FINE STRUCTURE OF NORMAL
AND PATHOLOGICAL GUT

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Volume 1

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STATEMENT

The studies described in this thesis are my own original investigations.

[Signature]
ACKNOWLEDGEMENTS

I would like to express my thanks to Dr. A.A. Barton, who supervised this work, for his constant interest and helpful advice throughout the course of this study. Both he and Mrs. Barton also gave invaluable linguistic help.

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CHAPTER 1

REVIEW OF THE LITERATURE - NORMAL SMALL INTESTINE

Introduction:

The appearance and relationships of the many different types of cell seen in the intestinal wall, particularly those in mucosa, are controlled by an interplay of many factors. Knowledge of these is important in considering the functional significance of mucosal structure (Padykula, 1962).

An important factor in the differentiation of mucosa is the presence of a physiological microbial flora. The importance of this was only realised after a comparison of the intestinal morphology in conventional and germ-free animals had been made. The gastro-intestinal tract of germ-free rats has a relatively low weight (Gordon and Wostmann, 1960), and the surface area of the mucosa is decreased about 30% (Gordon and Bruckner-Kardoss, 1961), mainly in the ileum. The stroma of the mucous membrane of germ-free mice in comparison to that of normal conventional animals is reduced in volume and contains only a few lymphocytes and mononuclear cells; similarly lymphoid tissue in the intestinal wall is reduced (Abrams, Bauer and Spring, 1963). The migration time of the epithelium as measured by tritiated thymidine increased and the villi are slightly shortened. The degree of incorporation of the label into the cells of the lamina
The propria mucosae is lower than in conventional animals. Similar appearance has the mucosa in germ-free guinea pigs (Sprinz, 1962). The normal pattern is acquired after the contamination with Escherichia coli (Sprinz, Kundel, Dammin, Horowitz, Schneider and Formal, 1961).

A factor important in determining the structure of the absorbing surface and the functional capability of the epithelium is the ratio between the epithelial cell proliferation and loss. Experiments with labelled precursors of DNA have established that there is constant renewal, as in other epithelia (Leblond and Walker, 1956). The only regenerative zone in the normal adult animal is the crypt epithelium (Leblond and Messier, 1958; Leblond and Stevens, 1948; Leblond, Stevens and Bogoroch, 1948). After a life cycle of 2-3 days, the enterocytes are extruded from the tip of the villi. The normal rate of turnover may be an inherent characteristic of the epithelium rather than a result of continued cell loss (Hooper, 1956). It is of interest in this connection that the estimated duration of DNA synthesis in some epithelia of the gastrointestinal tract is constant and similar to that in other mammalian cells, despite different durations of the cell cycle (Cameron and Greulich, 1963). There is no evidence available for mammals to suggest at what stage of foetal life the pattern of renewal develops; in the mouse (17 days after gestation) the only mitotic activity
observed was seen in the area of the future crypts. Extrusion zones gradually developed after birth (O'Connor, 1966). According to findings in developing chicken duodenal mucosa, the mature pattern of mitotic activity is reached before late foetal development (Overton and Shoup, 1964). In adult life, the rate of cell renewal is likely to be influenced by many natural and experimental factors, some of which are age (Lesher, Fry and Kohn, 1961); hormones (Leblond and Carriere, 1955); starvation (Hooper and Blair, 1958; Brown, Levine and Lipkin, 1963); microbial flora (Abrams, Bauer and Sprinz, 1963); radiation (Sherman and Quastler, 1960); partial resection (Loran and Althausen, 1960; Loran and Crocker, 1963); vagotomy (Silen, Peloso and Jaffe, 1966) and immunosympathectomy (Dupont, Biggers and Sprinz, 1965).

Some of these observations, like the effect of radiation where the target affected is the crypt epithelium, indicate that cell proliferation and loss are to some extent independent; migration of the epithelium along the villus proceeding independently of crypt cell damage. The changed migration period of the epithelium may be associated with change in the length of the villi and reflect the ratio of epithelial cell renewal to cell loss. Whether all conditions associated with the atrophy of the villi (review of Collins, 1965) change the migration time remains to be determined. Some authors studying the proliferation of the intestinal epithelium (Quastler and Sherman, 1959; Cairnie, Lamerton and Steel,
1965a, 1965b) have pointed out that there is a critical decision phase of differentiation. Their theoretical model seems to be of considerable importance, it appears useful therefore to discuss the findings which led to its construction. According to the results of Cairnie, Lamerton and Steel (1965a) the cell cycle throughout the crypt remains the same, and lasts about 10 hours. At the bottom it is slightly longer. Mitoses in the crypt are asynchronous and do not appear in the uppermost segment. At the level of the crypt where half of the total number of the mitoses is reached, about 60% of the cells are labelled with tritiated thymidine. Most of the mitoses in the lower portion of the crypt produce two proliferative cells, the reverse being true in the upper portion. These authors developed the "slow cut-off" model and using this, it was found that the critical "decision phase" to produce non-proliferative cells takes place with increasing probability at the 10th-18th cell position above the bottom of the crypt. The factors operating in the critical "decision phase" may be either an environmental change or some endogenous mechanism. Increased cell production can be explained by a shift of this critical region upwards in the crypt; it is not necessary to postulate a shortening of the cell cycle.

The functional maturation of absorbing epithelium on the villus can be demonstrated using visible substances which
are normally absorbed. The greatest accumulation is seen in cells near to the tip of the villus; the rate decreased downwards to the base. This well-known phenomenon of gradient was observed during fat absorption in animals by Palay and Karlin, (1959b); Ashworth and Johnston (1963); Palay and Revel (1964); in vitro by Strauss (1963); in humans by Ladman, Padykula and Strauss, (1963); and during in vitro absorption of labelled sugars and aminoacids by Kinter and Wilson (1965).

For the purpose of this review, the bibliography dealing with the fine structure of the gut will be considered according to the architecture of the gut wall. Even a casual examination of the bibliography shows an unbalanced proportion of papers dealing with the fine structure of enterocytes. Many other tissue components have not been adequately examined. Moreover, some older reports are short and based on outdated techniques. So far, only a limited number of species have been examined, and although the general appearance of most structures appears to be essentially the same, there are no detailed or systematic comparisons available which could permit conclusions to be drawn about species difference.

Reviews of the fine structure of the gut have appeared several times before (Laster & Ingelfinger, 1961; Trier, Phelps and Rubin, 1963; Trier and Rubin, 1965). However, these were intended mostly for clinical purposes.
**Enterocytes:**

The earliest electron microscope studies of the intestine dealt with the structure of the enterocyte particularly with the brush border (Granger and Baker, 1949, 1950; Dalton, Kahler, Striebich and Lloyd, 1950; Dalton, 1951; Dalton, Kahler and Lloyd, 1951; Dalton, 1953). These observations settled a much disputed question in light microscopy, namely the nature of the striated border. This was shown to consist of densely arranged cytoplasmic projections. The first detailed descriptions of enterocytes were made by Zetterqvist (1956) and Palay and Karlin (1959a, b). These studies established points of reference for further observations, most of which have aimed at clarifying the structures associated with absorption.

The *plasma membrane* along the lateral and basal surface of an enterocyte differs in appearance from that covering the microvilli. Along the lateral surface, the thickness of the membrane was estimated by Zetterqvist (1956) to be 70Å; Palay and Karlin (1959a) found it to be 32Å, 42Å or twice this thickness where the membrane was triple-layered. The studies of Sjostrand (1963a), Millington (1964) and Farquhar and Palade (1963) have clearly shown that it is asymmetrical. The cytoplasmic opaque layer is 30Å wide, the peripheral opaque layer, 25Å and the intermediate less dense layer, 25Å. The plasma membranes on the lateral cell surface are highly
folded (Zetterqvist, 1956; Palay and Karlin, 1959a; Trier, 1962; Sjostrand, 1963a, b; Strauss, 1963). The intercellular space showed considerable variation in width. In regions of close contact, the plasma membranes of the neighbouring cells were separated by a gap of 95Å - 120Å (Zetterqvist, 1956; Ashworth, Chears Jr., Sanders and Pearce, 1961; Palay and Karlin, 1959a). Sjostrand (1963a) stated that the minimum value in the starving animal was considerably higher: 200-300Å. In places, especially near the cell base, there is a considerably larger intercellular space which varies in width (Zetterqvist, 1956; Palay and Karlin, 1959a; Sjostrand, 1963a, b; Strauss, 1963). Sjostrand (1963b) remarked that the cell surface remains folded, even when the intercellular space is enlarged during fat absorption. The basal cell surface is usually quite flat.

Intercellular spaces reach maximum size in the apical area of a villus (Hartman, Smith, Hartman and Butterworth Jr., 1959).

The most investigated structure of the enterocyte is the brush border.

The dimensions of microvilli estimated by different authors are given in the following table.
<table>
<thead>
<tr>
<th>Authors</th>
<th>height u localization</th>
<th>width</th>
<th>number</th>
<th>surface increase</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granger</td>
<td>0.53</td>
<td>0.075</td>
<td>1000/</td>
<td></td>
<td>free surface of cell = 15u^2; diameter tends to vary inversely with length; actual number lower (calculation assumed infinitely close packing)</td>
</tr>
<tr>
<td>Baker</td>
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<tr>
<td>(1949)</td>
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<tr>
<td>Granger</td>
<td>0.62</td>
<td>0.08</td>
<td>3000/</td>
<td>30x</td>
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</tr>
<tr>
<td>Baker</td>
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<tr>
<td>(1950)</td>
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<tr>
<td>rat</td>
<td></td>
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<td>Dalton,</td>
<td>0.9</td>
<td>0.14</td>
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<tr>
<td>Kahler,</td>
<td>villus</td>
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<tr>
<td>Striebich,</td>
<td>0.44</td>
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<tr>
<td>Lloyd</td>
<td>crypt</td>
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<td>(1950)</td>
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<tr>
<td>mouse</td>
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<td>2.0</td>
<td>0.33</td>
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<td>(1955a)</td>
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<tr>
<td>mouse</td>
<td></td>
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</tr>
<tr>
<td>Zetterqvist,</td>
<td>0.91</td>
<td>0.1</td>
<td>47 per u^2 14x</td>
<td></td>
<td>35% of surface covered by microvilli</td>
</tr>
<tr>
<td>(1956)</td>
<td></td>
<td></td>
<td>per 700 cell</td>
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<td></td>
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<tr>
<td>mouse</td>
<td></td>
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</tr>
<tr>
<td>Palay,</td>
<td>1.0</td>
<td>0.07</td>
<td>75 per u^2 24x</td>
<td></td>
<td>- occupy approx. 60% of surface - taller and thinner at the apex of a villus</td>
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<tr>
<td>Karlin</td>
<td></td>
<td></td>
<td>1125 per cell</td>
<td></td>
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<tr>
<td>(1959a)</td>
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<tr>
<td>rat</td>
<td></td>
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<tr>
<td>Haubrich,</td>
<td>0.85</td>
<td>0.1</td>
<td>49 per u^2</td>
<td></td>
<td>length of microvillus independent of diameter</td>
</tr>
<tr>
<td>Watson,</td>
<td>1.60</td>
<td></td>
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<tr>
<td>O'Driscoll,</td>
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<td>Valentine</td>
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<tr>
<td>(1959)</td>
<td></td>
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<tr>
<td>man</td>
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<td>height</td>
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<td>surface increase</td>
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<tr>
<td>Hartman,</td>
<td>0.8</td>
<td>0.12</td>
<td></td>
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<tr>
<td>Butterworth, Jr.</td>
<td>1.3</td>
<td></td>
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<tr>
<td>Hartman,</td>
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<td></td>
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<td>Crosby,</td>
<td></td>
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<td>Shirai (1960)</td>
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<tr>
<td>Ashworth,</td>
<td>1.3</td>
<td>0.08</td>
<td>1800</td>
<td>per cell</td>
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<td>Chears,</td>
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<td>Sanders,</td>
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<td>Pearce (1961)</td>
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<td>Williams (1961)</td>
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<td>Shiner,</td>
<td>1.03</td>
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<td>Birbeck (1961)</td>
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<td>Millington, 1.0</td>
<td>0.1</td>
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<td>Finean (1962)</td>
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<tr>
<td>Shearman, Girdwood, 1.00</td>
<td>0.75-</td>
<td>1.00</td>
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<td>Williams, Delamore (1961)</td>
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<tr>
<td>Bergener (1962)</td>
<td>duodenum</td>
<td>0.187</td>
<td>0.187</td>
<td></td>
<td>duodenum 26x</td>
</tr>
<tr>
<td></td>
<td>jejunum</td>
<td>1.08</td>
<td>0.187</td>
<td></td>
<td>jejunum 38.1</td>
</tr>
<tr>
<td>hamster</td>
<td>1.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown (1962)</td>
<td>villous crest:</td>
<td>0.08</td>
<td>10.7</td>
<td>2 per u²</td>
<td>cross sectional area of micro-villi 57% of area of free surface of the cell</td>
</tr>
<tr>
<td>het man</td>
<td>1.36</td>
<td></td>
<td></td>
<td>1717 per cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inter-villous space</td>
<td>0.10</td>
<td>4.7</td>
<td>per u²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td></td>
<td></td>
<td>331 per cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td>crypt</td>
<td>0.15</td>
<td>3.9</td>
<td>2 per u²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.67</td>
<td></td>
<td></td>
<td>225 per cell</td>
<td></td>
</tr>
<tr>
<td>Laguens, Briones (1965)</td>
<td>1.0</td>
<td>0.07</td>
<td></td>
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<td></td>
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<tr>
<td>man</td>
<td></td>
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</tr>
<tr>
<td>Kraehen-buhl, (a)</td>
<td>3.0-</td>
<td>0.07-</td>
<td>0.07-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gloor,</td>
<td>3.5</td>
<td>0.08</td>
<td>0.08</td>
<td></td>
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<tr>
<td>Blanc (1966)</td>
<td>(b)</td>
<td></td>
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<tr>
<td></td>
<td>1.2-</td>
<td>0.09-</td>
<td>0.09-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) rabbit</td>
<td>1.3</td>
<td>0.11</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b) rat</td>
<td></td>
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</table>
Some deviations of the old measurements might be explained by imperfect technique. Brown (1962) showed, however, that differences in the height of microvilli occurred at various levels of the villus and suggested that this had been the cause of the disparity of the results. Another factor may be the position of the area examined, since we do not know exactly how the microvillus dimensions vary along the small gut. The greater length of the microvilli in the rabbit suggests that there is a species difference.

Few observations have been made on the development of microvilli. In the mouse, the greatest increase in height is observed before birth. After birth, the height increase is slower and is associated with a reduction of diameter; hence the volume of a microvillus remains approximately the same (Overton, 1965).

One of the recognised factors influencing the height of the brush border is the intestinal flora. A comparison of the brush border of pigs maintained under limited intestinal bacterial contaminations shows about a three-fold increase in height in comparison with multicontaminated animals (Kenworthy and Allen, 1966).

The plasma membrane covering the microvilli appears to be different in structure to that covering the rest of the cell. The early observations of Sjostrand (1956) and Zetterqvist (1956) had already resolved the plasma membrane of the brush
border region as triple-layered, measuring either 105Å or 120Å (Sjöstrand, and Zetterqvist, 1955) in total thickness. This is in contrast to the width of 70Å measured at the lateral cell surface. Later values are 70Å (Suganuma, 1961), 105Å (Palay and Karlin, 1959a), 110Å (Farquhar and Palade, 1963), 115Å (Millington and Finean, 1962). Sjöstrand (1963a) found the membrane to be asymmetric; the cytoplasmic opaque layer measuring 40Å, the peripheral opaque layer 30Å and the middle electron transparent layer 25Å in thickness. The stainability and definition of the peripheral opaque layer decreased with absorption. Earlier lower resolution studies did not resolve three layers under such circumstances, (Sjostrand & Zetterqvist, 1957). Deviations of measurements were in Sjöstrand's opinion (1963a) due mainly to differences in resolution and section thickness. Millington's (1964) measurements in the mouse were in close agreement with those of Sjöstrand, i.e. 94Å. In rat, however, the membrane was 101Å in mean thickness, both opaque components being of approximately the same density or sometimes with the peripheral layer of greater density. To explain this, Millington pointed out that the difference he observed between mouse and rat under otherwise identical conditions might be explained in terms of stainability, suggesting a species difference. These observations indicate that the "unit membrane" concept, as expressed by Robertson in numerous reports (see Robertson, 1964) is not entirely valid. The plasma membrane between
individual microvilli shows occasional invaginations
(Palay and Karlin, 1959a; Strauss, 1963; Ladman,
Padykula and Strauss, 1963; Ashworth and Johnston, 1963;
Clark, 1959; Millington and Finean, 1962) to form narrow
tubules. In some cases these appear to form vesicles
550-600Å (Palay and Karlin, 1959a) or up to 700Å in diam­
eter (Ashworth and Johnston, 1963). These findings could
indicate pinocytotic activity. However, the interpretation
of vesicular structures in an apical portion of the enter­
cyte is a matter of considerable controversy, especially
in connection with lipid absorption. This problem will be
examined later.

Millington and Finean (1962) described in the rat intest­
ine pore-like interruptions in normal plasma membranes cov­
ering the brush border. Similar interruptions were observed
by Mukherjee and Williams (1966) in the mouse. It appears
likely that these are artificial as is the appearance of the
brush border plasma membrane after saponin treatment (Muir,
1962).

By means of histochemical methods using the electron
microscope several phosphatases have been demonstrated in
association with the plasma membrane of the brush border.
The precise relation of this activity to the membrane remains
to be determined, since the concentration and diffusion of
the reaction product makes interpretation difficult. This
was the case with alkaline phosphatase (Brandes, Zetterqvist and Sheldon, 1956; Clark, 1961a, 1961b; Chase, 1963; Reale, 1962; Goldfischer, Essner and Novikoff, 1964; Hugon and Borgers, 1966c; Ito, 1965a). Overton (1965) reported that the reaction product was associated with the inner layer of the plasma membrane. Other phosphatases demonstrated in the plasma membrane were adenosine triphosphatase (Ashworth, Luibel and Stewart, 1963a, 1963b; Goldfischer, Essner and Novikoff, 1964) and in lesser amounts nucleoside diphosphatases and thiamine pyrophosphatase (Goldfischer, Essner and Novikoff, 1964). The localisation of acid phosphatase (Sheldon, Zetterqvist and Brandes, 1955; Ogawa, Masutani and Shinonaga, 1962) is now regarded as artificial. Using improved techniques, Goldfischer, Essner and Novikoff (1964), Barka (1964) and Ito (1965a) were not able to demonstrate a positive reaction.

Hampton and Rosario (1966) described an accumulation of granular material in cytoplasm close to the thickened inner component of the plasma membrane of some enterocytes in normal mice. The cause is unknown.

The outer surface of the plasma membrane covering the brush border is coated with a finely filamentous material, named fuzzy layer, which is seen with different grades of clarity in different animal species (Ito, 1964, 1965a), and in man, (Laguens and Briones, 1965). The filaments are continuous with the outer component of the plasma membrane and
may be several thousand Å long. Acid mucopolysaccharides were demonstrated in this layer by Revel (1964) and Ito (1965a). This coating incorporates tritiated acetate, glucose and Na$^{35}$O$_4$ in vitro within 15 min. (Ito, 1965b). The filamentous material seems to be resistant to the action of mucolytic substances and is disrupted only when the cell is degenerated and extruded. Pease (1966a) recently developed a method of inert dehydration and anhydrous sectioning and staining (1966b) which, when applied to intestine (1966c) showed that this surface polysaccharide layer is stained with phosphotungstic acid. In one electron micrograph of an enterocyte, examined in an unfixed freeze-fractured specimen (Bullivant and Ames, 1966, Fig. 8) short filaments on the surface of the brush border are seen. However, no mention of this is made in the text. The appearance suggests, however, that these filaments correspond to the fuzzy layer seen in conventional specimens. It is generally accepted that this layer represents localisation of a polysaccharide of widespread occurrence (Rambourg, Neutra and Leblond, 1966) termed glycocalyx by Bennett (1963). The function of the furry layer is unknown. The location of enzyme activities demonstrated in association with the plasma membrane does not allow any conclusions to be drawn as to whether the reaction product seen externally to the plasma membrane represents the true presence of enzyme in the fuzzy layer or
a diffusion artefact. However, the outer portion of the layer appears to be inactive. The resistance of the furry layer and its apparent similarity in individual species seems to indicate that the layer should be regarded as a part of the external component of the plasma membrane (Ito, 1965a). Pease (1966c) showed that polysaccharide is present in the tubular invaginations of the plasma membrane. These were interpreted by him as intracellular glands. He stressed the general importance of this layer in maintaining hydration of the cell surface.

An additional specialisation of plasma membranes is the junctional complex. This structure was referred to in older descriptions using the light microscopical term "terminal bar". The first observation of two different kinds of attachment zones in the electron microscope was made by Zetterqvist (1956). The existence of three components forming the junctional complex was first described in epithelia by Farquhar and Palade (1963). These were named zonula occludens (tight junction), zonula adhaerens (intermediate junction) and macula adhaerens (desmosome). The desmosomes occur as isolated units along the lateral margins of the cell. It is of interest that earlier reports describe only two components. Zamboni's (1961) description which appeared in abstract only was unnoticed by Farquhar and Palade, but obviously corresponded to zonula adhaerens, and desmosomes
(the former being treated as a separate kind of the latter).

Sjostrand (1963a) described the components which correspond to tight junctions and intermediate junctions in the terminology of Farquhar and Palade; in another paper (Sjostrand, 1963b) he states explicitly that no elaborate structures of desmosomes are seen in these attachment zones. The extracellular polysaccharide layer covering the plasma membrane demonstrated by the method of inert dehydration was observed in the region of zonula adhaerens but it was excluded in zonula occludens (Pease, 1966c). Few observations are available to demonstrate functional differences of the components of a junctional complex. Overton and Shoup (1964) using chicken intestinal mucosa, observed in early stages of development complete dissociation after tryptic digestion. At later stages, the dissociability was incomplete and while desmosomes were disjointed, tight junctions remained. The result of calcium and magnesium depletion in the intestine caused by chelation (Cassidy and Tidball, 1967) is essentially the same as reported in the parietal cells in frog stomach (Sedar and Forte, 1964); there is a loss of dense intercellular material in the desmosomes and zonula adhaerens with their dissociation. Functional measurements indicate increased permeability (Cassidy and Tidball, 1967) and increased electrical conductance (Sedar and Forte, 1964). In both cases, the changes are reversible.
Further structures forming part of the surface specialisation of the enterocyte are filaments seen in the core of the microvilli. These filamentous structures were observed in earlier studies (Zetterqvist, 1956; Palay and Karlin, 1959a; Haubrich, Watson, O'Driscoll and Valentine, 1959). However, they were not resolved with clarity. Millington and Finean (1962) observed that in the core, the filaments which were concentrated in the centre formed lateral connections with the plasma membrane. Their experiments with hypotonic and hypertonic saline showed that the core of the villus reacted in a different fashion from the region beneath the plasma membrane. Sjostrand (1963a) presumed that filamentous structures formed a feltwork rather than individual defined filaments. These filaments are more clearly seen after aldehyde prefixation, followed by osmium tetroxide. In an intestine fixed by this method, distinct filaments about 60Å in width were observed along the length of the microvillus extending to the apical cytoplasm (McNabb and Sandborn, 1964). The upper end of the filaments is associated with a dense region of the cytoplasmic surface of the plasma membrane covering the tip of a microvillus. In a cross section of the microvillus, the number of filaments is about 50 and their packing may be in hexagonal array. Filaments do not appear in a 200-300Å wide zone beneath the plasma membrane. Beneath the microvilli, the bundle of
filaments forms a rootlet. It is of interest that in the earlier observations, these structures were found to be prominent in unsuccessfully preserved cells and were considered to be artifacts (Zetterqvist, 1956; Palay and Karlin, 1959a). In human enterocytes the structures seen in the microvilli were interpreted as microtubules ranging from 60-150Å in diameter (Laguens and Briones, 1965). Both filamentous and tubular structures (60-110Å in diameter) were observed in mouse by Mukherjee and Williams (1966). These authors observed, like Millington and Finean (1962), transverse connections of filaments with the plasma membrane. Filaments and tubules, which were often paired, can be demonstrated after freeze substitution (Kjaerheim, 1963).

There is no information concerning the development of the microvillus core in mammalian enterocytes. In the chicken duodenum the height of microvilli at early stages of development increased after the formation of the core. The rootlets were formed after core formation. This suggests that the onset of differentiation of the core takes place in the surface area. At later stages of development, the width of the core remains unchanged, (Overton and Shoup, 1964).

Immediately below the brush border, there is a zone referred to as the terminal web. This term was reintroduced by Puchtler and Leblond (1958), in their light microscopical study of enterocytes and since then has been used in electron
microscopy. Earlier observations (Zetterqvist, 1956) interpreted this zone as an expansion of microvillus cores in a plane parallel to the free surface. Palay and Karlin (1959a) first observed lateral connections of filaments to the "terminal bar". Their finding confirmed the assumptions of Puchtler and Leblond (1958) which were based on light microscopical observations. These authors state that "If the web is made of filaments these would then end in the terminal bars." According to more recent observations, the majority of filaments in the terminal web appear to condense and terminate in an intermediate junction (Zamboni, 1961; Farquhar and Palade, 1963; McNabb and Sandborn, 1964). A less dense layer of coarser filaments is seen beneath, and was named the desmosomal web (McNabb and Sandborn, 1964) since these appear to be connected with the apical desmosome (Farquhar and Palade, 1963). The rootlets of microvillus filaments terminate at the level of either terminal or desmosomal webs. Some filaments of the rootlets are continuous with the web. Zamboni (1961) stressed the architectural continuity of all these filamentous structures using the term "cell web" for the whole complex. The significance of this complex in maintaining the shape of the apex of the cell was demonstrated by Millington and Finean, (1963); Eichholz and Crane, (1965); Overton, Eichholz and Crane, (1965). After isolation the terminal web remains attached to
the brush border. The evidence obtained in these and other studies (Overton, Eichholz and Crane, 1964) confirmed the existence of the microvillus core as an entity which was separable as a fraction by centrifugation. Negative staining applied to this fraction showed parallel structures corresponding to the filaments. A further fraction was formed by vesicles with a unit membrane substructure. Miller and Crane (1961a) isolated brush border and several enzymes in it, particularly disaccharidases (Miller and Crane, 1961b). Further fractionation of the isolated brush border made it possible to localise the alkaline phosphatase and maltase activities into a fraction consisting of vesicles which corresponded to the isolated plasma membrane. Particles covering the surface of these membranes may be compared to those coating mitochondrial membranes, (Fernandez-Moran, Oda, Blair and Green, 1964) and suggest that there is also an orderly arrangement of enzymes on the microvillus plasma membrane serving hydrolytic and absorptive functions.

The isolated microvillus cores of hamster enterocytes showed a helical chain-like structure (Overton, Eichholz and Crane, 1964).

The isolation of brush borders involves a binding of calcium by chelation, a procedure which appears to be necessary for complete separation (Millington, Critchley and Tovell, 1966). This seems to reflect the important role of
calcium in the maintenance of epithelium integrity.

The ground cytoplasm of an enterocyte beneath the terminal web contains cellular organelles.

The endoplasmic reticulum occurs as the granular and a-granular varieties. Some descriptions state that granular endoplasmic reticulum is seen only in moderate amount (Palay and Karlin, 1959a; Sjostrand, 1963b). Others do not mention the amount specifically. The granular endoplasmic reticulum is seen in the supranuclear zone as flat cisternae and lies parallel to the long axis of the cell, branching occasionally. In some places the reticulum forms small groups of cisternae (Palay, Karlin, 1959a). In Sjostrand's opinion, (1963b) the amount of endoplasmic reticulum is slightly greater in the infranuclear cytoplasm. In addition to cisternae, some ribosome-covered membranes form rounded vesicles or tubules in the supranuclear cytoplasm. The occurrence of this form varies with absorption (Palay and Karlin, 1959b). Frequently the membranes of vesicles and tubules are devoid of granules representing a transition to the smooth surfaced endoplasmic reticulum. Vesicles are seen in large numbers in the apical part of the enterocyte (Sjostrand, 1963b; Ashworth and Johnston, 1963; Padykula, Strauss, Ladman and Gardner, 1961). They are described as being more numerous and larger in areas of cytoplasm away from the surface (Palay and Karlin, 1959a; Ashworth, Chears,
Sanders and Pearce, 1961). The tubular form of agranular reticulum is often associated with vesicles. It is particularly well developed in the enterocytes of the hamster (Strauss, 1963) where it is seen to have a fairly dense content. These tubules may communicate with rough endoplasmic reticulum and Golgi complex. A similar development of smooth-surfaced tubules was seen in partially differentiated enterocytes of the bandicoot (Van Lennep, 1964). In this species, the membranes of these tubules are characterised by increased thickness (85 Å) and density. They seem to originate from vesicles which arise at earlier stages from the Golgi complex.

In contrast to the tubular forms of agranular reticulum, the significance of the vesicles and especially those seen in the apical portion of the cell is uncertain. However the invaginations of the plasma membrane which may be taken as evidence of pinocytosis, have special dimensional, structural and histochemical characteristics (see above). On the other hand, vesicles in the apical zone have a symmetrical triple-layered membrane about 70 Å thick, which makes their derivation from the plasma membrane doubtful (Sjostrand, 1963a, b). Sjostrand (1963a, b) suggested their origin from the Golgi complex. This seems to be the case in differentiating gut epithelium in the bandicoot (Van Lennep, 1964) in which species, the vesicles have thicker walls (85 Å) and are later
transformed into tubules. This mode of development may be, however, peculiar to this species or to marsupials in general.

Small vesicles in the apical zone may show alkaline phosphatase activity. This has been demonstrated in some smooth surfaced saccules of the reticulum and in the Golgi complex. This makes the interpretation of the mode of origin of the membranes on the basis of alkaline phosphatase activity difficult. The findings of Pease (1966c) using the method of inert dehydration, indicate that many of the tubular structures seen in the apical cytoplasm beneath the terminal web (see Fig. 12 in Pease, 1966c) have functional connections with the plasma membrane.

The narrow tubules penetrating the terminal web and frequently traced in continuity with the plasma membrane (Palay and Karlin, 1959a; Ashworth and Johnston, 1963; Strauss, 1963; Ladman, Padykula and Strauss, 1963; Strauss, 1966) were never seen to be continuous with the endoplasmic reticulum, although occasional continuity with vesicles beneath the web was observed (Ashworth and Johnston, 1963). Continuity of vesicles with endoplasmic reticulum was rarely seen (Strauss, 1966).

Two factors which might influence the form of smooth surfaced membranes should be pointed out. In evaluating the difference in dimensions of plasma membranes and vesicles, the possibility of the transformation of membrane has to be
considered. It is known that the symmetrical triple layered membranes bounding the zymogen granules of pancreatic acinar cells acquire an asymmetric character and thickness similar to that of the plasma membrane before extrusion (Sjostrand, 1963b; Malhotra and Van Harreveld, 1965). This indicates that some mechanism determines changes of structure before one membrane is incorporated into another. In endocytosis the change of membrane structure may be assumed to take place long before the membrane is incorporated into the interior of the cell. The events which accompany this "membrane flow" (Bennett, 1955) are not known. Another factor determining the proportion of vesicles seen in the cytoplasm may be the fixation. It is known that tubules and cisternae of agranular endoplasmic reticulum in various cells are transformed into vesicles when fixation is not optimal, (Ito, 1961; Christensen, 1965). Tubules of smooth endoplasmic reticulum in the enterocytes may presumably react in a similar fashion. Few observations of autolysis in enterocytes (Zetterqvist, 1956; Hartman, Smith, Hartman and Butterworth, 1959) indicate that vacuoles are formed. The descriptions however, do not allow any firm conclusion to be drawn as to their mode of origin.

Free ribosomes are numerous according to most observations.

The Golgi complex of the enterocyte is well developed
(Zetterqvist, 1956; Palay and Karlin, 1959a; Padykula, Strauss, Ladman and Gardner, 1961; Trier, 1962; Sjostrand, 1963b), and is situated above the nucleus. Additional complexes were seen in paranuclear and infranuclear positions (Sjostrand, 1963b). The complex consists of 4-8 flat cisternae stacked closely together in parallel fashion, surrounded by numbers of vesicles. The membranes are symmetrical and 60-70Å in thickness (Sjostrand, 1963a). Some cisternae usually show vacuolar dilations. The Golgi complex is occasionally vacuolar in appearance (Lacy and Taylor, 1962). Elements of the Golgi complex, even in fasting animals, often contain dense granules (Sjostrand, 1963b) of the same appearance as the lipid droplets formed during absorption (Palay, Karlin, 1959a). Striking numbers of thick-walled vesicles were observed in the Golgi complex at the earliest stages in the differentiation of bandicoot enterocytes (van Lennep, 1964);

The reaction products of nucleoside diphosphatases and thiamine pyrophosphatase activities were located in the Golgi complex by electron microscopy. Weak activity of these enzymes was apparent in the endoplasmic reticulum of enterocytes (Goldfischer, Essner and Novikoff, 1964). A strong reaction was observed in the endoplasmic reticulum, especially beneath the terminal web when thiol substituted acids were used to demonstrate "esterase" activity (Bell and
Alkaline phosphatase activity is present in Golgi cisternae and vesicles (Hugon and Borgers, 1966c). Golgi cisternae show some activity of acid phosphatase (Barka, 1964), but most of the activity of this enzyme in the Golgi area is present within lysosomes.

Ultrastructurally the most investigated activity of the endoplasmic reticulum and Golgi complex of enterocytes is that associated with fat absorption.

Briefly, the biochemical evidence indicates that fats are absorbed as free fatty acids or monoglycerides, which are present in the lumen of the gut as a micellar solution (Senior, 1964; Isselbacher, 1965; Hofmann and Børgstrøm, 1962). The triglycerides are resynthesised in the mucosa.

Electron microscopical investigations have brought conclusive evidence concerning the transport of the lipid within the cell, interpretations as to the mode of entry are, however, a matter of controversy.

