Physiologically Significant Specializations of the Cell Surface

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This paper presents a brief review of the ultrastructure of some of the structural elaborations of the plasma membrane that may be regarded as adaptations for specific cell functions. Among the relatively stable specializations of the free surface, it considers the striated border of the intestinal mucosa, the brush border in the nephron, and filamentous coatings on the membranes of certain cells of the gastric mucosa. It then turns to those transient configurations of cell surfaces involved in the dynamic processes of pinocytosis, phagocytosis, and liberation of secretory products and considers the turnover of membranes associated with these phenomena. Discussion of specializations of the contact surfaces includes the desmosomes and terminal bars and the present status of intercellular cement and interfacial canals. A section on specializations of the cell base considers the basement membrane and basal infoldings of the plasma membrane in cells engaged in active transport. And finally, there is a description of the terminal web and the marginal band of nucleated erythrocytes—specializations of the superficial cytoplasm concerned with maintenance of cell shape.

OTHER PAPERS in this symposium on the ultrastructure of the plasma membrane have reported important new observations bearing upon the molecular organization of the plasmalemma or on the fine structure of its extraneous coatings as revealed by high-resolution electron microscopy. The present paper takes a lower power view of the cell and undertakes to draw together a number of examples of modifications of the cell membrane and elaborations of its surface topography that seem to be structural adaptations for particular cell functions. Although the illustrations are drawn from my own work and that of my colleagues in the Department of Anatomy at Harvard, no claim is made to priority in discovery or description of the structures to be discussed here. They are, for the most part, quite familiar to electron microscopists, but for those who study the cell membrane by biophysical and biochemical methods, a review of the ultrastructure of the common specializations of the free, attached, and basal surfaces of cells may be informative.

Specializations of the Free Surface

The refractile, vertically striated border on the luminal surface of the absorptive epithelium of the intestine was described by Henle in 1841 and in greater detail by Kölliker in 1856. There was general agreement among the cytologists of that period that this striated or brush border was a nonliving, extracellular coating, but there was a divergence of opinion as to its finer structure. Some regarded it as a layer of hyaline material that formed a protective coating penetrated by narrow vertical canals. Others attributed the vertical striations to the presence of delicate parallel rods embedded in the amorphous surface layer. This controversy remained unresolved until Granger and Baker, a century later, examined the border with the electron microscope. It is now known that the intestinal brush border is composed of a vast number of closely packed, cylindrical cell processes called microvilli (fig. 1). It is estimated that each cell of the duodenal epithelium bears as many as 2,000 such processes. The extraordinary uniformity in their distribution and the constancy of their diameter can be appreciated best in horizontal sections parallel to the cell surface (fig. 2). The microvilli are said to vary in length and in number at different points on the surface of the intestinal villus, being longer and more numerous near the tip and shorter and less abundant near the base. They may also be subject to some size variation in different nutritional condi-

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Figure 1
Electron micrograph of a vertical section through the striated border of the hamster jejunal epithelium, showing the closely packed, cylindrical microvilli characteristic of these absorptive cells. (Courtesy of Dr. S. Ito, Harvard University.)

Figure 2
Electron micrographs at high magnification of horizontal sections through the brush border of the intestine (above) and the nephron (below). The space between the circular cross-sections of the microvilli of the intestinal epithelium appears empty, while the microvilli of the renal epithelium appear to be embedded in an amorphous matrix of considerable density. This material is believed to be an extracellular, mucopolysaccharide coating of the cell surface. If it is present in the intestinal striated border, it is more easily extracted during specimen preparation.

...tions of the animal. The space between the microvilli generally appears empty in electron micrographs of the intestinal striated border (fig. 2), and a question which remains unresolved is the localization of the strong reaction for carbohydrates exhibited by this border in histological sections stained by the periodic acid Schiff reaction. The reactive material may possibly reside within the microvilli. It seems somewhat more likely, however, that an extracellular mucopolysaccharide normally occupies the interstices between them but is extracted by the methods of specimen preparation commonly used for electron microscopy.

