MUCOSA IN SECONDARY INTESTINAL STEATORRHOEA

Introduction.

It is interesting to extend the studies of intestinal mucosa to certain secondary malasborptive syndromes. In this chapter I include steatorrhoea secondary to -

I. Whipple's Disease.
II. Progressive systemic sclerosis.
III. Zollinger-Ellison Syndrome.

I. Whipple's Disease.

A male, D.C. (51), was admitted to the Western General Hospital, Edinburgh with a history of episodes of fuzziness and a vague cervical ache during the past four years. He also complained of pain in wrists, knee joints and cervical spine during the past 3 years. He developed "looseness of the bowel" two years ago and during the past year the stools have been pale, bulky and buoyant. On examination there was generalised wasting and a petechial rash over the thighs. Examination of the stools showed intermittent steatorrhoea (up to 40.5 g. fat per day), impaired xylose, B_{12} and ? folic acid absorption. A barium meal and follow through examination showed that the rugae of the jejunum and upper ileum were extremely coarse and hypertrophic. Liver function and pancreatic function were normal. There was

no hyperacidity. Sigmoidoscopy and rectal biopsy were normal. Jejunal mucosa was obtained for microscopic examination by the peroral biopsy method.

Microscopic appearances of the jejunal mucosa.

Under the dissecting microscope, a thick layer of mucus was found to cover the mucosa but short treatment with a mucolytic agent removed this. There were no true finger like villi over the mucosa; instead, it was full of convolutions (Fig. 132). An occasional ridge was noted among these convolutions.

Under the light microscope the villi were found to be short and broad (150μ to 300μ in height). Over some areas the villi were replaced by mucosal folds. At the same time the height of the glandular layer was 350-400μ, about twice normal. The columnar absorbing cells were normal in height and the dimensions of the nucleus were also normal. The refractile border of the epithelial cell was well preserved, but the cytoplasm had a distinct foamy appearance. A peculiar type of mononuclear cell, probably a macrophage, was found in the lamina propria: in haematoxylin and eosin stained sections these cells had a frothy appearance, they were strongly PAS positive, but were non-sudanophilic. (Fig. 133). Under the high power of the light microscope these cells appeared to contain closely packed granules or irregular clumps of a homogeneous material. In places these PAS-positive substances were so numerous that they pushed out the tips of the villi and made them club shaped. There was also

a slight increase in the infiltration of mononuclear cells,mostly lymphocytes, into the lamina propria, and in addition to this the lamina propria showed large irregular spaces. There were few mitotic figures compared with the similar mucosal picture in primary malabsorptive disease.

Electron microscopic examination showed that the microvilli on the free surface of the absorbing cell was shorter. They varied in height from 0.3μ to 0.5μ but they maintained regularity in spacing and uniformity in packing, and were present parallel to the long axis of the cell. (Fig. 136). There were very few pino-cytotic vesicles over the apical part of the cytoplasm. In general the cytoplasm of the cell was more electron opaque than normal. In places it presented a vacuolated appearance. Some lamellated membranous structures were noted in the cytoplasm. In the connective tissue of the lamina propria, there were spaces of various size and shape in which curious aggregations of electron dense particles were seen. (Fig. 137). Some of these had smooth surfaces and others had uneven surfaces.

The present case has shown remarkable clinical improvement with a broad spectrum antibiotic (tetracycline). After undergoing this treatment for six weeks the patient was readmitted to hospital and biopsy of jejunal mucosa was obtained by peroral route.

Rudimentary finger like villi were noted under the dissecting microscope and some of these had rather a broad

base. The convolutions which were noted in the pretreatment mucosa were absent. Under the light microscope the villi were rather shorter in height than normal, varying from 150-250µ. The absorptive cells, goblet cells and refractile striated border appeared normal. (Fig. 134). There was no increase in the cellular infiltration into the lamina propria. The glandular layer was not hypertrophied. On staining the sections with PAS, very little positively stained substance was noted in the stroma of the villi. (Fig. 135). This would have escaped notice were one not aware of the enormous amount of PAS positive material in the pretreatment specimen.

Ultra thin sections of this mucosa when examined under the electron microscope presented microvilli of uniform size and dimension. They varied in height from 0.9-1.12µ and their distribution was uniform. (Figs. 139 and 140). On transverse section their hexagonal array and multiple row system were evident. The mitochondria and the endoplasmic reticulum appeared normal: no abnormal structures were noted in the cytoplasm of the cells. The nuclei of the cells, subepithelial basement membrane and the structures in the lamina propria appeared normal. There was no evidence of bacilli like bodies in the post-treatment specimen.

Discussion.

Mucosal atrophy as noted in the present case under the dissecting, light and electron microscopes, has not

been reported in the literature. Haubrich et al (1960) reported blunting of the villi in Whipple's disease when viewed under the light microscope, but the microvilli on the free surface of the intestinal epithelial cells appeared normal in number and configuration. Fisher (1962) gave a similar account of the absorbing epithelial cell in Whipple's disease under the electron microscope. The microvilli of these cells were normal in length and width, and there was no abnormality in the packing of the microvilli. However in the present case abnormal villous architecture and hypertrophy of the glandular layer of the mucosa were seen. These features are noted in non tropical sprue but as a contrasting feature there was no accompanying increase in the mitotic activity of the epithelial cells of the mucosa. Under the electron microscope although the microvilli were shorter there was no irregularity in their packing. The cytoplasm of the cell was uniformly electron opaque. At this stage it can only be suggested that an accumulation of some unknown substance affects the molecular arrangement of the apical cytoplasm, thus altering its acceptance of nutrients entering the cell. The same could account for the absence of pinocytotic vesicles in the apical part of the cytoplasm.

The original observation that the mucosa of the small intestine and the regional lymph nodes contain large lipid-laden cystic areas in Whipple's disease gave it the alternative and popular name of "intestinal lipodystrophy". Sieracki and Fine (1959) made an

elaborate study of the cytoplasm of the frothy cells mentioned by Whipple, and found that these cells are strongly PAS positive. Black-Schaffer (1949) drew attention to the fact that the cells are non-sudanophilic. Since this time the nature of these cells has received great attention. Examination of thick and thin sections of the PAS positive granules showed that these are aggregations of membranes, vesicles and amorphous substances. Cohen (1962) with the aid of the electron microscope probed into the detailed structure of these granules. He noted that some of them appeared as single rods, some as multiple rods and some rods appeared as if partly digested. From these observations he concluded that these substances are probably products of digestion of some materials phagocytosed by macrophages. Although the exact nature of these 'rods' is not known, their structure is consistent with bacillary bodies (Haubrich, 1960; Chears and Ashworth, 1961; Curran and Creamer, 1963).

These macrophages with unique morphological features may be found outside the intestinal tract, in various organs of the body (Sieracki and Fine, 1959). Hellwig and coworkers (1961) suggested that because of some metabolic defect, abnormal substances are produced in the lumen of the intestine or in the tissue spaces of the lamina propria and are phagocytosed by macrophages. These macrophages accumulate in the jejunal mucosa and travel to distant organs of the body. His alternative suggestion was that these abnormal cells are mutant

'clones' on the reticulo-endothelial system which is distributed throughout the body.

Previously this disease was thought to be always fatal, the patient surviving only for a few months or years after its onset. Chears and Ashworth (1961) reported a case of Whipple's disease which responded to adrenocorticotrophic hormone. Gibb et al (1964) have described a case which improved with adrenocortico-trophic hormone although the mucosal picture of the small intestine remained unaltered. Speedy clinical improvement and disappearance of the bacillary bodies from the intestinal mucosa has been reported by Ashworth et al (1964), by combined treatment with tetracycline and cortisone.

There is no report in the literature regarding the effect of treatment on the absorptive surface (micro-villi) of the intestinal mucosa. In the present case, after treatment with tetracycline only for six weeks, the patient was found to have gained two and a half stones in weight, and an improved mucosal picture was evident on microscopic examination. Although the improvement of the mucosa and its absorptive surface was not so evident under the dissecting and light microscopes its appearance under the electron microscope was most striking. The dimensions and distribution of the microvilli were those of normal epithelial cells of the small intestine.