Lipid droplets 30-100 μm in diameter occur with different degrees of frequency between the microvilli of the brush border (Palay and Karlin, 1959b; Strauss, 1963; Lacy and Taylor, 1962; Ladman, Padykula and Strauss, 1963; Ashworth and Johnston, 1963; Thomas and O'Neal, 1960; Ashworth and Lawrence, 1966). Sjostrand (1963b) and Strauss (1966) did not see any. Increased pinocytotic activity manifested by the occurrence of lipid droplets within vesicles close to the bases of microvilli and terminal web was seen by Palay.
and Karlin (1959b); Palay and Revel (1964); Napolitano and Kleinerman (1964). Others found fat globules in this location only occasionally or rarely (Ashworth, Stembridge and Sanders, 1960; Thomas and O'Neal, 1960; Strauss, 1963; Phelps, Rubin and Luft, 1964; Jones, Thomas and Scott, 1962). Ashworth and Lawrence (1966) pointed out that there was an association of micellar lipid particles (50-150Å) with the fuzzy layer. Similar particles rarely occurred in "spherules" (300-1000Å in diameter) in the region of the terminal web. These represented pinocytotic vesicles, but were only slightly increased in number during lipid absorption. Strauss (1966) observed vesicles with droplets only rarely in the lower portion of the terminal web without any increase in pinocytosis during in vitro fat absorption in hamster. Still others (Ashworth and Johnston, 1963; Ladman, Padykula and Strauss, 1963; Sjostrand, 1963b) noted that in general the terminal web was free of lipid. There is universal agreement that the fat globules appear within vesicles beneath the terminal web which have membranes 70-80Å in thickness (Sjostrand, 1963b; Ashworth and Lawrence, 1966). Sjostrand (1963b) is of the opinion that the lipid appears in those vesicles of the fasting animals which originated hypothetically from the Golgi complex. Napolitano and Kleinerman (1964) reported that the fat-containing vesicles of the terminal web which occur with highest
frequency at an early stage of absorption have a membrane similar in structure to the plasma membrane. This is in direct opposition to Sjostrand's findings. Other authors hold that there is continuity of these vesicles with smooth and rough surfaced endoplasmic reticulum.

Since the origin of the vesicles is not determined, the importance of pinocytosis in fat absorption is regarded differently. Palay and Karlin (1959b) pointed out that there was a discrepancy between the amount of lipid entering the cell and in the terminal web, and that accumulating more deeply in the endoplasmic reticulum. In order to explain the finding in terms of pinocytosis, it was necessary to assume that the entry and passage through the terminal web was very rapid. They did not, however, conclusively determine the importance of pinocytosis in absorption. Porter (1961) suggested that lipid seen entering the cells by pinocytosis represents the residues of incomplete hydrolysis, while the fat in the endoplasmic reticulum was resynthesised after hydrolysis. Other observations (see above) remained either inconclusive in deciding the mechanism of absorption, or questioned but did not completely exclude the significance of pinocytosis.

The process of fatty acid absorption (administered in the same fashion as fat) (Ashworth and Johnston, 1963), cannot be distinguished by electron microscopy from fat absorption. The morphological site of their conversion to the
triglycerides which appear in the chyle, has not been determined. Differences in structure of droplets associated with the absorption of corn oil, butter and some other lipids, have been observed (Thomas, and O'Neal, 1960; Jones, Thomas and Scott, 1962) but these did not contribute to the mechanism of absorption.

The absorption of polystyrene latex particles paralleled that of lipid. The material rarely occurs in the terminal web and is enclosed within vesicles (Sanders and Ashworth, 1961). However, the validity of these findings was subject to criticism (Trier and Rubin, 1965) since some particles might have been fat.

Palay and Revel (1964) confirmed the original observations and after examining the earlier stages of lipid absorption, found lipid in invaginations of the plasma membrane and terminal web, more often than in the previous investigation. In their opinion, the frequency of this process could account for the accumulation of fat in absorbing cells and suggested that the concept of the rapid uptake and passage through the terminal web was no longer necessary. However, an examination of their published photographs shows that the amount of lipid seen in this region is comparatively low.

Further doubts about the significance of pinocytosis in fat absorption followed experiments in which tracer materials
were suspended in the fat to be absorbed (Cardell, Badenhausen and Porter, 1966). Absorbed fat was seen in the endoplasmic reticulum, while tracer material (ferritin) was trapped in apical vesicles which were free from fat. These were apparently of pinocytotic origin.

Rubin (1966) observed in newborn puppies that there was no relationship of fat absorption to pinocytotic activity by which colostrum protein was being absorbed simultaneously.

Sjostrand (1963a) was the only person to describe increase in width of the peripheral component of the plasma membrane and the appearance of dense material in the intermediate layer evident during lipid absorption. His observation of irregularities of the outer surface of the plasma membrane seems to correspond to a description of the fuzzy layer.

Some observations suggest that lipid is absorbed in micellar form. Lacy and Taylor (1962) saw dense particles about 250Å in diameter within the plasma membrane, in microvilli and rarely in the terminal web.

Particles ranging in diameter from 50-1000Å were demonstrated on or within the microvilli essentially by procedures for demonstrating phosphatases when the substrate from Wachstein-Meisel medium was omitted (Rostgaard and Barrnett, 1965). The particles increased considerably in number after fat feeding. In the region of the terminal web,
only the smallest particles were seen. The fat appearing beneath the terminal web in the vesicles was non-reactive. In the opinion of these authors, this evidence indicated that some fat is absorbed in a particulate or micellar form which penetrated through the plasma membrane. It seems difficult, however, to explain the great size of some globules entering the microvilli; in some instances the appearance may be due to the superposition of structures.

Ashworth and Lawrence (1966) proposed that micellar particles become attached or incorporated into the plasma membrane and that the released lipid is transported by means of membrane flow within "spherules" or becomes hydrolyzed to form molecules which diffuse through the plasma membrane.

The passage of fat through the enterocyte is relatively well understood. Fat droplets accumulate in the cisternae of both agranular and granular reticulum. Few were demonstrated in the perinuclear cisterna (Palay, 1960). Fat droplets within the endoplasmic reticulum show an increase in size, suggesting coalescence. The endoplasmic reticulum surrounding fat droplets usually appears as vesicles or vesicular expansions (Palay and Karlin, 1959b; Ashworth, Stembridge and Sanders, 1960; Strauss, 1963). At this stage, fat appears within the Golgi complex which can be distended to a considerable degree. Fat droplets within the Golgi complex coalesce to form large ones (Ladman, Padykula
and Strauss, 1963; Strauss, 1963; Jersild, 1966b). Strauss (1966) on the other hand, denies that there is any coalescence of droplets. In cells loaded with droplets, larger masses of fat were observed in the cytoplasmic matrix, free of membranous investment (Palay and Revel, 1964; Jersild, 1966b; Strauss, 1966).

The synthetic activity of endoplasmic reticulum during fat absorption was recently shown by autoradiography with labelled glucose (Jersild, 1966a). This showed that the glucose was incorporated into the fat droplets inside the endoplasmic reticulum.

Radioautographic investigations disclosed that the fat absorption is very rapid. Labelled fat was observed in the endoplasmic reticulum and the Golgi complex one minute after administration and considerable quantities had accumulated within 8-30 mins. This sequence of events suggested that the rate of fat discharge is slower than absorption. The apparent concentration of fat in the Golgi area may explain this retardation (Jersild, 1966b).

The discharge of the fat into the intercellular space takes place at the level of the Golgi complex or nucleus. In this process reverse pinocytosis is assumed to operate. Most reports do not discuss the sequence of events in detail. The vesicles and channels of the endoplasmic reticulum which were assumed to be involved in this form of
activity (Palay and Karlin, 1959b) were never seen in direct communication with the cell surface. Lacy and Taylor (1962) observed vacuoles containing fat, in the Golgi complex, orientated towards the plasma membrane. Their evidence suggested that transport of fat is effected by this organelle. Ladman, Padykula and Strauss (1963) observed fat droplets in the endoplasmic reticulum of the infranuclear portion of the cell which indicated that fat discharge might also occur from the basal surface, although this was not actually seen. In other observations (Ashworth and Johnston, 1963) fat droplets were seen between the base of a cell and the basement membrane.

There is no doubt that most of the fat is discharged through the lateral borders of the cell and all observations agree that intercellular spaces are markedly dilated at this stage. Small gaps then appear between the bases of the cells which open into spaces directed towards the basement membrane. Sjostrand (1963b) discussed the possibility that some surface active agent is secreted by the cells.

Fat droplets in an extracellular location are devoid of any membranous investment. They correspond in size to chylomicra. In addition, Rubin (1966) observed, in man, smaller particles which had an appearance of lipoproteins. They pass through the basement membrane into the lamina propria. This passage through the basement membrane has not
received particular attention, but Palay and Karlin (1959b) and Ladman, Padykula and Strauss (1963) have pointed out the paucity or absence of droplets in this location. Trier and Rubin (1965) and Rubin (1966) laid particular stress on the presence of discontinuities in the basement membrane, through which they believe the passage of fat takes place.

An azo dye is formed in vivo by coupling naphthol and diazonium salt during absorption. Naphthol is administered as a phosphate and hydrolysed by alkaline phosphatase present in the plasma membrane. The azo dye formed is absorbed in a similar fashion to lipid. Particles of insoluble dye are observed in vesicles beneath the striated border; later they accumulate in the Golgi complex and are discharged through the lateral plasma membrane (Barrnett, 1959). However the possibility that diffusion takes place before the dye forms detectable particles must be taken into consideration.

It seems appropriate to mention the ability of neonatal enterocytes to absorb proteins such as antibodies and colloidal materials. This ability, which is lost in the rat after approximately 20 days of postnatal life, is associated with the presence of large, apparently pinocytotic vacuoles beneath the terminal web (Clark, 1959) in which these substances may be demonstrated after their administration. At the same time, the activity of alkaline phosphatase in the
brush border is lower than in adult animals (Clark, 1961). Numerous pinocytotic vesicles and vacuoles were observed in the enterocytes of newborn dogs (Rubin, 1966) and pigs (Sibalin and Björkman, 1966) during colostrum absorption; in the latter species, these contained a foamy substance. Kraehenbuhl, Gloor and Blanc, (1966) showed, however, that the vacuoles occur also in the postnatal enterocytes of species, like rabbit, which do not absorb protein. Vacuoles were seen frequently in direct continuity with the plasma membrane. This indicates that the ability to absorb protein may be determined by the membranes of vacuoles rather than by the process of pinocytosis in itself. To prove this, it would be desirable to determine if there is any difference in the occurrence and accumulation of proteins and colloids in the vacuoles of species which are able to absorb these and those which cannot.

The absorption of proteins in adult animals cannot be readily demonstrated by electron microscopy. Recently the uptake of ferritin by pinocytosis was demonstrated by Bockman and Winborn (1966); Casley-Smith (1967); Cardell, Badenhausen and Porter (1966). An increase in the number of vesicles was reported after sucrose and casein administration (Hampton, 1961) but because these observations are available in abstract form only, they cannot be reasonably interpreted.
An enterocyte contains fairly numerous mitochondria (Dalton, 1951; Zetterqvist, 1956; Palay and Karlin, 1959a; Padykula, Strauss, Ladman and Gardner, 1961; Ashworth, Chears, Sanders and Pearce, 1961). They are most frequently filamentous or rod shaped. Spherical or branched forms have been described, though less frequently (Palay and Karlin, 1959a). Palay and Karlin (1959a) pointed out that mitochondria are more frequent in supra-nuclear cytoplasm; Sjostrand (1963b), on the other hand, stated that the mitochondria and endoplasmic reticulum are abundant below the nucleus. In the supra-nuclear cytoplasm, mitochondria are orientated parallel to the cell length (Zetterqvist, 1956; Palay and Karlin, 1959a; Padykula, Strauss, Ladman and Gardner, 1961) consequently elongated profiles are commonest in longitudinally orientated sections. Cristae are generally transverse. Intramitochondrial dense granules are considered by some authors to occur frequently (Palay and Karlin, 1959a); Padykula, Strauss, Ladman and Gardner (1961) stated that they are present occasionally. During the absorption of sodium and potassium intramitochondrial granules increase in number in the enterocytes of the duodenum (Weiss, 1955b). These observations seem to represent a parallel to the later demonstrated role of dense granules in accumulation of divalent cations (Peachey, 1964; Greenawalt, Rossi, Lehninger, 1964). A different type of granule appears
during iron absorption in duodenal and jejunal enterocytes (Oki, Yoshioka, Hayashi, Masuda, 1965). The granules were located in the intracristal space and were composed of more dense particles which differed from ferritin.

With the exception of Zetterqvist (1956), nuclei are hardly mentioned in electron microscopic observations of normal enterocytes. He observed infrequent irregularities of the nuclear margin. After osmium fixation chromatin is rarely condensed. The nucleolus, nuclear pores and envelope are not remarkable.

Lysosomes and lysosome-like bodies are frequently encountered in enterocytes. Zetterqvist (1956) classified these lysosomes under the heading, "component I"; others merely refer to them as dense bodies (e.g. Lacy and Taylor, 1962). Lysosomes seen in the intestinal epithelium exhibit the same pleomorphism as in other locations. They frequently contain segments of membranes (Barka, 1964; Lacy and Taylor, 1962). Lysosomes demonstrated by the acid phosphatase reaction are most frequent beneath the terminal web and also occur around the Golgi complex. Many bodies corresponding to lysosomes, either by the appearance or location, give also a positive reaction for alkaline phosphatase (Hugon and Borgers, 1966c; Chase, 1962), E-600 resistant esterase and arylsulphatase (Hugon and Borgers, 1967). Cardell, Badenhausen and Porter (1966) suggest that pinocytotic vesicles take part in the
formation of "apical lysosomes". At present, it is generally accepted that lysosomes may be the final stage of cytolysomes (Glinsmann and Ericsson, 1966; De Duve, 1963; Ericsson, Trump and Weibel, 1965). In enterocytes, altered mitochondria have been seen in a cytoplasmic body by Zetterqvist (1956). Similar structures were described by Takano (1964) as complex bodies. Cytolysomes were seen in great numbers in the enterocytes of newborn rats (Moe and Behnke, 1962) and in foetal life during the change of the stratified epithelium to form a single layer of cylindrical cells, (Behnke, 1963a). The cytolysomes contained a variety of constituents which showed signs of repeated incorporation and various degrees of disintegration. Some cytolysomes predominantly included pinocytotic vesicles. The origin of the limiting membrane was not determined.

In human foetal intestinal epithelium, lysosomes, which correspond to meconium corpuscles were demonstrated by electron microscopy. These appear to be formed by the condensation of vacuoles which are of pinocytotic origin (Bierring, Anderson, Egeberg, Bro-Rasmussen and Matthiessen, 1964).

*Multivesicular bodies* were first seen in enterocytes by Zetterqvist (1956), who used the term "component II" to describe them. Later they were shown to contain the enzymatic activities of lysosomes (Barka, 1964; Hugon and Borgers,
1966c; 1967; Bjorkman and Sibalin, 1967) and to accumulate ferritin (Bockman and Winborn, 1966).

A smaller homogeneous single membrane-limited body, similar to microbody, was observed in porcine postnatal enterocytes by Sibalin and Bjorkman (1966). This body does not show acid phosphatase activity (Bjorkman and Sibalin, 1967).

Microtubules are frequently seen in enterocytes. The structural characteristics of microtubules are the same as in other cells (Behnke, 1964; Sandborn, Koen, McNabb & Moore, 1964). In the enterocyte they are usually orientated longitudinally (Behnke, 1964) but many microtubules run within the cell web (Sandborn, Koen, McNabb and Moore, 1964). In the opinion of these authors, there are areas of contact between microtubules and plasma membranes. These relationships must be interpreted with caution, however, and the illustrations presented are far from convincing.

In normal human enterocytes, moderately opaque bodies containing ferritin were found in the supranuclear cytoplasm (Hartman, Conrad Jr., Hartman, Joy and Crosby, 1963). The nature and origin of the bodies has not been determined with certainty, but it may play the role of receptor in the regulation of iron absorption (Wheby and Crosby, 1963; Conrad, Jr. and Crosby, 1963). On the other hand, iron containing bodies in the rat duodenal enterocytes occur only in iron-fed animals (Lever, 1961). In both man and rat, ferritin
was seen to be dispersed in the cytoplasm.

Crypt epithelium:

Undifferentiated crypt epithelium received less attention from electron microscopists than mature enterocytes.

The lateral surface of crypt cells is regular and the plasma membrane usually straight. Junctional complexes are the same as in other epithelia. Desmosomes are numerous (Trier, 1963) and expansions of the intercellular space are generally absent.

Apical structures are present though in a simplified form. Microvilli are less numerous, rather irregular and shorter (Brown, 1962; Gottlieb, Robertson and Zamcheck, 1962; Padykula, Strauss, Ladman and Gardner, 1961; Trier, 1962; 1963). The fuzzy layer is well marked (Trier, 1963). Microvilli have the cores formed by filaments, the fascicles of which may be traced into deeper layers of the cytoplasm than in mature enterocytes. In places, the apical surface of the cell forms lobe-shaped protrusions, some of which were seen free in the lumen at a considerable distance from the cell (Trier, 1963; 1964). Padykula, Strauss, Ladman and Gardner (1961) described the terminal web in crypt epithelium. The most detailed study available, however (Trier, 1963) reported its absence. Microtubules are seen in the cytoplasm (Behnke, 1964; Sandborn, Koen, McNabb and Moore, 1964), and free ribosomes are very numerous (Trier, 1963; Moe, Rostgaard and Behnke, 1965). The endoplasmic
reticulum is sparse (Trier, 1964; Moe, Rostgaard and Behnke, 1965). Mitochondria are not numerous and occur without any preferential localisation in the cell (Moe, Rostgaard and Behnke, 1965). They are larger and contain many dense bodies of greater size than those in mature enterocytes (Trier, 1962; 1963; Padykula, Strauss, Ladman and Gardner, 1961).

In the apical region a centriole of typical structure is found (Trier, 1963). The Golgi complex occurs above the nucleus or beside it and seems to have a variable size (Trier, 1962; 1963; Moe, Rostgaard and Behnke, 1965). Thick walled vesicles are numerous in the Golgi complex of the bandicoot (van Lennep, 1964). Sometimes the cisterna of the endoplasmic reticulum lies close to and parallel with the Golgi complex. This relationship suggests a transitional stage in their formation. Some components of the Golgi complex exhibit an acid phosphatase activity (Moe, Rostgaard and Behnke, 1965; Hugon and Borgers, 1965). Lysosomes are seen in association with the Golgi complex. Their shape suggests that they develop from Golgi cisternae and vesicles (Moe, Rostgaard and Behnke, 1965).

The apical portion of the human crypt cells contains numerous membrane-bounded granules which reach a considerable size and are presumed to be secretory (Trier, 1963). After pilocarpine administration, they were secreted in a merocrine or apocrine fashion (Trier, 1964).
In some crypt cells, glycogen deposits were observed. Cells located at the base of crypts may contain a highly lobulated nucleus and accumulations of mitochondria in the cytoplasm (Trier, 1963).

**Goblet cells:**

The fine structure of goblet cells varies to some extent with the phase of secretion. The microvilli and infoldings of the lateral plasma membrane are similar to those seen in non-secreting cells (Freeman, 1962; Palay, 1958) but with distension of the cell they flatten out. The ground cytoplasm is denser than that of other cell types in the intestinal epithelium (Trier, 1963; Palay, 1958). The density is thought to be caused by compression by accumulated secretory products (Freeman, 1962, 1966), or to the number of ribosomes (Bierring, 1962). Mitochondria in goblet cells are round and oval (Bierring, 1962; Freeman, 1966) or filamentous (Palay, 1958) and less numerous than in enterocytes (Bierring, 1962). The nucleus, when not compressed is similar to that of neighbouring cells.

Some authors hold that the earliest sign of goblet cell development is enlargement of the Golgi complex, (Freeman, 1962; 1966; Hollman, 1963). In the opinion of Palay (1958), this is preceded by proliferation of granular endoplasmic reticulum. Later, both organelles increase in volume. The granular endoplasmic reticulum may be arranged in a concentric
fashion round the nucleus and extends at the base and along lateral surfaces of the cell (Freeman, 1962; 1966; Palay, 1958). Most observations agree that the mucous granules originate by accumulation in Golgi vacuoles, which are formed by dilatation of the Golgi cisternae (Freeman, 1966; 1962; Hollman, 1963; Florey, 1960; Palay, 1958; Bierring, 1962). The mucous material seems to be finely granular (Hollman, 1963; Freeman, 1966; Trier, 1963).

Jennings and Florey (1956) demonstrated in goblet cells of colon by light microscopy that inorganic sulphate appeared in Golgi zones before being demonstrable in mucous granules. This was also seen with labelled glucose (Peterson and Leblond, 1964). Electron microscope autoradiography confirmed these observations (Lane, Caro, Otero-Viliardebo and Godman, 1964; Neutra and Leblond, 1966) and showed that the labelling started to appear in the Golgi cisternae within a few minutes. Subsequently, the sulphate label became associated with the membranes of mucous granules. The glucose label appeared within granules and reached the apex of the cell within one hour. The reaction for acid mucopolysaccharides with thorotrast, was positive in the peripheral cisternae facing the vacuoles and in mucous granules (Berlin, 1967). This provides additional confirmation of the polarity of the Golgi complex in elaboration of the secretory product. The autoradiographic evidence suggests that the
process of mucus elaboration and membrane renewal is continuous and quite rapid in colonic goblet cells. Some authors described a merocrine type of secretion in goblet cells (Trier, 1963; Neutra and Leblond, 1966) while others believe that it is apocrine (Freeman, 1966; Palay, 1958). The discharge of mucin caused by stimulation in colonic goblet cells is not complete (Florey, 1960).

The direct evolution of mucous granules from the endoplasmic reticulum (Taylor, 1959, abstract) has not been established.

The apparent similarity of the basic features of goblet cells and absorptive cells favours the assumption that they are the progeny of one cell type. **Paneth cells:**

The fine structural organisation of **Paneth cells** confirms that these are secretory. The plasma membrane of these pyramidal cells forms microvilli on the apical surface (Behnke, and Moe, 1964; Hally, 1958; Trier, 1963) while there are only minor interdigitations of the lateral surface. Junctional complexes and desmosomes are seen constantly. The endoplasmic reticulum or ergastoplasm occupies the basal, lateral and in part, supranuclear portions of the cell. The contents appear slightly dense after glutaraldehyde prefixation (Behnke and Moe, 1964). The Golgi complex in the supranuclear region is very well developed
and forms multiple stacks of cisternae. Secretory granules are seen in the neighbourhood of the Golgi complex and towards the apex of the cell. Among these granules are seen vesicles about 400\text{"Å"} in diameter (Hally, 1958; Trier, 1963; Behnke and Moe, 1964) which apparently belong to the Golgi complex. Some granules near the Golgi complex show a less dense finely particulate contents than those seen near the apex (Trier, 1963). In rat, the density of granules varies greatly after glutaraldehyde fixation (Behnke and Moe, 1964). In mouse, the early electron microscope study of Hally (1958) revealed a clear space around many granules. Later it was shown that there is an acid mucopolysaccharide capsule present around the granule which consists of a neutral polysaccharide-protein complex (Selzman and Liebelt, 1962). In the electron microscope, this appears as a finely granular material around the granule within a limiting membrane (Staley and Trier, 1965) and shows a positive reaction with colloidal iron for acid mucopolysaccharides (Spicer, Staley, Wetzel and Wetzel, 1967). The wide range of electron density of the capsule is due to different degrees of condensation. The expulsion of granules does not affect this appearance. In Paneth cells of all species dense bodies were frequently observed containing myelinic figures (Hally, 1958; Trier, 1963; Staley and Trier, 1965). These bodies in rat proved to be lysosomes (Behnke and Moe, 1964). Mitochondria are seen between cisternae of the endoplasmic reticulum.
Centrioles were observed in Paneth cells of all species examined. The nucleus of Paneth cells is unremarkable. Differentiating Paneth cells can display at certain stages, strikingly straight cisternae of endoplasmic reticulum, which contain periodically striated material (Behnke and Moe, 1964).

**Argentaffin cells:**

The most prominent feature of Kulitschitzky cells are granules which appear to be concentrated in the basal portion of the cell. These are membrane-bounded and variable in shape and density. The Golgi complex is supranuclear. The process of granule formation and secretion remains to be elucidated, (Trier, 1963). The other cytoplasmic organelles are not remarkable.

The ultrastructural changes which occur in wandering cells in the epithelium has not received much attention. Lymphocytes were most frequently observed. A decreased number or organelles were seen in these cells (Andrew, 1965). However, the cells referred to in this study as lymphocytes in the lamina propria do not fit the generally accepted criteria for this cell type; the identity is left in doubt. The re-utilisation of lymphocyte DNA by the crypt epithelium suggests, however, that the relationship is close (Bryant, 1963)
Lamina propria mucosae:

The fine structure of the lamina propria has scarcely been investigated. Most reports refer to this tissue as connective without emphasising its rather special character. Palay and Karlin (1959a) were the first to mention the components of the lamina propria; since then only a few reports have appeared. Deane (1964) examined the lamina propria in mouse and noticed that its most remarkable characteristic was its compact nature, with only sparse intercellular material. Some cells present were not identified for certain because of the lack of characteristic features. The close apposition of plasma cells and macrophages was thought to suggest immunological interaction.

Behnke (1963b) demonstrated acid phosphatase activity in the dense granules of plasma cells and classified them as lysosomes.

A rather peculiar cell type seen in small intestinal and other mucous membranes is the globular leucocyte. The granules of this cell are believed to be characteristic (Kent, 1966), though the differentiation from mast cells can be difficult.

Blood capillaries in the lamina propria are of the fenestrated type (Palay and Karlin, 1959a; Bennett, Luft and Hampton, 1959). Their ultrastructure is similar to that of other capillaries of this type (Majno, 1965).
diaphragm of the fenestra was shown to consist of the outer layer of plasma membrane. This might be a result of the "collision" of caveolae with the plasma membrane (Lüft, 1964; 1965).

The endothelium of lymphatic capillaries has no fenestrations and the basement membrane is poorly developed (Casley-Smith, 1962; Palay and Karlin, 1959a) or absent (Papp, Rohlich, Rusznyak and Toro, 1962). The arrangement of cells is more irregular and sometimes gaps occur (Palay and Karlin, 1959a; Casley-Smith, 1962). However, in the cat no gaps were seen even in distended intestinal lymphatics (Papp, Rohlich, Rusznyak and Toro, 1962). It appears likely that processes of lymphatic endothelia act as valves during the intercellular passage of fat into the lumen in cooperation with the action of smooth muscles. The intracellular passage suggested by the presence of particles in caveolae seems to be of much less importance (Casley-Smith, 1962; Rubin, 1966).

The boundary of lymphoid tissue with the lamina propria is not sharply delineated. Its appearance suggests that migration of other cell types in addition to lymphocytes, occurs (Clark, Jr., 1963).

Only one short report concerning innervation of submucosal vessels is available (Devine, 1966).
**Tunica muscularis:**

Although electron microscopic observations made on smooth muscle are not infrequent, those relating to the small intestine are sparse. The shape of smooth muscle cells was first appreciated with accuracy with the electron microscope. In the relaxed state, smooth muscle cells appear cylindrical along the greater part of the length, rather than fusiform (Rhodin, 1962; Lane, 1965). Caveolae and micropinocytotic vesicles characteristic of the smooth muscle cell are seen very frequently in the intestine. Their diameters are between 300Å (Prosser, Burnstock and Kahn, 1960) and 600Å (Rhodin, 1962). The caveolae were demonstrated to selectively contain several diphosphatases and triphosphatases (Rostgaard and Barrnett, 1964). Dense areas in the cytoplasm, interpreted as the site of myofilament attachment, lie adjacent to segments of the plasma membrane which are free of pinocytotic vesicles. In the contracted state, in these areas invaginations are seen (Lane, 1965). Cell organelles are concentrated above the poles of a nucleus. The endoplasmic reticulum is sparse and the Golgi complex is small (Rhodin, 1962; Lane, 1965). Mitochondria are also predominately seen in this area, but also occur in relaxed cells between myofilaments. The longitudinal orientation of mitochondria in relaxed cells disappears in contraction. The nucleus changes its contours and shape considerably.
during contraction and becomes deeply invaginated. Most of the cytoplasm of the smooth muscle cells is filled with myofilaments. Reports concerning their dimensions differ. Rhodin (1962) gave a figure 80Å while Lane (1965) observed myofilaments with two diameters; thin, with a 20-30Å diameter and thick, with a diameter of 50-60Å. During contraction, the myofilaments appeared to be more compactly arranged in discrete bundles. Dense areas between filaments, which are probably composed of non-contractible protein, (Prosser, Burnstock and Kahn, 1960) were seen in contracted cells to be more numerous and longer than under relaxation (Lane, 1965).

The intercellular relationships of intestinal smooth muscle reflect physiological properties such as low propagational velocity and good conductivity. The intercellular space is relatively large (Prosser, Burnstock and Kahn, 1960; Lane and Rhodin, 1964b) and the tight junctions of plasma membranes described as the nexus (Dewey and Barr, 1962; 1964) are less frequent than in multiunit smooth muscle tissue (Lane and Rhodin, 1964b). Cytoplasmic connections between smooth muscle cells originally reported in the gastrointestinal tract (Thaemert, 1959) were not later confirmed by other authors. The junctions observed between cell processes consist of tight and intermediate junction types (Oosaki and Ishii, 1964). Elsewhere, all cells are
invested by a basement membrane. A looser arrangement of cells occurs in the muscularis mucosae, the only contacts being between long processes (Lane and Rhodin, 1964a).

Differences in cellular interrelationships reflect the different functional properties of smooth muscle and are closely related to the pattern of innervation.

The early study established that the components of autonomic nerve plexuses in the intestinal wall are the same at various levels or organisation (Richardson, 1958). Interstitial cells appear to be identical with perineural fibroblasts (Richardson, 1958; 1960; Rogers, Burnstock, 1966). The close apposition between nerve fibres and smooth muscle cells is very rare, (Richardson, 1958; Lane and Rhodin, 1964b; Taxi, 1961). They consist of a simple apposition of the plasma membranes to a minimum distance of 150-180Å (Lane and Rhodin, 1964b; Taxi, 1961; Rhodin, 1962; Thaemert, 1963). Aggregates of "synaptic" vesicles and mitochondria occur in widened segments along the axons without relation to the points of apposition with smooth muscle cells. Nerve fibres in intestinal smooth muscle are sparsely scattered and consist of large aggregates of axons (Lane and Rhodin, 1964b). It appears likely that axons form synapses "en passant" (Richardson, 1958; Taxi, 1961; Burnstock and Merrillees, 1964; Lane & Rhodin, 1964b) and that transmitters diffuse through the intercellular space.
Tunica serosa:

There are a limited number of observations on mesothelium (see Rhodin, 1963). Special features of mesothelial cells are microvilli, pinocytotic vesicles and junctional complexes. A basement membrane underlies the mesothelium.
CHAPTER 2

MATERIAL AND METHODS

The animals employed in this study were either young adult New Zealand white rabbits weighing 2-3 kg, or adult albino rats weighing 180-350 g, as supplied by the animal breeding unit of the John Curtin School of Medical Research. Animals of both sexes were used. Four adult grey rabbits were used for comparison. These animals were given a standard laboratory diet and water ad libitum. The animals were starved but not deprived of water for the last 24 hours before killing. No provision to prevent coprophagia was made. In rabbits, material was removed under anaesthesia, consisting of the intravenous injection of Nembutal (35 mg per kg), supplemented with ether. For the rats, a short ether anaesthesia was used. Some of the animals were killed by cervical dislocation and material removed immediately after death, in order to ascertain whether anaesthesia affected the structure of the cells. This was not so. A segment of the upper ileum about 1½ cm in length was removed together with an adjacent short strip of mesentery. Care was taken to minimise manipulation of the intestine before removing the tissue. This segment was immediately divided lengthways and the mesentery and adjacent intestinal wall discarded. The remaining portion was kept
under the fixative for 2 minutes, after which the central portion of the tissue was removed and minced into cubes 1-2 mm across. These were then transferred to fresh fixative for 5-20 hours, usually 11 hours. In most cases, the fixative consisted of a 2.5% solution of glutaraldehyde, buffered with 0.1 M sodium phosphate at pH 7.3, (Sabatini, Bensch and Barrnett, 1963; Sabatini, Miller and Barrnett, 1964). In about \( \frac{1}{3} \)rd of the cases, the fixative consisted of 5% glutaraldehyde in 0.05 M cacodylate buffer. Fixation began at room temperature and was continued at 2-4°C. The tissue blocks were then washed for 2-4 hours in 0.2 M sucrose, buffered identically with the fixative, which was changed 4 times. After washing, the material was post-fixed in 2% osmium tetroxide, buffered with veronal acetate at pH 7.3 with sucrose added (Caulfield, 1957). The fixation time was 4 hours. During the last 30 minutes of fixation, the vials containing the material were left at room temperature. After a short rinse with tap water, the tissue was dehydrated in a graded ethanol series.

The following schedule was used during infiltration:

- **Absolute ethanol:** 4 changes of 30 mins. each
- **Propylene oxide:** 2 changes of 30 mins. each
- **Propylene oxide/araldite,**
  - 2:1 1 hour
  - " " " 2:3 1-2 hours
  - " " " 2:5 2 hours.
araldite: 13-15 hours, at room temperature
freshly-prepared araldite: 1 hour at 60°C.

CIBA araldite was used. This was hardened at 60°C for 3 days.

1-2 μ thick sections were cut using an LKB Ultrotome or Reichert OMU-2 ultramicrotome and glass knives. These were stained with alkaline 1% toluidine blue in 1% borax solution and examined under the light microscope for purposes of orientation. Thin sections which appeared grey to gold by reflected light were cut from selected areas using glass knives on the same ultramicrotomes. The sections were picked up on 100-mesh parlodion-carbon coated or 400 mesh uncoated grids and stained in a saturated solution of uranyl acetate in 50% ethanol, followed by undiluted lead citrate (Reynolds, 1963). A Siemens Elmiskop I electron microscope, operated at 80 kV or Phillips EM 200 electron microscope, operated at 60 kV were used to examine the sections. Both instruments were operated with a double condenser lens and 200 μ condenser and 30 or 50 μ objective apertures. The electron micrographs were taken on Ilford N.50 plates, which were developed in Kodak D-8 developer.

Evaluation of methods:
The method of double fixation was used for a number of reasons. The maximum available preservation of structure
is given by this technique (Pease, 1964). Furthermore, the results of double fixation are less influenced by factors, like buffer composition and duration of fixation, the effect of which is apparent in single osmium fixation (Trump and Ericsson, 1965; Wood and Luft, 1965). The greater flexibility associated with the use of glutaraldehyde for prefixation also makes it useful in the processing of clinical material, hence it was felt that the study of animal tissues prepared in this way would provide a basis of comparison. Few artifacts are caused by glutaraldehyde prefixation. In some places, cellular shrinkage is seen with the formation of wide intercellular spaces which contain irregular membranous profiles or small myelinic figures. Some of the shrunken cells also showed disruption of the plasma membrane. Interestingly enough, the rabbit gut was consistently more prone to shrinkage than that of the rat. The internal structure of some mitochondria was extracted but the defect was usually local. Both matrix and inner mitochondrial membranes were affected. The outer membrane occasionally burst. Usually an irregular membranous profile formed in the site of the defect. This indicates the imperfect stabilisation of membrane lipids by glutaraldehyde treatment prior to osmium tetroxide post fixation. The same explanation applies to the membrane-layer sometimes seen to cover the cleft-like tears which
are encountered in some cells. These artifacts were in most cases readily recognisable though they presented some interpretative problems in cases where they were combined with mitochondrial swelling.