When these details of the ultrastructure of the striated border were first revealed, it was quickly concluded that the substantial increase in surface area which would result from such a configuration was directly related to the absorptive function of the intestinal epithelium. It was regarded as simply an extension of the sound engineering for maximal surface with minimal mass that is evident in the structure of the gastrointestinal tract at all levels of organization. Surface amplification is achieved at the gross anatomical level by elongation of the tubular viscus (fig. 3A), then by corrugation of the mucosa to form the plicae circulares or valves of Kerkring (fig. 3B). These plications are, in turn, covered with villi of microscopic dimen...
sions (fig. 3C). The individual cells on the villi are covered with the microvilli visible with the electron microscope (fig. 3D). If one wishes to belabor the point still further, one finds, in the mitochondria within the cell, the cristae mitochondriales (fig. 3E), which again represent a surface amplification by plication. The strategem of increasing the efficiency of organs by amplifying the area of the physiologically significant interfaces, appears to be one of nature’s commonest morphological devices for “miniaturization” of the metabolic machinery.

Several enzymes have been detected in the striated border by histochemical staining methods. Among the earliest of these was alkaline phosphatase. More recently, Miller and Crane, in an admirable correlated biochemical and ultrastructural study, have isolated from homogenates of intestinal mucosa a centrifugal fraction, consisting of intact striated borders. They found virtually all of the invertase and maltase activity of the homogenate concentrated in this fraction. In the digestive process, disaccharides and sugar phosphatases are believed to be hydrolyzed to monosaccharides, which are then presented to an active-transport mechanism located in or near the brush border. As a result of its surface configuration and the enzymatic activity of its membranes, the brush border thus appears to achieve an efficient coupling of digestive hydrolysis and active absorption. Long thought of as an important absorptive surface, the striated border has now been shown to be an important digestive surface as well.

A highly developed brush border occurs also in the proximal convoluted tubule of the kidney. Here, the structure of the microvilli is essentially the same as on the intestinal epithelium, but the space between them is occupied by an amorphous, extracellular substance of appreciable density (fig. 2). The presence of a mucopolysaccharide matrix between microvilli was postulated above to account for the periodic acid Schiff (PAS) staining reaction of the striated border of the intestine. If such a substance is present there, it is more easily extracted than is that of the brush border in the kidney. An extracellular matrix between the microvilli of renal tubular cells is easily demonstrated in routine electron micrographs. There seems to be no free space around the microvilli; indeed, in well-fixed mammalian kidney, the brush borders of the cells lining the tubule are often in such close contact that they obliterate the lumen. Some investigators accept this as the condition commonly prevailing in life and suggest that the glomerular filtrate is not free to flow rapidly through an open lumen but instead may be obliged to percolate slowly through a column of mucopolysaccharide matrix in the interstices of the coapted brush borders.

There is another significant difference between the intestinal and renal brush borders. At the bottom of the clefts between adjacent microvilli of cells in the proximal convolution of the nephron, invaginations of the cell surface form slender tubules that extend into the apical cytoplasm, where they run a sinuous course and therefore usually appear in electron micrographs as discontinuous tubular profiles of highly variable shape. These apical canaliculi have a thicker limiting membrane than the endoplasmic reticulum and have a content that, in texture and density, resembles the amorphous material in the inter villous spaces of the brush border. In the differentiation of the cells of the proximal segment of the nephron, invagination (apical canaliculi) and evagination (microvilli) of the cell membrane have been combined to achieve a further increase in surface. A similar combination of microvilli and apical canaliculi is found in at least two other absorptive epithelia, namely, the visceral layer of the mammalian yolk sac and in the ductuli efferentes of the testis. Whether the absorption of the same or different classes of substances takes place on the microvilli and in the canaliculi remains to be determined in future studies.

Although there seems to be no doubt that the highly developed borders on the renal and intestinal epithelium are structural adaptations related to the absorptive function of these organs, we cannot always equate the

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The presence of microvilli with absorptive function. As more organs have been studied with the electron microscope, a sparse covering of short microvilli has been found on a wide variety of cell types, many of which are secretory rather than absorptive in function. Among the secretory cell types provided with microvilli are the surface mucous cells and parietal cells of the gastric mucosa and the goblet cells of the intestinal epithelium. It is true, however, that on none of these are the microvilli quite as numerous, as uniform in size, or as orderly in their arrangement as they are in typical brush borders. Significant differences are detectable among the cell types in the character of the outer surface of the plasma membrane covering the microvilli. In the gastric mucosa, for example, the limiting membrane of the short microvilli on the mucous cells has a fuzzy appearance due to the presence of a naplike coating of exceedingly fine filaments (fig. 4). These are similar to the filaments described on the surface of cells of the toad bladder by Peachey and Rasmussen and on ameba by Brandt in another paper in this symposium, and they need not be discussed in detail here. It is of interest, however, to note that the microvilli of the mucus secreting cells of the gastric mucosa have such a fuzzy appearance, while the microvilli of the immediately adjacent acid-secreting parietal cells are smooth surfaced (fig. 4).
This observation suggests that the filamentous coating is not merely a result of nonspecific adherence of a mucus secretion to the cell surface but is probably a characteristic feature of the membrane of these cells. The functional significance of this specialization of the plasma membrane remains to be clarified.