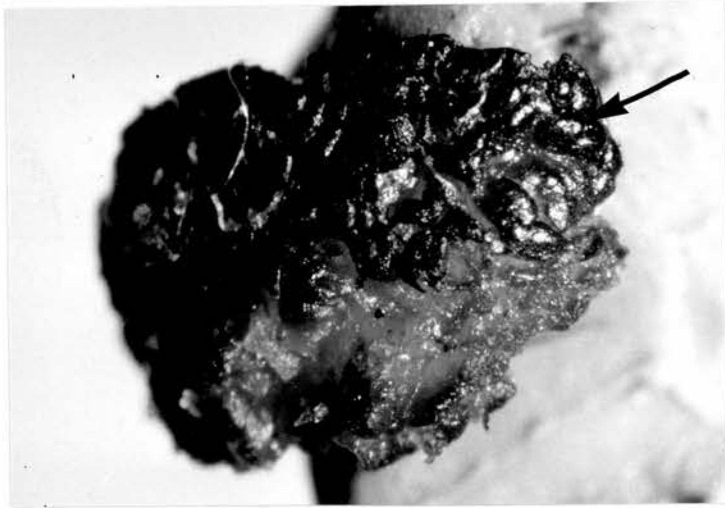


Fig. 132. Jejunal mucosa from a patient with Whipple's disease showing convolutions (arrow) x 30

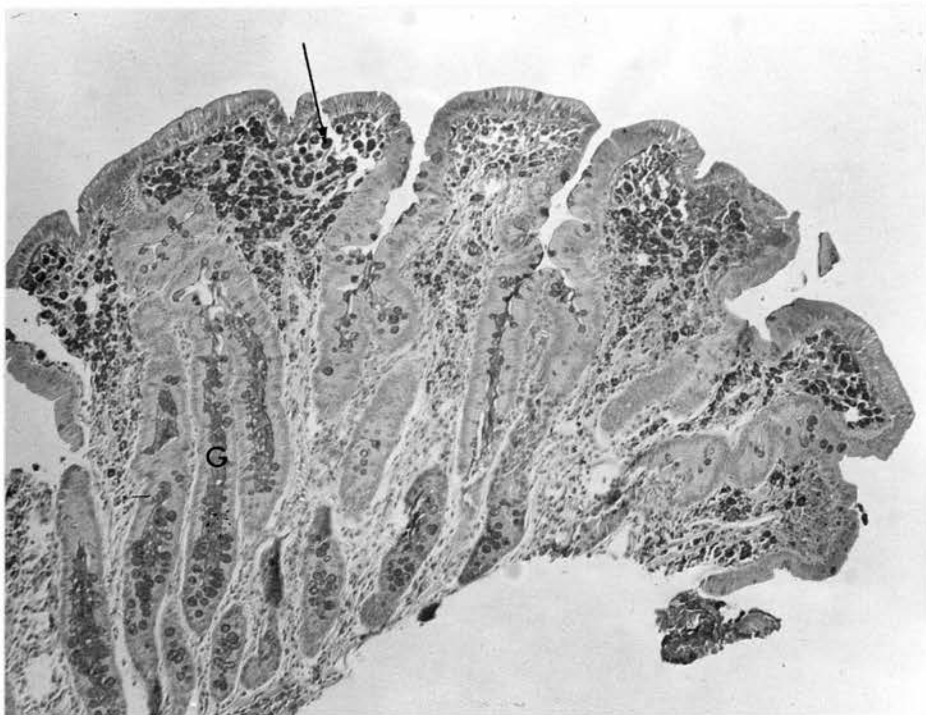


Fig. 133. Histological section from the area shown in above picture. The villi are blunted The glands (G) are hypertrophied. There is PAS positive material in the stroma of the villi (arrow) x 120

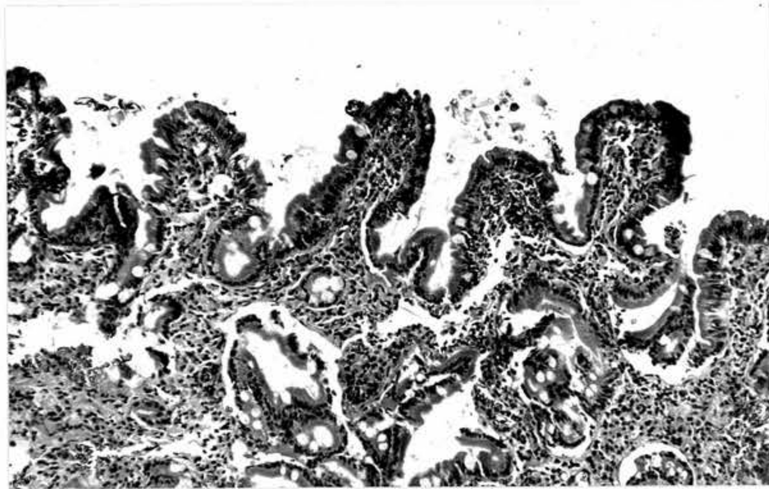


Fig. 134. Jejunal mucosa after treatment. The villi are shorter but there is no hypertrophy of the glandular layer. H & E x 120

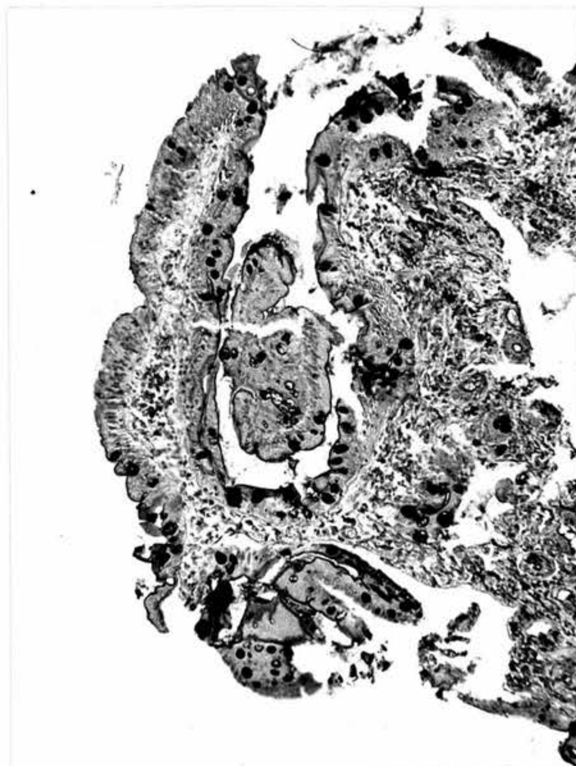


Fig. 135. Jejunal mucosa after treatment. Shows very little PAS positive material in the stroma of the villi. PAS x 120

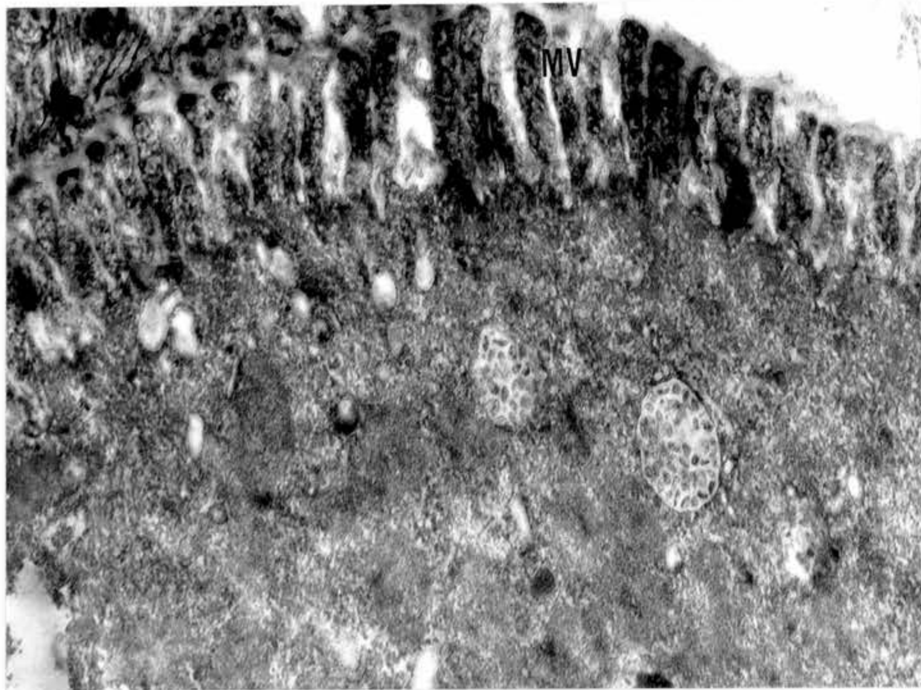


Fig. 136. Electron micrograph of jejunal epithelium showing microvilli (MV) which are shorter andmore densely packed than normal.
x 48,000

