ELECTRON MICROSCOPE STUDIES OF THE STRUCTURE OF THE MICROVILLI ON PRINCIPAL EPITHELIAL CELLS OF RAT JEJUNUM AFTER TREATMENT IN HYPO- AND HYPERTONIC SALINE

P. F. MILLINGTON, Ph.D., and J. B. FINEAN, D.Sc.

From the Department of Medical Biochemistry and Pharmacology, The Medical School, The University of Birmingham, England

ABSTRACT

Immersion of the intestinal tissue, from rat jejunum, in hypertonic saline produced very rapid changes in all regions of the epithelial cells, but the apical region was apparently unaffected by hypotonic solutions for at least ½ hour. In both cases, blistering of the microvilli was taken as the first sign of degenerative changes which finally resulted in a breakdown to large vesicular particles. Consideration of both normal and modified tissue indicates that the core of the microvillus contains either paired strands or tubular structures. Lateral cross-fibres extended from the core to the microvillus membrane and may be an essential part of the supporting structure of the microvillus. Densitometer traces across the microvillus membrane at various stages of modification indicated that this membrane might include a 75 A unit membrane structure with additional components associated at either surface. Interruptions in the membrane were apparently expanded by the hypotonic solutions and these might possibly be distinguished from preparative artefacts.

Observations made on the apical region of the epithelial cells of the small intestine by light microscopy have been reviewed by J. R. Baker (1, 2). Of particular interest in relation to this paper was his observation that canals appeared to pass through the brush border perpendicular to the surface (2). At about the same time, the earliest electron microscope observations were reported by Granger and R. F. Baker (8, 9). Their results showed that the brush border consisted of cylindrical projections, with rounded tips, oriented perpendicular to the cell surface. Further electron microscope studies confirmed and extended these general observations, (4, 5, 10, 25, 26).

Details of the structure of the membrane covering the microvilli have been reported by both Zetterqvist (27) and Palay and Karlin (20). After osmium tetroxide fixation and embedding in methacrylate, the membrane appeared as a double contoured line, that is, consisting of two opaque components separated by a less dense region. In mouse epithelium, the total width of this membrane has been found to be 105 A (27), but in a recent report by Suganuma (24) the microvillus membrane of frog epithelial cells appeared to be smaller (about 100 A maximum). In rat epithelium, the opaque components were estimated to be 40 A wide and separated by a less dense region 25 A across, (20). Studies of the rat microvillus membrane using different preparative procedures have shown that the over-all width may vary between 105 A and 125 A (17, 15) in normal tissue.

The microvillus membrane has been shown to be a continuous extension of the membrane cover-

ing the remainder of the cell (27), but the latter has appreciably different dimensions. In osmium-fixed tissue, the membrane on both the lateral and basal regions of the cell has been observed to be double contoured (27) but Palay and Karlin (20) found it to consist of a single layer 32 to 42 A thick, although for very short distances they found what appeared to be a doubling of the profile and here the membrane appeared twice as thick. After fixation in potassium permanganate, the double contouring has been consistently resolved (15, 22). Such a membrane has been shown to be characteristic of the surfaces of a wide variety of cell types (22), the 75 A unit comprising two parallel dense layers 25 A wide separated by a light zone also 25 A across. It would appear, therefore, that the microvillus membrane is not a typical "75 A unit membrane," (22), even though it does appear consistently as a double contoured structure.

It has been reported that pores (greater than 30 A) do not appear to be present in the microvillus membrane (27) and absorption of particulate material has been assumed to proceed *via* a mechanism involving invagination and vesiculation of the surface membrane (21). Following osmium tetroxide fixation, a fibrous core (believed to contain protein as its major component) has been seen in association with a subjacent web, the terminal web (3, 15, 17, 20, 27). However, the core has not been observed in tissue fixed in potassium permanganate (15, 22).

In the present study, the sequence of degenerative changes which followed immersion of the jejunal tissue in hypo- or hypertonic saline are described. New structural details were apparent after short periods of immersion in the hypotonic solutions and can be related to the structures seen in the control preparations.

METHODS

Male albino rats weighing between 200 and 230 gm body weight were starved for the 24 hours preceding the commencement of the experiment. Small segments of jejunum were removed immediately after killing the animal. The segments were cut open and immersed in different concentrations of saline at room temperature for various times (up to 24 hours) prior to fixation. The saline solutions were prepared at the following concentrations: 0.25 N, 0.33 N, 0.5 N, N, 2 N, 4 N, 5 N, and 10 N, (where N refers to the normal physiological concentration of saline, 0.9 per cent). After immersion in saline, the specimens were fixed for 4 hours in a 1 per cent osmium tetroxide (19) solution buffered with Veronal acetate buffer (14) to pH 7.2 and chilled to about 4°C. The ionic concentration of the fixative was adjusted to approximately 0.9 per cent w/v by the addition of sodium chloride. Control specimens of fresh tissue were also prepared and fixed immediately after death.

After fixation, the specimens were dehydrated in an ascending series of alcohol concentrations, stained for ½ hour in a 5 per cent solution (in alcohol) of phosphotungstic acid (PTA) and embedded in Vestopal W (13). Since the length and width of the microvilli change as cells migrate from the crypts to the villi, care was taken during trimming of the blocks of tissue to locate only the areas immediately below the tips of the villi, where the dimensions of the microvilli have been found to be constant. Thin sections were then cut on a Porter-Blum ultramicrotome and inspected in a Siemens electron microscope, type Elmiskop 1b.

Each series of experiments was repeated three times, giving a total of 108 specimens inspected by electron microscopy. Measurements were made on photographic enlargements of the negatives with the aid of a ×8 magnifying lens equipped with a graduated rule. The anticipated accuracy of this method for dimensions of about 100 A was about ± 10 per cent. Determinations of the over-all widths of the microvilli, the microvillus membrane, and the core were made on all specimens.

Densitometer traces were also made across the microvillus membrane of both normal and treated tissue. These were obtained from ×4 enlargements of the negatives onto Ilford half-tone plates.