The development of the concept of the "unit membrane" by Robertson and his demonstration of the very widespread occurrence of a trilaminar organization in cellular membranes has unfortunately tended to create the impression that all membranes are similar in chemical composition. It would be a mistake to lose sight of the fact that membranes having a similar appearance in electron micrographs may actually be highly diverse in their functions. At the present stage of development of our methods of specimen preparation, it is likely that we are visualizing mainly the phospholipid skeleton of the membrane. This may indeed have a structural organization that is common to most cellular membranes. The important functional differences between membranes doubtless reside in the protein enzymes associated with this phospholipid skeleton and in its mucopolysaccharide or glycoprotein extrinsic coatings. These components may well be lost in specimen preparation. The fuzzy coatings we have just been discussing were overlooked until recently because they were not preserved by earlier technical procedures. As our methods of preservation continue to improve, it is safe to predict that we will begin to detect more and more structural diversity among membranes that now look similar in electron micrographs.

Thus far, we have been discussing relatively stable structural differentiations of the free surface. We turn now to a consideration of those transient configurations of the surface membrane that are associated with the incorporation of extracellular fluid by pinocytosis, ingestion of particulate matter by phagocytosis, and liberation of cell products in secretion. Electron-microscopic investigations have revealed, in greater detail, the nature of these surface activities and have focused attention upon the very large turnover and translocation of plasma membrane involved in processes of bulk import and export from the cell. The presence of a limiting membrane on the pinocytosis vacuoles observed in living tissue culture cells by Warren Lewis in the 1920's could not be definitely established with the light microscope but was inferred from the sharpness of their limits and from the way in which they formed, which seemed to be by the impounding of a droplet of the fluid medium between folds of the thin, undulating membrane at the thinned-out peripheral portion of the cell. When capillary endothelium was first examined under the electron microscope by Palade, large numbers of small flask-shaped vesicles 70 to 100 mμ in diameter were found immediately beneath the surface. These submicroscopic vesicles were bounded by a membrane that was often continuous with the plasmalemma around the rim of a narrow stalk or neck, through which the interior of the vesicles was in open communication with the lumen of the capillary. Other vesicles were closed and appeared to have separated from the surface membrane. It was assumed that these vesicles arose by local invagination of the plasma membrane, and that they subsequently pinched off, carrying minute droplets of the plasma into the cell. Although the mechanism of their formation was rather different from that giving rise to the much larger vacuoles observed by Warren Lewis, this submicroscopic vesiculation of the endothelial cell membrane was nevertheless interpreted as a form of pinocytosis, and the suggestion was offered that it might have an important role in transport across the capillary wall. Whether any appreciable amount of fluid entering at the luminal surface in this manner can be moved across the cell and discharged from the vesicles at the basal surface is still a subject of dispute. A similar vesiculation of the surface membrane has now been observed in a variety of other cell types that are not specifically concerned with transport of substances from one surface to the other, and the idea that a submicroscopic order of pinocytosis is a common and
physiologically important mechanism for the active uptake of water and solutes for the cell’s own use is rapidly gaining acceptance.