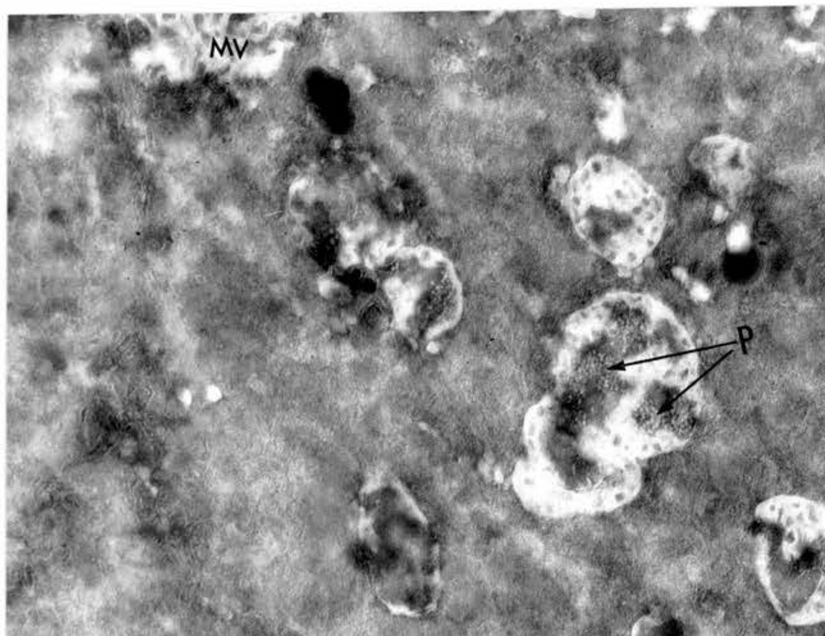


Fig. 137. Electron micrograph showing aggregated electron opaque particles (P) in the supranuclear cytoplasm. MV = cross section through the microvilli.
x 40,000

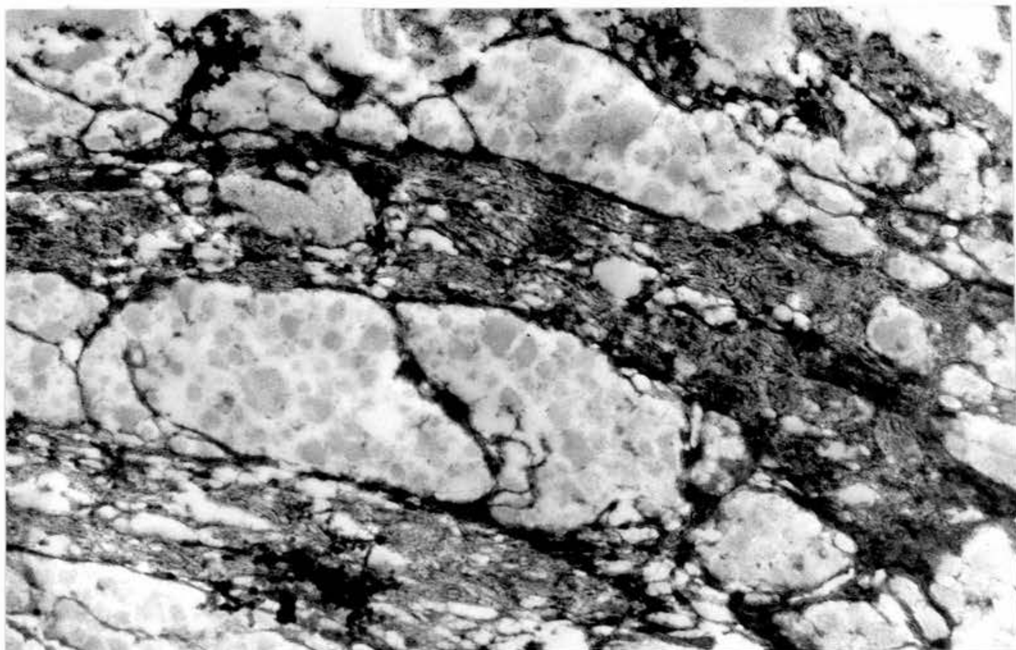


Fig. 138. Electron micrograph of the lamina propria of the villi showing islands of aggregated electron opaque material. x 28,800

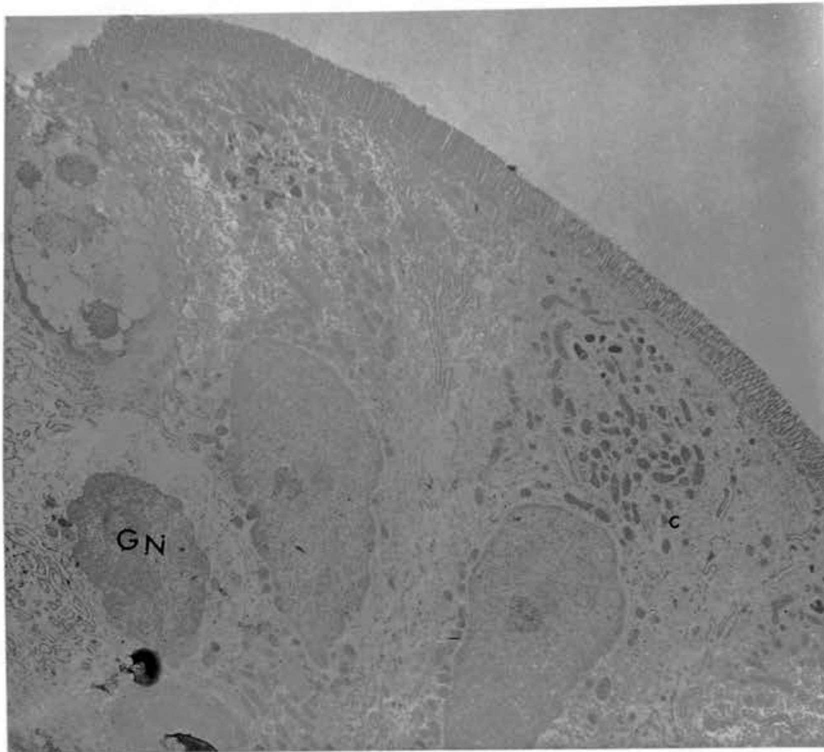


Fig. 139. E.M. of jejunal mucosa after treatment showing normal apical border, two absorptive cells and one goblet cell. GN = nucleus of goblet cell.
x 1,950

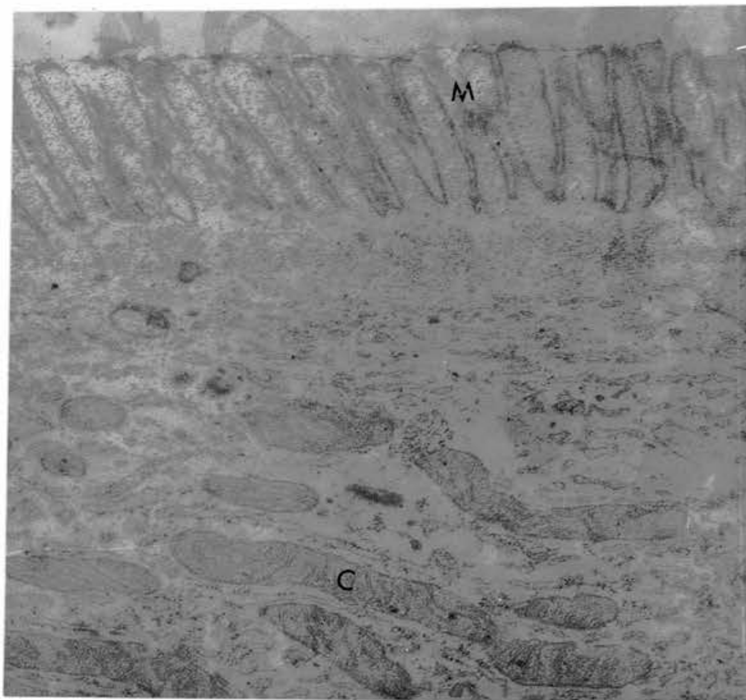


Fig. 140. Enlarged view of the above mucosa showing regularity of dimensions and spacing of microvilli (M). They are 0.9μ in length.
C = cristae of mitochondria are clearly seen.
x 24,000