RESULTS

1. *The Normal Structure of the Apical Region of the Principal Cell*

The microvilli on cells near the tips of the villi were generally about 1 μ in length, 0.1 μ in diameter, and had a core approximately 0.045 μ across (Fig. 2). In these preparations, estimates of the width of the microvillus membrane by the visual method were in close agreement with those made from densitometer traces. The average value was found to be about 115 A. The outer surface of the membrane was covered with a granular material and this made identification of the outer edge of the double contoured membrane difficult. In densitometer traces across the membrane, the dense line nearest the lumen appeared to be the wider of the two, and each was found to contain two peaks. The distance between the two inner peaks was about 45 A (Fig. 1 (*a*)).

Many interruptions were observed in the membrane either as dense lines traversing the lighter layer or as breaks in the dense lines (Fig. 4). The double contoured membrane covered the whole of the free surface of the cell, and invaginations of this membrane between the bases of the microvilli were common (Fig. 2).

The microvillus core and terminal web were cross-linking fibers of the terminal web. Where the horizontally aligned fibres of the terminal web approached the cell boundary they were frequently found to be associated with a terminal bar. The general structure of these bars has already been described (27) and is similar to that of the desmosomes of the cervix epithelium (11, 12), but in mammalian intestinal tissue the central dense line

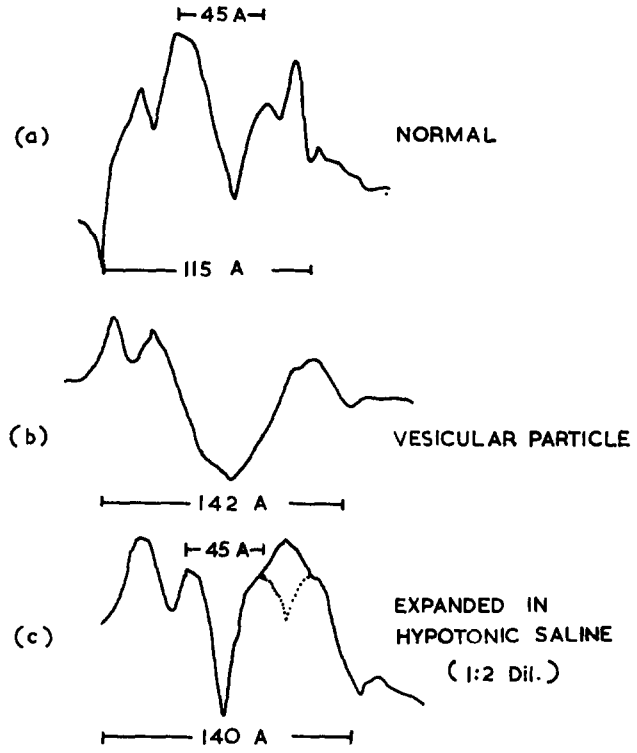


FIGURE 1

Densitometer traces across microvillus membranes. (a) From normal tissue; (b) From vesicular particles of degenerate tissue; (c) From microvilli expanded in hypotonic saline (0.5 N).

prominent in most cells. In the crypt, the core often extended some way into the cell giving the appearance of a root, but in the cells located on the sides of the villus, the roots were usually indistinguishable from other parts of the terminal web. However, it has been found that very small changes in the preparative technique (tonicity, pH, or early postmortem changes) produce very distinct roots at the foot of each microvillus. Fig. 5 shows part of a section through the terminal web region almost at right angles to the main axis of the cell. In this illustration the roots of the microvilli can be readily identified together with the of the system (*i.e.* the X-line of the desmosome (11)) is not resolved.

The dense lines of the microvillus core appeared to be arranged in pairs (Fig. 4) but the density of the surrounding material in these normal preparations made the observations uncertain. No consistent pattern could be ascribed to the fibres as seen in cross-section (Fig. 3) although in general they appeared to be concentrated towards the centre. In longitudinal and cross-sections, lateral strands could be seen which appeared to link the fibres of the core to the microvillus membrane.

In the present study, single contoured vesicles similar to those reported by Palay and Karlin (20, 21) were observed only in the basal region of the terminal web and these may have been part of the α-cytomembrane system. Double contoured circular profiles were frequently encountered in the apical region of the web (Fig. 5) but these were most probably sections through invaginations of the surface membrane.

2. *The Effects of Varying the Time of Immersion in Normal Saline prior to Fixation*

No abnormalities were noted in specimens which had been immersed in normal saline for ½ hour prior to fixation (Fig. 6). However, changes were apparent after immersion for 1 hour. The cristae of the mitochondria were swollen (Fig. 7), there appeared to be an increase in the number of vacuoles in the cytoplasm, and roots at the base of the microvilli had become prominent. There were no observable changes in the microvilli. After 2 hours' immersion, the cytoplasm contained many more vacuoles and fibres, and the microvilli were now noticeably distorted (Fig. 8). On many cells, the microvilli appeared to be abnormally long and all displayed a characteristic feature which was termed blistering. The blisters appeared at almost regular intervals along the microvillus length and appeared to be caused by a swelling of the region between the microvillus membrane and the core. After 5 hours, the blisters had increased in size and constrictions of many of the remaining regions of the microvillus were now observed. Finally after 24 hours, the tissue had partially disintegrated and some microvilli were broken into separate vesicular particles of sizes ranging from 0.1 μ to 0.3 μ (Fig. 9).

The measurements of the widths of the microvilli and of the microvillus membrane are shown in Table I, where they are presented along with similar results obtained from studies of the effects of hypotonic solutions. Each figure is the mean of ten measurements taken from at least four photographs of different areas of the specimen. The scatter of the results about the mean increased with prolonged immersion and where greater than 10 per cent but less than 50 per cent it is indicated by the letter "*v*" in the table. Eventually the scatter of the results was often greater than 50 per cent and no significance could be attached to the mean value. Thus, no figure is quoted in the table and the condition is designated by "*var.*"

The average diameter of the microvillus decreased steadily until breakdown. In contrast, the width of the membrane increased slightly during the first 2 hours and then became variable. Den-

FIGURE 2 TO 5

Electron micrographs from control preparations of rat intestinal cells.

FIGURE 2

The apical region of a normal principal epithelial cell. × 35,000.