In extreme instances, the number of vesicles being formed at the cell surfaces becomes astronomical (fig. 5). Although they are usually single, one vesicle opening at the surface may communicate with a second through a narrow constriction and this with a third, fourth, and fifth, forming a beaded strand of interconnected vesicles, extending for some distance into the cytoplasm. The interior of the cell body may become crowded with vesicles that have detached from the surface. If it is true that the vesicles form simply by invagination of a small area of the plasma membrane and its subsequent abscission, then the amount of surface membrane carried into the cytoplasm in such a dynamic process would soon use up the entire plasma membrane, if the latter were not being replaced at an equivalent rate. Since no site of compensating addition of preformed membrane to the surface can be identified in electron micrographs, one is led to conclude either that each vesicle forms as a result of local synthesis of new membrane or there must be a general and uniform addition to the plasma membrane of phospholipid and protein components from the cytoplasm, molecule by molecule, at such
A diagram illustrating the active exchange or circulation of membrane involved in secretion, phagocytosis, and pinocytosis. In secretion (A), the cell product acquires a membrane in the Golgi region. During release of secretory product, this membrane is added to the plasmalemma. The area enclosed in the interrupted line is intended to suggest that there must be a simultaneous degradation of membrane elsewhere on the surface and a reassembly of components into visible membrane in the Golgi region. In phagocytosis (B) and pinocytosis (C), considerable amounts of surface membrane are removed when particles or fluid are engulfed in membrane limited vacuoles that move into the cytoplasm. A compensating synthesis of membrane must be occurring elsewhere on the cell surface to replace the plasma membrane interiorized in these processes.

A rate as to counterbalance the bulk losses resulting from inward movement of pinocytosis vesicles (fig. 6C).

The mechanism of phagocytosis is not significantly different from pinocytosis. The membrane-limited vacuoles that envelop bacteria or large nonliving particles are vastly larger and fewer in number than the pinocytosis vesicles just described. The need to replace rapidly the considerable area of surface membrane taken into the cell is the same (fig. 6B). Biochemical investigations of cell metabolism and phospholipid turnover in cells active in pinocytosis have not yet been undertaken, but data are available for phagocytic cells. Karnovsky and coworkers\(^\text{18, 19}\) have shown that there is a substantial increase in metabolism and in the turnover of phosphatides in phagocytosing leukocytes compared to resting controls. This increased turnover amounts to a doubling of the incorporation of phosphate label while the total phosphatide remains essentially unchanged.

Electron-microscopic studies of pancreas, parotid, and other exocrine glands provide further morphological evidence for an internal circulation of membrane in relation to the process of liberation of the secretory product. The correlated fine structural and biochemical investigations of pancreatic secretion by Palade and Sickewitz\(^\text{20-22}\) indicate that the enzymes of the pancreatic juice are probably synthesized on or in close topographical relation to ribosomes that are associated with the membranes of an extensive endoplasmic reticulum. The product accumulates in the lumen of the reticulum and is somehow transported to the Golgi region. In the guinea pig
it may be formed into granules within the reticulum, but, in most species, segregation and concentration of the product into zymogen granules takes place largely within vacuoles in the Golgi region. Each zymogen granule stored in the apical cytoplasm is enveloped by a smooth-surfaced membrane acquired in the Golgi complex. In response to the stimulus of feeding, the digestive enzymes are released by a process in which the limiting membrane of the zymogen granule becomes continuous with the plasma membrane. As the membrane enclosing the granule is incorporated into the cell membrane, its contents become extracellular without having passed through the membrane in the usual sense (fig. 7). It is obvious that this mechanism of release involves addition to the plasmalemma of a considerable amount of new membrane formed originally in the Golgi region. Inasmuch as the cell does not increase in volume and the plasma membrane does not become redundant, there must be a concurrent withdrawal of an equivalent amount of membrane. This return of membrane to the cytoplasm may conceivably take place at an amicroscopic molecular level. The molecular components may then be reassembled into visible membrane in the Golgi region to complete the cycle.