II. Progressive Systemic Sclerosis with steatorrhoea.

A female, A.M. (50), was admitted to the Western General Hospital of Edinburgh with a history of difficulty in swallowing during the past 3 years. She also gave a history of long standing Raynaud's phenomena. There was no history of heartburn, vomiting or abdominal pain. During this period despite a normal appetite she lost about 2 stones in weight. On examination the skin of the hands and face appeared thickened. There was difficulty in lifting the skin over the forehead from the underlying structures. Her mouth appeared rather small. On investigation it was noted that she was passing excessive fat in the stools (up to 25 g. per day). Tests also revealed depletion with minimal malabsorption of folic acid. The Schilling test showed a normal absorption on one occasion and malabsorption on another. There was no excessive acid secretion. Barium meal examination showed dilatation of the first, second and third parts of the duodenum. Biopsy of the skin showed changes typical of scleroderma. At laparotomy the duodenum was found to be markedly dilated and was filled with stagnant fluid. Entero-anastomosis with resection of the afferent loop was performed along with duodeno-jejunostomy (feeding jejunostomy). A specimen for microscopic examination was taken at operation.

Microscopic appearances of the jejunal mucosa.

The mucosa under the light microscope presented villi mainly of normal height, although some appeared

shorter than normal. The epithelial cell height was normal and the refractile border of the cell was well preserved. There was no increase in the inflammatory cell content of the lamina propria. The height of the glandular layer was normal. The examined mucosa did not show any abnormality of the muscle or subserosal layers. The capillaries did not show any endothelial hyperplasia or any other change compatible with scleroderma. (Fig. 141).

Under the electron microscope the microvilli were found to be of uniform dimensions. They were parallel to the long axis of the cell and showed close hexagonal packing. (Figs. 142 and 143). The intermicrovillous space was found to contain electron opaque material. This was mostly amorphous but occasionally "strands" were noted. (Fig. 144). Those substances in the inter-microvillous space were present more in the duodenum than in the jejunum. The epithelial cell nuclei, mito-chondria and endoplasmic reticulum appeared normal. An electron opaque material was found to be deposited under the subepithelial basement membrane in a patchy manner. (Figs. 145 and 146). There was an increase in the collagen fibres in the lamina propria. (Fig. 147).

Discussion.

Involvement of the small intestine in progressive systemic sclerosis has received increased attention during recent years. Cooke (1942) reported steatorrhoea in this condition. Destruction of the muscle coat of the intestine is the only significant

morphological change which has been found to account for the lack of peristalsis and the stasis of food. (Rosenthal, 1959). The real disability in these cases is due to malabsorption secondary to the upper small intestine acting as a stagnant reservoir (McBrien and Lockhart Mummery, 1962). It has been realised that removal of the affected segment is accompanied by an improvement in the clinical condition (Marshall, 1956; Herrington, 1959). In this reported case the involvement of the small intestine was slight. However, following the operative procedure there was a remarkable improvement in the condition of the patient.

The only significant morphological change noted under the electron microscope was the accumulation of an electron opaque material in the intermicrovillous space which could have a functional significance in acting as a barrier and preventing the nutrients from coming in contact with the lipoprotein membrane covering the microvilli, this being the active site for absorption of nutrients. The accumulation of the electron opaque material below the subepithelial basement membrane could also act as a mechanical barrier to the passage of nutrients. These factors might account for steatorrhoea in this case.

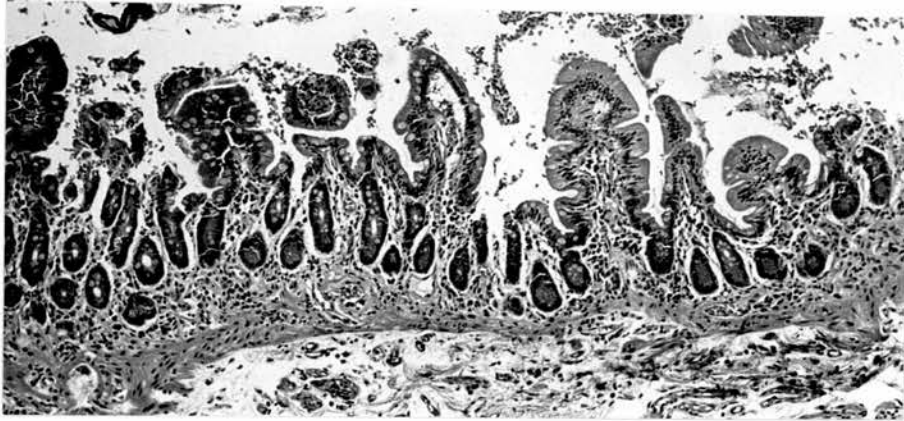


Fig. 141. Histological section from jejunal mucosa of a case with progressive systemic sclerosis. H & E x 80

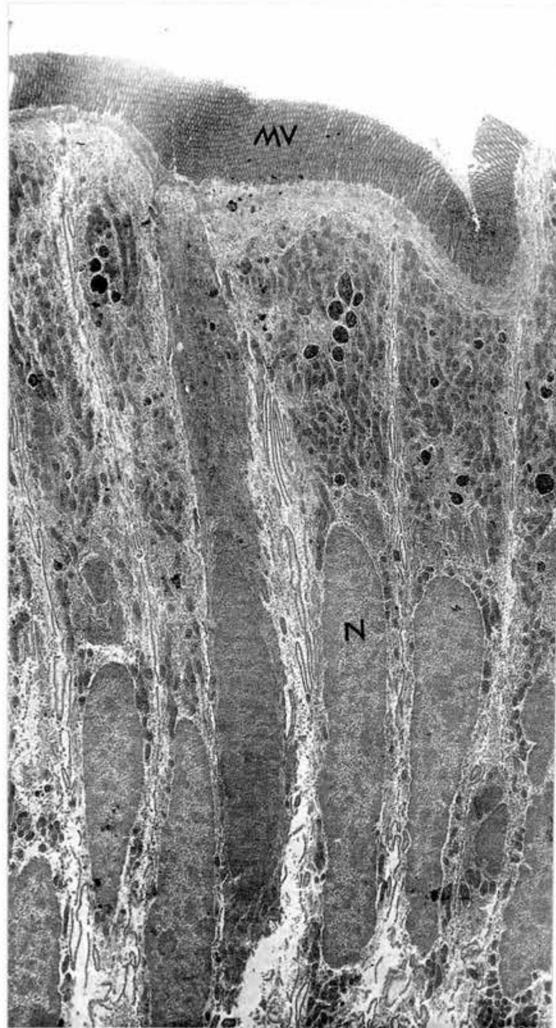


Fig. 142. Electron micrograph of epithelial cells showing normal microvilli (MV) and nucleus (N). x 3,000

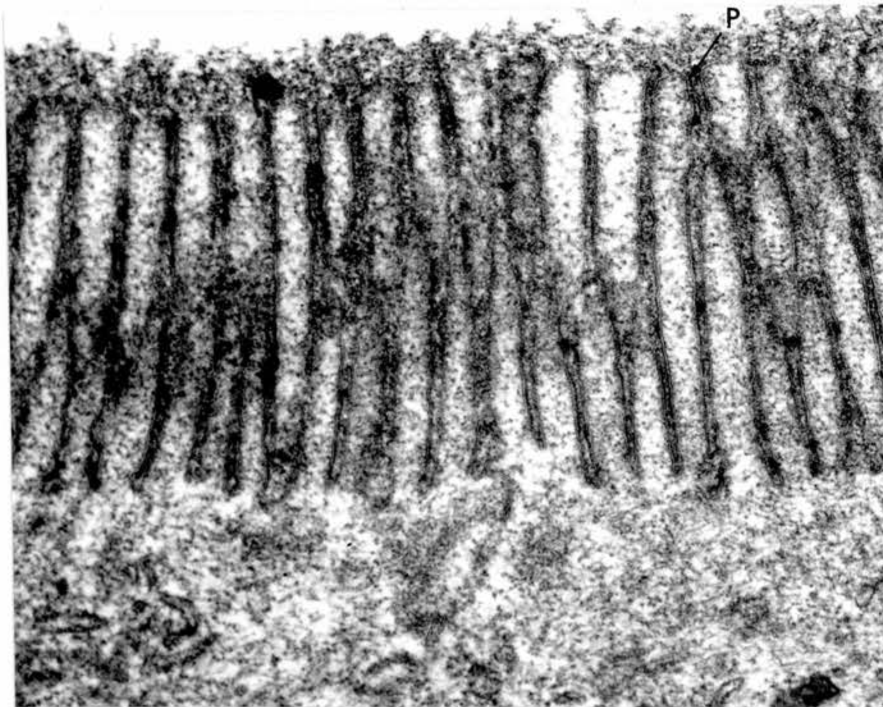


Fig. 143. E.M. showing normal microvilli of the epithelial cells of small intestinal mucosa. P = plasma membrane enveloping the microvilli. x 56,000