FIGURE 3

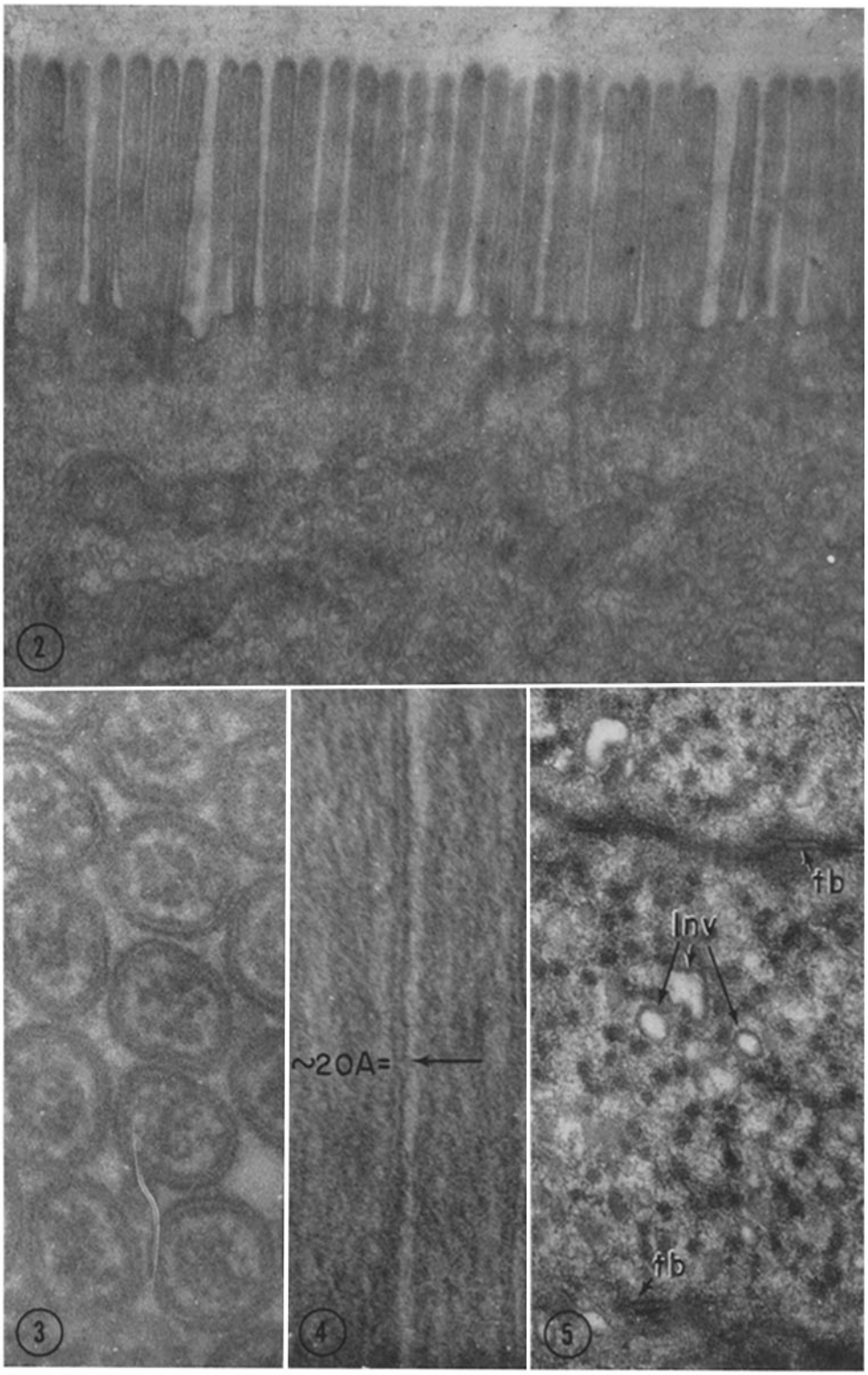
Microvilli in cross-section. The core can be seen as dense granules aggregated towards the centre of the microvillus. × 160,000.

FIGURE 4

Microvilli in longitudinal section. A pore-like interruption in the double contoured membrane is indicated by an arrow. × 190,000.

FIGURE 5

The terminal web region, sectioned almost at right angles to the main axis of the cell. The roots of the core are linked by the cross-fibres of the web. The terminal bars, *tb*, are numerous and extensive, and may also be connected to the terminal web. Sections through invaginations of the surface membrane can be seen as double contoured profiles (*Inv*). × 40,000.


2
3
4
~20A=
5
fb
Inv
fb

sitometer traces across the membrane were similar to that of the control until the tissue began to degenerate. After 24 hours' immersion, the membranes of the vesicular particles varied considerably in thickness and the densitometer traces showed that an expansion of the lighter region of the membrane was responsible for a large proportion of the over-all expansion (Fig. 1 (*b*)).

3. *Hypotonic Saline Effects*

The changes in the structure of the apical region of the principal cells after immersion in hypotonic solutions followed a sequence similar to that described in the preceding section, but in general the changes occurred more rapidly. In the most dilute solution (0.25 N) the changes were observed in the microvilli of most cells but not in a completely uniform manner in all of them. Thus, a cell was sometimes observed to possess microvilli that were only slightly distorted when all the adjacent cells were grossly damaged. After 2 hours' immersion, all the structures of the apical region were distorted and after 24 hours the tissue had largely disintegrated. The sequence of these changes and the types of distortion and breakdown are illustrated in Fig. 10.

Most of the detailed structural observations were made on tissue that was only slightly affected by the hypotonic solutions. Many specimens treated for ½ hour displayed a grossly vacuolated cytoplasm and the lengths of the microvilli varied from 0.5 μ to 2.2 μ, the majority appearing abnormally long (Figs. 11, 14). In contrast, the width of the microvillus was constant and its value close to that of the normal.

A noticeable feature of the specimens immersed for only ½ hour in the hypotonic solutions was the apparent increase in contrast between the fibres of the core and the adjacent material. In longitudinal section, the dense lines of the core were arranged in pairs (Figs. 13, 14) but, although the cross-sections now showed many circular profiles (Fig. 12, arrows), in general the irregular aggregation of the core prevented the positive identification of the fibres as tubular structures.