The findings described in Chapter 3 and 4 are based on the study of 15 rats and 10 rabbits.
CHAPTER 3

FINE STRUCTURE OF LAMINA PROPRIA MUCOSAE

General Features:

Lamina propria is a rather special type of mesenchymal tissue. The main purpose of this study was to examine the features which characterise this region and to determine the limits of structural variation. Electron microscopic investigation of the lamina propria reveals certain differences in the proportion of tissue-components at different levels in this layer. The typical features are shown in the villus core and around the lateral walls of the crypt while at the base the characteristic compact arrangement of tissue is less apparent. The cellularity of the lamina propria is best appreciated in low-power micrographs (Figs. 1, 2). These show the heterogeneity of the tissue. Some cells are seen in close contact, elsewhere there is an interstitial space containing small amounts of collagen. In places the cell processes occur in small groups. In Fig. 1 split-like spaces are seen between some cells. These are thought to be due to glutaraldehyde pre-fixation and appear to be more frequent in the rabbit intestine. At the level of the villus core the number of collagen fibres seems to be slightly higher in rabbit than in rat. Smooth muscle cells almost invariably occupy the central portion of the lamina
propria regardless of their relation to the central lacteal which is not always close. Where smooth muscle cells occur in a more lateral position they are invariably found close to the periphery of the lacteal. Smooth muscle cells were never seen immediately beneath the epithelial basement membrane. Non-myelinated nerve fibres occur fairly frequently but though constantly, are not closely associated with the smooth muscle cells.

Undifferentiated cells:

Undifferentiated cells form a minor proportion of the cell population and differ in appearance to some extent among themselves. The smaller of these cells are seen to occur more frequently in the lower portion of the lamina propria and are usually slightly elongated (Fig. 3), and approximately 10 μ long. The outer cell surface is smooth with occasional minute processes. The cytoplasm is of medium density and contains a moderate amount of ribosomes, usually associated to form small rosettes. A few short profiles of granular endoplasmic reticulum are present. Small mitochondria which are not numerous occur together with endoplasmic reticulum in the cytoplasm above the pole of the nucleus. In most cells, a Golgi complex was not included in the plane of section. Dense bodies are usually present in small numbers. The nuclei have a wavy contour and reveal peripheral condensations of the chromatin. These
elements are classified as reticulum cells. Larger undifferentiated cells are more numerous than small ones. They are of an elongated or irregular stellate shape. The ectoplasmic layer, which forms processes is more prominent (Fig. 4). Mitochondria and endoplasmic reticulum are more numerous and very frequently concentrated in the perinuclear cytoplasm near the nuclear poles (Figs. 4; 5). The mitochondria vary in dimension and shape in individual cells; elongated forms are frequent. The Golgi complex is well developed (Fig. 5). The nuclei are elongated with frequent undulations or indentations of the membrane. Chromatin is present in moderate amounts. Nucleoli are large (Figs. 4; 5).

Many cell types have an appearance intermediate between small and large cells.

Some cells lacking definite signs of differentiation contain additional features which warrant their separate characterisation (Fig. 6). These cells are generally larger than those previously described, possessing diameters which vary between 8-16 μ. The shape and surface is irregular with frequent broad-based processes. These cells may be in close contact with others but more frequently they tend to be isolated, mainly in areas rich in collagen fibrils. The cytoplasmic organelles, especially mitochondria, are rather sparse. Several bodies of very high electron density and irregular shape are present in the cytoplasm. The size
varies between several thousand Å units to one u. The smaller bodies appear homogeneous although electron density obscures the internal structure. The larger inclusions are characteristically heterogenous and can be classified as phagosomes or phagolysosomes. With increasing numbers of dense bodies, cells acquire a more characteristic appearance (Fig. 7). The layer of ectoplasm which is free of organelles, with the exception of sparse ribosomal rosettes, is more prominent. Multiple stacks of Golgi cisternae are present; the most prominent feature is the profusion of Golgi vesicles which are concentrated in large areas of the cytoplasm. These vesicles are either elongated or rounded; the latter are 400-1000 Å in diameter, and many show a dense coating of the membrane. Some of the elongated vesicles are totally occupied completely by homogeneous material of high electron density and there appears to be a fairly gradual transition between these and larger dense bodies. The largest bodies are, however, non-homogeneous. The rough endoplasmic reticulum occasionally forms flat, short, cisternae. These tend to be longer and more numerous along the border with the ectoplasm. The nucleus is elongated or in many instances, deeply indented and the peripheral concentration of chromatin is seen in a slight to medium degree only.

These cells can be labelled as histiocytes or macrophages
without attempting further classification of their origin.

The appearance of inclusions thought to have a phagocytotic origin is variable, but the internal components are essentially the same. When rat and rabbit are compared, it is seen that, in the rabbit, the larger inclusions are more numerous and tend to have a more complex internal structure. The size of the inclusions, which have irregularly rounded or ovoid shapes, varies from about 0.5 μ to more than 8 μ. Only in some instances are limiting membranes demonstrated, while at other times they are obscured by section geometry and internal density. Non-homogeneous inclusions (Fig. 8) consist of coarse granules and electron dense droplets about 50-380m μ in diameter. The largest of these droplets are irregularly extracted during processing. Further components formed by stacked segments of membranes (Fig. 8) are in most instances localised preferentially at the periphery of the inclusion. Each segment is formed by 3 dense layers, each about 30Å in width. The central dense layer is always more electron dense and is separated from those on either side by a clear interspace about 20-25Å wide. The matrix of the inclusion is usually more homogeneous in the rabbit (Fig. 9). Cylindrical formations appear frequently in cross-section as discs, formed by alternating electron dense and lucent layers about 20Å in width. Some of these concentric layers show a looser arrangement. In this case,
2 dense layers, each about 40Å in width, are separated by a clear intermediate layer of about 20Å. Smaller, more regularly shaped inclusions may be formed by membranes arranged in an irregular concentric fashion (Fig. 9 insert). In some cases, the matrix surrounding the membranes is partially extracted. Other inclusions seen only in the rat macrophages, are formed by electron dense coarse ill-defined granules which form irregular clumps or reticulated formations in an electron-lucent space, limited by a membrane. The topography of inclusions within the cell is variable. They occur mostly in cell processes, but in some cases may be found in the proximity of the Golgi complex.

Some cells which are free of phagocytic inclusions show in comparison with previously described types increased numbers of ribosomes and granular endoplasmic reticulum (Fig. 10a). The surface of these cells is extensively folded forming a great number of short pseudopods, which are often connected with the cytoplasm only by narrow necks. In the cytoplasm are seen numerous free ribosomes mostly arranged into small groups and narrow cisternae of granular endoplasmic reticulum which lack orderly arrangement. Dense bodies or cytosomes are fairly numerous. The nucleus contains a moderate amount of chromatin with peripheral concentration. There are many areas of the lamina propria within the villous core which consist only of interdigitated
processes (Fig. 11b). These usually contain only free ribosomes, but occasionally short cisternae of rough endoplasmic reticulum. The plasma membranes of these processes exhibit a fairly straight course, and are parallel along some segments of the perimeter, in which the intercellular gap often narrows to 80-120Å. No junctional complexes were found in these areas. Some of these processes are large and contain occasional mitochondria. In such larger processes, the cytoplasmic matrix has a low electron density (Fig. 12).

Many larger cells within the lamina propria show an increase in rough surfaced endoplasmic reticulum (Fig. 13), while other features remain similar to those described above. The cisternae reach considerable length and show signs of orderly arrangement in parallel or concentric formations, which surround areas containing dense bodies and coated vesicles. The Golgi complex is very well developed (not shown in Fig. 13), mainly in its vesicular component. In Fig. 13 is seen a peculiar structure, several times encountered in these cells: a small elongated area of cytoplasm, surrounded by a few smooth membranes so that it may seem sequestrated in the plane of section. In other instances, however, there are thin bridges of cytoplasm between the membranes. The significance of this structure is unknown. These cells are thought to represent developmental stages in the formation of plasma cells.
Plasma cells:

Plasma cells are very numerous in the lamina propria. They are seen quite frequently in a group consisting of a few cells in close proximity to one another, (Figs. 14; 17). The fine structure of plasma cells is well known and only additional features are described here. The cells differ in the degree of dilatation of cisternae of endoplasmic reticulum (Fig. 14). In a few cases, the cisternae show only moderate dilatation, so that the cross-sectional area of the ground cytoplasm exceeds that of the cisternae. In most instances, however, the opposite is the case, and then the orientation and degree of dilatation is rather irregular in a given plane of section. After glutaraldehyde prefixation, the contents of the cisternae are seen to be finely granular and more electron dense than the ground cytoplasm (Figs. 15; 17). The Golgi complex is particularly well-developed in plasma cells (Fig. 15). The cisternae of the endoplasmic reticulum are seen in close proximity to the elements of the Golgi complex, and some appear as vesicular profiles. The transitional zone between both organelles is occupied by small vesicles of rather irregular shape, about 400-600Å in diameter. The content of Golgi cisternae is more electron dense than that of the ergastoplasm. Further vesicles, many of which are coated, occupy the area between the stacks of cisternae. Some vesicles
appear to be in the process of pinching off the cisternae or fusing with the electron dense granules seen mostly in this area (Fig. 15). The granules which are bounded by unit membranes are rounded or oval and about 200-300 nm in diameter. The orientation of the Golgi complexes is frequently rather irregular. Occasionally a more polarized arrangement is seen, with a stack of Golgi cisternae flanked on one side by endoplasmic reticulum and coated vesicles on the other. Occasionally, Golgi cisternae are arranged in a concentric fashion instead of in stacks.

Some plasma cells exhibit an enormous dilatation of cisternae of the endoplasmic reticulum, which form either single or multiple sacs, containing a moderately dense homogeneous material.

No signs of discharge of secretory products by the plasma cell were seen. Sometimes the plasma membrane forms caveolae with a dense coating of membrane.

Lymphocytic series:

The cells which are identified as members of this series show features varying between lymphoblasts and small lymphocytes (Figs. 11; 12; 16; 17). The main characteristics appears to be the increased concentration of chromatin and grouping of sparse cytoplasmic organelles close to nuclear indentations. Mitochondria are large. The number of ribosomes varied being usually greater in the smaller forms of
lymphocytes. The cell surface was rather uneven, even in places where this does not seem to be determined by close apposition to other cells.

"Clear fibroblast-like" cells:

Clear fibroblast-like cells occur frequently in the lamina propria and although varying in size and shape, exhibit characteristic features which seem to justify their identification as a cell type which has not been described in reports published so far. The cells are elongated in shape, and frequently form long, rather flat processes (Fig. 18), which may reach about 10 μ in length. The plasma membrane is undulating in places and forms deep, narrow invaginations which circumscribe pseudopod-like lobes of cytoplasm (Fig. 19). Occasionally caveolae are seen with dense membrane coating and pinocytotic vesicles about 100μ in diameter. The ground cytoplasm shows, characteristically, a very low electron density which is usually in striking contrast with neighbouring cells (Figs. 18; 17). Many areas of the cytoplasm have an "empty" appearance. These are traversed by well-defined filaments, 50-80Å in diameter which are of low electron density, (Figs. 20a; 20b). In the superficial layer of the cytoplasm, similar filaments may be concentrated (Fig. 20b). Finely precipitated substance forms a cytoplasmic background in other areas. This is also slightly concentrated in the superficial layer. The
endoplasmic reticulum has a quite characteristic appearance of irregular voluminous sacs (Figs. 18; 19; 20a) with frequent invaginations of the ground cytoplasm. The ribosomes covering the membranes are distributed very evenly. The perinuclear cisterna forms frequent sacs which communicate with those in the cytoplasm. Some free ribosomes form rosettes in the ground cytoplasm. Mitochondria, which are infrequent, are unevenly distributed. They are rounded, measuring 0.3-0.6 μ, with transverse or curved cristae. A constant feature is the mottled appearance of the matrix, which is moderately dense (Figs. 17; 19; 20a). The Golgi complex is very well developed and multiple stacks of irregular cisternae (Figs. 18; 19; 20b) are surrounded by many vesicles, some of which are coated. The Golgi complex is sometimes seen in a superficial location (Fig. 20b). Few irregular cytosomes or very rarely small myelinic figures are present, usually without relation to the Golgi complex. Occasionally a centriole is included in the plane of section in a few cells times a typical cilium was observed. The nucleus contains a moderate amount of chromatin, which shows a marked peripheral concentration (Figs. 18; 20a).

In some cells only a thin rim of cytoplasm was observed surrounding a nucleus (Fig. 10b). In these instances, cytoplasm forms a short process above the nucleus which is occupied by an expansion of perinuclear cisterna. It is
difficult to decide whether such an appearance is due to the plane of section or to a true reduction of cytoplasmic volume.

The character, location and relationship of this type of cell shows no consistent differences in both species examined. In the villus core the most common localisation is immediately beneath the epithelium (Figs. 17; 18), while at lower levels of the lamina propria these cells occur at random. Clear cells may be seen in close relation to other cell types present in the lamina propria; the gap between the plasma membranes is in some cases, only 100-200Å in width. Plasma, smooth muscle, reticulum, mast cells and eosinophils are the most frequent examples of such neighbours. Some portions of the clear-cell plasma membrane, however, face intercellular spaces (Figs. 18; 20b) which contain an ill-defined substance of mottled appearance with collagen fibrils. This is particularly the case beneath the epithelial basement membrane. Rarely, the condensation of extracellular ground substance along the plasmalemma gives an impression of a rudimentary basement membrane. This is, however, always incomplete and the layer is very poorly defined. Oblique or tangential sections of the cell reveal in some instances bundles of thin filaments applied to the cell surface which encroach upon the intercellular space. The apparent continuation into the cytoplasm is most likely
to be a result of section geometry. Some processes of clear cells abut on a thin layer of connective tissue around the capillaries (Fig. 18).

**Fibroblasts:**

Fibroblasts form a minor proportion of the cells in the lamina propria, though they increase in number towards the base of the mucosa. The shape in most cases is irregular and the outlines are stellate, owing to narrow processes which project between the other cells. Beneath the epithelium the cells appear to be elongated in section (Fig. 14). Most of the processes are seen to be surrounding the crypts (Figs. 21a; 21b) or close to lymphatics (Figs. 5; 27a). Fibroblasts in the lamina propria have similar characteristics to those described elsewhere (Porter, 1964; Movat and Fernando, 1962a; Chapman, 1962); i.e. dense cytoplasmic matrix, extensive endoplasmic reticulum with sinusous cisternae and a well-developed Golgi complex. Nuclei may be irregular in outline and in such instances peripheral concentration of chromatin is more prominent. Cytoplasmic processes contain few small mitochondria and elongated channels of rough endoplasmic reticulum which contain material of medium electron density (Fig. 21b). The cytoplasm is in superficial areas of the processes occupied by fine filaments approximately 40-80Å in diameter and shows indistinct areas of density, beneath the plasma membrane (Figs.
21a; 21b). In places, groups of caveolae or pinocytotic vesicles are present, approximately 800-1,000Å in diameter (Figs. 21b; 50d). The tapering ends of processes are, with the exception of vesicles, free of organelles. Filaments in the ground cytoplasm appear more concentrated and less distinct (Fig. 21a). Cell processes frequently overlap and in some places both plasma membranes are parallel and the intercellular gap is reduced to a width of approximately 200Å. In these areas there is an increased density of the plasma membrane and of the intercellular gap. In a few cases, a disc identical to the intercellular contact layer of desmosomes was found (Fig. 21a insert). Areas of cytoplasmic density beneath the plasma membrane sometimes abut on a wide intercellular space, where there may be a fibrillar structure in the extracellular space running parallel to the plasma membrane (Figs. 21a; 21b at arrows).

Although there is some resemblance, the fibroblast processes differ from the processes of smooth muscle cells. They have no basement membrane; pinocytotic vesicles are less frequent and irregularly distributed. Filaments occur unevenly and are less clearly defined. Finally, there is a well developed endoplasmic reticulum.

**Smooth muscle cells:**

The appearance of smooth muscle cells in the lamina propria corresponds to descriptions of these cells in tunica
muscularis, which were reviewed in Chapter 1. They form discrete bundles near the lacteal vessels (e.g., Fig. 1). At the base of the lamina propria, they appear to be continuous with muscularis mucosae. The cells in these bundles and and within the muscularis mucosae are loosely arranged. Few smooth muscle cells are seen in the neighbourhood of blood capillaries (Fig. 22a) or venules.

Little difference in the appearance of smooth muscle cells is seen in the lamina propria. A few isolated cells show tongue-like processes containing pinocytotic vesicles approximately 500-1,000Å in diameter (Fig. 22a). These processes alternate with invaginations of the plasma membrane in which there are no pinocytotic vesicles. The dense areas close to the plasma membrane do not exactly correspond to these invaginated areas and are seen less distinctly in the processes. It is difficult to decide whether this appearance is due to the contraction of isolated cells. Cells which are loosely arranged in bundles form small finger-like processes (Fig. 24). These usually make contact with those of neighbouring cells or end without any connections in the extracellular space. Pinocytotic vesicles occur irregularly and their diameters vary between 650-1,000Å. Few bundles derived from the muscularis mucosae were seen to follow the course of lymphatic vessels in the submucosa (Fig. 24) and then to spread over the inner aspect of the
muscularis propria. In such cases, smooth muscle cells derived from muscularis mucosae were more electron dense but did not show an appreciable difference in the degree of contraction (Fig. 28c). Slight differences in electron density of smooth muscle cells are frequently observed within the lamina propria (e.g. Fig. 14). These variations are due to the differences in the number of myofilaments. There is a focal absence of myofilaments in areas adjacent to the plasma membrane. A rarely seen manifestation of exaggeration of this change is seen in Fig. 22b. Some myofilaments are preserved close to the periphery of the cell showing dense areas next to the plasma membrane. The interior of the cell is occupied by a finely precipitated substance with few vesicles, ribosomes and mitochondria. In smooth muscle cells of the muscularis mucosae these filament variations in density occurred more rarely than within the lamina propria. The association of cells remains loose (Figs. 23; 24) and the appearance of a definitive layer is due more to the confinement of a few bundles than to a compact arrangement (Fig. 23). Frequently, but not invariably, dense areas subjacent to the plasma membrane are found over a greater proportion of the cell surface (Fig. 23). The intercellular contacts remain confined to processes.

Nerve fibres:

As mentioned in the general description, almost all nerve
fibres in the lamina propria were seen in association with smooth muscle cells or small blood vessels. This association does not imply a close contact but rather a proximity of both structures. In some instances, thin processes of cells, which belong to fibroblasts or extracellular tissues intervene between both structures, while more frequently the association is closer (Fig. 5). Schwann cell processes were identified only rarely. With a few exceptions, there was not any close contact between nerve fibres and other structures in which the basement membrane was missing. The diameter of nerve cell processes within a fibre is variable (Figs. 25b; 25c) ranging between 170m u and 700m u. Some profiles of greater diameter appear to be a focal enlargement of smaller processes. These can be identified as axons with neurotubules (Fig. 25c). There is no relationship between fibre diameter and any components such as "synaptic" vesicles (cf. Figs. 25b; 25c). The dense-cored or granular variety of synaptic vesicle appears to be wide-spread in distribution and occurs without obvious regularity. The shape of these vesicles is most frequently oval, the smaller diameters ranging between 700-900Å and in extreme cases, reaching approximately 1,200Å. The membrane surrounding the dense core is frequently incompletely preserved. The agranular vesicles of diameters 400-550Å are seen in aggregates (Fig. 25d) of different sizes. These contain a minor proportion of larger granular vesicles, which occur interspersed.
Rarely, recognisable junctions between individual processes within a fibre were found and consisted of an electron-dense area adjacent to both sides of the plasma membrane (Fig. 25c).

The largest nerve fibres seen in the lamina propria are confined to its basal portion. An unusual accumulation of vesicles with cores of varying opacity and irregular dense bodies and mitochondria may be present.

**Lymphatic vessels:**

The fine structure of lacteal vessels or lymphatic capillaries reveals no differences between villi and pericryptal connective tissue. Most of the lymphatic vessels are widely patent but some are collapsed with a reduced lumen. The endothelial cell bodies contain a very limited amount of cytoplasm (Figs. 26; 27a). A few cisternae of rough endoplasmic reticulum are present and mitochondria are very rare. Pinocytotic vesicles and caveolae are frequent. Short bundles of filaments may be seen in the perinuclear cytoplasm (Fig. 26). Occasionally there is a cytoplasmic process towards the lumen in which the organelles appear in a slightly greater number; the Golgi complex is also seen in this position. The nucleus has a rather uneven membrane (Fig. 27a) and it is fairly rich in chromatin. The processes of lymphatic endothelia are attached to the cell body without changing their thickness very much - hence the transition of perinuclear cytoplasm to the process is rather well defined.
(Figs. 26; 27a). The processes contain a very limited number of organelles in which the endoplasmic reticulum constantly occurs. Dense bodies limited by a single membrane are present. Pinocytic vesicles are more frequent than in the cell body. These are usually elliptical, with the shortest diameter ranging between 600-1,200A. Many of these structures appear as open caveolae (Fig. 26). The outline and course of the endothelial processes differ; the majority show angular deflections and variations in thickness (Fig. 27b) with frequent cytoplasmic projections.

Thin processes maintain approximately uniform thickness and even outlines and they show a reduced number of pinocytic vesicles (Fig. 5). All processes overlap each other over a variable length (Fig. 27a). In many instances the end of a process is bifurcated and interdigitates loosely with its counterpart of similar form. Many processes after overlapping over a short distance, deflect towards the lumen and form flaps. Shorter flaps are constituted by portions of bifurcated ends which do not contact others. Cellular contacts are seen fairly frequently (Fig. 27a). These may be classified in many cases as of primitive desmosomal character because of the presence of an intercellular contact layer (Fig. 27d). In the region of a junction increased density of cytoplasm is seen.

The overlapping processes often circumscribe a
subendothelial space communicating with the exterior which contains an accumulation of poorly visible chylomicra (Fig. 27c). Desmosome-like junctions appear constantly between cell processes.

The lumen of some lacteal vessels is sometimes free of any demonstrable material. More frequently droplets are seen which correspond to the chylomicra (Casley-Smith, 1962), and which have a very low electron density (Fig. 26). These are dispersed in a finely precipitated substance which is more opaque.

The lymphatic endothelial cells rest upon a layer of connective tissue which contains collagen fibrils (Figs. 26; 27a; 27b). The lacteals are constantly associated with smooth muscle cells but the extent of this relationship is variable. Smooth muscle cells are seen immediately beneath the interstitial layer which may narrow to 0.15-0.25 μ (Fig. 5). Finger-like processes of smooth muscle cells form occasional connections with the endothelium (Fig. 28d). Elsewhere pinocytotic vesicles may be seen in both cells. A relationship between groups of vesicles in contiguous cells is sometimes seen (Fig. 27b). Processes of fibroblasts occur frequently at some distance from the lymphatic capillaries. These appear rarely in the proximity of the endothelium; without the intervention of further cells (Fig. 27a). These intervening cells are poorly
differentiated (Fig. 5). Characteristics of the lymphocytic series are sometimes present.

Blood vessels:

Fine structure of blood capillaries corresponds to that described in the bibliography (Majno, 1965) and only additional observations are mentioned here. Usually cell processes intervene between both epithelium and capillary basement membranes. The gap separating both basement membranes usually measures between 0.3-0.7 μ. In the rabbit there are occasional defects in the capillary basement membrane through which protrude small processes of endothelial cytoplasm. Such processes may extend for a short distance through a gap in the basement membrane of the epithelium (Fig. 29c) and lymphocytes are seen between the epithelial cells, above the gap in the basement membrane.

Contrary to the observations of Horstmann (1966), fenestrae were seen to occur around all the perimeter of the capillaries. They do not occur only in the immediate vicinity of the cell body. This is invariably located on the side opposite to that facing the epithelium, so that the total number of fenestrae is greater towards the epithelium. The surface of some endothelial cells forms numerous thin processes which may form bridge-like structures with fenestrations.

The fine structure of capillaries in the lamina propria
surrounding the crypts differs only in a few respects from that seen within the villi. The endothelial cell surface and capillary lumen are usually more uniform (Fig. 30b). The gap between the epithelium and capillary basement membranes may be narrower than in the villus, the shortest distance was about 100 μm (Fig. 30b). Endothelial cells contain cell organelles in which the Golgi complex is most prominent.

Vessels occur in the lamina propria with more complex walls. The larger can be identified as arterioles or venules. The terminal arterioles are present at the base of the lamina propria (Fig. 24). Some appear contracted like that in Fig. 31a. Endothelial cells bulge into the lumen and show cytoplasmic projections near cell junctions. Basement membrane is common to both the inner aspect of the endothelium and the smooth muscle cells. In places, however, it is split (Figs. 24; 31a) and a space is formed which contains traces of elastica. This has variable staining affinity. The media is formed by a single layer of smooth muscle cells. Connective tissue components surrounding terminal arterioles show no particular relation to smooth muscle cells; however nerve fibres are seen usually close to the perimeter (Fig. 31a).

The smaller vessels are characterised by the absence of fenestrae and are completely or almost completely surrounded by pericytes (Figs. 32; 33). The cytoplasm of the pericyte
processes is fairly electron dense and shows a distinctly fibrillar structure (Fig. 33). The processes occasionally approach the endothelium through the gaps in the basement membrane. These vessels show very irregular profiles (Fig. 33). Sometimes it is difficult to decide whether such a tortuous profile does not already represent a capillary. Furthermore, it is not certain whether they represent pre- or post-capillary segments of the vascular tree. The smallest vessels identified as venules are seen near the centre of a villus core. The outline of the vessel is regular with a diameter greater than that of the capillaries (Fig. 30a). The inner surface of the endothelium, which lacks fenestrae, shows only slight unevenness. Short projections are constantly present at cellular junctions. The larger venules or smallest veins were seen at the base of the lamina propria. These have an endothelium of increased thickness and the pericyte investment is replaced by a single layer of longitudinally orientated smooth muscle cells (Fig. 31b).

The endothelial cells of blood vessels larger than capillaries, normally contain dense bodies. These are usually elongated, dise-like or drop-shaped, of regular outline (Fig. 31b). The length varies between approximately 250-400μm; their width is 90-200μm. These are bounded by a single membrane closely applied to the homogeneous contents
which have a high electron density. Rarely indistinct longitudinal striations are seen. Cytoplasmic filaments, 60-80Å in diameter are usually present in the endothelium. The most common orientation of bundles which can be seen is parallel to the cell length (Fig. 31b).

**Eosinophilic leucocytes:**

Eosinophilic granulocytes are frequently encountered within the lamina propria. There were no differences in fine structure. After glutaraldehyde prefixation, externum of the granule appears usually, though not invariably, more dense than the internum. The internum exhibits a crystalline structure, consisting of parallel dense lines, with a periodicity of 25-30Å.

**Mast cells and globular leucocytes:**

Further cells with granules are frequently seen in the lamina propria. These are globular leucocytes and mast cells which are sometimes difficult to distinguish. Granules which occupy most of the cell volume are spherical, elliptical or polygonal in profile when close together (Fig. 34a). A definite variation in the granule-density occurs in some cells. Large vacuoles sometimes contain an electron-dense substance which suggests it may be a granule residuum. Less dense granules reveal a finely particulate sub-structure (Fig. 34b). Cells with granules of varying density are thought to be mast cells. All granules have an electron
dense membrane. Sparse organelles and free ribosomes are seen between granules.

A similar type of cell seen in the rabbit shows distinctive and uniform features (Fig. 34c). The vacuolar membrane-bounded spaces contain irregular fine filaments, while the granules with more coarse structure are only rarely present.

**Cellular regression in the lamina propria:**

Some cells in the lamina propria show changes which can be classified as degenerative. Affected cells or their remnants usually undergo phagocytosis before this is complete. Some features of the cell type remain recognisable in the early stages, though modified in a characteristic manner. Further structures appear as a non-specific result of degradation.

The description of different static images seen in electron micrographs would appear meaningless if an attempt to interpret them as a dynamic process was not made.

In very rare instances, a cytoplasmic change like the one in Fig. 35 is encountered. Altered mitochondria, myelinic figures and lamellated cylindrical bodies are seen. This area may be incompletely limited by a cisterna of the endoplasmic reticulum. It is conceivable that such a portion may be sequestered externally and subsequently phagocytosed by other cells. If internal sequestration occurs,
the process is identical with cytolysome formation.

It is unlikely that the changes illustrated in Fig. 35 can be interpreted as the phagocytosis of a degenerated cell remnant by the process of a macrophage. When the material is more condensed (Fig. 36), it is difficult to determine the origin.

In the rabbit intestine, part of a cytoplasmic process may be completely filled with material which is continuous with the cytoplasmic constituents. The material consists of two components which are loosely arranged (Fig. 37c). The first is formed by cylinders which may measure more than 0.8 μ in length and 65-300 μ in thickness. These are composed of dense lamellae each about 30-35 Å in width separated by a less dense space approximately 20 Å. In places lamellae are seen to consist of two sublayers divided by a space the thickness of which is just within the limits of resolution (Fig. 37c insert).

The lamellae are arranged in a concentric fashion but sometimes a cylinder is seen to be formed by a continuous lamella in the form of a tight spiral. The second component is formed by groups of tubules, each of 600-640 Å in diameter (Fig. 37c). The tubular wall is triple-layered with two dense layers about 25-30 Å thick, separated by a clear space of the same thickness.

Similar components may occur in phagosomes, but here the
arrangement is more dense.

A further characteristic type of degenerative process affects plasma cells and is seen frequently in both the species examined.

Phagocytosed plasma cells show coagulation necrosis (Figs. 40a; 39a). The density of the cytoplasm is strikingly increased. Mitochondria are swollen and partially disintegrated while the cisternae of the endoplasmic reticulum are preserved and usually appear as rounded sacs which contain a substance less dense than the cytoplasmic matrix. Ribosomes are preserved but because of increased background density appear to have reduced contrast. Other plasma cell bodies which may be supposed to be in a more advanced stage of degradation, consist of poorly defined membranous sacs compressed together (Fig. 41a). Between these sacs numerous dense particles are seen, some of which appear identical with ribosomes, while others are larger and irregular. Few mitochondrial remnants are recognisable.

These variations in appearance suggest different stages of a process which consists of a gradual loss of cytoplasmic matrix with concentration of the sacs of the endoplasmic reticulum and either condensation of ribosomes or formation of electron dense substances in the remains of the matrix between the sacs. This sequence of events is thought to provide an explanation of the final stages of degradation of
these cells in lamina propria.

Some of the phagocytosed remnants though they still retain recognisable traces of the original cell organisation, contain homogeneous areas at the periphery (Fig. 41c). Myelinic figures may be seen in these areas.

In other cases, the origin of the degenerated cell cannot be recognised with certainty. The cytoplasm is converted into an electron dense, finely granular substance which contains twisted membranes which in places show a parallel arrangement. Close to the membranes or between them, clumps of poorly defined electron dense substance occurred (Fig. 38). A similar substance probably replaces the nucleus.

Fragments which show such changes, may be seen occasionally isolated. After becoming phagocytosed, they have a more condensed appearance (Fig. 39b). These fragments are thought to be derived also from plasma cells. Under pathological conditions, similar changes appear to be related to the degradation of phagocytosed plasma cells (see Chapter 7).

Other inclusions consist of membrane limited sacs which contain finely particulate substance. The membranes are disrupted in many places and sacs communicate with one another. The spaces between sacs contain clumps of dense material forming, together with membranes, a reticulated pattern (Fig. 41b).
Some inclusions reach a considerable size and display pleomorphism. These bodies consist of matrix enclosing irregular areas of increased density, myelinic figures (Figs. 42a; 42b; 37a) and cylindrical bodies of the appearance already described. Occasionally, dense parallel lines, about 25Å in width, were seen in the matrix, separated by 45-90Å. Different arrays intersect at variable angles (Fig. 37b). In the periphery, the matrix is continuous with myelinic figures (Fig. 37a). The dense lines in these appear with approximately 45Å periodicity (Fig. 37a, insert). Small, partially extracted droplets of lipid, occur near the inclusions (Fig. 42b) or in their periphery.

The position of the largest inclusions is difficult to determine. They appear to be surrounded by a thin layer of cytoplasm, the origin of which is not obvious. Sometimes the relation suggests that the inclusions are being enclosed between the cell processes. In some cases red blood cells were seen close to the inclusions and possibly participate in their formation.

Degenerative changes were seen in eosinophilic leucocytes. Affected granules show irregular rarefaction and loss of the externum. The crystalloid internum remains partially isolated in a vacuolar space surrounded by a granule membrane (Fig. 40b). Some of the membranes disrupt and the spaces fuse.
DISCUSSION:

The fine structural appearance of most of the cells within the lamina propria suggests that it subserves a definite function. The main activity consists of phagocytosis and secretory activity. The former cannot be defined on the basis of ultrastructural appearance of the cells alone if we exclude the signs of phagocytosis. The latter involves synthesising cells of two different types, plasmocytic and fibroblastic cells. Vessels and smooth muscles with further characteristic cell types are present.

Undifferentiated cells:

Undifferentiated cells in the lamina propria do not form a clearly defined group. They seem comparable to undifferentiated cells of lymphoid tissue. The lack of precise morphological definition by light microscopy has lead to a confused nomenclature in the cytology of lymphoid tissue (c.f. review Gall, 1958; Lennert, 1961; Marshall, 1956) which has limited objective substantiation in relation to fine structure. It is possible, however, to compare the fine structure of these undifferentiated cells in the lamina propria without attempting a rigid classification.

The small cells resemble the primitive reticulum cells in lymph nodes (Han, 1961; Bernhard and Leplus, 1964). However, the higher chromatin density does not compare with the description of Bernhard and Leplus (1964) based on
aldehyde prefixed material.

Occasionally the identification of cells with similar organelles is uncertain. These cells often resemble lymphoblasts but it is difficult to make identification certain.

The larger undifferentiated elements in the lamina propria are thought to be variants of reticulum cells. This interpretation seems most plausible if the criteria of Bernhard and Leplus (1964) are adopted. The classification of Movat and Fernando (1964) would preclude so unified a classification. The large numbers of ribosomes and rough endoplasmic reticulum in some cells of this category indicate synthetic activity and a tendency towards plasmocyte development. A transition towards lymphocytes is less certain but is not excluded. Many of these cells in the lamina propria may be regarded as potentially immunologically competent (Nossal, 1962).

**Macrophages:**

The features of cells in the lamina propria which show the signs of phagocytosis display some variability. This is in agreement with published studies of phagocytes in other tissues (Clark, Jr., 1962; Han, 1961; Movat and Fernando, 1964; Bernhard and Leplus, 1964; Karrer, 1960; Essner, 1960; Galindo and Imaeda, 1962). The functional definition
based only on the presence of phagocytosis does not exclude the possibility that other cells of mesenchymal origin can act as phagocytic cells. In the lamina propria there is however, no valid reason to use terms other than macrophages. The close spatial relations and developmental potentialities in this tissue, suggest that the term "phagocytic reticulum cell" is pleonastic, although such may be found. On the other hand, the cell illustrated in Fig. 6 may be labelled as histiocyte or migrated monocyte, since it is isolated and shows little phagocytosis.