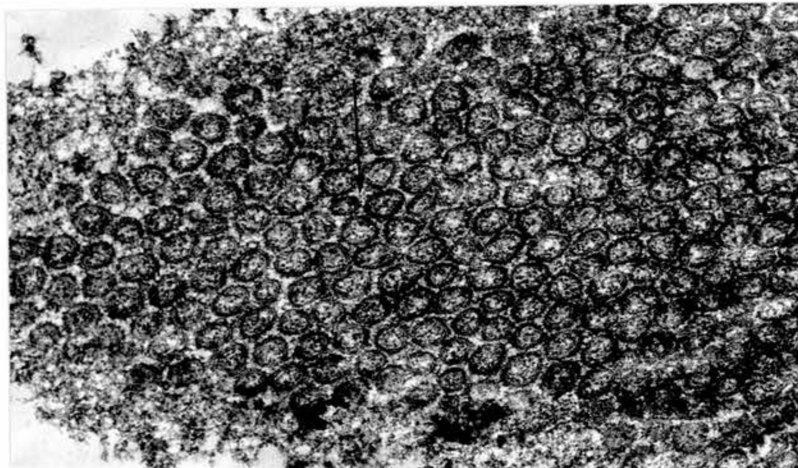


Fig. 144. Electron micrograph of section through the microvilli. Arrow points at intermicrovillous space filled with electron opaque material. x 40,000

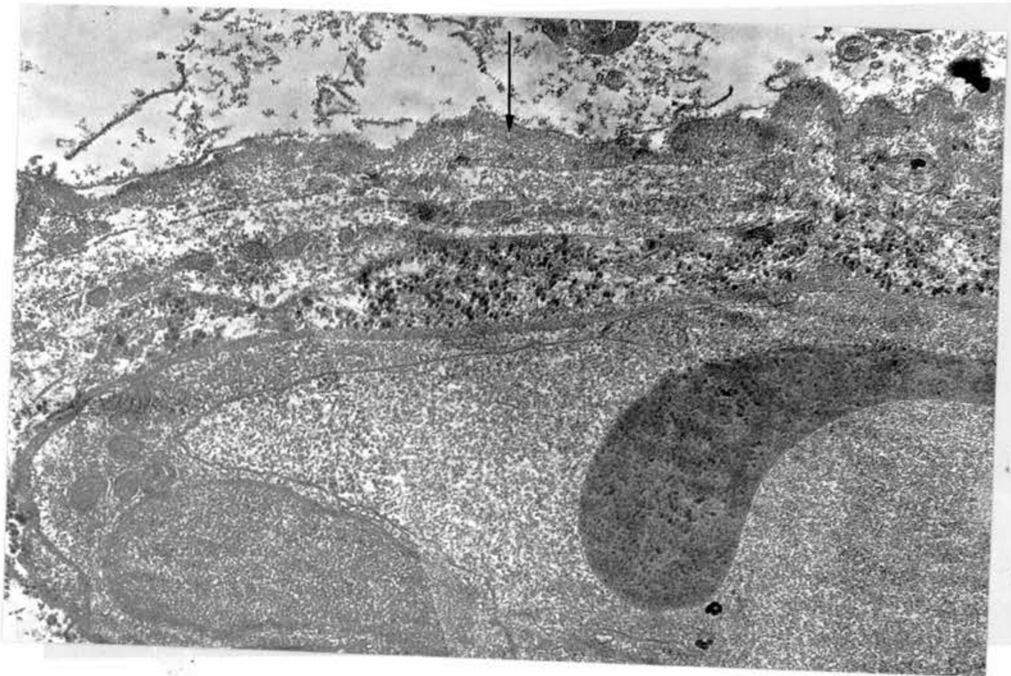


Fig.145 a. Electron micrograph of basal part of epithelial cells and blood vessel in the lamina propria which contains a red cell. Note patchy thickening of the subepithelial basement membrane (arrow) x 16,800

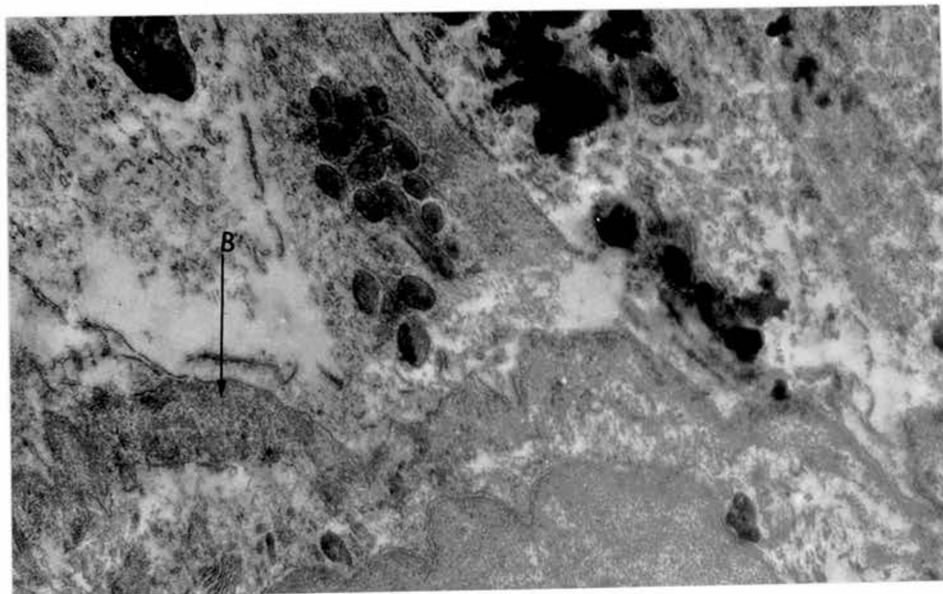


Fig.145b. Electron micrograph of basal part of the cell. B = electron opaque material which is deposited beneath the subepithelial basement membrane and making it thicker. x 21,600

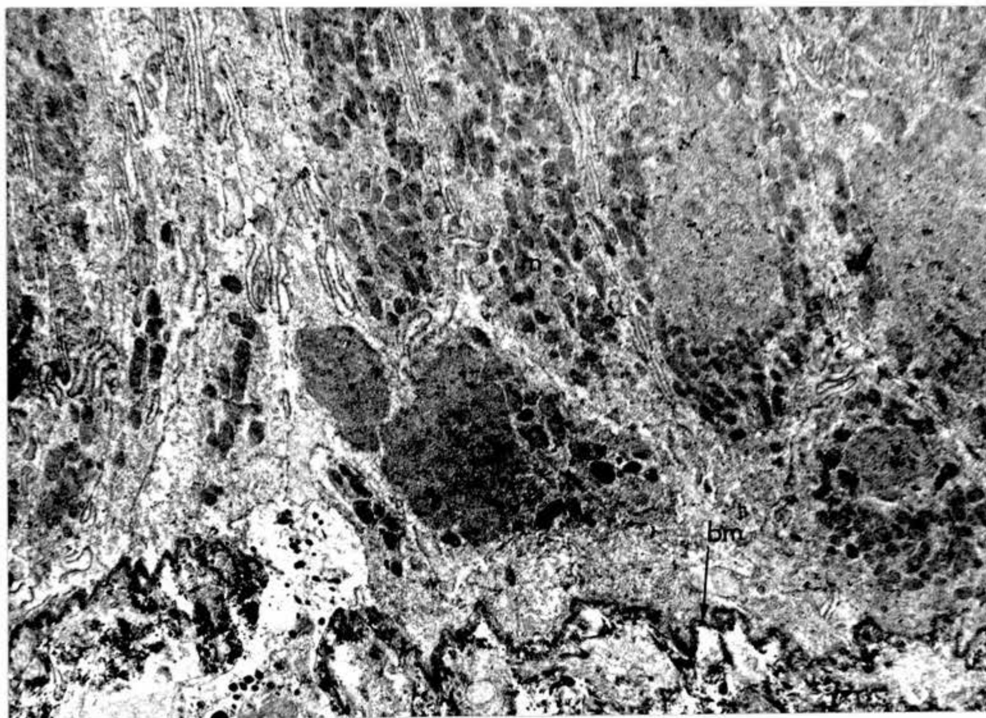


Fig. 146. E.M. showing basal part of normal epithelial cells of jejunum. m = infranuclear mitochondria. bm = subepithelial basement membrane. x 12,000