Before blistering appeared, there was often a constriction at the base of the microvilli (Figs. 13 and 14) and interruptions in the membrane were common. Many of these interruptions were larger than in normal preparations (up to 50 A across) and the two dense layers were frequently found to be continuous at the periphery of the pore (Fig. 15).

Figures representing the over-all widths of the microvilli and the microvillus membrane are shown in Table I. It is apparent that the changes in the membrane thickness are not directly proportional to the corresponding changes in the

FIGURES 6 TO 9

Electron micrographs of intestinal epithelial cells after immersion in normal saline (0.9 per cent w/v) prior to fixation.

FIGURE 6

The cytoplasm after immersion for ½ hour. The appearance is normal. × 10,000.

FIGURE 7

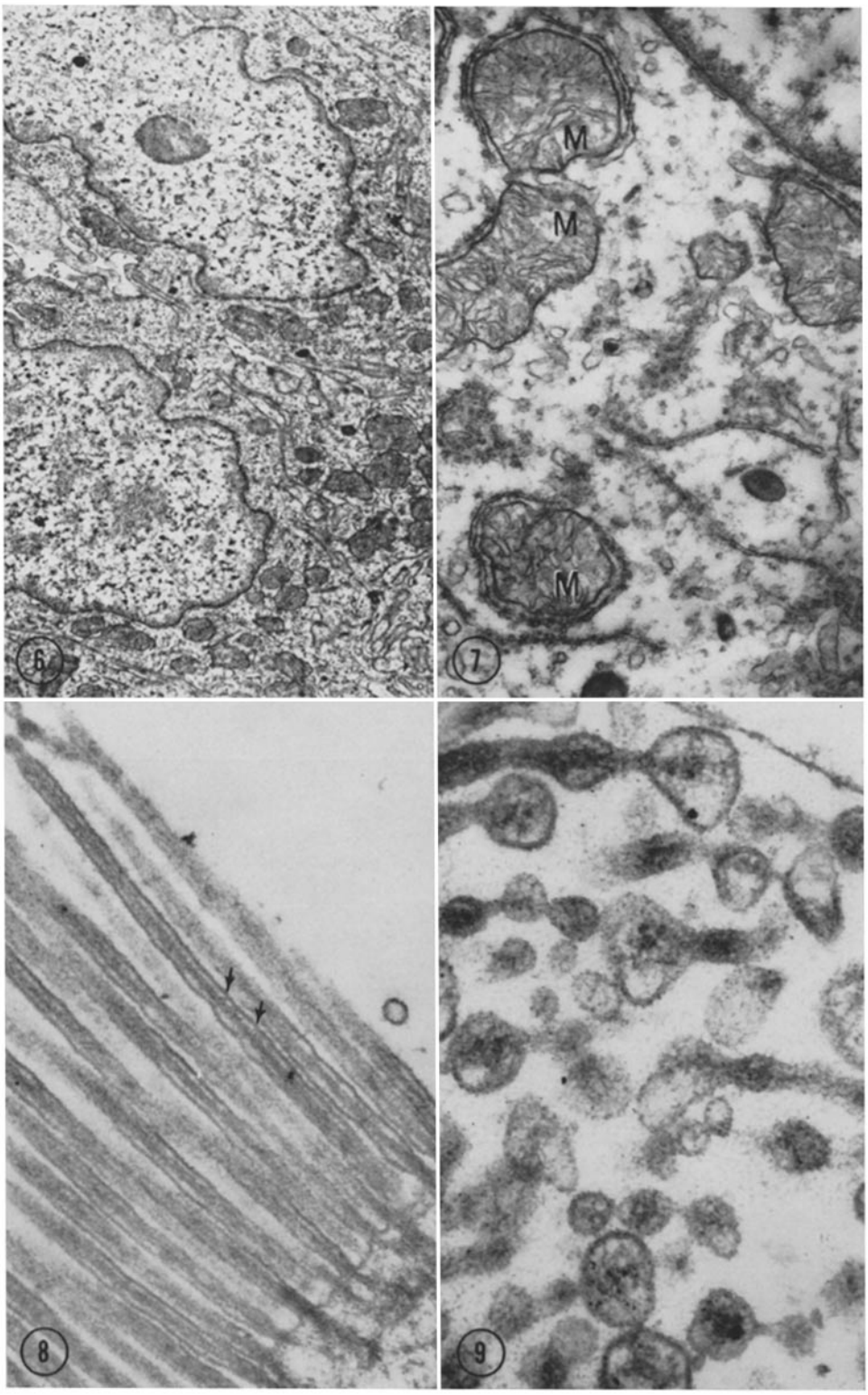
The cytoplasm after immersion for 1 hour. The cristae of the mitochondria, *M*, are swollen but in other respects the tissue appears normal. × 30,000.

FIGURE 8

Elongated microvilli observed after 5 hours' immersion. Blistering can be seen at this stage and lateral fibres extending between the core and the microvillus membrane can also be identified, (arrows). × 40,000.

FIGURE 9

Degenerate microvilli observed after 24 hours' immersion. The double contoured surface membrane can still be seen (over short distances) on the blisters and vesicular particles. The latter range in size from 0.1 μ to about 0.3 μ. × 40,000.


M
M
M
6
7
8
9

microvillus width nor simply related to the breakdown of the microvillus itself. Considering only the mean values, the maximum expansion (40 A) occurred after immersion for 1 hour in 0.33 N saline. However, after this period the variability of the measurements increased. It is interesting to note that where the preparations showed enlarged discontinuities in the membrane structure there was also an increased thickness and a direct correlation seemed probable.

Densitometer traces across the swollen membranes showed that the distance between the two inner peaks remained at about 45 A despite the over-all increase in width (Fig. 1 (*c*)). However, densitometer traces across the membranes around the vesicular particles showed that in most cases the central trough had expanded, but by now the over-all width of the membrane was extremely variable.

guished in this expanded core but on further immersion there was a shrinkage of the core so that it no longer filled all the space enclosed by the membrane (Figs. 17 and 18), and the fibres again became visible. The terminal web was also seen initially as an electron-opaque mass in which the individual fibres could not be distinguished. However, when the tissue began to disintegrate the cytoplasm throughout the cell became fibrous (Fig. 20).