A further question relates to the significance of fine structural variations in different macrophages. Greatly increased numbers of vesicles were described in macrophages in lymphoid tissues by Movat and Fernando (1964). In intestinal macrophages the evidence is conclusive that these vesicles are formed from the Golgi complex, and suggests that the increased elaboration of vesicles which may act in some cells as primary lysosomes (Novikoff, 1963) provides the requirements for enzymatic digestion. Vesicles containing dense material resemble closely "virgin" lysosomes in the crypt epithelium described by Moe, Rostgaard and Behnke, (1965).

In the lamina propria it appears that there is no correlation between phagocytic activity and the development of a granular endoplasmic reticulum and ribosomes. The variability in the development of the endoplasmic reticulum and/or
ribosomes suggests further possibilities. Mesenchymal cells in different stages of development towards more differentiated forms may become phagocytic. It appears that macrophage-type phagocytosis can be regarded as functional capability of undifferentiated cells.

The fine structure of phagosomes will be discussed later in connection with cell degradation in the lamina propria.

The cells of the lymphocytic series which are seen in the lamina propria are similar to those described by Han (1961); Galindo and Imaeda (1962); Movat and Fernando (1964); Bernhard and Granboulan (1960); Bernhard and Leplus (1964). Some difficulties appear to arise however, when an attempt is made to find the position of a given cell along the developmental line of lymphocytes. A comparison of the different descriptions in the bibliography shows a similar situation.

Plasma cells:

The fine structure of plasma cells seen in the lamina propria corresponds to the published descriptions of these cells in other tissues (Movat and Fernando, 1962a; Thiéry, 1960; Han, 1961; Bessis, 1961; Bernhard and Granboulan, 1960; Bernhard and Leplus, 1964). Occasionally plasmocytes are found in small groups which may be analogic to the plasmocytic islet of Thiéry (1962) in a less developed form.
The degree of dilatation of the cisternae of the endoplasmic reticulum (ergastoplasm) varies in a similar fashion with that described by others in normal and neoplastic plasma cells (Maldonado, Brown, Bayrd, Pease, 1966a). Although there occurred dilatations of cisternae, concentration of accumulated material corresponding to Russell body formation (Thiery, 1960; Welsh, 1962; Bessis, 1961; Movat and Fernando, 1962b) does not occur.

The role of the Golgi complex is of considerable interest in the elaboration of the secretory product now known to be immunoglobulins (Mellors and Korngold, 1963; Nossal, 1962; Crabbe and Heremans, 1966; Crabbé, Carbonara and Heremans, 1965). The polarity of the Golgi complex was pointed out in some observations of the plasmocyte (Stoeckenius, 1957; Bessis, 1961) but the transport mechanism was not studied in detail. Our findings indicate that the material is transported to the Golgi complex over a very short distance by vesicles separating from the endoplasmic reticulum, though continuity is not observed. Products condensed in the Golgi cisternae are contained in coated vesicles which are pinched off the cisternae and in turn they fuse and are incorporated into dense granules.

Migration of synthesised protein from the endoplasmic reticulum to the Golgi complex was demonstrated in plasma cells by electron microscopic autoradiography (Clark, Jr.,
1966); antibodies have been previously demonstrated in both organelles (de Petris and Karlsbad, 1965). This confirms that the Golgi complex in the plasmocyte has an identical role to that in the other protein secreting cells (Caro and Palade, 1964; Ross and Benditt, 1965; Revel and Hay, 1963; Zeigel and Dalton, 1962). The mode of transport of condensed material from the Golgi cisternae by coated vesicles which has not been described in the plasmocyte so far appears to be identical to that described in the hepatocyte (Bruni and Porter, 1965). Their further fate is different; in the hepatocyte the coated vesicles are incorporated into multivesicular bodies subsequently transformed into microbodies. In the plasmocyte coated vesicles are discharged directly into granules, which are homogeneous. These granules which are of an unknown nature were described to occur near Golgi apparatus (Bessis, 1961; Thiéry, 1960; Movat and Fernando, 1962b) but rarely likened to secretory (Bernhard and Granboulan, 1960). There appears only one description which suggests the observation of coated vesicles (Han, 1961) near the Golgi cisternae but the transport to the granules was not recorded. Transitions between the Golgi apparatus and granules without a description of coated vesicles were seen in myeloma cells (Maldonado, Brown, Bayrd and Pease, 1966b). Our observations suggest a transport of protein from the Golgi complex to the granules. The
nature of these substances and their further fate are not known, though it may be inferred that these are immunoglobulins. Behnke (1963b) demonstrated acid phosphatase activity in the granules of plasmocytes in the lamina propria and considered them as lysosomes. This does not invalidate the possibility that protein is transported to them. On the other hand, the presence of acid phosphatase activity does not preclude the possibility that the granules are secretory in nature (de Duve, 1963; Novikoff, 1963).

In the plasmocyte, further mechanisms of secretion are unknown. Granules were not seen to be discharged. Signs of other mechanisms of secretion suggested by Thiéry (1960) were not observed.

Fibroblasts and fibroblast-like clear cells:

Fibroblasts seen in the lamina propria are similar to those described by Porter, 1964; Chapman, 1962; Ross and Benditt, 1961; Movat and Fernando, 1962a; Peach, Williams and Chapman, 1961). The comparison indicates that they may be regarded as moderately active. Fibroblasts have a different appearance from reticulum cells, which were considered by some authors to be similar (Movat and Fernando, 1964). Their relation to collagen fibrils is most remarkable in the cell processes. The plasma membrane is clearly defined unlike the cells where synthesis is
active (Porter, 1964). Caveolae and pinocytotic vesicles (Movat, Fernando, 1962a) are unusually frequent. Fibrils flank the outer aspect of the plasma membrane in accordance with the observations of Porter (1964) but their superposition with densities in the fibrillar cortex of the cell has not so far been recorded. When confined in length, this apposition resembles hemidesmosomes which may occur in mesenchymal cells where these are elongated and subject to mechanical stress as in blood and lymphatic vessels (Stehbens, 1966; Leak and Burke, 1966).

Cell junctions of the primitive desmosome type were found in processes along crypts and in situations where some more stable arrangement of cells in forming sheaths may be important. The only description of cell junctions in cells of this type is that recently published by Greenlee and Ross (1967) in foetal fibroblasts of developing tendon.

Fibroblast processes resemble smooth muscle cells in possessing prominent filaments and pinocytotic vesicles, although the two may be readily differentiated. One is tempted to speculate as to whether this similarity indicates the presence of a cell with some transitional properties, as they might occur between smooth muscle cells and pericytes in the vessels.

Two features distinguish clear cells from ordinary fibroblasts in the lamina propria. The first is the very
low density of the cytoplasm. This is rather unusual after glutaraldehyde prefixation, since cells whose structure indicates synthetic activity show, in most cases, a rather dense cytoplasm. This low density can be compared to that generally observed in foetal cells. The total surface area of rough endoplasmic reticulum is limited since there are only a few large cisternae present. The common occurrence of communication with the perinuclear cisterna suggests that the formation of endoplasmic reticulum is in progress and that the cells are young or increasing their activity according to evidence available in other cells (Behnke and Moe, 1964; Parks, 1962). One can thus conclude that the modification is of a progressive nature. Further features are similar to those seen in active fibroblasts (Movat and Fernando, 1962a; Porter, 1964), particularly the fibrillar cortex and the relation of extracellular fibrils and matrix to the plasma membrane. The possibility that these cells are a collagen-producing variant of reticulum cells or of the transitional stages into fibroblasts must be considered.

Smooth muscle cells and nerve fibres:

The finger-like processes and minor surface modulations of smooth muscle cells are apparently associated with their loose arrangement. It is thought that these processes can act in some instances as devices for exerting traction on structures which abut upon them (e.g. lymphatic endothelium).
Cells containing few myofilaments are believed to be retrogressive. The possibility that they are as yet not fully differentiated, is unlikely since this is precluded by the poor amount of organelles. The nerve fibres observed in this study were only related to the smooth muscle cells and vessels. Structurally defined synapses were not detected however, and it may be supposed that they are rare. This parallels the observations in the muscularis propria as well as that in some other organs (Garrett, 1966). The presence of "synaptic" vesicles is not a sufficient criterion for synapsis (de Robertis, 1964). The relationship of nerve fibres to other structures in the intestinal mucosa appears to be functional and has no precise morphological definition.

Lymphatic vessels:

Filaments in the cytoplasm of the lymphatic endothelium have not been described in the lamina propria previously, though they occur in the same cells of other tissues (Casley-Smith and Florey, 1961; Leak and Burke, 1966). No intercellular gaps were seen. This finding seems to be at variance with the results of Palay and Karlin (1959a) and in agreement with Papp, Rohlich, Rusznyak, Toro (1962), who examined, however, cat intestine. These authors described long cytoplasmic projections of the endothelial cells into surrounding tissue which, though present in other locations
(Leak and Burke, 1966; Fraley and Weiss, 1961) were not observed in this study, nor by Palay and Karlin (1959a) in similar species. The bifurcation of cell processes is usually described in terms of interdigititation. Primitive desmosomes or cell junctions are rarely mentioned in connection with lacteals (Casley-Smith, 1962) though they are often seen together with other types of junctional complex in lymphatic vessels elsewhere (Casley-Smith and Florey, 1961; Leak and Burke, 1966). The basement membrane is reported as incomplete (Casley-Smith, 1962; Casley-Smith and Florey, 1961) or absent (Palay and Karlin, 1959a; Papp, Rohlich, Rusznyak and Toro, 1962). No structure which can be classified as a basement membrane was seen in this study.

Subendothelial accumulations of chylomicra, seen also in human intestine (Rubin, 1966), indicate that there is impedance of inlet valve function of lacteals as proposed by Casley-Smith (1962).

**Blood vessels:**

The fine structure of blood vessels in the lamina propria appears to be very similar to that described elsewhere (see Majno, 1965). A few observations, however, are worth mentioning. The finding of small gaps in the capillary basement membrane with protrusions of endothelial cytoplasm is unusual in adult tissue. These occur in foetal and regenerating vessels (Donahue, 1964; Schoefl, 1963) and in
the human placenta (Rhodin and Terzakis, 1962). Endothelial protrusions seen in the lamina propria seem in some instances to be the sequellae of diapedesis of leucocytes through the capillary wall.

The significance of dense bodies occurring in many endothelial cells within the lamina propria is unknown. Whether they contain acid hydrolases and represent lysosomes which they resemble structurally, remains to be determined. They differ in size, density and absence of recognisable internal structure, from bodies described in the arterial endothelia (Weibel and Palade, 1964). A relation, however, cannot be ruled out since some differences may be due to glutaraldehyde prefixation.

**Mast cells, globular leucocytes and eosinophils:**

Mast cells and elements which correspond to the description of globular leucocytes as given by Kent (1966) can be usually identified. The granules in mast cells correspond to those observed by Bloom and Haegermark (1965). A small number of organelles is present. This is similar to the description of mast cells by Fernando and Movat (1963). These authors, however, did not observe any membranes around granules with a coarser substructure. The criteria for distinguishing globular leucocytes (Kent, 1966) appear insufficient in some cases. In rabbit, the cells, believed to represent mast cells, constantly exhibit poor granule preservation. This is
likely to be due to dissolution during fixation and processing and may indicate a species difference in granule structure which could explain variances in the appearance of granules (Bloom, 1965).

The only granulocytes observed in the lamina propria of both the species examined in this study were eosinophilic. The crystalline internum in granules is a constant feature (review Hirsch, 1965; Wetzel, Horn and Spicer, 1967). In rat the longitudinal periodicity of the crystal resolved corresponds to the recently published observations of Miller, de Harven and Palade (1966). Transverse periodicity described by these authors was not identified.

**Cellular regression:**

Cellular regression in the lamina propria is accompanied by phagocytosis which supervenes the degenerative change. Most of the continuous process of degradation is seen within macrophages and the appearance preceding phagocytosis may be only inferred. The classification of described changes indicates that different types of regression occur. The whole cell may show the features of coagulative necrosis. This interpretation is supported by observations of similar changes in other cells, mainly hepatocytes, under pathological conditions (Wachstein and Besen, 1964; Klion and Schaffner, 1966; Cossel, 1965; Biava and Mukhlova-Montiel, 1965). In many of these observations, the necrotic cell was
surrounded or phagocytosed by others; this was the case in our material. In the normal lamina propria only plasmocytes are seen to be affected by coagulative necrosis. The contention that this basic change of cell appearance precedes phagocytosis is supported by observations of ischaemic lamina propria, where similarly changed cells are seen before being phagocytosed (See Chapter 7) and by the report of Clark (1966) who described degenerated plasma cells of similar appearance in normal lymph nodes. Phagocytosis of plasmocytes under abnormal conditions has already been observed (Weiss and Aisenberg, 1965; Swartzendruber and Congdon, 1963).

Limited cytoplasmic alterations form a more heterogeneous group and present problems in interpretation. The prerequisite for phagocytosis is the external sequestration of an altered area which is demonstrated exceedingly infrequently and difficult to assess. Some isolated fragments of the cytoplasm occur and show the transformation described. Whether these are only sequestrated or belong to a cell, which degenerated completely, is usually difficult to decide. The external sequestration of the cytoplasm has been described only a few times in liver cells (Steiner, 1961; Lane and Becker, 1966). The external limitation of the altered cytoplasm, indicating subsequent sequestration was described in tumour cells (Barton and Barton, 1965).
Macrophages very rarely show limited cytoplasmic alterations. These are different in character from plasmocytes and likely to disintegrate after sequestration before phagocytosis.

The intracellular phase of degradation seen in phagosomes exhibits some common features. The loss of cytoplasmic matrix in phagocytosed plasmocytes may be explained by the presence of proteolytic activity in the phagosome. The sacs of endoplasmic reticulum persist longer; hypothetically, the membranes may impede the digestion of the contents. The appearance of dense coarse particles which suggest ribosome condensation remains unexplained and does not parallel the disappearance of ribosomes in autolysis experiments (Trump, Goldblatt and Stowell, 1965d). If some inclusions are erythrocyte remnants the dense material may be ferritin. Homogeneous areas or whole inclusions of unidentifiable origin contain myelinic figures at the periphery. This resembles phospholipid in the process of hydration and there period of the myelinic formations is compatible with this assumption (Stoeckenius, 1959; 1962; Revel, Ito & Fawcett, 1958; Mercer, 1961). Crystalline arrays within the matrix consisting of dense lines are similar to those observed in phagosomes by Karrer (1960). Large complex inclusions containing further lipid droplets and membranes resemble ultrastructurally ceroid deposits (Hartroft and Porta, 1965).
This suggests the possibility of erythrocyte participation in their formation.

The origin of smaller phagosomes or phagolysosomes cannot be determined but it is reasonable to assume that some of these represent further condensed stages of larger inclusions. The components of these phagosomes appear similar to those described by Karrer (1960). The fusion of membrane pairs resulting in compound multilayered membrane formation is very frequent. A characteristic component is the cylindrical body which is similar in general appearance to that described by Sun (1965); Athanassiades, Herman and Hennigar (1965) but lacks the filamentous substructure described by the latter authors.

The presence of continuous lamellae in cylindrical bodies suggests that the material is again lipid in character. The tubules, which occur commonly together may be of a similar or identical composition. Evidence to support their lipid nature is found in the report of Thomas and Sheldon (1964), who observed tubular arrays in the peripheral nerve during Wallerian degeneration.

Dimension differences occurring between observations and particularly between in vivo and in vitro lipid systems (see Refs. above) are likely to be due to a presence of additional components such as proteins, which were found to alter the thickness of the artificial membrane (Stoeckenius quoted by.
Samuels, Gonatas and Weiss, 1964), or to the differences in lipid composition.

Macrophages loaded with cylindrical bodies and tubules within the cytoplasm are believed to be in exhausted or degenerative stages. Whether all the material is of phagocytic origin cannot be determined. Hypothetically, cytosome residues would have an identical appearance.

Material forming the larger inclusions or present within phagosomes was found to be PAS positive.

While this is only of secondary interest in laboratory animals, in man, the evaluation of PAS positive material in the lamina propria is of considerable importance.

Individual macrophages which contain PAS positive substances are not infrequently seen in human small bowel biopsies. These cannot be confused with the classical manifestations of Whipple's disease although the relationship may be speculated upon. A demonstration of a similar pattern of degradation in human lamina propria would corroborate the view that the presence of these cells is completely unrelated to Whipple's disease. Macrophages with non-specific PAS positive material of different character occur in rectal biopsies (Gonzalez-Licea and Yardley, 1967).
CHAPTER 4

FINE STRUCTURE OF THE EPITHELIUM, TELA SUBMUCOSA, TUNICA MUSCULARIS AND SEROSA

EPITHELIUM:

The present description is limited to features which have not been described before. For this reason a separate discussion has not been made.

Enterocytes:

In order to provide a comparison with the pathological changes to be described later the appearance of a normal enterocyte is illustrated in Fig. 43.

The lateral cell surfaces have been described as convoluted and interdigitating with neighbouring cells. When viewed, however, in oblique or transverse section, it may be seen that the lateral cell surface consists in part of finger-like processes which protrude into the cytoplasm of the adjacent cells (Fig. 44c at arrows). Caveolae with a coated membrane occasionally occur along the lateral plasma membrane.

Filaments of the fuzzy layer of the brush border plasma membrane after glutaraldehyde prefixation show a distinct type of insertion which is more prominent, but not basically different to that seen so far. The filament base appears in longitudinal section to be a triangular electron density
abutting on the outer leaflet of the plasma membrane (Figs. 44a). Grazing sections of the surface of microvilli reveal dots of similar appearance.

No systematic measurements of microvillus length have been made in this study. In rat, the maximum height of microvilli was approximately 1.4-1.5 u. In rabbit, however, consistently higher microvilli ranging between 2 u - 3.3 u were seen. These are difficult to measure, since adequate orientation is rare. Kraehenbuhl, Gloor and Blanc (1966) give a value 3-3.5 u for microvillus length in adult rabbit. The evidence suggests a species difference.

The desmosomal tonofilaments at paranuclear levels often form discrete bundles running parallel to the plasma membrane.

A striking number of coated vesicles is present in the Golgi complex of enterocytes (Fig. 45a) after glutaraldehyde prefixation. Most of these are found close to the Golgi cisternae. The larger vesicles contain material of increased electron density and seem to be transitional to the smallest cytosomes, believed to represent primary lysosomes. Slightly larger cytosomes show vesicular areas of decreased electron density, which become more prominent with increasing size. The fully developed structure will be described later in the crypt epithelium.

There is an apparent species difference in the occurrence of material which is thought to be the product of
cytoplasmic degradative processes. In the rat, larger residual bodies are observed in moderate numbers (Fig. 45b). In rabbit, material of a less condensed polymorphous appearance occurs with a much higher frequency and in larger amounts. These accumulations are usually located in a supranuclear position and when sufficiently large, deform the nuclear contours. The limiting membrane is usually seen around segments of the perimeter (Fig. 46a). The contents have an irregular pleiomorphic appearance, but the components are basically the same. They consist of lipid droplets (Fig. 46b) and non-homogeneous dense bodies (Fig. 46a) and membranes which usually form several concentric layers or short stacks around them (Figs. 46a; 46b). The matrix between these structures is in part, extracted. In some instances, the membrane arrangement suggests mitochondrial remnants which contain clumps of dense material (Fig. 46a). This appearance leaves little doubt that the material is predominately lipid in character. Some membranes are likely to be derived from amorphous lipid while others may be the residues of organelles.

It is difficult to explain the absence of earlier formative stages which would be recognisable cytolysones. This may be associated with the rapidity of the degradation.

The appearance of enterocyte nuclei after glutaraldehyde prefixation has not been described so far. The peripheral
The concentration of chromatin is rather variable but mostly slight. There is a fair amount of nucleolus-associated chromatin present. Perichromatin granules are prominent.

In normal tissue, degeneration of enterocytes was not often seen but showed fairly characteristic features (Figs. 47a; 47b). The electron density of the cytoplasmic matrix is decreased, microvilli of the brush border are constantly and uniformly increased in width and decreased in length so that they do not reach the height of those of neighbouring cells. In other instances, the apex of the affected cell and is partially superposed by projections of neighbouring cells. Straight bundles of filaments in the cytoplasm of degenerating cells are constantly present (Figs. 47b; 47c). Mitochondria may appear dense, as in rabbit, while in rat, swelling was seen. Later stages of cell extrusion were not observed.

It is difficult to compare these observations with the bibliography since degenerating cells have been rarely given attention (e.g. Ito, 1965a). The degenerating enterocytes observed by David (1967) in the process of desquamation were pyknotic. Kraehenbuhl, Gloor and Blanc (1966) described in rabbit intestine "cellules claires" with short microvilli and distinguished them by the absence of nuclear pyknosis from degenerating cells to be extruded. Similar cells observed in this study are thought retrogressive, though the
nucleus appears normal. In rat the signs of degeneration are obvious.

Crypt epithelium:

In rat, in addition to features described by others, glutaraldehyde prefixation reveals frequent bundles of tonofilaments in association with desmosomes (Fig. 48). The desmosomes are of normal structure but occasionally reach more than 0.5 μ in length (Fig. 49). An unusual observation is the presence of tight junction at cell base (Fig. 50d).

In the apical portion of the cell, frequent dense homogeneous granules of ovoid or ellipsoid shape are present (Fig. 49). These are bounded by a single membrane and their dimensions vary approximately between 75-150 μ. No signs of secretion were observed. They are believed to be primary lysosomes. In addition, larger cytosomes of a typical appearance are frequently seen in the supranuclear cytoplasm. The size varies usually between 230 μ to 350 μ. The greatest dimension seen was 1 μ. The shape is usually almost circular. The "unit" membrane-bounded cytosomes contain moderately electron dense homogeneous matrix in which are embedded less dense vesicles, several hundred Å in diameter. Many vesicles contact each other. The greater the number, the closer the apposition of vesicles and the reduction of the matrix (Fig. 49). "Multivesicular cytosomes" of the same appearance are frequently seen also in the mature enterocytes (c.f. Fig. 43), where they frequently occur beneath the
terminal web. A similar structure named the "apical lysosome" was illustrated by Cardell, Badenhausen and Porter (1966) and reported to have some relationship to pinocytosis.

The rabbit crypt epithelium has consistently different appearance to that of the rat because of the high numbers of electron dense homogeneous granules (Fig. 50a). They are circular, elliptical or drop-shaped and of variable dimensions, within the range of 100-200 μm. Quite frequently, similar membrane bounded granules are seen within the nucleus. In this position, however, some granules reach greater size and appear less electron-dense. In addition to granules, some nuclei contain unidentified circular profiles of membranes and clustered irregular dense particles several hundred Å in size (Fig. 50b). In the late telophase, the positions of granules suggest that these may be entrapped within the reforming nucleus (Fig. 50c).

Neither the nature nor the mode of elaboration of the granules is known. There is at present no evidence of their secretory nature, which might be suggested by an analogy to Trier's observations of human crypt epithelium (1963; 1964).

Goblet cells:

Goblet cells in normal control animals were examined in order to compare their appearance after glutaraldehyde prefixation, since the bibliography is largely defective on this point. The density of the cisternal content only rarely
equals or slightly exceeds that of the cytoplasmic matrix (Fig. 51c). Mucus contained in granules appears more finely precipitated than the substance contained within cisternae of the endoplasmic reticulum (Fig. 51a). Occasionally, dilated cisternae appear almost empty without the cell being depleted of mucous granules (Fig. 51b). The cytoplasmic matrix contains sparse bundles of tonofilaments. The filaments of the fuzzy layer appear identical to those in absorptive cells (Fig. 44b).

**Paneth cells:**

Paneth cells in the rat contain frequent myelinic figures and cytosomes (Fig. 52); in rabbit, Paneth cells contain inclusions of a striking appearance which have not been described before. Although inclusions were seen in all locations, they occurred most frequently near the cell base (Fig. 53). The number and size vary considerably in individual cells; the largest measured up to 6-7 μ in diameter. The smaller inclusions are roughly circular or irregularly rounded and approximately 1.0 μ in diameter. Most of the inclusions were bounded by a triple-layered membrane about 80Å in thickness (Fig. 56), while only segments of a limiting membrane were seen around the larger inclusions. Where cells contained many inclusions, some of these were formed by myelinic figures without a surrounding membrane (Fig. 53, asterisk).
The inclusions consist of two main components arranged together without apparent regularity and cut in different planes and angles. In the small inclusions, the components are packed closely together (Fig. 54), while in the larger, the arrangement was usually looser. The first component consists of lamellated cylindrical bodies (Fig. 55) of the same appearance as those in macrophages of the lamina propria. The thickness of the lamellae and of the less dense spaces is practically the same as in macrophages: \(30-40\AA\) and \(15-20\AA\) respectively. In most cases, lamellae are arranged in a concentric fashion, as in macrophages. Occasionally, however, the whole cylinder is formed by a continuous lamella in the form of a tight spiral (Fig. 55 insert). Very rarely a form is observed where both the lamellae are fused together at the inner end of the spiral. When cut longitudinally, the cylinders appear either as rectangular profiles or else taper gradually.

The second component is represented by tubules, again identical in appearance to those in the macrophages (Fig. 56). In transverse sections, the groups of tubules are arranged in hexagonal array (Fig. 55). In oblique and longitudinal section, the clear spaces in the walls and lumen of the tubules are made indistinct by section geometry and the whole array appears as a reticular network or banded structure (Fig. 56). In some inclusions, tubules
form small groups or lie in isolation. In such cases their course is not straight, but bent in places at wide angles. Occasionally, connections between the cylinders and tubules are seen. These connecting structures resemble segments of unfolded lamellae. In places, fusion of peripheral lamella with the outer layer of a tubular wall is seen. This also occurs in the rare instances where the tubule is seen to be within the lumen of a cylinder (Fig. 56, arrow).

The smaller inclusions are bounded by a unit membrane and resemble lysosomes containing paracrystalline structures. Whether, however, these inclusions have a corresponding enzymatic equipment remains to be determined. The structure of the components, similar to those in macrophages, suggests that they contain lipids. The spiral configuration of some cylinders and the hexagonal arrangement of tubules is, however, more obvious in Paneth cells. The spiral arrangement is very similar to that described in "cylinders" of similar dimensions in macrophages (Athanassiades, Herman and Hennigar, 1965) and mast cell granules (Weinstock and Albright, 1967). The substructure of the spirals in Paneth cells is different however, since it lacks clear "dotting". Hexagonal phases are formed by some lipids or lipoproteins (Luzzati and Husson, 1962; Stoeckenius, 1962; Miller, 1961) but lack the tubular structure observed in some lipids by Thomas and Sheldon (1964).
The significance of the inclusions in Paneth cells remains to be elucidated. Their similarity to material within the macrophages suggests cytoplasmic degradation as a source of material, although this was not observed. The presence of inclusions does not seem to interfere with elaboration of secretory granules.

Argentaffin cells:

Argentaffin cells possess few additional features to those reported in the bibliography. In both species after glutaraldehyde prefixation there are constantly present filaments approximately 60-80Å in diameter (Fig. 57) which form bundles interspersed between granules or running parallel to the cell surface. The cytoplasmic matrix contains many free ribosomes. Differences in the density of granules and their non-homogeneous appearance are infrequently seen (Fig. 57, insert), while variations in shape and size are consistently present (Fig. 57). In a few cells, accumulations of glycogen in the cytoplasmic matrix were seen. A very rare appearance of argentaffin cells is illustrated in Fig. 58. The cytoplasm is unusually dense and contains few lipid droplets. The membrane limited spaces around granules are dilated and mitochondria are somewhat swollen. These changes are believed to be degenerative.
Migratory cells in the epithelial layer:

Migrating cells are seen very frequently in the epithelial layer. Their number is consistently higher in rabbit. Lymphocytes account for the great majority of these cells. They do not show any changes of appearance when compared with those in the lamina propria (Fig. 59). This is contrary to the observations of Andrew (1965). Cell processes passing through the gap in the basement membrane are not seen frequently and neither these nor the basement membrane show any abnormalities (Fig. 29b). The shape of lymphocytes between the epithelium is rather irregular. Macrophages are occasionally seen in this locality and do not exhibit unusual features (Fig. 60a).

Some migratory cells are degenerating and their type cannot be identified with certainty. Most of them are likely to be lymphocytes. Some features of degeneration are increased density of the remaining cytoplasmic matrix, swelling and relative concentration of mitochondria and separation of nuclear components (Fig. 60b). This nuclear change resembles that seen in lymphocytes of involuting thymus (Cowan and Sorenson, 1964). Different types of degeneration (Fig. 29a) appear as fine flocculations of ground cytoplasm with decreased density. Recognisable cisternae of the endoplasmic reticulum show close apposition to mitochondria which have disintegrated inner membranes. Dense
granules, probably ribosomes, are clustered in the cytoplasm or are irregularly attached to vesicular profiles. Rounded, homogeneous masses, limited by membranes, are probably remnants of nuclear material.

Degenerating cells of this appearance are consistently seen only in the extracellular space.

In very rare cases, degenerated masses occur as inclusions in the crypt epithelium. Their relation to the cell cannot be determined with certainty from a single section. The inclusion seen in Fig. 61 contains only clumped coarse granules, suggesting a degenerated nucleus and irregular membranes. A less likely interpretation is that this body is a cytolysome.

Addendum:

In rabbit, lipid material occurs more frequently than in rat and often appears to be a product of cytoplasmic degradation, although the origin is difficult to demonstrate. The increased storage in rabbit mucosa suggests the possibility of species difference which cannot be decided morphologically.

Intestine is known to possess a considerable degree of cholesterol synthesis. Since sterol synthesis is associated with smooth endoplasmic reticulum in steroid-secreting glands and in the liver (Christensen, 1965; Jones and Fawcett, 1966) it was thought to be of interest to attempt
its localisation in intestine. No cells showing striking development of smooth endoplasmic reticulum were found.

It is thought that an enterocyte may be capable of this function.

The content of the crypts and intervillus spaces was studied in order to provide a basis for comparison of pathological material in rat. In the crypt lumen, numerous bacteria are present which do not show any close relations to the cell surface. Further structures, rarely encountered in crypts and very variable in distribution, are peculiar crystals, with an oblique periodicity of 70-90Å. Their nature is unknown. Parasitic protozoa are seen frequently in intervillus spaces and the lumen of the crypts. Their ultrastructural appearance is very similar to Friend's description (1966) of giardia muris. In a normal rat, the presence of these parasites is not associated with any ultrastructural change of epithelium.

**Fine structure of tela submucosa, tunica muscularis and tunica serosa:**

The cells of the intestinal wall appear similar to those described elsewhere. Electron microscopic examination supplements some relationships too detailed for light microscopy.

The most frequent cell type in the submucosa is the fibrocyte (Fig. 62a). Most of the submucosa consists of
bundles of collagen fibrils interspersed with thin fibrocyte processes similar to those described by Porter (1964). Cells with more prominent synthetic equipment were seen in perivascular positions. Such active fibroblasts may have a cilium (Fig. 28b). Lymphatic capillaries, which are numerous, exhibit different degrees of patency; where the lumen is collapsed it narrows in places beyond the resolution of light microscopy (Fig. 62a). Endothelial cell processes are quite narrow, being only 500-1,000Å in thickness (Fig. 62b).

Some pinocytotic vesicles or caveolae reach almost 300μm in diameter. Occasionally small caveolae show a dense coating. The endoplasmic reticulum, unlike that of the endothelium of mucosa lacteals, forms vesicular profiles. The endothelium of submucosal lymphatic vessels lies directly upon connective tissue and the basement membrane is absent. Collagen fibrils may be in the immediate vicinity of the plasma membrane (Fig. 62b). In other places a thin layer containing connective tissue microfibrils (Low, 1962) intervenes (Fig. 62c). Sometimes poorly defined extracellular material observed near the cell surface coincides with intracellular density subjacent to the plasma membrane (Fig. 62b, arrow). These structures are believed to be hemidesmosomes which were recently described in lymphatics (Leak and Burke, 1966). Thin processes of
fibrocytes which run parallel to the endothelium are separated by a thin layer of fibrils (Fig. 62b). They are consistently distinguished by means of this from overlapped endothelium processes. Small elastic fibrils rarely occur between the endothelium and the collagen.

Blood vessels in the submucosa are represented by arterioles and small veins. The smallest vessels observed have a very similar appearance to those pericapillary vessels described in the lamina propria (Fig. 28a). The layer of connective tissue which separates arterioles from ensheathing lymphatics contains processes of fibrocytes (Fig. 63a) with the appearance of "veil cells" (Majno, 1965). Their thinness is in places beyond the resolution of light microscopy. The small veins (Fig. 63b) contain a single layer of longitudinally oriented smooth muscle cells representing media surrounded by collagen and a discontinuous layer of fibroblastic processes. There may be fairly well developed elastic fibrils present.

Nerve fibres forming larger plexuses almost invariably contain Schwann cells though the sheath is sometimes incomplete; otherwise they are similar to those in the lamina propria (Fig. 25a).

Fine structure of smooth muscle cells and nerves in the tunica muscularis is similar to that described in the bibliography (cf. Fig. 64b) and hence only additional observations
are recorded. General relations of smooth muscle cells in our material are characterised by the paucity of their close contacts. Tight junctions (nexuses) were not observed. The intercellular gap is in narrowest places, 250-350Å in width and is frequently bisected by a dense line which varies in thickness and show discontinuities. In these places of contact, dense areas are constantly present beneath the plasma membrane of both cells. Occasionally such a junction extends over a considerable length and its course is wavy (Fig. 64c). Around most of the cell perimeter collagen intervenes between both basement membranes. The collagen fibrils have inconsistent staining characteristics (cf. Figs. 64a; 64d) and appear more frequently in negative contrast. Somewhat thicker bundles of collagen fill the intercellular space near places where three cells meet. Tapering processes or the ends of cells which bordered such intercellular spaces showed reduction of myofilament density and frequently contained pinocytotic vesicles (Fig. 64d). The fibroblasts were observed only near capillaries (Fig. 64a) and close to nerve plexuses (Fig. 64b). The identity of fibroblast and interstitial cell is confirmed in this material.

Segments of basement membranes shared by endothelium and smooth muscle cell are rarely observed.

Small nerve fibres between muscle cells are very sparse,
and are seen in interstitial space which is continuous with the capillary (Fig. 64a). Schwann cells occur only where large numbers of axons are present, which is rarely seen (Fig. 65a).