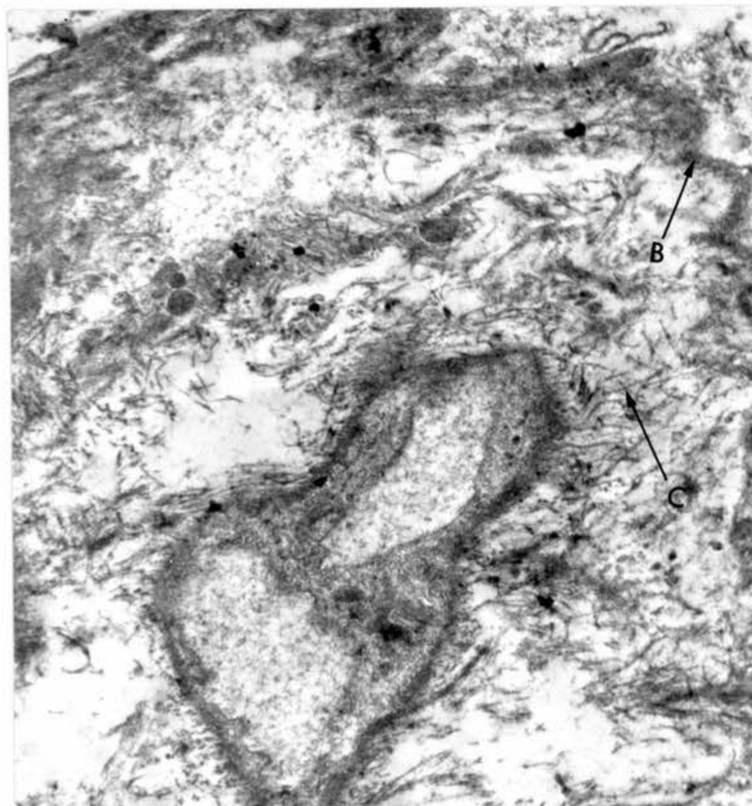


Fig. 147. Lamina propria showing increase in number of collagen fibres (c) which bind structures within it to the subepithelial basement membrane.(B). x 15,000

III. Zollinger-Ellison Syndrome.

A female, J.L. (45), was admitted to the Western General Hospital, Edinburgh with retrosternal discomfort and intermittent left subcostal gnawing discomfort during the past 4 years. The symptoms were relieved with antacids. During the past 3 years she developed also diarrhoea (2 to 6 liquid or semisolid stools per day). There had also been bouts of vomiting since the onset of diarrhoea. She had lost about 1 stone in weight. Her father and brother had abdominal operations. There was no history of any endocrine disturbance in the family. Investigations showed that in addition to steatorrhoea there was an increase in the volume of gastric juice, which had a high content of total and free acid. After operation the case was diagnosed as "Zollinger-Ellison Syndrome" with hyperacid secretion, duodenal ulcer, steatorrhoea, and intestinal hurry. Subtotal (7/8th) gastrectomy with total vagotomy and hemipancreatectomy was performed. After operation the acid secretion was reduced but the steatorrhoea persisted, and this was thought to be due to the operative manoeuvres. Specimens of stomach, duodenum and jejunum were obtained at operation.

Microscopic appearance.

(i) Stomach - the ultra structures of the parietal and zymogen cells are presented in figures 148 and 151.

(ii) Jejunum - the jejunal mucosa presented villi of normal shape and height. The epithelium covering the villi had a serrated appearance and the striated border

of the cell was well preserved and prominent. The epithelial cell height was normal. The goblet cells appeared slightly larger and more numerous. Their occasional emptiness even over the middle third of the villi suggested hypersecretion probably to neutralise the acid content of the intestine. The lamina propria did not show any increase in cellularity. The height of the glandular layer of the epithelium was normal.

Under the electron microscope, the brush border presented uniformly distributed and apparently normal microvilli with their long axis parallel to the long axis of the cell. (Fig. 152). They varied from 1μ to 1.25μ in height and were 0.1μ in width. The terminal web of the cells was well preserved. The mitochondria, cytoplasm and the nucleus of the cell were found to be normal. The subepithelial basement membrane was of normal thickness and was found to be continuous. (Fig. 153).

In short the jejunal mucosa was found to be normal under all microscopic examination.

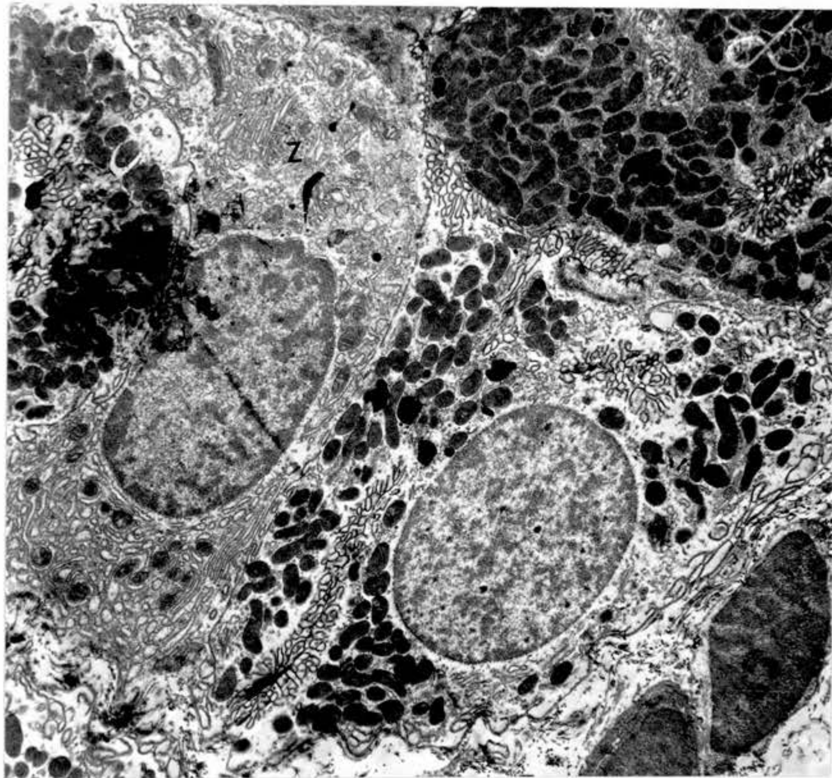


Fig. 148. Electron micrograph showing both parietal (P) and Zymogenic (Z) cells of gastric mucosa.
x 2,250