Degeneration of the microvillus was observed after immersion for only 1 hour in 10 N solution. In the less concentrated solutions degenerative changes were not seen until the tissue had been immersed for 2 hours. Blistering was again a feature of the initial degeneration (Fig. 17), and disintegration followed a pattern similar to that described above with alternating regions of expansion and contraction. Eventually the microvillus disrupted to form vesicular particles (Figs. 19 and 20).

TABLE I

Effect of Hypotonic Solutions on the Widths of the Microvillus, Mv, and Microvillus Membrane, Mem

	Concentration of saline							
	N		0.5 N		0.3 N		0.25 N	
Time of immersion	Mv μ	Mem A	Mv μ	Mem A	Mv μ	Mem A	Mv μ	Mem A
½ hour	0.10	115	0.094	118	0.098	126	0.086	127
1 hour	0.088	125	0.11	135	0.115	154	0.12	146
2 hours	0.086	126	0.107	125	0.103	177	var	117
5 hours	0.082v*	100v	0.090v	114v	0.097v	137v	var	130v
24 hours	var‡	137v	var	107v	var	var	var	var

* *v*, Variation between ±10 and ±50 per cent.
‡ *var*, Variation greater than 50 per cent.

4. *Hypertonic Saline Effects*

The mean values of the microvillus width, the thickness of the membrane, and the width of the core, determined after the tissue had been immersed in hypertonic saline for various times, are given in Table II. The presentation of the results follows the same pattern as that adopted for Table I.

The first noticeable feature was the increase in the width of the core by about 50 per cent but at the same time there was a contraction of the whole microvillus. Both the core and the cytoplasm had increased in density (Fig. 16), and the expanded core appeared to occupy all the available space in the microvillus. The fibres could not be distin-

DISCUSSION

Stability of the Apical Region of the Intestinal Principal Epithelial Cell

The mitochondria were the organelles which first showed signs of distortion and breakdown following immersion of the tissue in hypotonic saline solutions. Further changes in the structure of the cell included an increased vacuolation of the cytoplasm. However, even after immersion in the 0.25 N saline for ½ hour, no gross changes in the structure of the microvilli were noted. Thus it would seem that there may be a stabilising factor

present in the structures of the apical region of the epithelial cells which enables them to withstand short term gross hypotonic environments. On the other hand, the microvillus structures were affected very rapidly by immersion in hypertonic solutions.

The over-all contraction of the microvillus following immersion in the hypertonic solutions may reflect the removal of free water from the structures by osmosis. However, an unexpected feature of the early changes observed under these conditions was the apparent expansion of the microvillus core to occupy the whole of the space available within the microvillus. Failure to identify the individual fibres of the core may mean that they had expanded to fill the entire space available. Such an expansion of the fibres might be possible if bound water played some role in the core structure and ions diffused into the core to effect an equilibrium. Nevertheless, one cannot

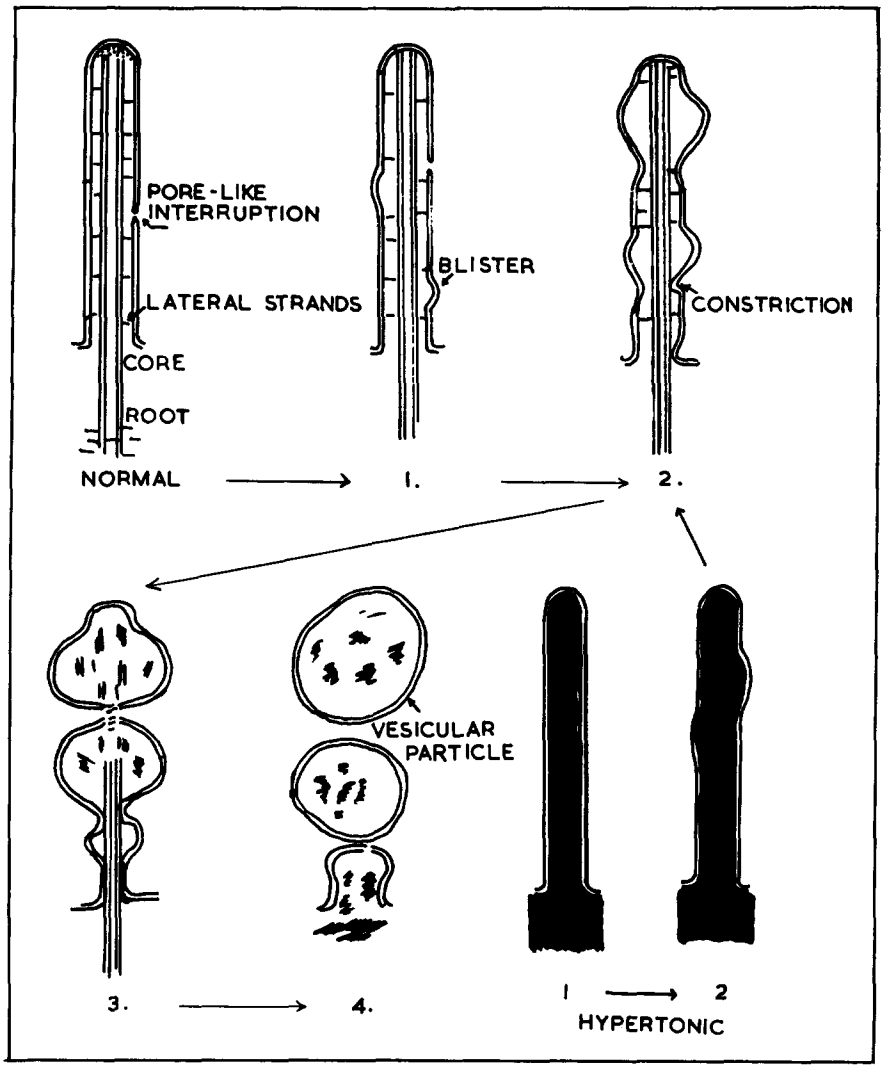


FIGURE 10

A diagrammatic representation of the sequence of degeneration of microvilli when intestinal tissue was immersed in saline solutions. The first two stages of the sequence in hypertonic solutions are shown separately.

rule out the possibility that the fibres may have been obscured simply by an increased staining of the intervening material.