The components of Auerbach's plexus are compactly arranged (Figs. 65b; 66) and separated from tunica muscularis by basement membranes superimposed by fibroblasts and collagen. The number of processes within a plexus which show accumulation of vesicles and/or membrane specialisations suggesting synapses is relatively small. Non-neuronal elements occur in the periphery of the plexus (Fig. 66). Neurons did not show an orderly arrangement of the numerous cisternae of rough endoplasmic reticulum; otherwise the fine structural features are typical of nerve cells (Fig. 67).

Mesothelial cells (Fig. 68a) form long thin microvilli projecting into the peritoneal cavity. Organelles in these cells are not prominent but pinocytotic vesicles are fairly frequent. Desmosomes and basement membranes are present. Subserosal connective tissue was observed in significant quantity only in rabbit. Fibrocytes, form tortuous thin process (Fig. 68b). Lymphatic vessels and nerve fibres are similar to those in the submucosa.
The chief difficulty in reviewing the literature dealing with pathological ultrastructure is the lack of a systematised classification on the cellular level. The classification used in general pathology can be applied only to a limited extent. For the purpose of review it is useful to adhere to aetiological criteria. Experimental studies using electron microscopy were concentrated mostly upon the study of the epithelium.

Many various substances have been studied experimentally in their effect upon intestinal epithelium when being absorbed. While these findings per se provide some important information, their relation to the natural conditions of absorption remains to be determined (Trier and Rubin, 1965), since the experimental conditions deviate grossly from the normal.

Striking cellular changes are seen after the instillation of hypotonic solutions into the segment of an intestine. Distilled water causes general swelling of cells with dilatation of the endoplasmic reticulum and mitochondria. Extracellular spaces are widely dilated (Ruska, 1960; Williams, 1963). In vitro, microvilli were found to be
shorter and wider or fragmented and vesiculated. (Millington and Finean, 1962). After hypotonic saline treatment, there was an initial increase in the length of the microvilli. At a later stage, blister-like dilatations of the space external to the microvillus core were seen. This change is essentially the same as that observed during simple autolysis, but more rapid.

The changes seen after hypertonic saline were completely opposite to those after hypotonic solutions. Shrinkage of cells, including organelles occurs. In vitro, observations parallel in vivo instillation studies. Dilatation of cisterna of the endoplasmic reticulum, Golgi complex and mitochondria occurs after the action of many substances (Ruska, 1961; Lischka, 1962; Birkhoff, 1962; Ruska, 1962; Williams, 1963). Although individual organelles may be affected in a different degree and to a variable extent, the alterations are not specific. The substances tested included $K^+$, $Ca^{++}$, $Mg^{++}$, $Cl^-$, $OH^-$, chloroform, diethylether, detergents, alcohol and acetone. Some substances, such as trypsin, glucose, sucrose, staphylococcal, enterotoxin, tween 80, and dilute ethanol, were not found to cause any appreciable change.

A number of reports deal with fine structural effects of agents which interfere with cell proliferation. The pattern of epithelial replacement provides an opportunity
to study the late effects of these agents upon cell maturation. Early structural alterations after X-irradiation were observed both in mature enterocytes and the crypt epithelium (Quastler and Hampton, 1962; Detrick, Latta, Upham and McCandless, 1963; Braun, 1960; Hugon, Maisin and Borgers, 1965). The former, as may be anticipated, are affected to a lesser degree. Changes however, appear in the microvillus plasma membrane and there is disarrangement of cristae in a few mitochondria (Quastler and Hampton, 1962). More pronounced early effects are observed in the crypt epithelium (Hugon, Maisin and Borgers, 1965). The swelling of microvilli leads to their destruction after 24 hours. Mitochondrial swelling with disruption of cristae and the outer membrane is most pronounced at early stages after irradiation. Later, a close association of mitochondria and endoplasmic reticulum is observed. This seems to persist during the recovery phase when cells migrate along the villus (Braun, 1960). An interesting, although not unexpected, feature of degeneration is cytolysome formation, which appears quite early (Hugon, Maisin and Borgers, 1965). The available evidence suggests that bodies which have the appearance of karyolytic bodies under the light microscope are either cytolysomes or phagosomes resulting from the phagocytosis of degenerating crypt cells or leukocytes passing through the crypt wall (Hugon and Borgers,
1966a). Both processes may be combined. A study of cytolysome formation in irradiated epithelium suggests that smooth endoplasmic reticulum participates in membrane formation (Hugon and Borgers, 1965a). Acid phosphatase appears to be brought in by Golgi vesicles (Hugon and Borgers, 1965b). Cytolysomes may be extruded from the cell into the crypt, while others form residual bodies similar to those of other tissues (Hugon and Borgers, 1965a).

Phagocytosis and inclusion of necrotic leukocytes by the crypt epithelium after irradiation is an unusual form of activity of these cells (Hugon and Borgers, 1966b). Cytolysome formation can be reduced or delayed by chemical radioprotection (Hugon, Maisin and Borgers, 1966a) which in turn affects the crypt epithelium (Hugon, Maisin and Borgers, 1966b; 1966c).

At later stages delayed damage to the crypt epithelium is seen and the affected cells migrate to the villi (Hugon, Maisin and Borgers, 1966d). Cells in the crypts are decreased in number and flattened. Only a few microvilli persist. Endoplasmic reticulum, ribosomes and Golgi complex are reduced; mitochondria are pyknotic and vacuolated. Fat droplets are seen in the cytoplasm. Nuclei are irregular, and frequently very large, while the amount of chromatin is reduced. Goblet and Paneth cells show marked alterations. Argentaffin cells are resistant to damage. The epithelial
cells which appear on the villi are cuboidal with short irregular microvilli. Further changes are similar to those in the crypt epithelium. Intercellular spaces are dilated (Quastler and Hampton, 1962; Detrick, Latta, Upham and McCandless, 1963). In areas of intercellular oedema the cells within the villus core including vessels showed vacuolisation. Bacteria were observed within the lamina propria (Quastler and Hampton, 1962). The granules of mast cells decrease in electron density or disappear (Detrick, 1963).

Similar changes occur in human intestine exposed to X-irradiation (Trier and Browning, 1966).

Damage of intestinal mucosa known to result after administration of cytostatics such as aminopterin was studied by electron microscopy, (Millington, Finean, Forbes and Fraser, 1962; Williams, 1961; Rybak, 1962; Trier, 1962). Both crypt epithelium and enterocytes are altered. Cytoplasmic changes consist of dilatation of the endoplasmic reticulum, Golgi complex and swelling of some mitochondria. Microvilli are swollen and fragmented. In human crypt epithelium, inclusions appear (Trier, 1962) which are similar to karyolytic bodies after X-irradiation. It is of interest that cytoplasmic changes were seen at early stages even in some cells of the villous epithelium of the human mucosa which suggests that primary damage, although prominent, is not
limited to the crypt epithelium. Later stages studied in animals show marked changes of microvilli which are shorter, sparse and irregular. Degenerating cells are extruded both from crypts and villi. Defects in epithelial continuity (Rybok, 1962) were seen following the extrusion of degenerated cells. In some cases preservation of epithelial continuity was maintained by means of overlapping of epithelial processes (Millington, Finean, Forbes and Fraser, 1962). Later stages show features of epithelial regeneration. Nuclei and nucleoli of these cells are large. An impaired absorption of fat is observed (Williams, 1961; Millington, Forbes, Finean, Fraser, 1962). Alterations of microvilli were observed after administration of 5-fluorouracil (Williams, 1963).

The physical relationship or attachment of bacteria to enterocytes has only been studied in the mouse after Streptobacillus moniliformis infection or superinfection (Hampton & Rosario, 1965). These bacteria appear to be attached exclusively near intercellular junctions. The altered enterocytes show, besides displacement of organelles and modification of cytoplasmic density around the invagination a change of plasma membrane contacting the microorganism.

Different relationships were observed when pathogenic bacteria such as Salmonella typhimurium invaded enterocytes
in guinea pig (Takeuchi, 1967). Here a degeneration of microvilli and the terminal web appears in a localised area of bacterial entry into the cell. Eventually a bacterium is enclosed in a vacuole containing degenerated parts of microvilli and cytoplasm, then dense material and sometimes organelles. Another mode of entry is between the cells through the junctional complex. In Shigella infection (Takeuchi, Sprinz, LaBrec and Formal, 1965) the bacteria in the enterocytes were seen either free or enclosed within a membrane-limited space. During this infection, enterocytes show degenerative changes which are not related to the presence of the bacteria and follow the inflammatory reaction in the lamina propria. Besides the loss of microvilli, swelling of mitochondria and the endoplasmic reticulum, inclusions appear which correspond to cytolsomes. An important feature is the tendency of the epithelium to maintain continuity and the persistence of the desmosomal junctions during the detachment and extrusion of degenerated cells.

Another intestinal infection investigated by electron microscopy is cholera. There appears to be an alteration of endothelial cells which precedes moderate epithelial changes (Goldstein, Merrill and Sprinz, 1966). Gaps between endothelial cells were observed (Goldstein, Merrill and Sprinz, 1966; Patnaik, Ghosh, 1966). Further changes which
were seen are apparently due to exudation. The vesicles and discontinuities in the plasma membrane of microvilli of the enterocytes described by Patnaik and Ghosh (1966) may be subject to equivocal interpretation.

An ultrastructure of epithelium in the atrophic intestinal mucosa was studied in protein-deficient rats (Takano, 1964). Besides reduction and irregularity of the microvilli, there was seen a decrease of RNP particles and swelling of mitochondria.

Vesiculation of mitochondrial cristae was observed in the duodenal epithelium of a mouse deficient in essential fatty acids (Leduc and Wilson, 1964).

Electron microscopical investigations of human small intestinal disease have focused so far on a few diseases, associated with the malabsorption syndrome, mostly coeliac disease or sprue and Whipple's disease.

Although relatively numerous fine structural studies of changes in coeliac disease are available, most of these are incomplete. Earlier reports stressed the sparsity and irregularity of microvilli (Shearman, Girdwood, Williams and Delamore, 1961; Shiner and Birbeck, 1961; Zetterqvist and Hendrix, 1960). Most observations are limited to the epithelium covering the atrophic mucosa. Some of these cells were seen to have, besides reduction of microvilli, further abnormalities, including a defective terminal web,
a reduced amount and dilatation of mitochondria and endoplasmic reticulum. They also showed a higher frequency of lysosomes, variations in density of cytoplasm, myelin figures and vacuoles. These changes vary in degree and showed some differences (Padykula, Strauss, Ladman and Gardner, 1961; Nunez-Montiel, Bauza, Brunser and Sepulveda, 1963; Ashworth, Chears, Sanders and Pearce, 1961; Ashworth and Chears, 1962; Curran and Creamer, 1963; Shiner, Lacy and Hudson, 1962; Rubin, Ross, Sleisenger and Weser, 1966). Alterations in overall shape, to form cuboidal or flattened cells with irregular position of the nuclei give the epithelium a pseudostratified appearance known from light microscopy. These may accompany the cytoplasmic changes. The extent of impairment of the lipid absorption in affected cells is variable (Ashworth and Chears, 1962; Shiner, Lacy and Hudson, 1962). Only a few reports described defects in the epithelium basement membrane (Ashworth and Chears, 1962; Ashworth, Chears, Sanders and Pearce, 1961). The evidence, which is limited, indicates that crypt epithelium has a normal ultrastructure (Padykula, Strauss, Ladman and Gardner, 1961; Rubin, Ross, Sleisenger and Weser, 1966). However, the interpretation of epithelial cells lining the upper portions of crypts which are transient to the atrophic surface is uncertain. Padykula, Strauss, Ladman and Gardner (1961) believe on the basis of histochemical criteria that
the cells in this zone are normal enterocytes. Because of orientation difficulties, they were not able to distinguish it from the superficial zone by electron microscopy. Rubin, Ross, Sleisenger and Weser (1966) consider cells in this zone to represent normal upper crypt epithelium. These interpretations are compatible with the increased turnover rate or shortened life span of the epithelium which is suggested by the high mitotic index (Padykula, Strauss, Ladman and Gardner, 1961).

The fine structural study of tropical sprue by Hartman, Butterworth, Hartman, Crosby and Shirai (1960) is hardly worth mentioning because of extensive artifacts. The most agreeable statement contained in this paper is that more observations are needed.

A human disease, the intestinal manifestations of which have attracted the particular interest of electron microscopists is Whipple's disease. In this review, the briefest account of pertinent findings will be given, many other interesting features of this disease must be omitted. Most reports agree in demonstrating bacteria in the lamina propria (Chears and Ashworth, 1961; Ashworth, Douglas, Reynolds, and Thomas, 1964; Rostgaard, 1964; Kent, Layton, Clifton and Schedl, 1963; Kurtz, Davis and Ruffin, 1962; Curran and Creamer, 1963; Cohen, 1964; Kojecky, Malinsky, Kodousek and Marsalek, 1964; Trier, Phelps, Eidelman and
Rubin, 1965). Phagocytosed bacteria in various stages of degeneration may be seen within the inclusions of macrophages present in the lamina propria. All descriptions of the PAS positive inclusions in these cells are similar.

With the progressive degradation, bacteria contained within the inclusions disappear and replaced by closely packed membranes, some granules and amorphous material. Most reports indicate that the inclusions in more advanced stages of degradation which are contained in more deeply localised macrophages or those seen during remissions after antibiotic therapy contain less membranous and more amorphous material with some granules and filaments (Trier, Phelps, Eidelman and Rubin, 1965; Kurtz, Davis and Ruffin, 1962; Ashworth, Douglas, Reynolds and Thomas, 1964; Kent, Layton, Clifton and Schedl, 1963). The bacteria disappear. Recently, minor changes of the enterocytes were reported, consisting of shortening of microvilli and increased numbers of lysosomes (Trier, Phelps, Eidelman, Rubin, 1965).

When the evidence based on these fine structural observations of intestinal mucosa is correlated with the clinical course, there is little doubt about the pathogenic role of bacteria. In Whipple's disease, pathogenetic considerations concerning other manifestations of this disease remain hypothetical.

Conclusions concerning the relation of inclusions to
extracellular particles have not been arrived at in other studies (Hollenberg and Jennings, 1962; Cohen, Schimmel, Holt and Isselbacher, 1960). A few others hold the opinion that the inclusions result from abnormal synthetic activity of histiocytes (Fisher, 1962; Adams, Wolfsohn and Spiro, 1963).

Fine structural changes have been described in patients with giardiasis and malabsorption. These mostly affect the crypt epithelium. The alterations are accentuated in areas of inflammation. The changes in crypts are believed to result from disturbance of the secretory processes by increased pressure in an obstructed crypt lumen; those of the enterocytes may be considered secondary to disturbed differentiation (Takano and Yardley, 1965).

Total vagotomy is denied to cause any changes in intestinal mucosa (Elliot, Barrnett and Elliot, 1966).

In cystic pancreatofibrosis the only abnormality detected by electron microscope was the discovery of a material consisting of thick filaments adherent to the outer aspect of the fuzzy layer (Frey, Kurtz, Spock and Capp, 1964).

In congenital β-lipoprotein deficiency an accumulation of lipid within the enterocytes is observed. Some of the lipid droplets which may attain a large size, occur without being surrounded by a membrane (Isselbacher, Scheig, Plotkin and Caulfield, 1964).
Dobbins (1966) observed that the accumulation of fat does not affect the Golgi complex and suggested that the "exit block" of fat is due to a defective function of Golgi or endoplasmic reticulum membranes. The failure of chylomicron formation seems to be a reflection of defective protein metabolism. Similar change may be induced experimentally by interference with proteosynthesis (Sabesin and Isselbacher, 1965).

Sensitisation of hamsters against ferritin increases its absorption after intraluminal instillation. Ferritin was observed in plasma membrane invaginations, vesicles, structures resembling multi-vesicular bodies and the Golgi complex of the enterocyte as well as within some other cells of the lamina propria (Bockman and Winborn, 1966).

The ultrastructural appearance of epithelium of the intestinal type present in atrophic gastric mucosa in pernicious anaemia is similar to that of normal intestinal epithelium. Lysosome-like bodies were, however, more frequent (Rubin, Ross, Jeffries and Sleisenger, 1966).

An accumulation of dense material is seen in the mitochondria of epithelial cells which are close to intestinal tumours in 20-methylcholanthrene fed mice (Burt, Killmeyer, Thompson and Grauer, 1961). This material seems to occupy the intracristal space.
CHAPTER 6

FINE STRUCTURE OF SMALL GUT DURING ISCHAEMIA AND REPAIR OF ISCHAEMIC INJURY I:
THE PRENECROTIC AND NECROTIC PHASE

Few systematic electron microscopic studies of disorders of the gut have been made. Increased attention is being paid, however, to clinical conditions where intestinal ischaemia is the main pathogenic factor. Vascular pathology shows that a variety of conditions can be responsible for intestinal ischemia. Examples range from embolism, arteriosclerosis, polyarteritis nodosa to rare entities like Degos' papulosis atrophicans (Sidi, Reinberg, Spinasse and Hincky, 1960; Naylor, Mullins and Gilmore, 1960; Nomland and Layton, 1960) and intimal hyperplasia (Aboumrad, Fine and Horn, 1963). While the clinicopathologic aspects of vascular occlusions are still discussed (e.g. Wilson and Block, 1956; Marrash, Gibson and Simeone, 1962) the existence of intestinal infarction without detectable vascular occlusion has only recently been emphasised (Ende, 1958; Berger and Byrne, 1961; Grosh, Mann and O'Donnell, 1965; Si-Chun Ming, 1965). The role of shock in producing intestinal ischaemia and its possible consequences has also been studied (inter alia Fine, 1965;
Sandritter, 1965; Marston, 1962; Rayner, MacLean and Grim, 1960). Other manifestations of intestinal ischaemia, like dyspragia or angina intestinalis, malabsorption, and secondary stenotic inflammatory lesions are a matter of renewed interest (e.g. Leymarios, 1966; Fry and Kraft, 1963; Shaw & Maynard, 1958; Pope and O'Neal, 1956).

Disturbances associated with alterations of intestinal position and lumen are frequent and intestinal ischaemia is usually the main, but not the sole, result.

A vascular etiology of potassium-induced ulcerations of the small gut was suggested recently (Allen, Boley, Schultz and Schwartz, 1965) and considered without reaching a positive conclusion in nonspecific intestinal ulcus simplex (Alexander and Schwartz, 1966).

Interest in intestinal ischaemia and anoxia has not, however, stimulated many morphological studies. Some fairly recent experimental studies are available (e.g. Cameron and Khanna, 1959; Khanna, 1959) but so far no reports dealing with fine structural changes have been published. This has prompted the present investigation.

**MATERIAL AND METHODS**

Adult white rats of both sexes as supplied by the Animal Breeding Unit of the J.C.S.M.R. were used. Under ether anaesthesia medial laparatomy was made and the trunk
of arteria mesenterica superior located, care being taken to minimize the manipulation of the intestine. Immediately afterwards, a polythene-covered clamp was applied to compress the artery before entering the radix mesenterii. The circulatory changes in the gut wall and mesenterium were observed for 1-2 minutes after compression. The abdominal wall was then closed and the animal allowed to recover from the anaesthesia. This occurred within 6-7 minutes after compression of the artery.

After 1 hour the laparatomy was quickly reopened under ether anaesthesia and the condition of gut and mesentery examined. After the clamp had been removed, a lapse of about 1 minute was allowed to elapse in order to check the re-establishment of circulation. The laparatomy was closed again. After the required time, the animal was re-anaesthetised and the intestine exposed for the third time. The circulatory conditions in the gut were checked and a segment of the upper third of the ileum was removed for examination. When recirculation was not desired, the previous stages coincided.

Animals without recirculation were studied after 30 mins. and 1 hour of ischemia. Others were studied 10 minutes, one, 2, 4, 8, 12, 16, 24, and 48 hours after the beginning of macroscopical ascertainable recirculation. At least two animals in each group were examined with the
exception of those at 12 and 48 hours when only one animal was examined. A total of 27 animals were examined. A few animals examined after 1 hour of recirculation were injected intravenously (intra venam cavam caudalem) with 1 ml of colloidal carbon (Gunther, Wagner, Pelikan, Werke, Hannover, Germany; batch C11/1431a) just prior to clamp removal.

In order to study postmortem changes, two isolated segments of intestine were ligated at both ends and placed in the peritoneal cavity of a littermate and examined after 24 hours. 2 sham-operated animals were used as controls.

**Light microscopy:**

Material for light microscopy was fixed in buffered formol (Lillie) and embedded in wax. Sections were stained with haematoxylin and eosin, P.A.S. and haematoxylin, PTAH.

To reduce the sampling error inevitable in studying processes which are variable in the limits of time and region, semithin sections of untrimmed blocks were stained with toluidine blue and examined with the light microscope. After establishing a range of lesions, blocks from selected animals were studied in detail by light and electron microscopy. Conditio sine qua non for electron microscopy was an appropriate orientation and location of the area to be examined. This was met by selective trimming.
RESULTS

Gross findings:

Within 20-30 seconds after mesenteric artery compression, anaemic palor fully developed. From that time on, the gut wall began to change colour slowly to a cyanotic shade. The arteries were pulseless and the veins darkened without initially changing their prominence. After 30 minutes, the gut wall was grey-violet. After 1 hour, it was dark violet with occasional ecchymoses. The gut was slightly distended and the content was of low viscosity and was slightly haemorrhagic. Despite intense changes the intestinal wall at this stage cannot be compared to the appearance of haemorrhagic infarction. These observed changes affected the lower jejunum, ileum and caecum and were not sharply defined. After removal of the clamp, pulsations reappeared and were initially more marked than in the normal animal. The colour changed within 1 minute to a vivid red. A gradual decrease of the arcuate and small artery pulsations was observed after 1-2 minutes. The intense red colour of the intestinal wall persisted for up to 4 hours. The serosa remained smooth. The mucosa showed a loss of normal relief and had a greyish surface. In places, the superficial layer of the mucosa had the character of a discrete pseudomembrane. The intestinal contents
were thin and pale with a yellowish tinge. Hyperaemia was noticeable on the serosal aspect at 8 hours. Later a less prominent mucosal relief was seen.

**Light microscopy:**

The changes observed in individual animals at different stages show some variations and overlapping in details. This diversity reflects the variation of the process in time and extent. The basic character of changes is constant enough, however, to establish a definite sequence of events.

**Ischaemic phase:**

**30 minutes**

The earliest changes seen after 30 minutes of ischaemia consist of oedema. This is manifested at the cell bases as vacuole-like spaces limited by thin bridges of cytoplasm. These are most prominent at the tops of the villi. Vacuole-like spaces are less conspicuous in semi-thin araldite sections. Simultaneously an increased capillary packing with erythrocytes appears.

The enterocytes covering the apical third of the villus become detached by disruption of cytoplasmic bridges between vacuole-like spaces (Fig. 69a) and those at the tip may be already desquamated. Desquamating cells show a cytoplasmic rarefaction which has an appearance of very fine vacuolisation. In araldite, semithin sections, this fine vacuolisation is less prominent.
One hour

After one hour of ischaemia, some areas appear almost identical but the changes extend to the base of the villi. Mostly, however, the apical halves of the villi are frankly necrotic (Fig. 69b) and only some cells including a few polymorphs remain discernible. Beneath the area of necrosis, a rim containing some pyknotic cells or oedema may be seen. Polymorphs are quite rare. This zone is not sharply delimited and minute areas exist where mesenchymal reticular elements are enlarged and have a rather compact arrangement. The basal portions of mucosa show sparse leucocytic infiltration.

Recirculation:

After 1 hour of re-circulation, the appearance was fairly similar but oedema of the villus core is prominent. In one animal, the extent of necrosis was fairly small and carbon labelled vessels were distributed irregularly within the villi, but not in their apical portions.

At 2 hours of re-circulation, necrotic zones show up distinctly and reach their maximum extent, frequently affecting the whole villus (Fig. 69c). The necrotic areas are eosinophilic and contain numerous erythrocytes. Numbers of pyknotic cells, debris and karyorrhectic polymorphs are concentrated in the spaces between the villi. At this stage, a basophilic irregular regenerating epithelium appears at
the bases of necrotic villi (Fig. 69d). Aberrant proliferation of the epithelium forms hyperplastic multilayered foci and "spurs" into the lumen (Fig. 70a). Epithelial bridges or synechiae with neighbouring villi frequently occur (Fig. 70b). Detachment of whole epithelial sheets also occurs. Basal portions of the mucosa exhibits reactive inflammation and there is some exudation into the crypts. Both crypt and regenerating epithelium contain basophilic bodies of differing sizes. Some epithelial cells appear shrunken or disintegrating.

4 and 8 hours

At 4 to 8 hours of recirculation, most of the necrotic areas are shed and the remains infiltrated with polymorphs (Fig. 70d). These frequently show karyorrhexis. In places where the necrosis has extended deeply and already been eliminated (Fig. 70e), there may be almost complete re-epithelisation. Complete re-epithelisation was also occasionally seen 4 hours after recirculation and resulted in the formation of mucosa completely devoid of villi; only crypts present (Fig. 70c). The surface is covered by the remains of necrosis and pyknotic cells. Where necrosis had been less extensive, the bases of villi could still be seen. The spaces between regenerating epithelium covering these remnants were almost obliterated. These areas do not show complete regeneration of the epithelium at 4 hours, but
frequently do so after 8 hours of recirculation.

At 8 hours, a serrated surface pattern appears which suggests incipient villus formation (Fig. 71a). Before regeneration has been completed the epithelium appears similar to that seen at earlier stages (Fig. 71b). In some areas, the re-epithelisation is complete and short neovilli are seen, the core of which still shows inflammation. In other instances, the villus core containing polymorphs is expanded and oedematous or is formed by a layer which appears to be like oedematous granulation tissue (Fig. 71c). In some places the reparative process is more advanced and similar to that seen later.

16 and 24 hours

After 16 or 24 hours of recirculation (Fig. 71d) the epithelium which covers the short villi increased in height but remained basophilic. The villus core showed either inflammatory residues or more frequently contained various mesenchymal cells in a losse reticular arrangement, separated by wide intercellular spaces. Wide spaces are also present between epithelial cells which are separated from the villus core by a continuous basement membrane. These features are observed to advantage in semi-thin araldite sections (Fig. 71e). The central lacteal is usually wide. The base of the mucosa shows very sparse infiltration, mostly with polymorphs, eosinophils and macrophages. At
this stage, some villi show less advanced reparation and re-epithelisation is incomplete. The appearance is similar to that which occurs predominantly 8 hours after recirculation.

The sequence of events in the rat, following the re-establishment of circulation is similar to that described by Cameron and Khanna (1959). The whole process can be divided into these three stages:

1. Prenecrotic and necrotic phase
2. Early reparative phase which comprises reactive inflammation and regeneration.
3. Residual phase after completion of re-epithelisation.

Of course these phases cannot be sharply separated, but the distinctions have been made in order to provide a background for a study of fine structural changes which follow this sequence.

Fine structural changes during the prenecrotic and necrotic phase:

**Epithelium**

**30 minutes**

Profound fine-structural changes preceding desquamation are seen 30 minutes after arterial occlusion. Changes of the enterocytes at this stage are striking, although not uniform (Fig. 72). Mitochondria are swollen with shorter irregular cristae. Some cisternae of the rough endoplasmic
reticulum are irregularly dilated and tortuous. A minority retain a normal appearance. The Golgi complex is dilated. The cytoplasmic matrix in many cells shows decreased density. This change is accompanied by a displacement of organelles in lower portions of the cells towards the perinuclear cytoplasm. This leaves behind large prominent cytoplasmic zones which only contain free ribosomes (Fig. 73). The intercellular spaces which usually occur between cell bases are obliterated. Some enterocytes show alterations of the brush border which consist of distortion and fragmentation of microvilli. This will be described at a later stage. Occasionally there is a partial loss of the brush border (Fig. 74a). The surface of the cytoplasm bulges and the protrusions which are limited by a membrane contain bundles of filaments in irregular positions. These bundles appear to be the residues of microvillus filaments or their rootlets. When these lie parallel to the plasma membrane, their appearance suggested that some microvilli were incorporated into the partially reconstructed plasma membrane.

In a few cases, microvilli are increased in width to approximately double their diameter and the filaments occupy the whole width. Their rootlets are abnormally long and unusually numerous microtubules accompany them (Fig. 74b). Between the rootlets, tortuous vesicular profiles are seen. These changes are an exaggeration of a variation occasionally
observed in controls.

One hour

One hour after arterial occlusion almost all villi show extensive defects of epithelium.

Changes of a different character are seen in the enterocytes of villi in which desquamation has not been detected. Some of the enterocytes exhibit a striking decrease in density of the ground cytoplasm (Fig. 75). This change affects the whole cell. The cytoplasmic matrix appears "empty" with only fine thread-like precipitates being present (Fig. 76). An increase in cell volume occurs which is best appreciated where the cell borders a less affected cell when a bulge towards it is observed. Small vacuoles are usually present beneath the level of the nuclei (Fig. 75). Mitochondria do not appear to be severely affected, but some seem to be enlarged. Only few stacks of cisternae of the endoplasmic reticulum are seen; others are dispersed. Some cisternae are narrow; there is, however, an irregularity of the attached ribosomes (Fig. 76). Free ribosomal rosettes are not altered. Many fat droplets are present. These are of variable size (approximately 75-250 m u) and are contained within smooth or, occasionally, ribosome-covered, vesicles (Fig. 76). The largest droplets are seen within the Golgi complex. Droplets of a similar size, free of membranes, which appear to correspond to chylomicra, are observed in
intercellular spaces beneath the level of the nuclei (Fig. 75). During the discharging process, these droplets are occasionally seen within caveolae, the membrane of which is dense.

The plasma membrane of many enterocytes shows discontinuities. Further changes result in a complete loss of microvilli (Fig. 77). In places, this defect is covered by an irregular plasma membrane. Subjacent to this, there are the remnants of the terminal web. Swollen mitochondria are seen beneath the terminal web. Occasionally lipid droplets are seen which lack a limiting membrane. Vacuole-like dilatations of the intercellular space occur above the level of a nucleus. Some vacuoles which resemble multivesicular bodies, increase in number.

Most villi show extensive defects of the epithelium. When the edge of a defect is examined it is seen that the enterocytes show graded alterations. Those which are located at the base of a villus display an increased formation of vesicles formed by endoplasmic reticulum at the expense of cisternae (Fig. 78). The vesicles contain finely granular material of low electron density or small fat droplets. Circumscribed dilatations of the intercellular space appear, which are located in segments between two desmosomes. Some spaces appear as vacuoles within the cell. Cisternae of the endoplasmic reticulum are frequently seen close to the
mitochondria. These show a rather peculiar alteration. In this the membranes of the mitochondrial envelope are of greater density around segments of the perimeter and appear in these places to be almost straight (Figs. 78; 79a). The thickness (50-60Å) and triple-layered pattern of these membranes is not altered markedly (Fig. 80c). In addition to the increased density of mitochondrial membranes, dense material seems to be present within the outer mitochondrial chamber. Cisternae of the Golgi complex show an increase in length.

In enterocytes located close to the defect, these changes are accentuated. The endoplasmic reticulum is completely transformed into vesicles which contain numerous fat droplets (Fig. 79a). Golgi cisternae form curved or concentric arrays which enclose and are in turn surrounded by vacuoles containing fat droplets (Fig. 80d). Microvilli of the brush border are distorted and vary in width. Filaments occupy the whole width of each microvillus. With progressive alteration, the microvilli become fragmented in place of constriction (Figs. 79a; 79b). The plasma membrane and fuzzy layer are preserved (Fig. 80b). The filaments of the terminal web are dispersed in a wide zone beneath the apical plasma membrane and are not clearly defined (Fig. 79b). The same change affects the rootlets of filaments. Immediately beneath this zone, elongated, finely-granular clumps of dense
material are seen (Figs. 79b; 81). A few small clumps of the same material occur deeply in the cytoplasm. The plasma membrane along the lateral cell surface is highly irregular and in places, cannot be detected. Some desmosomes are dissociated and abnormal intercellular spaces formed. Rounded cell processes, free or organelles, may be seen between the basal portions of the cells. These show disruption.

Cells which undergo desquamation are loosened, acquire an irregular shape and become surrounded by debris (Fig. 81). This is formed by the disintegration of the outer cytoplasmic layer. The plasma membrane is lost along most of the perimeter and the brush border fragmented. Besides advanced cytoplasmic changes the chromatin, regardless of the size of the nucleus, becomes markedly condensed (Fig. 81). These pyknotic masses display regular outlines and delineation towards the nucleoplasm. Sometimes the remnant of a cell which is being desquamated, remains attached to the nearest enterocyte. These remnants often possess organelle-free, irregular protrusions of cytoplasm (Fig. 80a). Other cells found in the edge of desquamation appear to have grossly defective apical areas, although they remain in contact with the basement membrane.

In sheets of epithelium which are lifted in continuity above the basement membrane the cells at the defect near the
apex are always severely altered. Remote cells in the sheet show a disruption at the base which is difficult to distinguish from artifact, since further alterations in these cells are comparable to those seen in undesquamating cells.

In many cases, the first cell encountered on the edge of a defect is a goblet cell (Fig. 82a). The cell shape may be changed and cell organelles show some displacement (Fig. 82b), but the endoplasmic reticulum in contrast to that of altered enterocytes, retains its cisternal configuration. While the Golgi cisternae are also dilated and mucous granules vary abnormally in electron density, other organelles do not show striking alterations (Fig. 82a). Defects of plasma membrane are rare and occur only above mucous granules.

Goblet cells, shed into the lumen, also show changes which are less severe than those of enterocytes in the same place. In these changes, endoplasmic reticulum is broken into fairly uniform vesicles which contain substances of medium electron density. Mitochondria are swollen. Golgi cisternae are transformed into membranous arrays of unusual length. This change is of the same character as that in enterocytes but because of the lack of fat accumulation, appears more prominent.

After one hour's ischaemia, the crypt epithelium shows only a swelling of mitochondria.
Lamina propria mucosae:

30 minutes

Fine structural alterations in the lamina propria are seen at the earliest stage examined. Most of the cells in the apical areas of villi have swollen mitochondria. The endoplasmic reticulum becomes transformed into large circular profiles. The cisternae of endoplasmic reticulum in some plasma cells are dilated to a degree which is very rarely seen in normal controls. Swollen processes which contain ribosomes or no organelles are interposed among the cells. Many cells show defects of the plasma membrane and organelles appear intermixed in the intercellular space (Fig. 84a). Capillaries show a marked swelling of the endothelium. This affects the cytoplasmic matrix and usually varies along the perimeter of a capillary (Fig. 85). The Golgi complex which is usually difficult to see in normal capillaries, appears to be prominent in the pale voluminous cytoplasmic areas. Microtubules and pinocytotic vesicles show increased relative contrast. The precipitated blood plasma within the capillary lumen is increased somewhat in density. The basement membrane in most cases appears to be less defined than in normal capillaries. Frequently erythrocytes fill the lumen almost completely.

Collagen and interfibrillar matrix, which in most controls stain poorly were in some instances seen to be unusually
dense in these altered areas (Fig. 73).