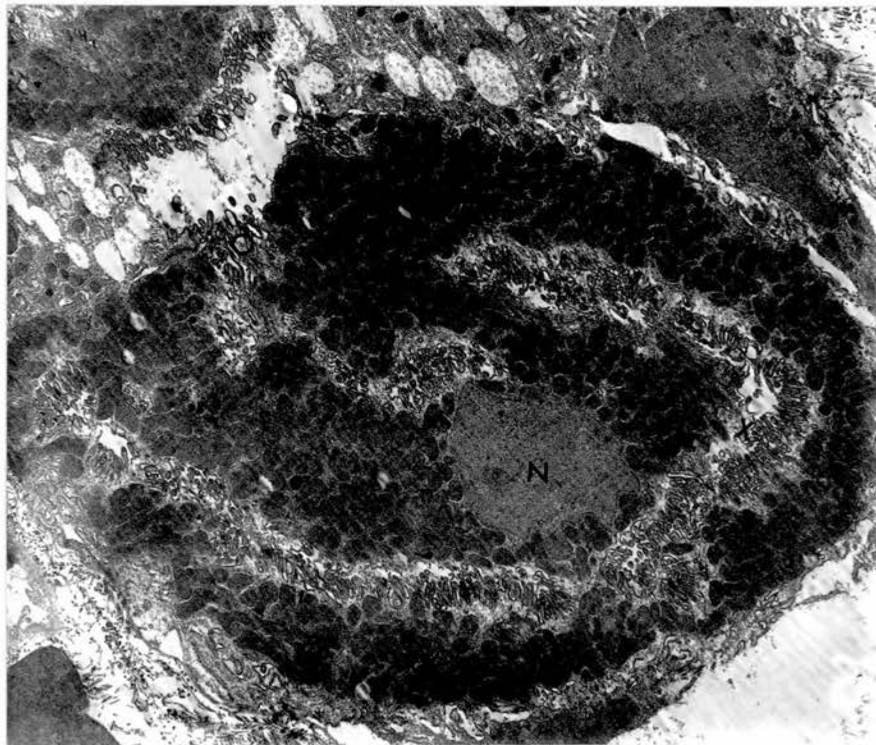


Fig. 149. E.M. of parietal cell showing micro-villi (x), nucleus (N) and intracellular canaliculi opening (o) into the gland lumen.
x 2,250

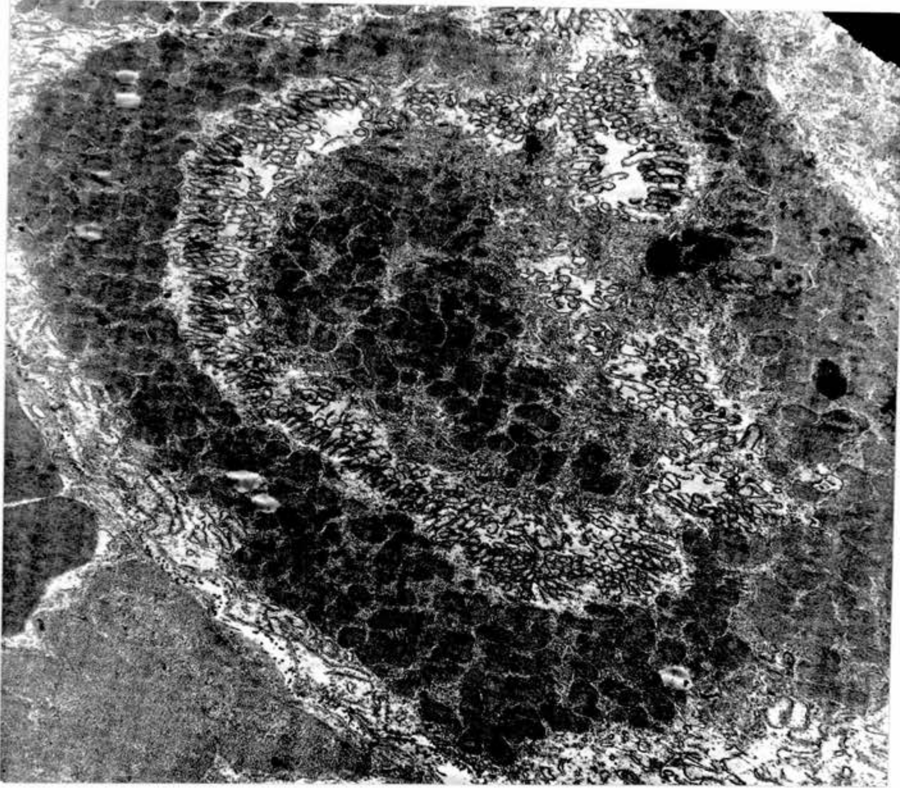


Fig. 150. E.M. of parietal cell showing microvilli (x). x 6,000

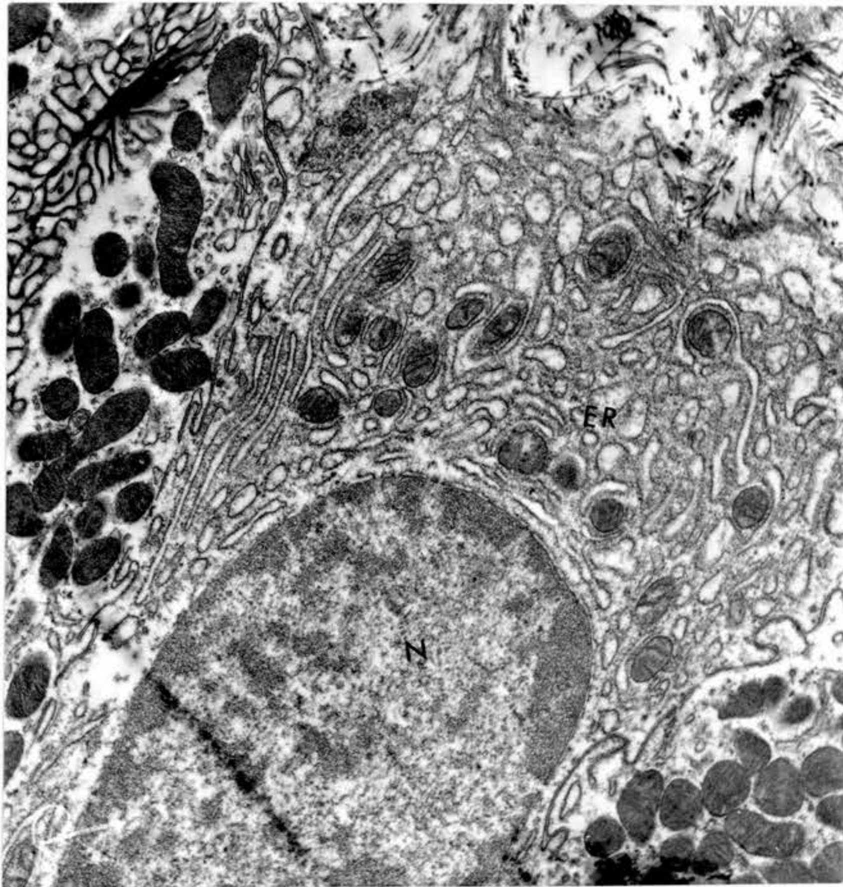
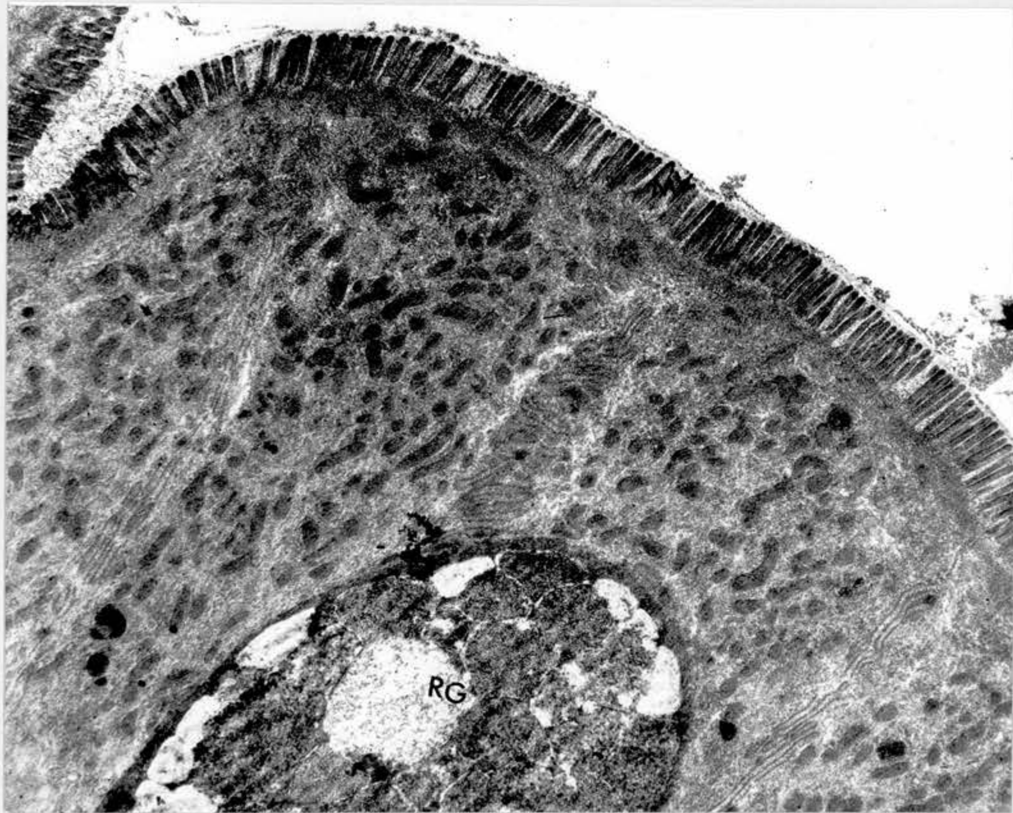
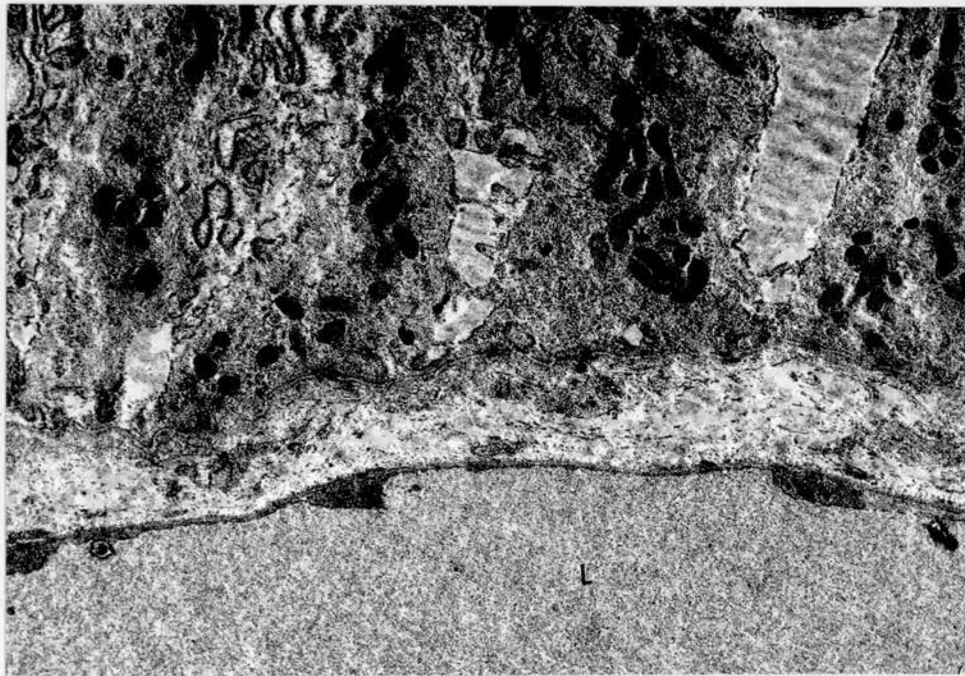