In all specimens the appearance of blisters along the sides of the microvilli was the first sign of breakdown, and although the cause of blistering cannot be deduced from the present data, it is possible to account for the distribution of blisters by assuming that the lateral cross-fibres which appear to link the core to the surface membrane are not precipitated protein but structural elements. Thus swelling may have taken place where the lateral fibres were weak and a contraction of the stronger fibres may have been responsible for the local constrictions which developed later. However, fibres similar to the lateral fibres also occurred outside the microvilli (Fig. 12) and these render the interpretation of the lateral fibres within the microvillus as structural elements somewhat uncertain. It is possible that both ionic exchange and osmotic forces have played some part in the development of the degenerate condition and further studies of the effects of non-ionic solutions may assist in distinguishing between these two possibilities.

The ultimate breakdown of the microvillus into vesicular fragments is also observed in cells which have been shed from the villus, either in certain pathological states, or following treatment with aminopterin (17, 18) and even occasionally when cells have been shed from the extrusion zone under normal conditions (16). The observed sequence of structural modifications therefore may be common to all degenerative conditions. The vesicular fragments have not been observed in the lumen of ileal tissue and it would therefore seem that they must undergo further breakdown in the lumen. However, positive identification of such fragments that may have been derived from the particles has proved difficult. Specific labeling of the microvilli with a radioactive substance and the use of autoradiographic techniques might facilitate an identification of these breakdown products.

FIGURES 11 TO 15

Electron micrographs of intestinal epithelial cells after immersion in hypotonic solutions prior to fixation.

FIGURE 11

Vacuolated epithelial cells after ½ hour immersion in 0.3 N saline. The microvilli are elongated but the width is about the same as in normal tissue. × 7,500.

FIGURE 12

Microvilli in cross-section after 1 hour in 0.25 N saline. In some microvilli the fibres of the core appear as circular profiles and the lateral fibres are more distinct than in the normal preparations, (arrows). × 70,000.

FIGURE 13

Shortened form of the microvilli. The specimen was immersed for 2 hours in 0.3 N saline prior to fixation. The pairing of the dense lines of the core can be seen clearly. × 90,000.

FIGURE 14

Elongated microvilli after ½ hour immersion in 0.3 N saline. The pairing of the dense lines of the core can be identified even after this short period of immersion. × 90,000.

FIGURE 15

Further enlargement of Fig. 14 to show details of the pore-like interruption (arrow) in the double contoured membrane. × 190,000.

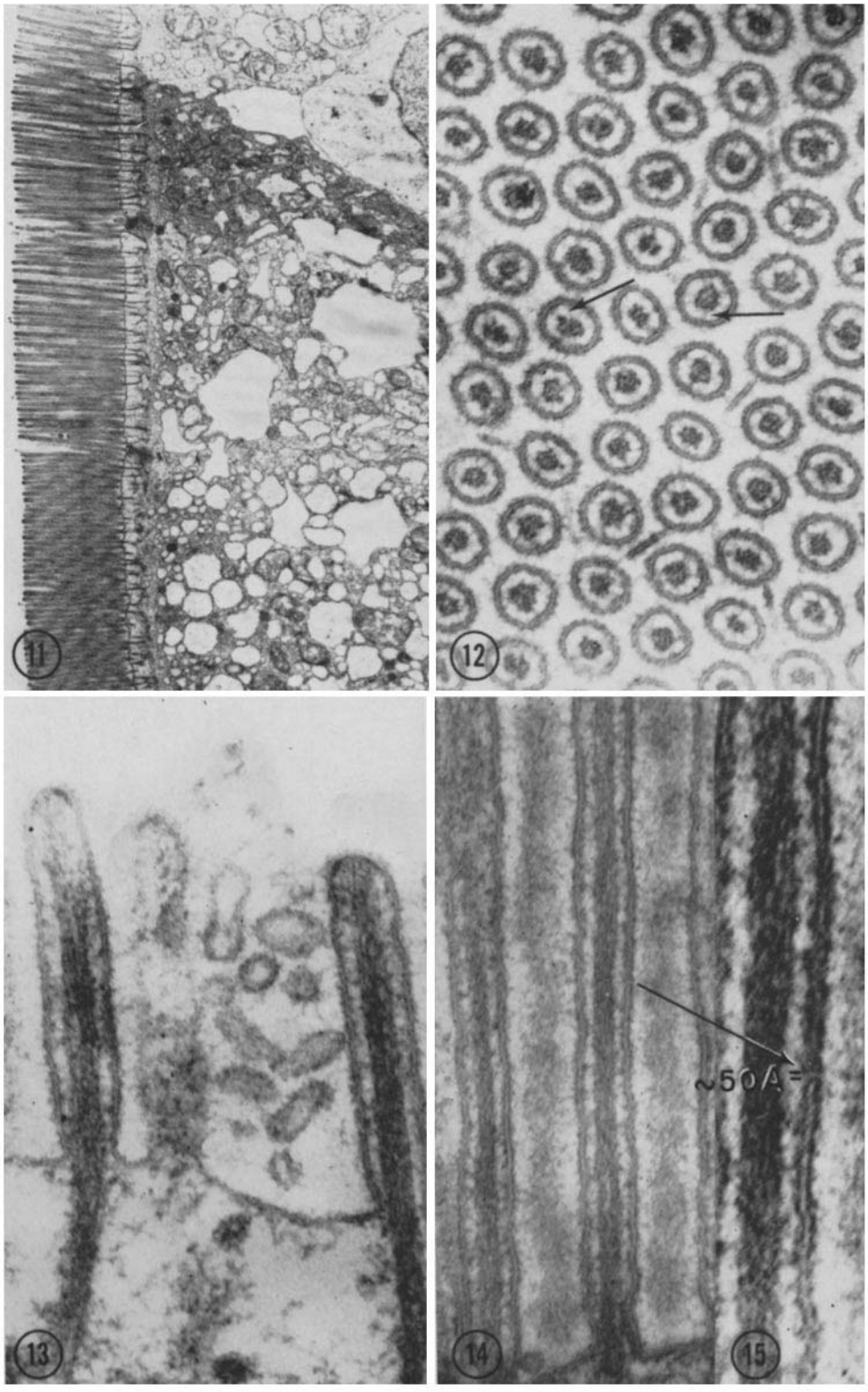

11
12
13
14
~50A
15

TABLE II

Effect of Hypertonic Solutions on the Widths of the Microvillus, Mv, Core, and Microvillus Membrane, Mem