Figure 7
Electron micrograph of the apical portions of several pancreatic exocrine cells around the lumen of an acinus. The apical cytoplasm contains numerous dense zymogen granules. The inset illustrates the mechanism of release of the cell product. The zymogen granule approaches the cell surface and its membrane becomes continuous with the plasma membrane (at arrows). The contents of the granule or vacuole thus become extracellular and are emptied into the lumen without having passed through the plasma membrane in the usual sense.
In spite of the dramatic cinematographic testimony of living cells, the plasma membrane has too long been thought of as a relatively inert lipoprotein film penetrated by pores of fixed dimensions that gave it certain permeability properties. Research on the passage of substances into and out of cells has focused mainly upon their molecular size, charge, and lipid solubility. A much more dynamic concept of the cell surface is now evolving. The idea of a circulation of membrane substance between the surface and the interior of the cell has steadily gained momentum in the past decade. It was first suggested by Palade's early observations on submicroscopic pinocytosis by capillary endothelium and deep infolding of the plasma membrane of macrophages. The hypothesis that membrane vesiculation and membrane flow from superficial sites of synthesis to deeper sites of degradation may be important mechanisms for cellular active transport was further developed by Bennett. The elucidation of the mode of release of zymogen from the pancreatic acinar cell led Palade to propose that an exchange of membrane also takes place between the Golgi region and the plasma membrane in the process of secretion. The morphological basis for this suggestion has been amply confirmed by our own observations on the pancreas and by those of Parks on the parotid. The increased turnover of phosphatides in the stimulated pancreas reported by Hokin is probably a biochemical correlate of this traffic in membrane components. Investigations of the cell surface in the future will probably be increasingly concerned with bulk translocation of substances in membrane-limited vesicles and with the cellular mechanisms for synthesis, degradation, and internal circulation of membrane.

Specializations of the Contact Surfaces

In the textbooks of 15 to 20 years ago, we read that columnar epithelial cells were separated from each other by narrow spaces filled with intercellular lymph or cement substance and that, near the free surface, each intercellular cleft was closed by a bandlike accumu-
In addition to the cohesive force that acts over the entire surface, the majority of epithelia have special attachment devices, the desmosomes, distributed at intervals along the cell boundaries (figs. 8 and 10). These are bipartite structures consisting of circular plaques on opposing surfaces of adjacent cells. In section, they are rectangular or lenticular in shape and have a characteristic fine structure which can be resolved into several layers of differing texture and density. These dense bands are flanked by two wider bands of lower density and less clearly defined limits. The dense bands seem to consist of a homogeneous material, which, while possibly distinct from the plasmalemma, is very closely applied to its cytoplasmic surface. The image recorded in electron micrographs could be produced equally well by a thickening of the innermost element of the trilaminar unit membrane itself. The broader bands are clearly not an integral part of the membrane but are made up of a feltwork of interwoven fine filaments running both parallel and perpendicular to the cell surface. In cells that have well-developed tonofilaments
in their cytoplasm, these tend to converge upon the desmosomes (fig. 10). At high magnifications a thin dense line is visible in the intercellular cleft midway between the two cell surfaces (see inset fig. 5), but no structure that could mechanically bind the two parts of the desmosome together is seen crossing the interspace. Although there is ample evidence that cells adhere more tenaciously at these sites than elsewhere on their surfaces, the nature of the binding force is unknown. It is noteworthy that the desmosome is formed as a cooperative enterprise between two cells. A half desmosome is rarely seen on a lateral cell surface. We know nothing about what determines the location of desmosomes or how the formation of one half induces the immediate formation of the complementary half in the adjacent cell.

The structures identified as terminal bars by classical cytologists are found to be quite similar to desmosomes in their fine structure but differ from them in their shape and location on the cell surface. Instead of circular plaques, they are long bands near the free surface of the epithelium and extend the full length of the interface between cells. In vertical sections of the epithelium, the terminal bars are more extensive and more variable in size than desmosomes, and their limits are less sharply defined (fig. 9). The cytoplasmic filaments of the terminal web in columnar
epithelia bear the same relation to the terminal bars that the tonofilaments of other epithelia bear to the desmosomes. The intercellular space at the terminal bar, and particularly between the terminal bar and the free surface, is often narrower than elsewhere on the boundary between cells. The possible significance of this closer approximation of the surfaces as a water-tight closure of the luminal end of the intercellular cleft was pointed out by Peachey and Rasmussen and has recently been re-emphasized by Farquhar and Palade.

The old concept of functionally significant intercellular channels, occupied by fluid or lymph, still appears to have some validity for the basal and spiny layers of stratified squamous epithelia and in certain stratified columnar epithelia, even though, in general, the results of electron microscopy have tended to minimize the physiological importance of the intercellular spaces by revealing that they are usually much narrower than was formerly thought to be the case and by showing that there are, at the terminal bars, special tight closures that seal off access from the lumen.