Fig. 151. E.M. of zymogenic cell showing rich endoplasmic reticulum (ER). Nucleus (N). x 15,000



<u>Fig. 152.</u> Electron micrograph of apical cytoplasm of intestinal epithelial cells, MV = microvilli; RG = resting goblet cell. x 7,200



<u>Fig. 153.</u> Electron micrograph of basal part of epithelial cell. L = lumen of capillary in the lamina propria. x 9,600

Summary.

1. In Whipple's disease morphological abnormality was noted under the dissecting, light and electron microscope. The dissecting microscope showed that the jejunal villi were replaced by convolutions. Light microscopy showed abnormal villi which were broad and club shaped. Electron microscopy showed a reduction in the height of the microvilli. Under the electron microscope the other striking features were absence of pinocytotic vesicles over the apical cytoplasm and intracytoplasmic organelles resembling bacterial bodies. The absorptive surface of the mucosa returned to normal following a short period of treatment with tetracycline. This was most obvious under the electron microscope. The bacterial bodies also disappeared, with treatment, from both cytoplasm of epithelial cells and stroma of the villi.

2. In systemic sclerosis there was deposition of an amorphous substance in the intermicrovillous space, along the cell wall and beneath the subepithelial basement membrane, which gave it an appearance of patchy thickening. These could be a mechanical barrier to permeability of nutrient materials.

3. In the Zollinger-Ellison Syndrome there was no noticeable morphological change in the absorbing cells of the jejunum. The hyperacidity and increased intestinal motility were probably the factors accounting for the steatorrhoea.

REFERENCES.

1. Ashworth, C.T., Douglas, F.C., Reynolds, R.C., Thomas, P.J. (1964). Amer. J. Med., 37, 481.

2. Black-Schaffer, B. (1949). Proc. Soc. exp. Biol., 72, 225.

3. Chears, C.W.Jr., and Ashworth, C.T. (1961). Gastroenterology, 41, 129.

4. Cohen, A.S. (1962). Fifth International Congress for Electron Microscopy, S.S-2 Academic Press, New York, London.

5. Curran, R.C. and Creamer, B. (1963). J. Path. Bact., 86, 1.

6. Fisher, E.R. (1962). J. Amer. med. Ass., 181, 396.

7. Gibb, W.E., Malpas, J.S., Parrish, J.A. (1964). Brit. med. J., 1, 417.

8. Haubrich, S.W., Watson, J.H.L., Sieracki, J.C. (1960). Gastroenterology, 39, 454.

9. Hellwig, C.A., Weiner, R.G., Wilkinson, P.N. (1961). Arch. Path., 72, 274.

10. Herrington, J.L. Jnr. (1959). Arch. Surg., 78, 17.

11. Marshall, I. (1956). New. Eng. J. Med., 255, 978.

12. McBrien, D.J., Lockhart Mummery, H.E. (1962). Brit. med. J., ii, 1653.

13. Rosenthal, F.D. (1957). Gastroenterology, 32, 332.

14. Sieracki, J.C. and Fine, G. (1959). Arch. Path., 67, 81.

CONCLUSION.

1. The examination of the intestinal mucosa by the electron microscope is accepted to be a method of established value. In the work described in this thesis, normal and abnormal material has been studied extensively.

2. So far as concerns the normal controls, specimens were obtained from several small laboratory rodents, and also from sheep and from a cat, in addition to those from human subjects without alimentary tract disease. In all those mammals, the findings confirmed the observations of other workers. (Chapter 2).

3. Autolytic changes were studied in portions of bowel allowed to remain unfixed for varying short periods. The earliest alteration seen by the electron microscope consisted of fusiform dilatation of the upper parts of the microvilli. The importance of those changes lies in their resemblance to pathological lesions for which they may readily be mistaken. Washing the lumen of the bowel with normal saline proved to be a simple way of delaying the onset of autolysis. (Chapters 3 and 4).

4. The effect of protein malnutrition was studied in rats maintained on a diet deficient in protein. As compared with normal controls, the protein-deficient animals developed atrophy of the intestinal mucosa in the upper part of the small intestine. (Chapter 5).

5. Experimental infection with V. Cholerae was examined in closed-loop experiments in rabbits. At 6

hours, although no lesions could be seen by the light microscope, with the electron microscope there was cytoplasmic vacuolation of the microvilli and rupture of their lipoprotein membranes. It is possible that those changes may indicate sites from which fluid is lost ultimately to the lumen. (Chapter 6).

6. The investigation of human primary malabsorptive disease comprises the major part of the thesis. Biopsies were obtained from 63 patients diagnosed as suffering from this disease and of those specimens, 14 were examined completely and comprehensively by the electron microscope. The earliest abnormalities included fusion of the microvilli one with another and, at a later stage, irregularity in the size and shape of the microvilli. These changes were shown to affect not only groups of cells in one area, but also individual cells scattered between normal cells, thus reproducing in miniature the well-recognised focal nature of the disease process. The limitations of the electron microscope in the diagnosis of primary malabsorptive disease are discussed, and the contribution of the low power binocular instrument for routine diagnosis is emphasised. (Chapters 7 and 8).

7. A hypothesis is propounded to account on a physical basis for the malabsorption of fat and other nutrient particles from the intestinal lumen in idiopathic steatorrhoea. (Chapter 9).

8. Secondary steatorrhoea is exemplified by three cases,

one of Whipple's disease, one of systemic sclerosis and one of Zollinger-Ellison syndrome. The findings are discussed in detail. (Chapter 10).