Time of immersion	Concentration of Saline														
	N			2 N			4 N			5 N			10 N		
	Mv	Core	Mem	Mv	Core	Mem	Mv	Core	Mem	Mv	Core	Mem	Mv	Core	Mem
	μ	μ	A	μ	μ	A	μ	μ	A	μ	μ	A	μ	μ	A
½ hour	0.10	0.041	115	0.096	0.048	91	0.085	0.066	96	0.092	0.070	83	0.085	0.061	106
1 hour	0.088	0.041	125	0.085	0.039	114	0.088	0.044	99	0.091	0.050	104	0.082	0.060	90
2 hours	0.086	0.035	126	var‡	var	90	0.089v*	0.044	108	0.087v	0.043	110	0.076v	0.052	75
24 hours	var	var	137v	var	var	88v	var	var	97v	var	var	108v	var	var	116v

*, ‡ See Table I.

The Structure of the Core

New details of the core structure which were not apparent under normal preparative conditions were observed after immersion of the tissue in hypotonic solutions. The pairing of the dense lines seen in the longitudinal sections could represent either paired strands or sections through tubular structures of the type seen in cilia and flagella (6, 7), but a detailed study of cross-sections through microvilli from the same specimens did not provide sufficient evidence to distinguish with certainty between the two alternatives.

There was a tendency for the fibres to be clumped about the centre of the microvilli but the variability of the appearance precluded any count of the fibres and thus a comparison with the number found in cilia and flagella has not been attempted. The lateral strands which appeared to link the core to the surface membrane were a consistent part of the core architecture and may play an essential role in supporting the microvillus.

The function of the microvillus core as a whole may extend beyond that of a supporting structure and such a core may possibly act as a contractile element which by deforming the microvillus assists in the transfer of material from the lumen of the cell. It is possible that a contraction of the core might lead to a blistering of the type observed in this study during the degenerative changes. In fact early electron microscope studies of the small intestine (8) contained reports of changes in shape of the microvillus during absorption but later studies have failed to confirm this observation. Blistered microvilli have only been observed under normal conditions on cells in the extrusion zone of the villi and were then accompanied by degenerate-type changes in the cytoplasm (17, 15)

The Structure of the Microvillus Membrane

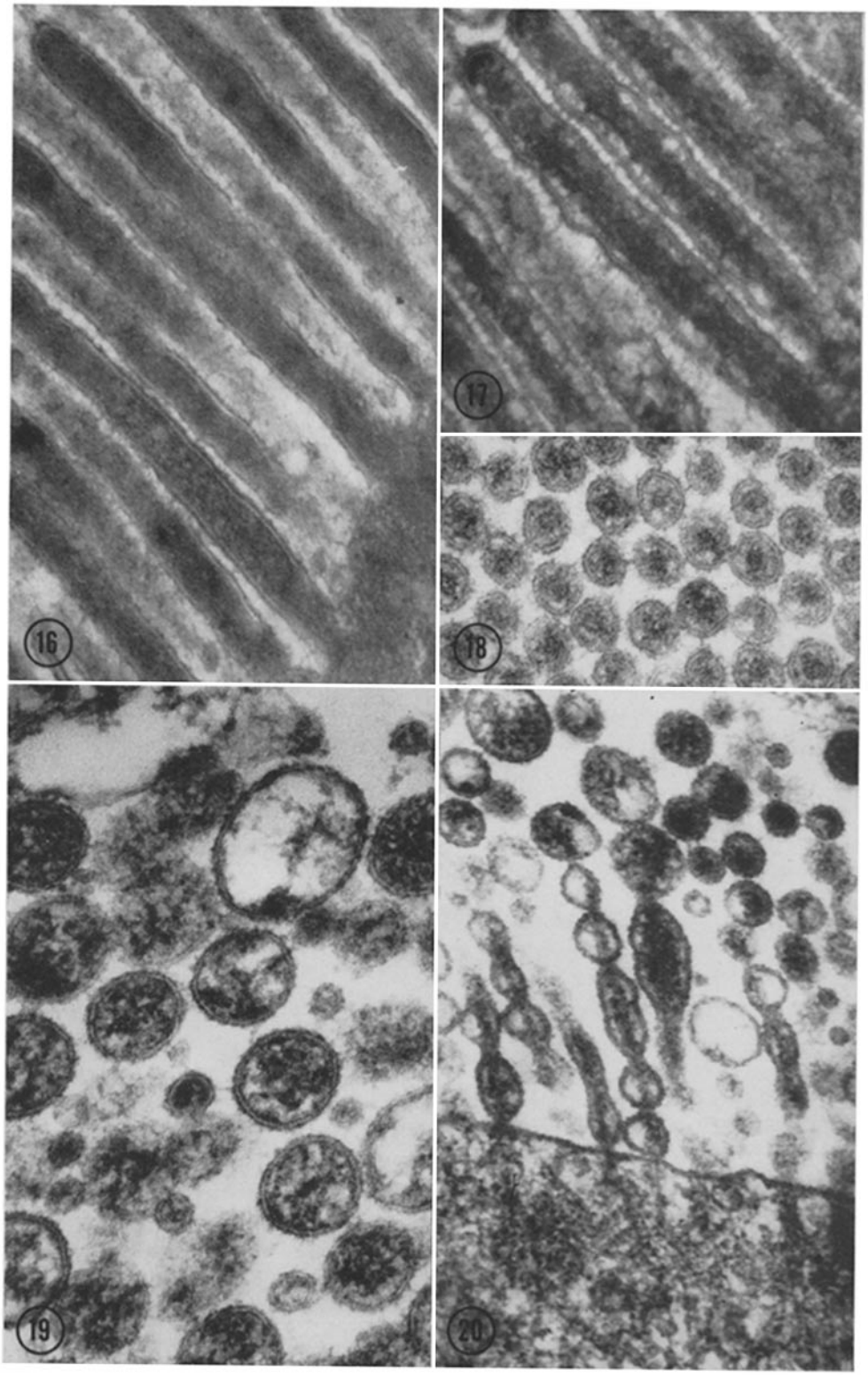
Visual estimates of the widths of the membrane have been found to give close agreement with the dimensions derived from densitometer traces. However, the over-all width of the membrane found in the present study is slightly greater than that reported by Zetterqvist (27) and Palay and Karlin (20). These earlier workers also estimated the widths of the dense and light components of the membrane (40 A and 25 A, respectively) but the densitometer trace shows that although there is a trough near the centre of the membrane the allocation of a width is somewhat arbitrary. The densitometer traces also showed that the membrane was asymmetric. If, in order to compare the earlier visual estimates with the densitometer trace, the 25 A low density region of the membrane is equated with the trough in the densitometer curves, then the widths of the membrane on either side would be 50 A and 40 A, respectively. Thus the difference between these results and those quoted by previous workers could reduce to a 10 A difference in the width of the outer dense line of the membrane. It is possible that this difference could be accounted for by differences in preparative technique employed. However, this does not invalidate the observation of an asymmetry in the membrane. Indeed it is probable that staining with PTA is necessary to demonstrate this asymmetry.