Figure 10
Portions of the adjoining cells in an electron micrograph of epidermis from an Amblystoma larva. The cells have short processes that meet end-to-end at desmosomes. These processes correspond to the "intercellular bridges" of light microscopy. Between them the cell surfaces are retracted to form a system of communicating intercellular spaces or interfacial canals. (Courtesy of Dr. Elizabeth Hay, Harvard University).
to these potential intercellular pathways. In stratified squamous epithelium, the cells are covered with numerous short processes. The processes of neighboring cells meet end to end and are firmly joined by desmosomes at these sites (fig. 10). The end-to-end junctions of these processes correspond to the so-called "intercellular bridges" of light microscopy. Between them is a system of interfacial canals which may have an important role in the nutrition of the more superficial layers of the epithelium. In the stratified columnar epithelium lining the ducts of the avian salt gland, the cells are surrounded by conspicuous intercellular spaces, and the lateral cell surfaces bear numerous microvilli or thin folds that increase the area of cell surface exposed to the intercellular fluid. Indeed, the cells appear to be attached only by small desmosomes on certain of the villi that come into contact (fig. 11). It is not known whether these prominent intercellular pathways are essential to the nutrition of the epithelium or provide avenues for transport of water to alter the osmotic properties of the secretion as it passes through the duct system.

Figure 11

The junction of three cells from a duct of the salt gland. There are prominent intercellular spaces into which project numerous slender folds or ridges on the surfaces of the neighboring cells. The presence of this surface amplification in relation to the intercellular clefts suggests that these spaces have physiological significance either for nutrition of the duct cells or for transport of water or ions to or from the duct lumen. The cells are evidently attached only by small desmosomes at points of contact of the folds or villi of adjacent cells (see at arrows).
Specializations of the Cell Base

At the base of most epithelia there is an extracellular layer that appears to be a feltwork of fine fibrillar material. The fibrillar component is more loosely organized, and therefore less dense, immediately adjacent to the plasma membrane but has a compact darker zone at a distance of 40 to 50 m. from the plasmalemma. Beyond this it grades off again in density and has a poorly defined outer limit. This layer which appears in electron micrographs as a continuous dark band about 50 m. away from the base of the epithelial cells is believed to be mucopolysaccharide or glycoprotein in nature and is now commonly called the basement membrane—a term which is unfortunate in at least two respects. First, it does not correspond exactly to the basement membrane of classical histology, which was a thicker structure, evidently including the layer under discussion here, plus a variable amount of ground substance and reticular fibers. Secondly, the word membrane has now come to have more precise and restricted meaning for the histologist using the light microscope. It generally describes an osmiophilic lipoprotein component of cells which, at relatively low magnification, appears as a dense linear profile 8 to 12 m. in thickness and which, at higher magnification, can often be resolved as a trilaminar structure consisting of two dense lines above 30 A thick, separated by a less dense layer of about the same thickness. To avoid confusion, it would be desirable to retain membrane to describe such lipoprotein components of cells and to refer to the external glycoprotein substrate of epithelia as the basal or basement lamina.

Traditionally, the "basement membrane" of epithelia was regarded as a condensation of the ground substance and fibers of the underlying connective tissue. Although more investigation is needed to determine its origin and properties, there is increasing morphological evidence that the basal lamina seen in electron micrographs is to be equated with other external cell coatings, such as those investing the Schwann cells of nerve, the sarcolemma of muscle, etc., and it is probably a product of the epithelial cells themselves, instead of the neighboring connective tissue cells.

In the majority of epithelia, the plasma membrane at the cell base is smooth, specialized, and runs parallel to the continuous basal lamina. However, in several cell types that are engaged in active transport, the plasma membrane turns inward at intervals along the cell base, forming narrow infoldings, or bilaminar septa, that extend for variable distances into the basal cytoplasm, dividing it into numerous compartments that are closed below, but which may be open above to the perinuclear cytoplasm. Other compartments appear in electron micrographs to be completely enclosed by a continuous pair of membranes that can be traced into the cell and then back again to the base (fig. 12B). These evidently represent processes of the same or of adjacent cells that have insinuated themselves between the cell body and the basal lamina and intermingle there with other such processes to form a labyrinthine system of intracellular compartments and extracellular clefts. Of the many pairs of membranes that extend into the epithelium from the basal lamina, some may thus represent the boundaries of two different cells, while others represent infoldings of the plasma membrane of the same cell. Which of these two possibilities applies in any particular case is difficult to establish without reconstruction from serial sections.