30 minutes - one hour

Alterations of the villus core beneath the area of the epithelial defect after desquamation are not uniform. The most extensive changes are seen in the apical portion. Most cells here are extensively swollen and disintegrated. At the same time, abnormal spaces appear between cell remnants which contain released organelles and debris (Fig. 86). Plasma cells and lymphocytes and eosinophils are more resistant to disintegration. The plasma cells, which are disrupted, show almost invariably, breakdown of the cisternae of the endoplasmic reticulum into rough-surfaced vesicles. Blood capillaries are distended by stasis of erythrocytes and the endothelium breaks down in many places (Fig. 87b). A dilated endoplasmic reticulum and enlarged Golgi complex is seen in perinuclear cytoplasm (Fig. 87a). Some capillaries show more extensive changes and contain irregularly paired membranes and dense granules or spherules which partially or completely, replace the endothelium. Erythrocytes are present in the lumen. These changes will be described in detail later. In other vessels, platelets are adherent to the endothelium.

Few features characterise the denuded surface of the villus core. The basement membrane has a strikingly uneven undulating course, consisting of both coarse and fine folds.
The zone of collagen fibrils beneath the basement membrane is wide. Breaks in the basement membrane are rare and were usually seen near capillaries. The folding of the basement membrane is seen also beneath the epithelium in the edge of the defect (Fig. 82a).

Towards the base of the villus the breakdown of the denuded connective tissue is less extensive. The cells here may show extensive fragmentation of the plasma membrane, but only some are further disintegrated.

The mast cells contain granules which vary in the density and prominence of internal structure. The spaces between granules and their membranes increase in width and some granules disappear. A few cells, however, have a normal appearance of granules and show only slight dilatation of endoplasmic reticulum.

The alteration of the granules of eosinophilic leucocytes consists of the disintegration of externum and membranes; the interum is invariably preserved.

Synaptic vesicles and neurotubules are present in nerve fibres and the axolemma is altered less than membranes of other cells.

Smooth muscle cells are devoid of pinocytotic vesicles and the plasma membrane breaks down in many places.

The area subjacent to the denuded villus core has a compact appearance (Fig. 88). The cells vary in appearance
between reticulum cells and macrophages and form prominent interlocking processes. Organelles are concentrated within the cell bodies and have a normal appearance; the Golgi cisternae are however dilated. In most cells, Golgi vesicles are extremely numerous and are crowded between organelles all over the cell body (Fig. 89). Most of these vesicles are coated. Microtubules in many cells are orientated in a radial direction or lie parallel to the border of the ectoplasm. Chromatin is concentrated in areas near the periphery of the nucleus. Cytoplasmic processes usually contain only a few ribosomes.

In those rare cases where there is no defect of epithelial continuity at 1 hour, no gross disintegration in the lamina propria occurs, the changes just described are seen in areas close to the apex of the villus. In some places, however, the arrangement of cell processes is less compact. Many caveolae and vesicles are formed and contain particles identical in appearance to chylomicra (Fig. 84b). These are also present in moderate amounts between the processes.

In areas close to drastically altered tissue the cell processes show swelling and sometimes disruption of the plasma membrane.

The plasma cells which occur in these zones retain their integrity and normal configuration of organelles. The cytoplasmic matrix however, increases in density to a degree
comparable with the coagulation necrosis (Fig. 88) discussed in Chapter 3.

There is also an increased number of phagosomes observed in this reactive zone. While their composition is similar to that of normal tissue, they are frequently larger. Large phagosomes show a complex arrangement of components (Fig. 90b). In some cases the periphery of phagosomes is not sharply defined and organelles are seen close to it (Fig. 90a). In rare instances, granules of eosinophilic leucocytes were observed within such inclusions.

The changes which occur in lower portions of the lamina propria are less intense. Some cells have swollen mitochondria. In endothelial cells mitochondria are sometimes seen in close association with lipid droplets. The Golgi complex is enlarged. There is stasis in these vessels, but distention of the wall is much less prominent than in villus capillaries. The plasma membrane of some endothelial cells forms folds and small processes on the luminal surface.

Changes seen in smooth muscle cells of the muscularis are slight. Mitochondria, though they are not swollen, occur in small groups in contrast to their being isolated in normal cells. Pinocytotic vesicles seem to be decreased in number. Nerve fibres in the muscularis do not differ in appearance from those in control animals.
One hour of recirculation:

**Epithelium**

The epithelium becomes detached from the basement membrane in long continuous sheets without cell disintegration. Single cells are extruded from areas of the epithelium which remain in situ. In these instances, the cell being extruded remains attached to those remains attached to those remaining in situ, and no defect in epithelium continuity was detected. The plasma membranes are tortuous, but the intercellular gap is only slightly irregular. Tight junctions are seen along the edges of adherent plasma membranes (Fig. 91).

Sometimes groups of cells remaining in continuity extruding into the lumen, are "anchored" to those left, by single cells, the plasma membranes of which are still adherent.

Both detached and persistent enterocytes show changes which differ from those seen before recirculation (Figs. 92a; 92b). The free surface bulges conspicuously, while the areas of intercellular contact are indented. The intercellular gap expands to form large spaces which are sealed by desmosomes. The brush border is distorted and stretched, but fragmentation of microvilli is rarely seen. The terminal web disappears as an organised structure, but some remnants of filaments are present. The endoplasmic reticulum becomes changed into long cisternae which may in places form
circular profiles enclosing invaginated cytoplasmic matrix. These formations are frequently dilated. Large lipid droplets are seen in the cytoplasmic matrix. Mitochondrial swelling, single membrane-bounded vacuoles, transformation of the Golgi complex, displacement of organelles from the cell base and nuclear changes, are similar to that described at 1 hour of ischaemia. Few cytolyosomes are present. Similar, but less profound, changes are seen in goblet cells. These may remain attached to the basement membrane, while neighbouring cells are detached.

**Lamina propria**

The degree of disintegration of cells in the necrotic zone of the villus core is greater, although cell remnants are still recognisable. Disruptions of the denuded epithelial basement membrane are more frequent and are accompanied by loss of debris from underneath. All capillaries within the necrotic zone are thrombosed. Many platelets, erythrocytes and some polymorphs are present. Membranes and spherules, together with some cytoplasmic remnants, are seen in the place of the endothelium. The thrombosed capillaries and smallest venules in the basal portion of the necrotic tissue and in an area adjacent to the necrosis, show colloidal carbon labelling which indicates that the thrombosis is secondary to recirculation. In some of these vessels, even those close to the denuded surface, the endothelium is
preserved. Some labelled thrombosed capillaries show small gaps between endothelium, which are covered by thrombocytes (Fig. 87c).

Cells in the basal necrotic area show less disintegration. Occasionally a small amount of debris may be seen entering the gap between lymphatic endothelial cells which do not show marked alterations. Although artifact cannot be excluded, this may represent a passage of debris towards lacteals.

In order to study the further stages of cell disintegration, the mucosa of a closed loop of the ileum was examined 24 hours after intraperitoneal implantation (see Material and Methods). Enterocytes, although severely altered, retain a distorted and fragmented brush border, which remains in continuity with the remnants of the cell. Disintegrating mitochondria are recognisable by means of cristae remnants. Small vesicles of varying size which occur in great numbers, are apparently derived from endoplasmic reticulum (Fig. 93a). Similar changes are seen in disintegrated mucosal stroma, although the remnants of organelles cannot usually be identified for certain. The appearance of nuclei is characteristic (Fig. 93b). A dense substance derived from chromatin, forms a meshwork with empty interstices. The remaining space inside the nucleus, is occupied by a less dense substance which contains some
poorly defined particles. These disintegrated cell remnants and numerous bacteria are phagocytosed by well-preserved polymorphs.

**DISCUSSION**

When a comparison of ischaemic changes seen in the intestinal mucosa with those seen in other organs is made, it becomes apparent that the former proceed with unusual rapidity to necrosis and disintegration. The appearance of enterocytes after 30 minutes of ischaemia leaves little doubt of their being severely altered. The diffuse decrease of electron density of cytoplasmic matrix, together with the increase of the cell volume and swelling of some cell organelles probably represents cell oedema. This was observed in electron microscopical studies of ischaemic liver (Bassi and Bernelli-Zazzera, 1964), myocardium (Caulfield and Klionsky, 1959; Jennings, Baum and Herdson, 1965), kidney (Thoenes, 1962; Totović, 1966) and accompanies simple post mortem autolysis (Latta, Osvaldo, Jackson and Cook, 1965; Cook, Osvaldo, Jackson and Latta, 1965; Trump, Goldblatt and Stowell, 1962). In some enterocytes, however, particularly those seen on villi which do not show marked epithelium defects after 1 hour of ischaemia, it could be seen that oedema of the cytoplasmic matrix was not accompanied by comparable degrees of swelling of the
endoplasmic reticulum and mitochondria such as is seen in the case of ischaemia and autolysis of other tissues. The mitochondria of enterocytes during ischaemia show some unusual features. Intramitochondrial dense granules remain at least in part, preserved for the first hour of ischaemia. This is at variance with their rapid disappearance in the early stages of ischaemia (Bassi, and Bernelli-Zazzera, 1964) or autolysis (Trump, Goldblatt and Stowell, 1962; 1965a) in hepatocytes. In dog myocardium they are rare or absent under normal conditions and large atypical granules appear in altered cells (Herdson, Sommers and Jennings, 1965; Jennings, Baum and Herdson, 1965). The peculiar changes in mitochondrial configuration seen at 1 hour which consist of densification and strengthening of the outer membranes have not, so far, been described in ischaemia. An exact parallel is difficult to find, even in a wide variety of mitochondrial alterations accompanying other processes (c.f. Trump and Ericsson, 1965b). The swelling, segmentation and vesicular transformation of the endoplasmic reticulum described during ischaemia, oxygen deprivation and autolysis (Bassi and Bernelli-Zazzera, 1964; Jennings, Baum and Herdson, 1965; Herdson, Sommers and Jennings, 1965; Caulfield and Klionsky, 1959; Trump, Goldblatt and Stowell, 1962, 1965d; Stenger, Spiro, Scully and Shannon, 1962; Webster and Ames, 1965) is a common and non-specific change.
In enterocytes during ischaemia the presence of fat droplets within the vesicles of endoplasmic reticulum is seen in addition. This seems to parallel fat absorption in normal cells. It is unlikely, however, that the process represents an incidental fat absorption, since no control animals, starved for the same time, showed comparable amounts of intracellular lipid. It can be assumed that the increase is a manifestation of the disturbed balance between fat uptake and discharge in enterocytes. Our observations suggest that altered cells discharge fat droplets through the plasma membrane of the lateral cell surface. The possibility that the process represents pinocytotic uptake is unlikely. Whether, however, the rate of fat discharge is decreased cannot be ascertained. As reviewed in Chapter 1, the evidence available indicates that triglycerides are resynthesised in the process of fat absorption by the endoplasmic reticulum. Changes in β-lipoprotein deficiency and after ethionine administration suggest that defective protein synthesis leads to fat accumulation in the enterocyte. These processes may not necessarily be destroyed at early stage of ischaemia, an assumption supported by the evidence of continuing protein, phospholipid and fatty acid synthesis in the liver provided adenosine triphosphate is available (Dawkins, Judah and Rees, 1959). A decreased rate or protein synthesis, though fat synthesis continues, would
lead to fat accumulation because of the decreased formation of a phase which can be discharged. Although this would be a hypothetical assumption in considering ischaemia of the gut, it is likely that this mechanism, causing fat accumulation in the enterocytes, operates during ethionine intoxication (Sabesin and Isselbacher, 1965) and in many instances of fat accumulation in the liver (Lombardi, 1965; 1966; Magee, 1966). The possibility that similar mechanisms operate during ischaemia is suggested by an increase of droplets in the endoplasmic reticulum and in the Golgi complex, which was observed in hepatocytes (Bassi and Bernelli-Zazzera, 1964). The structural manifestation of these changes is difficult to demonstrate. Irregularity of ribosomal attachment and vesiculation of the endoplasmic reticulum which might suggest a disturbed proteosynthesis and transport of material within cisternae is also seen during lipid absorption in normal animals. At later stages, the presence of lipid in severely altered enterocytes is likely to be due to its persistence rather than to any continuing formation of droplets.

The source of the lipid contained within enterocytes is unknown. Although it is (in the case of ischaemia) assumed to have an exogenous origin, the possibility of an endogenous release must also be considered. This is likely in the case of large lipid droplets seen without limiting
membrane, which increase in number with the progression of the ischaemic changes. Lipid droplets have been described in some other ischaemic tissues (Bassi and Bernelli-Zazzera, 1964; Stenger, Spiro, Scully and Shannon, 1962). Their appearance was unremarkable, however, and similar to that seen in various other conditions and normal tissues.

The origin of vacuoles which occur in some ischaemic enterocytes has not been determined. In some instances these vacuoles may not be easily distinguished from invaginations of extracellular space into the cell. In general, however, they are related to cytoplasmic oedema, as in the ischaemic liver (Bassi and Bernelli-Zazzera, 1964). Oudea (1963) described vacuoles in hypoxic liver which were of pinocytotic origin. Although this was not demonstrated in the enterocytes it must be considered.

A marked increase in the length of the cisternae of the endoplasmic reticulum with formation of circular cisternae and protrusions of cytoplasmic matrix seen at one hour after re-circulation were not described in other ischaemic tissues but to a much smaller extent were observed during autolysis in liver (Trump, Goldblatt and Stowell, 1965d). The evolution of this change in enterocytes may be associated with temporary cell recovery during an early stage of re-circulation. Most of the enterocytes at this stage show, however, further alterations which do not seem to be
compatible with survival and some are desquamated - hence these alterations may be associated with continuing autolysis, undisturbed by early cell disruption.

The dilatation of the Golgi complex which was observed at early stages is the change described in other ischaemic tissues (Bassi and Bernelli-Zazzera, 1964; Caulfield and Klionsky, 1959) or during autolysis (Trump, Goldblatt and Stowell, 1962; 1965d).

In some enterocytes dilatation of the Golgi complex is undoubtedly due to fat accumulation. Elongation and circular transformation of Golgi cisternae in enterocytes after one hour's ischaemia is an unusual change. A somewhat similar elongation was observed in cerebellar neurons as an early post mortem change (van Nimwegen and Sheldon, 1966; Karlsson and Schultz, 1966) but not in other ischaemic tissues or during autolysis. The mechanism of elongation of the membranes is not obvious but the rearrangement and subsequent fusions of cisternae end to end, seems a likely explanation.

The nuclear changes observed in enterocytes during ischaemia are of the same character as those seen in other studies of ischaemic changes referred to above and in liver subject to autolysis (Trump, Goldblatt and Stowell, 1965c). It is, however, difficult to make any comparison with osmium-fixed preparations as used by previous workers, because
glutaraldehyde prefixation enhances clumping of nuclear substances. Whether the regular outlines of clumped chromatin masses seen in severely altered cells represent a fixation artifact or true configuration of these is unknown. The appearance of nuclei in enterocytes subject to autolysis for 24 hours is similar to that in liver during "in vitro" necrosis, but the density of chromatin is higher, and more interchromatin substance remains. These differences can be easily explained because glutaraldehyde prefixation was used.

The early alterations of brush border are similar to those observed in vitro during autolysis and following the action of hypotonic solutions (Millington and Finean, 1965); in our experiments, however, the distinctiveness of the filamentous core is lost. Fragmentation is frequently observed in damaged enterocytes, particularly after the action of X-rays and cytostatic drugs, as has been reviewed in Chapter 5. It is of interest to compare alterations of analogic structures during ischaemia and autolysis. Microvilli of hepatocytes facing the space of Disse, disappear within 15 mins. of ischaemia and are replaced by oedematous cytoplasm while those projecting into bile canaliculari show slightly later changes (Bassi and Bernelli-Zazzera, 1964). During autolysis, concentric lamellar formations derived from plasma membrane are formed in place of
disappearing microvilli. Similar changes were seen in the brush border of the proximal kidney tubule (Trump, Goldblatt, and Stowell, 1962; 1965d; Ito, 1962a; 1962b; Latta, Osvaldo, Jackson and Cook, 1965). Similar alterations were not seen in the brush border of enterocytes. It seems unlikely that if they were present, lamellar formations were completely voided into the lumen or extracted during tissue processing.

Dispersion of the terminal web constantly accompanies brush border distortion. Widening of the zone beneath the distorted brush border may represent oedema. The origin of dense material beneath this region was not determined. Although with advanced cytoplasmic alteration, collections of ribosomes are observed, their relation to this material is not constant enough to suggest transformation into the latter. Some smaller densities occurring near the distorted plasma membrane are probably desmosomal remnants.

Occasional early changes consist of the uniform increase in the width of the microvilli with prominent microtubules and vesicles beneath the brush border. These may be incidental observations with no relation to ischaemia and appear similar to those seen in multivesicular cells of normal mouse colon (Silva, 1966). The secretory activity proposed by the quoted author is thought to be unlikely.

An important problem is the mechanism by which extrusion
and desquamation of the epithelium takes place in relation to the altered cell. The formation of an initial defect in the epithelium has escaped detection because of sampling errors, but is more likely to be formed by the partial disintegration of altered cells than to the extrusion of cells, the cytoplasmic continuity of which remains preserved. Prior to the disruption in outer layer of the cytoplasm, abnormal intercellular spaces are present in an insignificant extent and during this stage, there is neither gross subepithelial intercellular oedema, nor detachment of epithelial cells from the basement membrane. These changes seem to affect the outer organelle-free abnormal cytoplasm of the enterocyte first, and result in the separation of the cell remnant. At this stage, the basement membrane shows no defects. These events appear to be different to the findings of Sandritter (1965) that the opening of Grünhagen's space by exudation is a primary factor in the epithelial detachment, in intestinal lesions during haemorrhagic shock. When a comparison of the fine structural changes is made with that revealed by light microscopy it is seen that the vacuole-like spaces correspond to the disruption in oedematous outer layer of the cytoplasm and that this leads to desquamation. A similar mechanism of desquamation by disruption of the cell base was seen in the light microscope study of the effects of hypothermia (Black-Schaffer, Gall, Shimizu and Esparza,
It is suggested here that the vacuole-like appearance as seen in paraffin sections is exaggerated.

After one hour of recirculation the continuity of the exfoliated epithelium sheets and the extrusion of individual cells with the preservation of the continuity of the epithelium suggests that at this stage: (1) Epithelial loss is still in progress; (2) A different mechanism of extrusion is operating, presumably related to simple detachment from the basement membrane.

The affected cells are believed to have either temporarily recovered from ischaemia or experienced the process of a more protracted death. These events seem to occur less commonly at the end of the ischaemic period, but because of the combination with cell disruption, are difficult to distinguish from artifacts.

A striking feature of the changes of the villus core is their rapid progress. While early changes, particularly dilatation of the endoplasmic reticulum and mitochondrial swelling may be explained in terms of anoxia, disintegration of cells in the villus core is difficult to explain by anoxia alone. In the myocardium, for instance, no changes of connective tissue were seen after up to 5 hours of ischaemia (Caulfield and Klionsky, 1959). A major factor causing cell damage may be the lytic action of intestinal contents diffusing into the denuded tissue. The noxious
effect of intestinal contents upon damaged tissue was demonstrated by Khanna (1959) and Black-Schaffer, Gall, Shimizu and Esparza (1967) in light microscopic studies of ischaemia and changes associated with hypothermia. Enzymatic activities and ionic composition are responsible for this. Some displacements may be due to the movements of the intestine or may be artificial, but the nature of the changes rules out their being the sole cause of damage.

Endothelial oedema has been observed in ischaemic tissues (Hills, 1964). In the intestine, however, the same change also affects the pericytes and the alterations of cell organelles, are insignificant. The relation of cytoplasmic oedema to enlargement of the Golgi complex and changes in the basement membrane, remains to be determined. An increase in density of the basement membrane associated with other changes was described by Moore (1959) in tourniquet ischaemia, but the comparison of the published photographs with our results is difficult. The further fate of the swollen endothelial cells is also not known. Since it is unlikely that complete regression of oedema takes place before endothelial disintegration, it seems that endothelial oedema is not a constant feature of ischaemia in the gut. The appearance of capillaries will be discussed in Chapter 7.

In areas adjacent to necrosis the change of reticulum cells and macrophages to form pseudopods leaves little doubt
that these changes represent an active response which forms a prelude to phagocytosis. It is unlikely that the change of cell shape reflects an abnormal movement associated with "cell agony" as observed by Bessis (1964). The concentration of organelles seen in some cells is thought to be relative, due to the extensive formation of pseudopods, although an absolute increase of Golgi vesicles cannot be ruled out. The beginnings of necrosis in this zone are, however, indicated by a swelling and disruption of some cell processes.

The prominence of phagosomes within macrophages in this area may also be relative and seems to be due to their increased size rather than to their numbers. The structure of phagosomes is also not compatible with recent origins. Moreover, these cells occur in areas where the disintegration is slight or absent. A tentative interpretation of change in phagosomes might be that there is a fusion of smaller phagosomes to form larger ones. Incorporation of organelles during the formation of larger inclusions cannot be precluded.

Changes in plasma cells are indicative of the development of coagulation necrosis and will be dealt with later.

The alteration of the granules of eosinophilic leucocytes indicates sensitivity of the externum. It is known that acid phosphatase activity is present within granules and was localised in the externum of human eosinophils by
electron microscopy (Ghidoni and Goldberg, 1966). If this is also the case in rat, the appearance would suggest that there is a preferential disintegration of the acid phosphatase containing portions of the granules and possible enzyme release. The lysosomes do not play, however, a primary role in the autolysis of the liver (Trump, Goldblatt and Stowell, 1965b).

The changes of early degranulation in mast cells are similar to those described elsewhere (Bloom and Haegermark, 1965).

The absence of pinocytotic vesicles in smooth muscle cells in the altered zone of the lamina propria is not an unexpected finding. A decrease in the number of pinocytotic vesicles during ischaemia has been described in myocardial cells (Caulfield and Klionsky, 1959).

Alterations of intercellular connective tissue components do not appear to exert a significant role in the pathogenesis of ischaemic changes. The significance of the increased stainability of collagen seen at an early stage, and the increased width of the zone of collagen fibrils beneath the denuded basement membrane, can only be speculated upon. The latter cannot be satisfactorily explained by the mere presence of oedema. This is not compatible with the wrinkling and undulated course of the basement membrane. It is suggested that the cause is most likely to be an extrusion
of necrotic debris from beneath an occasional rupture of
the basement membrane with subsequent partial collapse.
CHAPTER 7

FINE STRUCTURE OF SMALL GUT DURING ISCHAEMIA AND REPAIR OF ISCHAEMIC INJURY II:
THE REPARATIVE PHASE

This phase is arbitrarily defined as the stage between the earliest recognisable form of epithelial regeneration to completion of the reepithelisation. The main processes involved are of both a retrogressive and progressive nature and reflect residues of ischaemic injury, regeneration and reactive inflammation. This stage spreads over a period of 2-8 hours. Most of the fine structural features remain similar throughout this time and differ only in distribution and quantity. The regenerating epithelium is defined for descriptive convenience as cells situated above the crypts which show abnormal features.

CRYPT EPITHELium:

The changes in crypt epithelium consist of a slight mitochondrial swelling and dilatation of Golgi cisternae. Many cells contain multiple large nucleoli. Abnormal inclusions are usually present within or between the cells. The appearance of those seen in the intercellular position leaves no doubt that they are cells undergoing necrosis.

Sometimes a whole cell is seen to be affected by coagulation necrosis (Fig. 94). The cytoplasmic matrix shows a uniform increase in electron density. Ribosomes and the
cisternae of endoplasmic reticulum are preserved, but the mitochondria appear swollen and the inner membranes are partially disintegrated. Some empty vacuole-like spaces in the cytoplasm are seen in the vicinity of indistinct smooth membranes which may represent Golgi cisternae. The nucleus is transformed into a dense homogeneous mass.

In the extra-cellular space, small inclusions of similar appearance are occasionally seen near to large ones (Fig. 94). It is, however, impossible to decide whether these originate from the same cell after breakdown into smaller fragments. In some instances, inclusions in the extracellular position are partially disintegrated (Fig. 95b). Some of these fragments contain dense masses and membrane-bounded granules about 100 μm in diameter.

These necrotic elements were seen in an apparent extra-cellular position only between the basal portions of the crypt epithelium. Some lie directly upon the basement membrane (Fig. 95b), or are separated from it by thin processes of neighbouring cells (Fig. 94); Others have, however, a different appearance. They are distinguished by the very low density of the cytoplasmic matrix. The scanty endoplasmic reticulum is transformed into vesicular profiles and mitochondria show dilatation of intracristal spaces. Free ribosomes form irregular clusters. Chromatin is clumped into meshwork which consists of fine particles. Sometimes the cytoplasm of the affected cell contains a few ribosomes.
Particles of identical appearance are contained in membrane-limited aggregates (Fig. 95a). These cells show more advanced nuclear changes. The mitochondria in these cells may not be severely altered.

The cells of the crypt epithelium contain abnormal inclusions of a variable appearance. The largest inclusions have an almost identical appearance as to free, coagulated cells. Organelle remnants are, however, less distinct (Fig. 96a). A single or double limiting membrane is seen around the perimeter. Although the relation of the inclusions to the cell cannot be determined with certainty, it seems likely that they occupy a true intracellular position. The smaller inclusions which occur in the crypt epithelium, are cytolyosomes (Fig. 96b). Some contain altered mitochondria, endoplasmic reticulum and ribosomes in an aggregate of high electron density. While most mitochondria are swollen, some appear to have increased density and are hardly recognisable. Swollen mitochondria seem, in some instances, to fuse. Narrowing of intracristal spaces in degenerating cristae is rarely observed.

The outer mitochondrial membrane is difficult to differentiate because of the presence of electron-dense material surrounding the mitochondria (Fig. 96b). Many cytolyosomes contain only ribosomes in variable amounts, irregular membranes and a small amount of dense substance. Some of these
cytolysomes fuse (Fig. 97b) and incorporate further structures, like degenerating mitochondria. In other cytolysomes, the clumped particles become ill-defined and fuse into masses which may be homogeneous in appearance (Fig. 97a). Most cytolysomes are situated close to the Golgi complex (Figs. 96b; 97a). The presence of Golgi vesicles in the immediate vicinity of the limiting membrane suggests a possibility of their being discharged into the cytolysome.

Abnormal structures occur in the vicinity of some mitochondria. These are larger and consist of irregular membranes and small vesicles. These, together with small amounts of amorphous substance are enclosed within a space, limited by a triple-layered symmetrical membrane about 80Å in thickness (Fig. 98a). The appearance of these structures indicates that they are altered mitochondria. However, the transitional stages of this process were not clearly identified. Furthermore, the membranes, particularly limiting ones, differ from those of mitochondria. The former are thicker and show a triple-layered pattern, at lower magnification, while the latter do not. These bodies may occur in the immediate vicinity of some cytolysomes and the relation suggests that they are being incorporated into them.

Many crypts contain necrotic cells in the lumen. These either show coagulative necrosis or undergo disintegration after a decrease of electron density and swelling of
organelles. Membranous debris may be seen in close contact with the plasma membrane of the surviving epithelial cell, the membranes forming in places, a tight junction (Figs. 99a; 99b). The microvilli of the epithelial cells underneath are distorted and the bundles of desmosomal filaments are disorganised.

The height of the epithelium in the crypts which contain debris, is uneven in different sections of the perimeter (Fig. 100). In many cases, this is difficult to explain by the amount of debris present and by the section geometry. Disturbed cellular relationships are indicated by the presence of cells overlapping at the luminal surface (Fig. 100). The microvilli of cells lining such dilated crypts are reduced in number. Some cells show decreased density of cytoplasmic matrix and dilatation of the sparse, endoplasmic reticulum.

The most severe changes seen in the crypts is the complete necrosis of segments of the epithelial lining. These are prominent at 2 and 4 hours of recirculation. Most of the cells affected undergo coagulation necrosis and are extruded into the lumen (Fig. 101). Cells seen at the edge show dilatation of the endoplasmic reticulum. They may be flattened. The basement membrane remains continuous. Numerous bacteria frequently occur among the debris (Fig. 102a). These are phagocytosed by macrophages. Polymorphs rarely
occur. Some macrophages outside the crypt send processes which penetrate the basement membrane and extend along the inner aspect to surround numerous bacteria (Fig. 102b). These have an appearance of gram negative bacteria with flagella.

Paneth cells show dilation of the endoplasmic reticulum which is fragmented into vesicular profiles. Mitochondria are swollen. The surface of secretory granules is depressed in some places and the space enclosed by the granule membrane widened (Fig. 103).

Argentaffin cells show severe mitochondrial swelling, while other cell components have a normal appearance.

REGENERATING EPITHELIUM:

Zones of regenerating cells appeared after 2 hours of recirculation above the crypt epithelium. The transitional zone to the crypt epithelium was narrow and difficult to define. Crypt cells near the zone of transition often show abnormalities as illustrated in Fig. 104. It may be seen that the cell apex protruded into the lumen and microvilli are irregularly distributed.

The cells of regenerating epithelium are irregularly arranged. The appearance of epithelial sheet is shown in Fig. 105 in one or two and occasionally more, layers. The relationships of cells in multilayered foci at 2 hours, indicate that their organisation is stratified without any
regular polar arrangement (Fig. 106a). At later stages, however, both stratified and pseudostratified arrangement seem to occur (Fig. 107). Since the cell components throughout the period of 2-8 hours show similar features, a general description will be given.

The cell shape at an early stage is an irregularly rounded one, oblong and slightly elongated (Fig. 106a). The greatest degree of elongation is seen along the advancing epithelial edge (Figs. 108; 109). Elongated cells occur irregularly near the basement membrane and spread along it (Fig. 115). At later stages, the cell shape becomes more regular and the profiles of cells are rectangular or pyramidal (Fig. 107) with a flat (Figs. 116a; 116b) or bulging (Fig. 119) apical portion. The perpendicular orientation of these cells towards the basement membrane indicates a restoration of cell polarity. This is not seen in advancing epithelial cells (Figs. 108; 110). Changes in cells associated with completion of the re-epithelisation are shown in Figs. 111a; 112; 113a. The regenerating epithelium lining the lateral surface of the villus appears to be multilayered and only the cell profiles close to the surface are more regular (Fig. 111a). The apex (Fig. 112) shows polarity of the cells despite the lack of regular orientation, towards the basement membrane. The arrangement shown in this figure is thought to result after the joining of advancing edges of the epithelium and
is an infrequent finding. The appearance of regenerating epithelium at the tip of a villus is shown in Fig. 113a. The apparent polarity, cylindrical shape and size of the cells, suggest that these represent a somewhat later stage than previously illustrated. The cells lining the lateral surface of the same villus show less orderly arrangement than those on the apex (Fig. 114). The cell bodies are orientated obliquely in both directions or elongated in a direction parallel to the basement membrane. The luminal surface is arranged in a more regular fashion.

Despite overlapping of measurements which reflect the grossly irregular shape of cells, it seems that the cell size increases gradually. This increase is most obvious when early and late stages are compared. The cell diameter of the former may be only 5-6 μ, and the latter 11-16 μ x 5-6 μ.

Intercellular spaces between regenerating epithelial cells vary in appearance with the time of regeneration. At early stages the intercellular gap along most of the perimeter measures about 100-250 Å (Figs. 106a; 115). Larger intercellular spaces are not very frequent and occur between cells situated in the basal layer (Fig. 115). They may contain some debris. At a later stage there is a striking increase in the prominence of these spaces (Figs. 112; 113a). The plasma membranes limiting intercellular spaces converge at the sites of desmosomes. Some spaces contain fat
droplets (Fig. 126).

Relation of the epithelium to the sub-epithelial tissue: in most instances, regenerating cells lie upon a basement membrane left after the desquamation of necrotic epithelium. The presence of denuded basement membrane in front of the advancing epithelium, confirms this view (Fig. 109). Defects of the basement membrane are limited in extent. In these places, the cells lie directly upon necrotic debris (Fig. 115). This may also occur at the advancing edge of the epithelium (Fig. 108). Other cells send thin branching processes through defects in the basement membrane to the necrotic tissue (Fig. 98b). The closeness of the relationship between cells and basement membrane is variable. Spaces of limited size occur between cell and basement membrane in places of separation (Fig. 106c). Some cells send small processes by which they become anchored to folds in the basement membrane (Fig. 106b). Large spaces between the basement membrane and epithelium are frequently present at the later stages (Figs. 111a; 112).

Plasma membrane and cell surface specialisation: two hours after recirculation the plasma membranes of regenerating cells were even or slightly undulating and formed only a few short, irregular microvilli which lack filaments in their cores (Figs. 106a; 115). Junctional complexes were rarely seen between cells situated at the base of regenerating zone. Their arrangement and occurrence shows
irregularities (Fig. 122c). Near the advancing edge, and in the intermediate zone of regenerating epithelium, some junctions of a primitive type are present. These consist of dense material subjacent to the plasma membrane and to a smaller extent present in the gap between them. The parallel arrangement and close apposition of plasma membranes appears to precede their formation (Fig. 106d). These junctions apparently differentiate into desmosomes although these are rarely seen at this stage. At 4 hours, and at later stages, junctional complexes are often seen (Figs. 116a; 118). Their presence is associated with a more regular cell shape and orientation. The arrangement is sometimes irregular. Under these circumstances, zonulae occludentes occur along the lateral plasma membrane in many places or do not reach the lumen, nor are they accompanied by further components of the junctional complex (Fig. 122b).

The number of microvilli increases with the progress of regeneration (c.f. Figs. 106a; 116a; 111a; 113a; 121a). While at 2 hours, none, or only few, are present in the plane of section at four hours, many cells have more than 10 microvilli and at 8 hours, 20-30. The increase in height is much less conspicuous. At two hours, the highest microvilli are approximately 0.3-0.4μ long; at 8 hours, they are 0.5-0.6μ long. At the later stages, a greater proportion of microvilli are orientated perpendicular to
the cell surface. Formation of microvilli and the associated modification of surface cytoplasm is best seen in the early stages of regeneration.

In places which lack microvilli, the ectoplasm forms a very thin layer (Fig. 116b). Microvilli appear to be formed in ill-defined groups. The first sign of microvillus formation is a slight elevation of the plasma membrane with some condensation of the fuzzy layer (Fig. 122a). Filaments in the core of the microvillus are rather indistinct and are not seen until the microvillus reached a cylindrical form of about 0.25 μ in height. The concentration of filaments towards the axis of the microvillus is first apparent at 4 hours (Fig. 118). The rootlets of filaments, however, are very short.