Another significant detail of the densitometer trace is the doubling of the peaks. This feature has also been recorded from frog intestinal epithelium (24). The separation of the inner peaks of the microvillus membrane (Fig. 1 (*a*)) is remarkably similar to that recorded between the peaks of a unit cell membrane (45 to 50 A). It is possible, therefore, that the microvillus membrane can be considered as a unit membrane with additional material associated with both surfaces. The identification of the central region of the microvillus membrane as a unit membrane would be consistent with the observation that, although there is an over-all expansion of the membrane in hypotonic solutions, the separation of the inner peaks does not appear to be affected. This would be in keeping with the location of the hydrocarbon chains of the lipid component at the centre of the membrane.

The expansion of the pore-like interruptions normally present in the microvillus membrane suggests that these can be distinguished from preparative artefacts. Although there is at present little evidence of a definite pore structure in the membrane it is possible that the interruptions are of the type suggested by Stein and Danielli (23), with the non-lipid components lining the pore, and that these are of physiological significance.

We wish to thank Professor A. C. Frazer for his continued interest in this work and also the Welcome Foundation for the generous loan of the electron microscope.

Received for publication, March 15, 1962.


16
17
18
19
20

REFERENCES

1. BAKER, J. R., *Quart. J. Micr. Sc.*, 1942, **84,** 73.
2. BAKER, J. R., *Quart. J. Micr. Sc.*, 1951, **92,** 79.
3. CLARK, S. L., *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 41.
4. DALTON, A. J., *J. Nat. Cancer Inst.*, 1950, **11,** 439.
5. DALTON, A. J., *Am. J. Anat.*, 1951, **89,** 109.
6. GIBBONS, I. R., *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 171.
7. GIBBONS, I. R., and GRIMSTONE, A. V., *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 697.
8. GRANGER, B., and BAKER, R. F., *Anat. Rec.*, 1949, **103,** 459.
9. GRANGER, B., and BAKER, R. F., *Anat. Rec.*, 1950, **107,** 423.
10. GOL'DIN, L. S., *Doklady Akad. Nauk. S. S. S. R.*, 1956, **106,** 721.
11. KARRER, L., *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 181.
12. KARRER, L., *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 391.
13. KELLENBERGER, E., SCHWAB, W., and RYTER, A., *Experientia*, 1956, **12,** 421.
14. MICHAELIS, L., *Biochem. Z.*, 1931, **234,** 139.
15. MILLINGTON, P. F., Ph.D. thesis, Birmingham University, 1960.
16. MILLINGTON, P. F., unpublished results.
17. MILLINGTON, P. F., FINEAN, J. B., FORBES, O. C., and FRAZER, A. C., *Exp. Cell Research*, 1962, in press.
18. MILLINGTON, P. F., FORBES, O. C., FINEAN, J. B., and FRAZER, A. C., *Exp. Cell Research*, 1962, in press.
19. PALADE, G. E., *J. Exp. Med.*, 1952, **95,** 285.
20. PALAY, S. L., and KARLIN, L. J., *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 363.
21. PALAY, S. L., and KARLIN, L. J., *J. Biophysic. and Biochem. Cytol.*, 1959, **5,** 373.
22. ROBERTSON, J. D., *Biochem. Soc. Sym.*, 1959, **16,** 3.
23. STEIN, W. D., and DANIELLI, J. F., *Discussions Faraday Soc.*, (membrane phenomena), 1956, **21,** 249.
24. SUGANUMA, A., *J. Biophysic. and Biochem. Cytol.*, 1961, **10,** 292.
25. WEISS, J. M., *J. Exp. Med.*, 1955, **102,** 775.
26. WEISS, J. M., *J. Exp. Med.*, 1955, **102,** 783.
27. ZETTERQVIST, H., Thesis Karolinska Inst. Stockholm, Aktiebolaget Godvil, 1956.

FIGURES 16 TO 20

Electron micrographs of intestinal epithelial cells after immersion in hypertonic saline prior to fixation.

FIGURE 16

Microvilli after only ½ hour immersion in 10 N saline. The core appears to fill the entire space within the microvilli and the individual fibres cannot be distinguished. × 90,000.

FIGURE 17

Microvilli after immersion for 1 hour in 2 N saline. The core has contracted and a space is now visible between the core and the microvillus membrane. Blistering can also be seen in this illustration. × 90,000.

FIGURE 18

Cross-section of microvilli after immersion for 1 hour in 4 N saline. × 85,000.

FIGURE 19

Vesicular particles produced by breakdown of microvilli after 24 hours' immersion in 10 N saline. The vesicles are two or three times the diameter of normal microvilli. × 90,000.

FIGURE 20

Microvilli after immersion for 24 hours in 5 N saline. They have not yet completely disintegrated into vesicular particles and the cell cytoplasm though fibrous is still intact. × 60,000.