The degree of development of this kind of specialization of the cell base varies in different epithelia involved in transport. In the proximal convoluted tubule of the frog nephron, the ciliary body of the eye, and in the choroid plexus of the brain, the basal labyrinth is of limited extent, and the compartments of the cytoplasm are generally too small to contain mitochondria. On the other hand, in the distal convoluted tubule of the nephron, the striated ducts of the parotid gland, and certain other organs with a capacity to move sodium against a concentration gradient, the basal labyrinth penetrates more deeply into the epithelium. Numerous large mitochondria are lodged in the narrow compartments de-
Figure 12
A diagram showing varying degrees of development of the basal labyrinth of cells engaged in active transport. (A) The shallow penetration of basal infoldings such as is seen in the ciliary body, choroid plexus, or proximal convoluted tubule of the frog. (B) The deeper incursions of membranes delimiting compartments large enough to include numerous mitochondria. Such a configuration of the cell base is common in the distal convoluted tubule of the kidney and the striated ducts of the parotid gland. (C) The extraordinarily elaborate compartmentation of the cells of the salt gland. The entire epithelium is divided into thin leaflike compartments extending nearly to the free surface. A very large number of mitochondria are lodged in these slender compartments, and between them is an elaborate system of extracellular spaces.

Figure 13
Electron micrograph of a small area of the basal cytoplasm of a cell in the distal convoluted tubule of bat kidney. The elaboration of the cell base brings a very large area of membrane into close association with many mitochondria that presumably provide energy for active transport of fluid or ions. (Courtesy of Dr. S. Ito, Harvard University.)

Figure 14
An electron micrograph of a portion of the base of one of the principal cells of the salt gland in the herring gull. There is a continuous basement lamina, but the plasma membrane turns inward at very close intervals, dividing the cell base into compartments 50 to 200 mμ wide, except where they expand to enclose a mitochondrion. The extracellular clefts between compartments are of varying widths and may enlarge under certain physiological conditions.

limited by the interdigitating cell processes and by infoldings of the plasma membrane (fig. 13). The most extreme example of membranous compartmentation of the cell base is seen in the salt gland of marine birds. This remarkable organ is capable of excreting salt at concentrations of 0.5N and, because of its capacity to eliminate salt at these high concentrations, is believed to make it possible for marine birds to drink sea water and profit from it. The principal cells of the glandular epithelium have myriad basal infoldings that extend to, or nearly to, the cell apex, dividing the cell into countless thin, parallel compartments separated by narrow extracellular clefts. Many of these compartments are only 50 to 80 mμ in thickness, except where they expand to accommodate mitochondria, which are extraordinarily abundant in the epithelium of the salt gland (fig. 14).
We still know very little about the actual mechanism by which this specialization of the cell base promotes the specific function of cells engaged in transport. It is evident, however, that the peculiar surface configuration of the cells found in organs that are most active in this function brings a greatly extended area of plasma membrane into intimate relation with a very large number of mitochondria. The mitochondria are thus in a position to furnish energy for the transfer of ions across the membrane. In the case of the salt gland, the infoldings of the cell base also bound a labyrinthine system of extracellular clefts which may constitute sites of accumulation or pathways for movement of fluid or ions.

**Specializations of the Cytoplasm Concerned with Maintenance of Cell Shape**

Several of the early cytologists described a reticular or fibrillar component of the cytoplasm that seemed to constitute a sort of cytoskeleton with an important role in maintaining cell shape. The reticular appearance of the cytoplasm that was the basis of this idea later came to be regarded as a coarse precipitation pattern due to the action of certain fixatives. Thereafter, interest in internal, form-determining constituents of cells diminished. Recent cytological and ultrastructural investigations have focused new attention upon fibrillar and tubular components of cells that may influence their shape. On the basis of selective staining for the light microscope, Leblond et al.\(^3\) described a structure called the *terminal web*, extending across the apex of intestinal epithelial cells. This component and the associated terminal bars are believed to have a supporting or stiffening function. In subsequent light-microscopic studies by these authors, corresponding structures were found in many different types of cells. At the ultrastructural level, fine filaments about 50 Å in diameter have been found as a component of the cytoplasmic matrix in nearly all cell types that have been studied with the electron microscope. The terminal web of intestinal epithelial cells was shown by Palay and Karlin\(^3\) to consist of a feltlike condensation of these filaments extending across the apex of the cell and terminating in the terminal bars. A coarse-meshed, intracellular network made up of similar filaments aggregated in bands immediately beneath the plasma membrane has been described by Hay\(^3\) in the Leydig cells of amphibian epidermis. A conspicuous condensation of similar filaments in the cytoplasm of Müller’s cells was found by Ladman\(^3\) to constitute the so-called external limiting membrane of the retina. No doubt there will be forthcoming many other examples of mobilization of this fibrillar component of the cytoplasm at the periphery of cells to maintain special configurations of their surface.