At early stages the desmosomal tonofilaments show poor development and orientation and their bundles are ill-defined. These become more prominent and orientated parallel to the plasma membrane at the 4 hour stage mostly in the intermediate and basal zone of regenerating epithelium (Figs. 116a; 117; 118). Some cells also overlap one another at the luminal surface (Fig. 120) and the overlapping projections show filamentous material in the cytoplasm. The cells near the advancing edge of the epithelium even at later stages, are irregularly shaped and lack prominent bundles of desmosomal filaments (Fig. 119).
Thin, less dense filaments may be seen in the surface layer of the cytoplasm, external to the desmosomal filaments. Unlike the normal terminal web, these do not form a defined layer and remain sparse and poorly orientated.

Microtubules beneath the filamentous layer run parallel with it (Fig. 116a).

Cells with polar orientation form frequently processes of variable length near the base which are usually parallel to the basement membrane and partially limit the intercellular space sealing it against basement membrane (Fig. 121b). The interdigitating processes show a regularity of the gap between apposed plasma membranes (Fig. 121b).

The lateral plasma membrane of cells which show polar orientation is straight or slightly irregular (Fig. 121a). The number of desmosomes is small. Their filaments run parallel to the lateral cell surface; microtubules in the superficial layer of the cytoplasm show a similar orientation (Fig. 118).

The cytoplasmic matrix in regenerating epithelium varies in electron density (Figs. 118; 127). These differences are not observed at the earliest stages of regeneration nor are they obvious after re-epithelisation is complete. The increase in density is due to ground cytoplasm and ribosomes. Ribosomes are generally numerous (c.f. Figs. 108; 115; 116a; 117; 118; 120; 121a). They form rosettes which
consist of 3-8 particles. Rarely, small groups of ribosomes resemble helicosomes. Ribsomal rosettes are distributed evenly and with the exception of the zone of filaments occur even in the most superficial layers of the cytoplasm (Fig. 116b). Fat droplets without limiting membranes occur frequently and are variable in size (Figs. 110; 115; 119). They may be seen in contact with mitochondria.

Rough endoplasmic reticulum is sparse. Small numbers of narrow cisternae of irregular course are present some of which show a close relationship to the mitochondria (Fig. 115). Some cisternae have a circular profile. The cytoplasmic matrix enclosed does not appear to be altered (Fig. 110). At later stages, occasional cisternae show slight dilatation (Fig. 117). Some narrow cisternal profiles reach abnormal length and their close relation to the mitochondria is exaggerated (Figs. 116a; 121a).

Mitochondria are not numerous and the arrangement is not regular. They have a round or ovoid shape. Cristae are irregular and intramitochondrial dense granules are rare and the matrix is about the same density as is the cytoplasm or shows mottling (e.g. Figs. 109; 115). At the earliest stages, only a small proportion of the mitochondria are swollen. Later, the swelling is more severe, though variable in distribution (c.f. Figs. 116a; 117; 119; 120). Occasionally, swollen mitochondria occur in groups (Fig. 118).
In severely swollen mitochondria matrix disappears, the cristae are shortened, detached and partially defective.

At the 8-hour stage when complete re-epithelisation has taken place, an increase in density of mitochondrial matrix is consistently seen (Fig. 121a).

Golgi complex. This is well developed. At early stages, its position is irregular. In cells with polar orientation, it is supra-nuclear. The stacks of cisternae are often multiple and appear in various places without regular orientation. Some may be seen near to the lateral cell border (Fig. 117). Dilatation of some cisternae is consistently found (Figs. 120; 125). The number of vesicles, most of which are coated, is variable. Some coated vesicles were seen in positions remote from the Golgi area in the superficial layer of the cytoplasm. Fat droplets are occasionally seen within the cisternae. At later stages of reepithelisation, many cells show a heavy accumulation of fat droplets within Golgi cisternae (Fig. 121a). With increasing size and coalescence of droplets, cisternae are transformed into vacuoles which extend towards the intercellular space (Fig. 126). Near by cisternae of the endoplasmic reticulum are free of lipid.

Inclusion bodies. Inclusion bodies of different types are very numerous. They are similar to those seen in crypt epithelium and only additional features are described here.
They can be classified as phagosomes or cytolysomes. The origin of polymorphous residual bodies or cytosomes, which are formed during degradation, cannot be determined with certainty. The largest inclusions result apparently from phagocytosis of other necrotic cells (Fig. 123a). These inclusions may reach up to 5-6 μ in diameter and are usually rounded. A limiting membrane is constantly present. The remnants of necrotic cells show different degrees of swelling, clumping and disintegration of organelles. Most of them contain pyknotic nuclei or smaller rounded masses, probably resulting from karyorrhexis. This material is always homogeneous. In some instances, many rounded membrane-limited dense granules of 200-300 μ in diameter of unknown origin are scattered between other remnants. The abnormal phagocytic activity of regenerating epithelium is demonstrated by the presence of inclusion seen in Fig. 123b. This can be identified as parasitic protozoon with cilia.

The most common component of cytolysomes are ribosomes and mitochondria. The appearance of some cytolysomes indicates that they increase in size by the repeated incorporation of components. Most cytolysomes show advanced stages of degradation and condensation of the contents to form residual bodies or cytosomes (Figs. 115; 117; 118; 125). These consist of dense non-homogeneous material, membranes,
vacuoles and vesicles in various proportions. Cytosomes are occasionally formed by dense aggregates of vesicles (Fig. 116a) and they resemble, in appearance, bodies described in the normal crypt epithelium (See Chapter 4).

Large inclusions frequently have a vacuolar appearance. Some of them contain varying numbers of vesicles, 300–500Å in diameter (Fig. 125) and appear similar, apart from their larger size, to the abnormal inclusions described in crypt epithelium (see this Chapter). Smaller inclusions with densely accumulated vesicles appear to be transitional stages in the formation of cytosomes (Fig. 118). The relationship of inclusions to the Golgi complex is similar to that seen in the crypt epithelium.

The nuclei of the regenerating epithelium are relatively large and irregular in outline (c.f. Figs. 106a; 110; 112; 113a). The indentations of the nuclear membrane are wide and not usually deep. Chromatin is condensed at the periphery of the nucleus in medium amounts. Frequently large or multiple nucleoli are seen. Usually, however, the nucleolus is obscured by a well-developed nucleolus-associated chromatin. Inter-chromatin granules are prominent and may form in some nuclei small aggregates (Fig. 119). Some cells are bi-nuclear.

Goblet cells. These are seen in various degrees of development from the 4-hour stage of recirculation onwards.
The first signs of differentiation are indicated by increased amounts of regularly arranged profiles of rough endoplasmic reticulum and dilatation of Golgi cisternae to form vacuoles (Fig. 113b). These contain a finely filamentous material representing early mucous granules. Coated vesicles are seen between cisternae and granules. A well developed endoplasmic reticulum is present over the whole cytoplasmic volume or limited to an area round the Golgi complex. The cisternae are slightly dilated by contents which are usually denser than the cytoplasmic matrix. Cisternae may form concentric layers around the nucleus (Fig. 126). Many goblet cells show abnormalities. The irregularities of cell shape and position are similar to those seen in regenerating epithelial cells. In abnormally-shaped cells, mucous granules occur along the luminal surface without forming a "goblet" structure. Some granules may be abnormally dense. The endoplasmic reticulum is seen close to granules without an intervening Golgi complex (Fig. 129a). The cytoplasmic matrix is dense and contains numerous free ribosomes. In some goblet cells, the cytoplasm shows a marked decrease in density and the endoplasmic reticulum is irregularly dilated (Fig. 129b). Mitochondria may be altered. Cytosomes of polymorphous appearance occur frequently. They may be closely associated with mucous granules and some are seen to coalesce with them (Figs. 129a; 129b).
Argentaffin cells. A few argentaffin cells are found in regenerating epithelium. Some contain small numbers of granules in a clear cytoplasm. The cytoplasm of some argentaffin cells does not show this decrease in density and is more voluminous. The granules are numerous and concentrated at the cell base. Their shape is, however, irregular. Cytoplasmic filaments are interwoven to form bundles and are present in large amounts (Fig. 130).

Many cells in regenerating epithelium show degenerative changes which prelude cell death. The initial change consists of gross mitochondrial swelling and dilatation of the endoplasmic reticulum (Figs. 107; 128). The proportions vary; in severely altered cells the cytoplasmic matrix shows a marked decrease in electron density and the cell disintegrates. An increase in fat droplets and cytolysomes is not consistently seen and nuclear changes may be variable. Most of the degenerating cells occur in the surface layer of the epithelium where two layers are present (Fig. 105). Some cells became detached without showing a decrease in density and disruption (Figs. 110; 127).

Most of the cells which appear to be first in an advancing row of the epithelium form flat cytoplasmic projections which spread along the basement membrane in front of the cell body (Figs. 109; 110). These projections may show invaginations of the plasma membrane (Fig. 108). Signs of
the development of an internal cell organisation such as may be seen at later stages in adjacent cells are absent.

At the 2-hour stage, altered goblet cells are occasionally seen at the front of the epithelium sheet. These cells are thought to be left from the original epithelium. At the 8-hour stage, goblet cells were sometimes found to be in the advancing edge of the epithelium sheet (Fig. 124). These are similar to goblet cells, formed in other places of the regenerating epithelium. In some instances a detached goblet cell is situated close to the advancing projection of the first cell (Fig. 108).

The aberrant nests of epithelial cells vary in size. The largest, which may contain up to three layers of cells, may be distinguished with difficulty from tangentially-cut adjacent epithelial sheets. The organelles of cells forming these hyperplastic foci do not show appreciable differences from cells present elsewhere (Fig. 127). They lack, however, signs of arrangement which would indicate the development of internal polar organisation and degenerative changes are commonly observed. In multilayered hyperplastic sheets, these degenerative changes affect cells which form the outer layers; those centrally situated may be spared (Fig. 127).

The relationships of cells which form bridges or synechiae are variable, for the area of contact has a different
width, occasionally forming only a thin bridge (Fig. 125). The cells facing a space limited by synechiae are often orientated towards this, resulting in the formation of crypt-like structures (Fig. 128). Well-differentiated goblet cells with their free surface facing the recesses of the lumen are present in these places. The shape of goblet cells may be atypical.

A relationship between cells of adjacent epithelial sheets which is difficult to interpret is seen in Fig. 111b. Here both cells are separated by a narrow gap containing small amounts of debris. Whether this relationship precedes bridge formation or results from the disintegration of a cell forming an anastomosis, is difficult to determine. A vacuole within a cell seems to communicate with the extracellular debris, suggesting that there is engulfment or release of this material.

In order to follow the progress of changes in necrotic enterocytes, the pseudomembrane formed at 2 hours after recirculation was examined. The enterocytes altered show mitochondria and vesiculated endoplasmic reticulum with some ribosomes attached. Many fat droplets are present in vesicles and in the dilated perinuclear cisternae (Fig. 83a). The pyknotic nucleus has a homogeneous appearance. In contrast to the enterocytes, goblet cells usually show fewer changes and are comparable to those seen at the end of the
Lamina propria

The appearances of necrotic tissue show minor variations which cannot be precisely correlated with a time scale. This seems to be of little importance since the character of changes seen underneath the area of necrosis does not seem to be related to time. In some places, outlines of disintegrated cells can still be traced in the debris, while in others, disintegration is more homogeneous. Non-haemolysed and haemolysed erythrocytes are very numerous (Fig. 131a). Some membrane-limited vesicles are seen to be remnants of endoplasmic reticulum; others, consisting of the twisted segments of membranes, which occur in irregular pairs and occasionally fuse, seem to be derived from mitochondria. The segments of membranes show a symmetrical, triple-layered "unit membrane" substructure, approximately 90Å in thickness. Amorphous debris is interspersed or partially enclosed within membrane segments (Fig. 131b). Some particles in this debris are identical to ribosomes. The membranous and amorphous debris contains numbers of fat droplets about 150-200μm in size. The amount of fibrin is extremely small (Fig. 108). Granules of disintegrated eosinophil leucocytes present in the debris are readily identified. Disintegrated parasitic protozoa were occasionally found.
At later stages, the remnants of the necrotic tissue are permeated by polymorphs and macrophages. The former are more numerous and show a higher density of the cytoplasmic matrix than the latter (Fig. 132a). Both contain numerous phagosomes, which attain a larger size in macrophages. The debris contained in most of the phagosomes has a more condensed and homogeneous appearance than that which occurs outside the cell (Fig. 132b). The phagosomes in macrophages are usually situated near the Golgi complex and vesicles are seen between both. Other phagosomes contain granules. This is also seen in polymorphs. Polymorphs show frequently degenerative changes. The number of "specific" granules in some cells is decreased. This change is associated with the presence of vacuole-like spaces. These lack limiting membranes and show contents which consist of irregular short filaments about 150-200Å in width which appears as dots when cut transversely. Some of these abut upon the wall of the space (Fig. 133a). Similar dot-like structures in the cytoplasm are seen to be surrounded by a thin halo, but cannot be differentiated from ribosomes. "Specific" granules may be seen close to these vacuole-like spaces. These spaces vary in size and their configuration indicates that their fusion has occurred. Membrane-limited vacuoles of an empty appearance may be seen in addition. Another change frequently seen in exuded polymorphs is
pyknosis and karyorrhexis (Fig. 133b). The nuclear lobes are rounded and condensed, the chromatin sharply delimited from small amounts of nucleoplasm which form a crescentic mass adjacent to the nuclear membrane.

The rounded nuclear segment is displaced towards the periphery of the cell and the layer of cytoplasm, which intervenes between it and the plasma membrane may become extremely narrow. However, the rupture of the cell and extrusion of the pyknotic segment was not observed. Some cells show membrane-bounded vacuoles and contain free, large lipid droplets.

Macrophages show degenerative changes less frequently. These consist of dilatation and vesiculation of the endoplasmic reticulum, the appearance of free lipid droplets in the cytoplasm and disruption of the plasma membrane. Clusters of vesicles surrounded by vacuoles are occasionally seen, derived, possibly, from the Golgi complex. These degenerative changes do not seem to be associated with phagocytosis.

Blood vessels in the zone of necrosis show characteristic changes. The lumen is obstructed by non-homogeneous irregular masses of varying electron density. The surface of these masses which are apparently haemolysed erythrocytes is partially surrounded by membranes (Fig. 134a). Numerous membranes are seen external to these remnants. The appearance
is characteristic. The membranes are triple-layered, symmetrical, approximately 80 Å in thickness. They are arranged in pairs which, when running parallel, enclose a layer about 90-150 Å thick (Fig. 134b). This consists of material similar to that seen in erythrocyte remnants. The paired membranes form, in places, large arrays which consist of whorls or are concentric. Close to the remains of the erythrocytes and membranes, granule-like structures or "spherules" are seen, the size of which is variable, but mostly about 100 μm in diameter, (Fig. 134a). These are bounded by a triple-layered membrane and contain material, the density of which varies between that of erythrocytes and their remnants (Fig. 134b). In most cases, "spherules" are concentrated in the places where the endothelium is absent. Sometimes, remnants of endothelial cytoplasm, lying nearby "spherules" are seen (Fig. 135a). Preserved erythrocytes are, however, rare in thrombosed vessels. The basement membrane shows poor definition (Fig. 134b). "Spherules" may be seen external to the basement membrane (Fig. 135a). Further similar structures occur in the debris near to vessels, but these are not thought to have migrated from the vessels.

The delimitation of necrosis towards surviving tissue is not sharp. The transitional zone shows less advanced disintegration of cells and the degree of alteration in
individual cells may vary grossly. Most of the preserved cells in this area are macrophages.

Plasma cells show a characteristic change which can be classified as coagulation necrosis. Such cells are occasionally seen in the debris of the upper zone of necrosis, but most frequently they are being phagocytosed in its edge (Fig. 136). Both free and phagocytosed fragments of plasma cells appear as round, dense bodies, most of which contain recognisable cell constituents. They are similar to those observed in necrotic plasma cells in normal lamina propria, but their features are more variable (Fig. 137a). Occasionally the mitochondria appear normal, but in most instances they are swollen and contain only remnants of cristae and matrix. Endoplasmic reticulum varies from a normal cisternal to a purely vesicular appearance. The Golgi complex is transformed into vesicles which may be displaced to the periphery of the fragment. Granules associated with the Golgi complex remain unchanged. The nuclear remnants are usually very dense and homogeneous. In rare instances, when the density does not obscure the structure, discrete bundles of thin filaments may be seen in the nucleoplasm. Some free plasma cells with a slightly denser cytoplasm than normal have a nucleus with condensed chromatin which shows regular delineation from the nucleoplasm (Fig. 137b). Whether this change precedes necrosis is uncertain. The
macrophages which contain phagocytosed plasma cells frequently have a dilated endoplasmic reticulum and the layer of the cytoplasm which intervenes between the phagosome and the plasma membrane, may be very thin (Fig. 138b). Some macrophages contain several large inclusions of variable appearance (Fig. 138a). Loss of definition between the endoplasmic reticulum and the dense matrix or its disappearance is usually associated with the further degradation of the phagocytosed plasma cell. Some inclusions consist of coarse particles about 300-400\AA in diameter which may coalesce, membranes and amorphous dense material. Particles and membranes are sometimes arranged into parallel arrays (Fig. 138b). Their appearance is similar to that which was seen in the cell fragments of normal lamina propria, (c.f. Fig. 38).

It is thought that some of the less characteristic inclusions may represent phagocytosed lymphocytes.

Surviving plasma cells contained few membrane-bound inclusions which can be classified as cytolysomes (Fig. 139). These contain rounded fragments of the cisternae of endoplasmic reticulum with a thin rim of cytoplasmic matrix. Cytolysomes are situated close to the Golgi complex. Some phagosomes, which contain plasma cells may be seen lying close to surviving plasma cells. This relationship is distinguished from the formation of large cytolysomes or external
extrusion of an altered cell portion by the presence of a thin layer of a macrophage cytoplasm which intervenes between both. The distinction may be obscured by dilated endoplasmic reticulum around the inclusion in the cytoplasm of the macrophage.

A few plasmocytes, on the other hand, undergo necrosis and disintegration which has not a coagulative character. The endoplasmic reticulum of these cells is transformed into vesicles.

Mast cells usually show advanced degranulation. Vacuoles which result from this process contain traces of amorphous precipitate or some granule remnants (Fig. 140a). The limiting membrane of these vacuoles is discontinuous and they frequently fuse. Discontinuities in the cytoplasm surrounding the vacuoles are seen; these may in part be artificial. The remaining granules are homogeneous and electron dense, but a decrease in density with the appearance of substructure may occur in some granules. The sparse endoplasmic reticulum is dilated (Fig. 140b). Some mast cells in the margin of the necrotic zone show similar changes but the cytoplasm is dense. These cells seem to undergo necrosis, resembling that of plasmocytes.

The granules of eosinophilic leucocytes show alterations similar to those described during the ischaemic stage. Advanced disintegration of the externum results in release
of the crystalline internum (Fig. 141b). The longitudinal periodicity of the crystal is retained. Alterations of granules, thought frequent, do not seem to be related to the cell damage. Released, unaltered granules may be seen in the debris. Some eosinophils, on the other hand, are not, apart from granules change, severely altered (Fig. 141a). Some cells show marked nuclear pyknosis with separation of the chromatin from nucleoplasm.

The appearance of capillaries and small venules varies. Some are unchanged and contain individual polymorphs or platelets. Endothelial cells sometimes show numerous small projections into the lumen and caveolae which are larger than normal. These may have an appearance of large vesicles. In other vessels there is a wide gap between endothelium and pericyte in which deformed erythrocytes or their large fragments, amorphous precipitates, "spherules" and membranes identical in appearance to those described above, were seen (Fig. 142a). The gap may communicate with the perivascular space. Continuity with the lumen is rarely detected. The basement membranes show defects and their remnants are, to a variable degree, obscured by precipitated material. This material consists in places of indistinct fibrils which did not show a periodicity, which would indicate fibrin deposition (Fig. 142a, arrow). The endoplasmic reticulum in endothelial cells is dilated and some mitochondria swollen.
The vessels which show gaps are mostly situated in the zone of severely altered tissue. Polymorphs were seen migrating between endothelium and pericytes.

Vessels adjacent to the zone of necrosis show platelet thrombosis. Rarely an agglutinate of platelets and polymorphs are seen without obstructing the lumen completely. Any gaps between the endothelium were covered by cell processes or thrombocytes (Fig. 142b). Regardless of the condition of the vessel wall, the "spherules" were seen in all stages of their passage through the basement membrane into surrounding tissues, (Fig. 135b).

Lymphatic vessels which were seen in this zone show dissociations of endothelial cells. The gap is filled by debris.

The changes in smooth muscle cells underneath the necrotic zone, consist mainly of the prominent dilatation of endoplasmic reticulum. The ribosomes attached to the membranes are distributed irregularly and are usually absent along the aspect of the cisternae facing the zone of myofilaments (Fig. 143a). Further changes consist of gross mitochondrial swelling and formation of cytolysomes (Fig. 143b). The number of pinocytotic vesicles along the plasma membrane seems to be decreased in affected cells.

Under the light microscope, the core of some villi after complete reepithelisation is formed by a layer of oedematous
granulation tissue or contains fluid with polymorphs, macrophages and erythrocytes. Electron microscopy shows that the transition of the lamina propria into the area of oedema is rather sharp, and only a few cells and some processes are detached from the zone of compact tissue. Some endothelial cells in the transition zone are found which show many processes projecting both into the lumen and surrounding tissue. The basement membrane is absent (Fig. 144).

The changes present in the zone of reactive inflammation recede in the base of the lamina propria. Many areas appear normal apart from frequent phagosomes (Fig. 145). These are sometimes of large size, but their origin cannot be determined. Occasionally early stages of cytolysomes were found in reticulum cells after 2 hours of recirculation.

Changes in the smooth muscle cells of the tunica muscularis were observed after four and eight hours of recirculation. At four hours, abnormal large vesicles were seen beneath the plasma membrane. Some are rounded or irregular, but most are flattened (Fig. 146c). Narrow communications with intercellular spaces were seen. Mitochondria in some cells are swollen. At 8 hours, the plasma membrane shows extensive invaginations resulting in the formation of large spaces which apparently form vacuoles after a separation of the membranes (Fig. 146b). These vacuoles were only seen in the periphery of cells. In the perinuclear cytoplasm,
cytolysomes occurred which contained cytoplasmic matrix or mitochondria in various degrees of degradation (Figs. 146d; 146e).

Neurons of Auerbach's plexus show swollen mitochondria, moderate dilatation of the Golgi cisternae and the endoplasmic reticulum (Fig. 146a). A few Golgi cisternae contain dense material which resembles primary lysosome formation. Unlike neurons no consistent changes were seen in the nerve fibres of the plexus.

A discontinuity was rarely observed in the layer of mesothelium. The defect may be artificial.

DISCUSSION:

Epithelial changes:

The factors which determine the rate of repair depend on the extent of the preceding cell loss and kinetics of epithelial proliferation. Completion of reepithelisation takes place most frequently 8-16 hours after recirculation. At this stage, the height of the zone of the regenerated epithelium is lower than the depth of crypts. Provided that cell cycle of an epithelial cell averaging $10\frac{1}{2}$ hours (Cairnie, Lamerton and Steel, 1965a) is not grossly changed as a result of ischaemia, one cell cycle in the proliferative crypt zone is sufficient to produce all the cells in the regenerated zone. There is a striking rarity of mitoses in the zone of regenerated epithelium, suggesting that most
of the mitotic activity takes place in the crypts. In terms of the "slow cut-off" model proposed by Cairnie, Lamerton and Steel (1965) (See Chapter 1) the zone associated with the "critical decision phase" is displaced upwards or absent to meet the increased demand for cell production. Another factor which may influence the rate of regeneration is the synchronisation of mitotic activity. It is unknown whether this occurs.

The influence of different factors on the appearance of regenerating cells is difficult to determine. Similar difficulties occur in all observations and the general interpretations of fine structural changes, associated with regeneration, are not unanimous. The cell changes were thought to be the result of the injury associated with the operation (Fisher and Fisher, 1963). Jordan (1964), Becker and Lane (1965), and Bernhard and Rouiller (1956) believed that changes preceding and associated with the stage of the proliferation represent an active response. The secondary influence of disturbed tissue relationships and changes in tissue components such as those caused by inflammation have to be considered. The changes in regenerating cells at later stages reflect differentiation. The literature offers little help in terms of fine structure, and the evidence available mostly concerns regenerating liver.

The crypt and the regenerating epithelium can be regarded
as one system, so that separate discussions are not made unless necessary. The term "regenerating epithelium" is used to describe cells which are situated above the crypt epithelium. A comparison of the cells of the regenerating epithelium at an early stage with those of crypts indicates that the most striking change is a loss of a regular organisation and simplification of surface structure. This modification can be classified as dedifferentiation. The mechanism of surface simplification is difficult to determine. In liver cells at early stages of regeneration, microvilli are lost by fusion and sequestration into the space of Disse. The ectoplasmic layer disappears at the same time (Lane and Becker, 1966). No signs of a similar process were seen in the intestine where the surface structures are more complex. Here the disappearance of microvilli seems to be associated with the loss of a polar organisation of cells in the border between crypt and regenerating epithelium. Microvilli may disappear by being distorted during the change of the cell shape and orientation. Simplification of the surface in liver cells was thought to be a sign of dedifferentiation preceding cell division (Lane and Becker, 1966). This assumption is less plausible in the intestine. The first cells of regenerating epithelium to show simplification of the cell surface have migrated from the uppermost zone of the crypts. According to Cairnie, Lamerton and Steel
(1965a; b), these cells are mostly nonproliferative elements after a "critical decision phase". On the other hand if the first cells migrated into the regenerating zone before the "critical decision phase" these are normally proliferative. This may be decided by labelling with tritiated thymidine. Abnormalities of the cell apex in the upper zones of the crypt, however, suggest that cellular modification takes place in this area, though whether this change preludes loss of microvilli is uncertain. These changes somewhat resemble those seen in sprue in the same zone (Rubin, Ross, Sleisenger and Weser, 1966) where cells above this area also have defective microvilli.

The representation of organelles of cells in the regenerating zone resembles that seen during regeneration in other tissues. There is an abundance of free ribosomes and the endoplasmic reticulum is present in reduced amounts (Stenger and Confer, 1966; Jordan, 1964; Price, Howes and Blumberg, 1964). This is known to be common to undifferentiated and proliferating cells (See Porter, 1961). Increased RNP synthesis demonstrated in the regenerating liver (See Bucher, 1963) is correlated with the increased free ribosomes. A close association of endoplasmic reticulum and mitochondria was frequently observed in regenerating liver (Bernhard and Rouiller, 1956; Jordan, 1964; Stenger and Confer, 1966; Virágh and Bartók, 1966) but is not specific to this process
for it may be seen to some extent in normal cells or in other conditions. Dilatation of the endoplasmic reticulum was seen in regenerating liver (Virágh and Bartók, 1966; Stenger and Confer, 1966). In the cells of regenerating intestinal epithelium, dilatation is moderate and inconsistent unless the cell shows further signs of degeneration. Appreciable increases in the amount of the endoplasmic reticulum were not seen during the period examined, although at the end of reepithelisation cisternae are frequently longer. Enlargement of the Golgi complex of regenerating epithelium has been seen in hepatocytes (Stenger and Confer, 1966; Virágh and Bartók, 1966). The frequent association of this finding with autophagocytosis in intestine suggests that enlargement may be related to this process (see below). An accumulation of fat droplets within the Golgi complex during regeneration was described in liver (Jordan, 1964; Trotter, 1965). Here it accompanied the presence of fat in the endoplasmic reticulum or within small vesicles. The fat was believed to have entered the cells by pinocystosis. In the intestine, the sparse endoplasmic reticulum is free of lipid and only a few vesicles, which contain fat, are present above the Golgi complex. This will be discussed in the next Chapter. The location of fat-containing vesicles and vacuoles close to the plasmalemma, suggests a discharge of fat into the intercellular space.
Mitochondrial swelling may accompany regeneration in liver (Virágh, and Bartók, 1966; Jordan, 1964; Fisher and Fisher, 1963) and enzymatic activity is decreased (see Viragh and Bartok, 1966). In our material the evidence suggests that swelling is a secondary change due to disturbed relationships between proliferating epithelium and the altered circulation in the stroma. In some cases, however, the epithelium covering oedematous villi which lack any vessels does not show mitochondrial swelling. The increase in the density of mitochondrial matrix which occurs later, appears to be associated with the later phases of reepithelisation and will be discussed later. The close relationship of mitochondria to lipid as seen at the early stages, was described in regenerating hepatocytes (Stenger and Confer, 1966). This relationship is non-specific, however, and has, since Palade's report (1959) been observed in many conditions. Increased numbers of fat droplets in the cytoplasm have been described frequently in liver regeneration (Stenger and Confer, 1966; Jordan, 1964; Virágh and Bartók, 1966; Fisher and Fisher, 1963), but their relationship to other changes is not precisely known.

Variations in electron density of the cytoplasm of regenerating cells were described in liver by Viragh and Bartok (1966), in association with differences in the contents of organelles. Regenerating intestinal epithelial
cells do not show similar differences in the representation of organelles.

The appearance of the nuclei is similar to that of regenerating liver cells (Stenger and Confer, 1966; Jordan, 1964) where ultrastructural features were correlated with biochemical changes of nucleoproteins. Nuclear changes appear to be associated with proliferation. Aggregation of interchromatin granules, such as were seen in regenerating cells, has been seen to a larger extent in cancer cells (Bernhard and Granboulan, 1963) and in preneoplastic liver during ethionine intoxication (Miyai and Steiner, 1965).

The retrogressive changes during regeneration which are represented by autophagocytosis and cell necrosis, affect crypt and regenerating epithelium. Cytolysomes cannot sometimes be distinguished from phagosomes. In liver, regenerating after partial hepatectomy, lysosomes increase in number (Jordan, 1964). This is held to be the result of the experimental injury (Fisher and Fisher, 1963). Autophagocytosis observed by Becker and Lane (1965) in liver, was believed to represent a form of dedifferentiation preceding mitotic activity but later the same authors concluded that it is secondary, probably due to the mitotic stimulus (Becker and Lane, 1966). In the present study, there is little reason to doubt that cytolysomes result from sublethal ischaemic injury to the epithelium, similar to that seen in
in liver during hypoxia (Glinsmann and Ericsson, 1966; Confer and Stenger, 1964). The appearance of cytolysomes is similar to that observed in the crypt epithelium by Hugon and Borgers (1965a; 1966a) after X-ray irradiation.

Some circular membranous profiles which enclose matrix suggest the formation of cytolysomes, but their further fate is not obvious. The derivation of the limiting membrane was not determined. The relationship between the cytolysomes and the Golgi cisternae and vesicles is similar to that observed in the crypt epithelium by Hugon and Borgers (1965a). These authors demonstrated increased acid phosphatase activity in Golgi vesicles during cytolysome formation (Hugon and Borgers, 1966d). The opinion prevails that the membranes of cytolysomes in various cells are derived from the endoplasmic reticulum (Novikoff and Shin, 1964; Novikoff, Essner and Quintana, 1964; Hugon and Borgers, 1965a; Ericsson, Trump and Weibel, 1965; Ericsson and Glinsmann, 1966; Glinsmann and Ericsson, 1966). The relationship of the Golgi complex to cytolysomes and the role of Golgi vesicles as primary lysosomes in the transfer of enzymatic activity has also been recognised (c.f. Novikoff, 1963; Brandes, Buetow, Bertini and Malkoff, 1964). Whether any pre-existing cytosomes (= lysosomes) were incorporated into cytolysomes, as was suggested in the crypt epithelium by Hugon and Borgers (1965a) was not determined. Cytolysomes
which mostly contain ribosomes correspond to the "les amas ribosomiques" of Hugon and Borgers (1965a). Their frequency may be due to the high number of ribosomes present in the crypt and regenerating epithelium. More complex bodies may result from the fusion or incorporation of altered organelles, such as mitochondria. This suggests that mitochondrial alteration may progress during the period of regeneration.

The large vacuolar bodies which contain vesicles are believed to represent a form of cytolysomes. Cytolysomes containing aggregates of vesicles were observed in foetal intestinal epithelium by Behnke (1963a). Some small bodies may be derived from altered mitochondria, although transitional stages are difficult to find. Multivesicular bodies of normal appearance were not present.

Cytolysomes in the intestinal epithelium appear to develop with the progressive degradation into cytosomes. This parallels observations in other cells (Glinsmann and Ericsson, 1966; Ericsson and Glinsmann, 1966; Confer and Stenger, 1964; Ericsson and Trump, 1964; Ericsson, Trump and Weibel, 1965; Novikoff and Shin, 1964; c.f. de Duve, 1963). It is possible that extrusion of cytolysomes into the lumen takes place as was suggested by Hugon and Borgers (1965a) in crypt epithelium and Swift and Hruban (1964) in pancreas. The material seen extruded in the crypts had
however, the appearance of necrotic cells.

The necrotic cells were seen to be incorporated into the epithelium as phagosomes after X-irradiation by Hugon and Borgers (1966a; b). In our material similar inclusions appear in the regenerating epithelium and are less complex than those described by these authors. In crypts, densified cells are thought to be epithelial in origin, while those which have a decreased density or are disintegrated, are likely to represent degenerated migratory cells. The phagocytosed cells with granules seem sometimes to be polymorphs.

The appearance of the crypt epithelium affected by coagulation necrosis is similar to that seen elsewhere. The bibliography relevant to the fine structural features of this process was discussed in Chapter 3. Our evidence suggests that lytic necrosis with cell disintegration is more frequent in regenerating epithelium, while the coagulative necrosis is seen more commonly in the crypts. Whether this reflects a cell or environmental difference cannot be determined. Flattening of the crypt epithelium in the colon was observed by Florey (1960), after stimulation of the mucosa. In our material this is thought to accompany the more severe changes in other segments of the same crypt. Extensive necrosis of the epithelium is believed to be the result of ischaemic injury. There is little tendency to cause secondary inflammation around the affected crypt.
Unfortunately no fine structural observations are available to compare changes where the alterations of the crypt epithelium are associated with marked exudation, as in "crypt abscesses".

The comparison of sequential stages of regeneration indicates that differentiation takes place with regeneration. The double-layered arrangement of cells during regeneration may be taken as evidence of hyperplasia and suggests uncoordinated mitotic activity in the regenerating epithelium. Hyperplasia is reduced at later stages, by degeneration and necrosis of cells. Cell necrosis in hyperplastic regenerating epithelium does not necessarily represent a "pathological process". Evidence is now available that cell death has a general significance in normal morphogenesis during foetal development (see Saunders and Fallon, 1966).

A clear sign of differentiation is the restoration of polar organisation and development of surface specialisations in regenerating epithelium.

Intercellular adhesion is a feature of epithelial regeneration. Intercellular spaces which are present at early stages, appear to be the result of abnormal cell shape.