Another supporting element beginning to receive some attention consists of straight hollow tubes about 30 μ in diameter. These occur immediately beneath the pellicle of trypanosomes,\(^3\) where they may conceivably play a role in maintaining the characteristic shape of the organism. They appear transiently during mammalian spermiogenesis at the time of elongation of the spermatids and form by their lateral association a cylindrical structure called the *manchette*, or caudal sheath.\(^4\)

Because of the prominent place which erythrocytes have had in the discussion at this symposium on the plasma membrane, I would like to present a brief account of the *marginal band* of nucleated erythrocytes as a further example of this tubular cytoskeletal element. Although it was recognized earlier by Dehler\(^4\) in fixed and sectional material, Meves\(^4\) appears to have been first to demonstrate, by supravital staining, an equatorial band around the periphery of the nucleated erythrocytes of birds, amphibia, and reptiles. He observed that the band seemed to be made up of multiple parallel filaments and carried out experiments which led him to conclude that this structure had elastic properties which probably were important in maintaining the flattened elliptical form of the erythrocytes in these species. Other investigators, notably Weidenreich,\(^4\) rejected this interpretation.
and considered the images obtained by Meves to be staining artifacts. This view prevailed, and references to the marginal band disappeared from the hematological literature. Incidental to electron-microscopic studies on other fish and amphibian tissues, it has been possible to verify the reality of the marginal band of the erythrocytes.\(^4^4\) In accord with Meves’s remarkably keen observations, it is made up of a number of finer subunits. In electron micrographs it is found to consist of a bundle of about two dozen, parallel tubular elements about 30 m\(\mu\). in diameter encircling the erythrocyte immediately beneath the plasma membrane (fig. 15). The hooplike form of these elements, their equatorial location, and their fine-structural resemblance to supporting elements in other cells lend plausibility to Meves’s suggestion that they may play a significant role in maintaining the flattened elliptical form of avian, amphibian, and piscian erythrocytes.

**Concluding Comment**

The histologist of a generation ago recognized certain obvious specializations of the free surface of simple epithelia, such as brush borders, stereocilia, motile cilia, and the like. He was aware of a few examples of highly irregular cell boundaries, such as those in lymphatic endothelium and the fluted margins of the cells of the proximal convoluted tubule.
in the mammalian nephron, but in general it was assumed that the shape of cells in the parenchyma of epithelial organs was largely the result of surface tension and of mutual deformation. By reconstruction of various animal and plant cells from serial sections, the late Prof. F. T. Lewis\textsuperscript{15, 46} showed that they were polygons with an average of fourteen sides—a number that is in accord with Lord Kelvin's solution to the problem of dividing space into uniform bodies of minimal surface. The observed approximation of the shapes of epithelial cells to the Kelvin tetra-kaidecahedron encouraged the belief that epithelial cells behaved as passive plastic bodies, whose shape was largely determined by their tendency to pack without interstices and to assume minimal surface in relation to volume.

The electron microscope has brought far-reaching changes in our concept of the shapes of cells and the factors which determine them. Many new features of surface topography have been disclosed, of which only a few have been included in this presentation. It is evident that surface tension and externally applied forces cannot explain all of the specializations of the free, lateral, and basal cell surfaces now revealed by the greater resolution of the electron microscope. These features suggest a degree of intrinsic determination of shape and adaptation of form to specific cell function that was entirely unsuspected twenty years ago. Cytology today is characterized by greatly expanded interest in synthesis and turnover of membranes, in the interaction between contiguous cell surfaces, the mechanisms of cell cohesion, and the fibrous components of the cytoplasmic matrix which may have a cytoskeletal function. There is a greater awareness of the heuristic value of morphological science and a growing appreciation of the fact that there is physiological meaning in the geometry of the cell surface as well as in its biochemistry.

References


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32. RUTBERG, V.: Ultrastructure and secretory mecha-


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