Adhesion is an important factor in differentiation (Mercer, 1965; Saxen and Wartiovaara, 1966; Moscona, 1962; Weiss, 1960). The relationships of cells of the regenerating intestinal epithelium are complex. It is possible, however, to
compare them with relatively simple systems as studied by Mercer (1965). Primitive cell contacts and desmosomes are formed at stages where there is no polar orientation of cells, suggesting that cell stabilisation occurs before further differentiation, similarly, as postulated by Saxen and Wartiovaara (1966). There is a tendency to sealing of the intercellular spaces between regenerating epithelium as measured by electrical means in urodelle epidermis (Loewenstein and Penn, 1967). The development of cell polarity is associated with the formation of junctional complexes and microvilli. The irregular arrangement of the components of the junctional complex may result from movement of cells during their formation. The development of microvilli suggests that the formative stimulus act primarily upon the plasma membrane since the core structure appears relatively late. This sequence is similar to that observed in normal chicken foetal intestine (Overton and Shoup, 1964).

The stimuli which determine the polar organisation of epithelial cells cannot be ascertained from static observations. Contact with the lumen, resulting in an environmental difference is suggested as a factor inducing cell polarity (Wolpert and Mercer, 1963; Saxen and Wartiovaara, 1966).

An inadequate environmental difference resulting from the
limited area of contact with further cells may account for the lack of differentiation in cells of the advancing edge. The appearance suggests activity associated with movement. Vaughan and Trinkaus (1966) demonstrated that adhesion of cells of the free edge of epithelial sheets in vitro to the substrate is an important factor in their spread. Plasma membrane activity is greatest at the top of the outgrowth. The movement of epithelial sheets over a denuded basement membrane may be similar.

The tips of narrow villi which show complete reepithelialisation after eight hours of recirculation, are lined by cells showing more advanced signs of differentiation than those seen on the lateral side. This suggests that differentiation of apical cells follows the establishment of polarity associated with the joining of the most advanced cells.

Synechiae or bridges are formed by the adhesion of epithelial cells of adjacent villi. They are thought to be formed at early stages, which lack polarity. The arrangement of the cells around the bridge suggests that it persists for some time. Signs of degeneration in cells forming the bridges suggest their regression. Whether some bridges disappear by stretching during the epithelial advance is difficult to decide. The hyperplastic spur-like foci are sometimes difficult to distinguish from epithelial sheets shed in toto. This may be the result of specimen preparation.
The differentiation of regenerating goblet cells in the epithelium is thought to indicate that these do not have an origin which is separate from that of other epithelial cells (c.f. Freeman, 1966).

Argentaffin cells in regenerating epithelium are rare and discussion is difficult; it is pertinent to note, however, that the extensive development of cytoplasmic filaments is not seen in other regenerating cells.

The apparent expansion of intercellular spaces seen at later stages of regeneration seemingly throws some doubt as to the importance of cell adhesion in the differentiation of the epithelium. Dilatation of intercellular spaces occurs in villi where the reepithelialisation is complete. At the same time, inflammation is still present in the lamina propria. It is thought that intercellular spaces result from exudation and damming of fluid in the epithelial sheet and that earlier the denuded surface does not impede the escape of fluid. In rabbit gall bladder epithelium, a relationship between the appearance of intercellular spaces and fluid transport during absorption was demonstrated. These spaces resulted from an accumulation of fluid between two barriers (Kaye, Wheeler, Whitlock and Lane, 1966). It is believed that they appear also when the direction of flux is reversed. Grossly dilated intercellular spaces, similar to those seen in our observations, were described in
ulcerative colitis (Gonzalez-Licea and Yardley, 1966). These were interpreted as a non-specific reaction to cell injury by the authors. It is possible that exudation due to the presence of inflammation was the cause of these dilations. The possibility that dilated spaces represent fluid absorption is thought unlikely.

Another explanation to be considered is that the spaces result from an insufficient volume of the cytoplasm of cells after their reorientation.

**Lamina propria:**

The rapid disintegration of necrotic tissue which leaves, in places, only membranous debris, seems to be associated with the lytic action of intestinal contents.

The characteristic appearance of the vessels in necrotic zones appears to result from intravascular haemolysis. Fragments of dense material incompletely surrounded by membranes are similar in appearance to the damaged and lysed erythrocytes of other tissues (e.g. Rifkind, 1966; 1965). The presence of large numbers of membranous formations in our material suggests that many are newly formed from the liproproteins of erythrocytes. Some of these paired membranes possibly represent tubular extrusions, demonstrated by negative staining after osmotic haemolysis (Dourmashkin and Rosse, 1966; Baker, 1964). Section geometry suggests that some formations are flat. The possibility that "spherules"
are formed from erythrocytes is indicated by the similarity in density of their contents and spatial relationship. These "spherules" apparently result from erythrocyte fragmentation (see Weed and Reed, 1966). Variations in the degree of haemolysis may account for the variation in density of the "spherules". It is likely that a rapid destruction of erythrocytes in necrotic tissue results from the action of surface active substances either present in the intestinal contents or released from necrotic tissue.

Signs of recent cell necrosis in the zone of inflammation suggest that there is a secondary alteration due to this process. A delayed cell death associated with original injury, seems less likely.

Vascular changes in the zone of inflammation are similar to those described elsewhere (Majno, 1964; Cotran and Majno, 1964; Marchesi, 1964). The reaction of some vessels may represent a delayed response (Cotran, 1965). This could not be decided in the case of gut, since injury is not exactly defined in time. The variable degree of alteration of their cells suggests that there is a combination of direct and mediated injury to the vessels (Cotran and Majno, 1964). Severe damage to the capillaries was almost invariably associated with thrombosis. The capillary damage indicates a direct vascular injury (Cotran and Majno, 1964; Ham and Hurley, 1965). Microtubules seen in platelet aggregates are
components of thrombocytes (Behnke, 1965). Inclusion of platelets within the cells of the endothelium as described by Marchesi (1964) was not observed. Occasionally the endothelial cells seem to surround the platelets.

The large caveolae and vesicles in apparently normal capillaries may not be related to inflammatory changes but rather to transitory ischaemia. Moore (1959) described an increased number and size of pinocytotic vesicles in muscle capillaries following tourniquet ischaemia. The variations of normal pinocytotic activity makes the appreciation of this change difficult in our material.

The appearance of the endothelium in the base of oedematous villi is thought to be associated with their proliferation. This was described in regenerating capillaries by Schoefl (1963; 1964) but only some features appear to be similar. Because of sampling difficulty more material needs to be examined to establish the character of this change.

Gaps between endothelial cells of lymphatic vessels which communicate with surrounding tissue were described in inflammation by Casley-Smith (1964).

Some aspects of the coagulation necrosis of plasma cells have been discussed in Chapter 3. Variations in their appearance indicate different stages of necrosis and degradation. Mitochondrial swelling is seen before the cells become phagocytosed and vesiculation of the endoplasmic reticulum occurs
later, but any time sequence is difficult to establish. Transitional stages in the degradation of phagocytosed plasmocytes occur, which indicate that inclusions consisting of coarse particles and membranes may result from this process. The plasmocytic origin of these inclusions is not always obvious. The coarse particles seem to be derived from ribosomes, but the mode of their formation is not clear. Similar formations, consisting of membranes and dense coarse particles were contained in cytolysomes of pancreatic exocrine cells (Weisblum, Herman and Fitzgerald, 1962; Volk, Wellmann and Lewitan, 1966). These originated from degradation of the endoplasmic reticulum and it is thought likely that degradation in plasma cells results in a similar appearance.

Cytolysomes in surviving plasma cells have not been described so far.

The polymorphs and macrophages which infiltrate the remnants of necrotic tissue show a few features which will be discussed. The fusion of granules with phagosomes is a process whereby there is release of lysosomal enzymes into phagosomes (Zucker-Franklin and Hirsch, 1964; Horn, Spicer and Wetzol, 1964). The appearance of empty spaces in the cytoplasm of polymorphs and the decrease of granules is thought to be a degenerative change. The occurrence of empty spaces and other gross alterations has been described after
the action of streptolysin in degranulated polymorphs (Zucker-Franklin, 1965). The difference in appearance may be accounted for by experimental conditions and species.

The rounded shape of pyknotic nuclear segments in polymorphs suggests that these behave like liquids, at least at the time of their formation.

The release and disintegration of granules was suggested to be potentially significant in cell and tissue injury (Hirsch and Cohn, 1964), but this is difficult to follow with this material.

Progressive degranulation of mast cells takes place and is similar to that described elsewhere (see Bloom and Haegermark, 1965). Signs of granule extrusion were not seen and it could not be decided whether associated cytoplasmic damage is only due to degranulation.

Some changes are apparent in smooth muscle cells of the lamina propria and tunica muscularis. Cytolysomes are observed in both locations which have not so far been described. Abnormal pinocytotic vesicles and vacuoles in the tunica muscularis may be the result of hypoxia. Pinocytotic vacuoles associated with hypoxia were described in liver by Oudea (1963). Vacuole formation in the smooth muscle cells of the tunica muscularis appears to be delayed, being most prominent after 4-8 hours of recirculation. The more severe alterations of smooth muscle cells in the lamina propria are believed to be due to secondary factors.
The degree of change in ganglion cells and nerve fibres suggests that the sensitivity of autonomic plexuses to oxygen deprivation is not high.
CHAPTER 8

FINE STRUCTURE OF SMALL GUT DURING ISCHAEMIA AND REPAIR OF ISCHAEMIC INJURY III:

THE RESIDUAL PHASE.

The residual phase comprises the changes which occur after completion of the reepithelisation. The events which followed reepithelisation after 8 hours of recirculation, were for the sake of continuity and relations described in Chapter 7. In this chapter changes seen from 16 hours of recirculation onwards are described.

The epithelium:

The epithelium shows restored polarity and has returned to a normal single-layer arrangement. The maximum height of the epithelium near the top of the villus was found to be approximately 16 u after 16 hours and between 28-30 u after 48 hours. The orientation and height of the cells show some minor variations hence the surface of the epithelium forms undulations and the lateral outlines of the cells are often difficult to trace. Microvilli of the brush border are between 0.45 u-0.85 u in height with overlapping measurements at 16 and 24 hours. After 48 hours the microvilli are more than 1 u in length. Their regularity increases in proportion to their length. Normal junctional complexes are present. Some desmosomes are dissociated. Interdigitations of the lateral plasma membranes are consistently seen. At 16 hours
these appear to be less well-developed than normal. Sometimes they are compressed by an expanded intercellular space underneath to form highly convoluted structures. Dilatation of the intercellular spaces occurs constantly. The degree of dilatation decreases from apex to the base of the villus. Extreme expansion is frequently seen between infranuclear portions of the enterocytes (Fig. 147). These form long processes which spread along the basement membrane and touch one another; gaps are small. Interdigitating cell processes traverse dilated spaces forming an irregular network (Fig. 149b). Between the upper portions of the cells, the spaces frequently have the appearance of very large vacuoles, which may lie close to the surface (Fig. 148). The extracellular spaces contain fine filamentous precipitates and fat droplets. Some particles, also presumably fat, are of irregular shape.

The microvillous filaments, rootlets and terminal web are of a normal appearance. Some caveolae and vesicles, 30-40mμ in diameter are present in the region of the terminal web. Smooth surfaced vesicles about 100mμ in diameter, are frequent in the supranuclear cytoplasm of some cells and contain fat droplets (Fig. 148). Very rarely vesicles show a few attached ribosomes. Very few fat-containing vesicles are seen in the region of the terminal web. Fat droplets accumulate within dilated cisternae of the Golgi complex.
Some vesicles are situated near the dilated intercellular space, but the signs of fat discharge were not seen (Fig. 148). Some cells only contain droplets within the dilated Golgi cisternae. Coated vesicles, derived from the Golgi complex, are frequent in some cells.

Free ribosomes are numerous. The cisternae of the endoplasmic reticulum are narrow and irregularly orientated and attached ribosomes are not evenly distributed. The amount of the endoplasmic reticulum and vesicular profiles after 16 and 24 hours of recirculation is less than in normal enterocytes. The reduction is most apparent in the supranuclear cytoplasm (Fig. 148). A more developed endoplasmic reticulum which was almost normal in appearance, was seen in animal examined after 48 hours.

Mitochondria are irregular in shape and size. They are more numerous in the infranuclear cytoplasm. The cristae are orientated irregularly and a uniform increased density of the mitochondrial matrix constantly occurs (Fig. 149b).

The outlines of nuclei are frequently indented and peripheral condensations of chromatin are more prominent than in normal enterocytes.

Many cells show signs of incipient differentiation into goblet cells similar to that described in Chapter 7. Fully developed goblet cells are also frequent. The nuclei of the crypt epithelium frequently contain abnormally large or
multiple nucleoli. Mitotic figures appear normal. Alterations of cytoplasmic organelles are rare.

**Lamina propria:**

The cores of the apical segments of the villi have very wide intercellular spaces. Cells are separated and contact one another by means of the processes. The spaces may appear empty. Usually, however, they contain a finely precipitated substance of low density, which shows variations in degrees of condensation. The presence of this substance makes the outer aspect of basement membranes less distinct (Fig. 147). The bundles of collagen fibrils are usually separated. Debris occurs in some places in small amounts. The space beneath the epithelial basement membrane is frequently free of cells and only a precipitated substance with some collagen separates the basement membrane of capillaries and the epithelium. Particles of lipid similar to those seen between the epithelium are present in small amounts.

Polymorphs, eosinophils and macrophages infiltrate the oedematous areas in some places (Fig. 150). All macrophages are loaded with phagosomes of polymorphous appearance. These often contain the granules of eosinophilic leucocytes. Other cells in this zone are poorly differentiated. Cells similar to the 'clear fibroblast-like cells' described in the normal intestine are frequent (Fig. 151a). Their shape is
irregular and some show disruption of processes. The number of free ribosomes in these cells seems to be increased and Golgi vesicles are numerous. Large fat droplets constantly occur in the cytoplasm. Mitochondria show frequent artifactual defects. Rarely other cell types are seen in the oedematous areas. Plasmocytes are absent. Usually capillaries and venules are dilated but occasional capillaries are narrow. The endothelium in capillaries is slightly thicker than normal; fenestrae occur irregularly. Most endothelial cells are surrounded externally by debris which consists of membranes and erythrocyte spherules (Fig. 151b). The basement membrane in these places is absent or poorly developed. A few thrombosed capillaries with defects of the endothelium are present.

The degree of oedema decreases towards the base of the villus. Abnormally dilated lacteal vessels in this zone are lined by a flat endothelium of normal appearance.

The base of the villi and adjacent areas of the lamina propria have a compact appearance. This zone contains many macrophages similar to those described in the previous chapter. The plasma cells contain occasional cytolyosomes. In places, small areas of disintegrating cells and debris occur. Blood and lymphatic vessels have a normal appearance but a few endothelial cells show a strikingly increased density of the cytoplasm. The smooth muscle cells contain
slightly dilated cisternae of the endoplasmic reticulum. Most of the cells in the basal areas of the lamina propria are normal.

Discussion:

Progressive differentiation takes place after completion of reepithelisation. An increase in the length of villi occurs and is seen under the light microscope.

The complete restoration of the cell and brush border seems to vary in individual villi and significant values cannot be obtained without large scale sampling.

The development of the terminal web is associated with the increase in number and regularity of the microvilli and suggests the same formative influence. On the other hand the presence of microvilli may hypothetically induce terminal web formation. It is of interest that development and alteration of these structures, proceeds in parallel. Differentiation of the internal cell structure is incomplete at 16 and 24 hours. The increase in quantity of the endoplasmic reticulum lags behind the increase in cell volume. Many cells contain fat droplets which resemble those associated with normal fat absorption, though the membranes of the vesicles which contain fat, are almost invariably smooth. Fat-containing vesicles in the terminal web are few and seem to be different from invaginations of the plasma membrane.

The mechanism of fat transport into the intercellular
spaces is uncertain. Some fat droplets apparently pass through the basement membrane. Their further path, through the extremely voluminous extracellular space, is difficult to trace.

According to a few reports density of the mitochondrial matrix increases with functional activity (Farquhar and Rinehart, 1954; Hess and Staubli, 1963). If this is the case in the intestine an increase in mitochondrial matrix density may be associated with the evolution of absorptive capacity. The "ballooning" of many intercellular spaces suggests that these are under pressure. A comparison with the preceding stage shows that the spaces increased in size. Fluid loss associated with abnormalities of vessels in the villi is thought to be the explanation.

The lamina propria:

The formation of extremely wide intercellular spaces may be due to a previous cell loss and oedema. There is no solid framework, like fibrin, present in significant amounts, to support the migration of mesenchymal cells. Collagen and the residues of necrotic capillaries which are left, do not appear to subserve this function. It is not however known where proliferation of mesenchymal cells takes place.

The presence of large numbers of "clear" mesenchymal cells in apical areas of newly-formed villi, seems to indicate that these cells are young fibroblasts or transitional
elements between reticulum cells and fibroblasts as suggested in Chapter 3.

The appearance of capillaries may be explained by migration of endothelial cells inside the original lumen. It can be argued that a similar appearance may result from escape of erythrocyte debris from the vessels at a preceding inflammatory stage. This is unlikely. Debris in similar amounts is only associated with the endothelial disintegration. Moreover, a massive leaking of the debris was not found.

The formation of new capillaries was not seen. Capillary outgrowth does not occur unless a solid substrate is available (Chalkey, Algire and Morris, 1946; Fulton, Parshley and Simms, 1949). The role of other factors (see Schoefl, 1964) cannot be determined by morphology.

The distension of lymphatic vessels is apparently functional and appears to be related to oedema. The changes in the base of the villi are thought to be associated with inflammation.
CHAPTER 9

FINE STRUCTURE OF THE MUCOSA OF SMALL GUT
DURING ACUTE OCCLUSION, CHRONIC STENOSIS
OF THE PORTAL VEIN AND ACUTE ILEUS.

Many conditions bring about changes of the intestinal circulation. It was thought to be of interest if a comparison of the fine structural changes associated with acute intestinal ischaemia was made, with those in which the circulatory disturbance had a different character. The conditions examined were acute occlusion, chronic narrowing of the portal vein and acute intestinal obstruction.

METHODS

White adult rats of both sexes were used. The animals were deprived of food 24 hours prior to killing. All operative procedures were done using ether anaesthesia. The upper third of the ileum was examined.

Acute portal occlusion was produced by clamping the portal vein near to its origin. Animals were examined after 30 minutes and one hour of occlusion (one at each interval). Another animal was examined after 2 hours of recirculation, which followed one hour of occlusion.

A silk ligature in the middle of the length of the portal vein was applied to narrow its diameter to approximately one half. Two animals were examined 50 days after operation.
Acute intestinal occlusion was produced by double ligature of the ileum, about 3-4 cm above the ileocaecal valve, care being taken to avoid vessel damage. Animals were examined 3, 4 and 6 days after operation, one at each period.

RESULTS AND DISCUSSION

Occlusion of the portal vein:

The characters of the changes following occlusion of the portal vein are generally similar to that seen after arterial occlusion though they are less extensive. After one hour, only a few enterocytes and mesenchymal cells are disintegrated. Some enterocytes show a striking swelling of the apical cytoplasm above the terminal web. The swollen area is traversed by elongated rootlets (Fig. 152a). Microvilli are shortened and in places, these disappear and the cytoplasm protrudes. Microvillous filaments may be incorporated into or pushed aside at the base of the protrusion. This suggests that the microvilli are incorporated inside the cell by swelling of the cytoplasm and that the plasma membrane between their bases shifts upwards; the elongation of rootlets is only relative. Protrusions are formed after the whole length of the microvillus has been incorporated into the cell. A similar transposition of the plasma membrane may be seen above the tight junction which becomes invaginated (Fig. 152b). These changes cause the loss of the brush border. Their frequency is thought to be the
result of the slower progress of changes which follow ven­
ous occlusion than those which follow arterial occlusion.
In some crypts there is an extensive loss of microvilli
which appear to be fragmented and shed into the lumen (Fig.
153a). Increased numbers of cytosomes are seen beneath the
abnormal cell apex. A crystalline material similar to that
seen in normal animals may be present in large amounts in
the crypt lumen (Fig. 153b).

The altered lamina propria contains numerous scattered
erythrocytes and small areas of haemorrhage. Distension of
the capillaries may be extreme (Fig. 154b). The diaphragms
of fenestrae are continuous while the basement membrane dis­
appears. The endothelial cytoplasm is usually dense (Fig.
154a). Erythrocytes which escape through the vessel wall
are partially surrounded by stretched and fragmented endo­
thelial cells and basement membrane (Fig. 154c). This change
apparently reflects increased intravascular pressure.
Aggregates of platelets are frequent in vessels but no gaps
were observed in the endothelium (Fig. 154a). Spherules
and membranes derived from erythrocytes are present and may
be separated from the endothelium by a gap, measuring
100-200Å in width. There is continuity of the paired mem­
branes with erythrocytes or their fragments.

Single extravasated erythrocytes occur between the cells
and collagen fibres. Some were seen within crypt epithelium
or crypt lumen. No membranes derived from extravasated erythrocytes were seen. The structures which surround erythrocytes show very little distortion.

Two hours after declamping the portal vein most cells appear normal. Numerous erythrocytes become phagocytosed.

The less severe changes associated with portal occlusion do not seem to be correlated with the condition of the animals which is more serious than that after artery occlusion. Khanna (1959) observed after ligation of superior mesenteric vein, changes similar to those following arterial occlusion. The former were also associated with more severe general alteration. The difference is explained in terms of alterations of the total blood volume.

**Acute ileus.** An increase in the number of inclusion bodies was observed in acute ileus. Those which occur in enterocytes after three days of occlusion have the appearance of large irregular vacuoles which contain small vesicles about 300-500Å in diameter (Fig. 155a). Dense bodies and some membranes may be present. Other inclusions are smaller and have a condensed appearance. The Golgi complex is dilated. After 6 days, similar inclusions rarely occur. Enterocytes show, however, a disorganisation and vesiculation of the endoplasmic reticulum. In some moderately swollen mitochondria a dense abnormal substance which forms clumps about 30-100μm in diameter is present (Fig. 156c). Occasional mitochondria are enlarged and very dense. Cytosomes
are numerous. Extrusion of the enterocytes is frequently seen.

After 6 days of obstruction, the intercellular spaces between enterocytes in the apical region of the villi are grossly dilated.

The appearance of the lamina propria varies. After three days the arrangement remains compact. At this stage, dilated lacteal vessels were observed with abnormally dense contents. The outlines of the endothelium may be uneven and some cells show abnormally electron-lucent cytoplasm. Numbers of caveolae and pinocytotic vesicles, many of which are coated are present and their contents are frequently of a density similar to the material in the lumen or to that seen in the narrow gap between the endothelium and other tissue components (Fig. 155b). Other cell changes are comparable to those seen at the 4th and 6th days of intestinal occlusion. At this stage, a wide dilatation of extracellular space appears, (Fig. 156a). Dilatation also affects the capillaries and venules and may be extreme. The number of macrophages is increased. These contain many large (2-5 u) inclusions, which mostly consist of twisted membranes rather evenly distributed in a moderately dense matrix. In some cells the inclusions are polymorphous. The Golgi complex of these cells is extensive. The endoplasmic reticulum may be dilated. The reticulum cells and clear fibroblast-like cells contain numerous cytosomes. Other cells show free
lipid droplets. In clear cells, however, some large fat droplets appear which are situated in the lumen of the endoplasmic reticulum (Fig. 157). Segments of membrane about 60Å thick separated by a gap of approximately the same thickness are seen around these droplets. Other cells show an increased amount of the endoplasmic reticulum which forms sinuous cisternae and multiple stacks of Golgi cisternae with associated vesicles.

The endothelium of venules shows markedly dilated cisternae of the endoplasmic reticulum and swollen mitochondria (Fig. 156b). The elements of the Golgi complex may be increased in number.

The signs of cell injury during acute intestinal obstruction seem to precede those of the oedema. The epithelial changes may indicate impairment of function but gross alterations of the absorptive surface were not found. The origin of the numerous phagosomes or phagolysosomes is unexplained. Increased numbers of cytosomes, believed to be lysosomes, indicates an increased catabolic activity. Lipid droplets inside the cisternae of the endoplasmic reticulum may enter these by invaginations of the membrane.

Dilatation of vessels and oedema were observed by light microscopy (Shields, 1965). The congestion resulting from bowel distention and compression of veins in the gut wall may be the cause of water and electrolyte loss into the
lumen (Shields, 1964; 1965). The changes observed by microangiography (Derblom, Johansson and Nylander, 1963) cannot be assessed by electron microscopy. It is, however, possible that changes of the endothelium in venules are associated with a more severe congestion than in capillaries. **Portal stenosis.** After 50 days of portal stenosis severe dilatation of venules is seen. This is less severe in capillaries. Pericytes are numerous. In capillaries, they are situated along the abepithelial section of the perimeter. Long, flat processes are formed in places around venules which are applied externally to the basement membrane, (Fig. 158). Indistinct filaments may be seen in the cytoplasm in moderate amounts. Occasionally a cell junction is found where two processes contact one another. These processes appear to be formed by reticulum cells although their continuity with cell bodies is difficult to trace. Some capillaries have a thickened basement membrane (Fig. 158 insert). In other places, the amount of perivascular collagen seems to be increased. The endothelium appears to be normal. Occasional macrophages contain pleomorphic dense inclusions which probably represent hemosiderin (Richter and Bessis, 1965).

It is thought that the large number of pericytes is associated with the increase in intravascular pressure. The appearance of cell processes applied to the venules suggests
that these are formed as a reaction to the dilatation of the vessel. The thickness of the basement membrane may be correlated with the intravascular pressure (see Majno, 1965). Obvious thickening is not, however, frequent, and the appreciation of this change would require a more extensive study. No conclusion can be drawn as to whether any endothelial changes preceded the stage examined. These were observed in the vena cava after partial obstruction (Tedder and Shorey, 1965a; 1965b).
CHAPTER 10

THE FINE STRUCTURAL CHANGES IN THE RABBIT
UPPER ILEUM AFTER SUPERIOR MESENTERIC SYMPATHECTOMY WITH SPECIAL REFERENCE TO THE MUCOSA

Morphological changes following denervation of the gut have rarely been reported. The mucosal atrophy observed by light microscopy after autotransplantation with denervation (Ballinger, Christy and Ashby, 1962) was later reported to be the result of vagotomy (Ballinger, Iida, Aponte, Wirts and Goldstein; 1964; Ballinger, Iida, Padula, Aponte, Wirts and Goldstein, 1965). Other authors detected no alterations after vagotomy in rat by light microscopy (Ellis, Pryse-Davies, 1967) or in dog, by electron microscopy (Elliott, Barnett and Elliott, 1966). No recent study of intestinal morphology after "surgical" sympathectomy seem to have been published. After immunosympathectomy a normal mucosa was observed. The migration time of the epithelium was, however, decreased (Dupont, Biggers and Sprinz, 1965). Lack of information at the fine structural level has prompted us to make this study.

MATERIAL AND METHODS

Adult New Zealand albino rabbits of both sexes, weighing 2.5-3 kg supplied by the Animal Breeding Unit of the
JCSMR were used. Standard laboratory diet and water was available and libitum. Under aseptic conditions with Nembutal anaesthesia (35 mg/1 kg) supplemented with ether, superior mesenteric neurlectomy of the postganglionic segment of about 5-7 mm in length was made. This was followed by superior mesenteric ganglionectomy and division of the communicating branches to the ganglion coeliacum. The ganglion coeliacum was resected partially. In the course of this preparation, some damage of the lymphatics was usually inevitable. Care was taken to avoid injury of blood vessels. Animals were examined 1, 2, 5 and 7 days and 1, 2, 3 and 5 weeks after the operation. Two animals were studied in each group with the exception of the one-day stage where only the one, and the three-week stage where three were examined. Three control animals were sham-operated. Further normal control animals are included in Chapter 3.

RESULTS

A few minutes after neurlectomy and ganglionectomy, a period of increased peristaltic movements was noted in many animals. For a few days after operation, almost all animals showed moderate diarrhoea. At this period, the intestinal wall was slightly hyperaemic, when viewed from the outer aspect. By light microscopy, the mucosal architecture and cellular composition appeared normal. In
animals examined 2 and 3 weeks after operation, finely granular PAS positive, diastase-resistant material was slightly more frequent.

**Fine structure:**

Changes of non-nervous components were found in animals examined 2 and 3 weeks after operation.

**Enterocytes.** Residual bodies similar to those seen in normal animals are frequent 3 weeks after sympathectomy. In some cells, many lipid droplets occur which occasionally form groups. Other formations are more complicated. Lipid droplets, usually membrane-limited and extracted, are enclosed with cytoplasm into membranous profiles. In other instances, large multi-layered cylindrical arrays are seen (Fig. 159a). Droplets may be seen in contact with the innermost layer of an array. In other places, this is flanked by a membrane of endoplasmic reticulum and the space between both is intracisternal (Fig. 161b). The varying appearance of the arrays may be explained by section geometry. The end segments of arrays are difficult to trace. Some membranes, however, are continuous with the endoplasmic reticulum (Fig. 159a).

These arrays of membranes may, together with lipid droplets and large membrane-limited heterogeneous inclusions, form ill-defined groups. Small (0.2-0.3 µ in diameter) dense bodies which occur near these components, appear to be
encircled by endoplasmic reticulum. Some bodies are identifiable as densified mitochondria (Fig. 160). Occasionally, dense bodies seem to fuse and are in continuity with vacuolated spaces which contain a few irregular lamellae. The endoplasmic reticulum in these areas frequently forms circular profiles. Sometimes a large vacuolar space is present, close to these formations. This contains thread-like precipitates with more densely-aggregated areas.

Some enterocytes show more generalised changes. These consist of mitochondrial swelling and polymorphism, dilatation of the endoplasmic reticulum and the appearance of the recently described dense bodies surrounded by cisternae of the endoplasmic reticulum (Fig. 161a). Another type of degeneration is associated with decreased density of the cytoplasm and appears similar to that described in the normal intestine (see Chapter 4).

Lamina propria. In some places of the lamina propria, large phagosomes occurred frequently. Some were mostly homogeneous, while others resembled the cell degradation described in the normal lamina propria. A large, complex inclusion body is shown in Fig. 162. Other inclusions consist of myelinic figures. A definite increase of cytosomes is seen in the clear mesenchymal cells (Fig. 159b). These vary in size from about 0.2 μ to more than 1.5 μ in diameter and show heterogeneous contents. These consist of a granular
substance, densely packed, irregular membranous segments, dense bodies and triple-layered membranes which frequently fuse and form multilayered parallel or concentric formations (Figs. 163a; 163c). Dense layers of these membranes are about 30Å in thickness. In multi-layered formations, the inner membranes are slightly thicker and denser (Fig. 163b). Some inclusions contain closely-packed tubules with a lumen of about 50-60Å in diameter which may form hexagonal arrays embedded in a dense substance, (Fig. 163d).

Rarely a clear cell containing cytosomes is partially surrounded by a layer which has the appearance of a basement membrane (Fig. 159b).

Nerve fibres. Changes in nerve fibres were very rarely found 3 weeks after sympathectomy. In the lamina propria, axons show a disintegration or disappearance of constituents (Fig. 164a). This may be accompanied by swelling (Fig. 165b). The basement membrane may be absent. Macrophages seen nearby contain phagosomes which consist of dense vacuolated aggregates with membranous formations (Figs. 164a; 165d). Membranes may be arranged into lamellae (Fig. 164b). These phagosomes are thought to be derived from altered axons. Phagocytosis was observed in very few instances. A few cells contain large masses similar to these phagosomes and complex layered inclusions which may have resulted from fusion. Conclusions as to their origin cannot be made. In the
tunica muscularis, a few nerve fibres show swelling of axons. These contain irregular filaments, a few neurotubules and irregular tubular profiles 200-400Å in diameter which may have dense cores (Fig. 165a). A few dense cored vesicles, 700-1,000Å in diameter are present. Similar changes were also seen in the submucosa. Most of the affected nerve fibres occur near vessels. Some axons contain myelinic figures with clumped neuro-filaments or small dense aggregates (Fig. 165c). Some axons show a variation of constituents which is difficult to classify as degenerative. Dense aggregates or tangles of membranes are rarely present. These were, however, also seen in a few axons in controls.

The myenteric plexus was examined only in a few animals. No changes were found. The significance of few enlarged axons cannot be assessed.

**DISCUSSION**

The increase in residual bodies in enterocytes indicates an acceleration of the degenerative process. Further abnormal components in the cytoplasm appear to be lipids or lipoproteins. The close relationship of lipid droplets and membranous arrays suggests that the latter may be derived from the former. The relations of lamellae to the endoplasmic reticulum suggest that there is a continuity of membranes. It is of interest that a close relationship of lipid droplets
to membranous formations was observed in rat liver in carbon tetrachloride cirrhosis and was interpreted as being due to the formation of membranes of the endoplasmic reticulum (Stenger, 1966). Here the arrays were much more irregular and extensive. Apart from the continuity of membranes, no accumulation of cisternae was observed around the lamellar formations in affected enterocytes. The endoplasmic reticulum surrounds dense bodies which occur nearby. Whether all these bodies are derived from mitochondria remains uncertain. The relationship of cisternae and dense bodies is thought to represent focal degradation by means of sequestration.

The changes which affect whole enterocytes are degenerative. The cause is not known. The increase is appreciable at a stage when functional changes following sympathectomy have presumably receded. Dupont, Biggers and Sprinz (1965) demonstrated a decreased turnover of intestinal epithelium in rat after immunosympathectomy. If this is also the case in rabbit after surgical sympathectomy, it may be speculated that the changes are related to ageing of cells or to a modified response of senescent cells. No conclusion can be drawn about the direct effect of denervation.

A consistent change in the lamina propria is an increase in the inclusion bodies or cytosomes of "clear mesenchymal cells". Some of these have a structure which is consistent with lipofuscin (Biava, 1965; Essner and Novikoff, 1960;
Samorajski, Ordy and Keefe, 1965). In others, the membranous component is more prominent. It is of interest that the arrangement of the membranes resembles that seen in some lipidoses of the nervous system (e.g. Gonatas and Gonatas, 1965). It may be speculated that the change of "clear cells" represents abnormal ageing. However, nothing is known about turnover of these cells. The presence of inclusions which are essentially lysosomes or residual bodies may be a result of an injury without relation to cell age.

Changes preceding the formation of phagosomes are not obvious and the relation to the experimental conditions is difficult to establish.

The changes in nerve fibres present problems in interpretation which appear to be common to the investigations of degeneration of nerve "endings" of non-myelinated nerves (c.f. Biscoe and Stehbens, 1967). Usually a long lapse of time is required before degenerative changes appear. A comparison of reports indicates that the appearance and rapidity of the development of degenerative changes may vary (Biscoe and Stehbens, 1967; Garrett, 1966). Our observations suggest that some axons in the lamina propria become phagocytosed. No consistent pattern of degeneration was seen.

The relationship of changes in nerve fibres to the non-nervous components is uncertain. The latter may be altered indirectly by the functional effects of sympathectomy. Their
retrogressive nature is however apparent. Conclusions about their significance may only be obtained by a long-term study.
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ADDENDA.

