INFOLDED BASAL PLASMA MEMBRANES FOUND IN EPITHELIA NOTED FOR THEIR WATER TRANSPORT*

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PLATES 67 TO 69

One of the most unexpected and extraordinary cytological discoveries of electron microscopy was the observation of Sjöstrand and Rhodin (9) and Rhodin (8) that the basal cell surface of the proximal tubule of the kidney is extensively and deeply infolded into the cytoplasmic mass. These folds penetrate between mitochondria and form multiple lateral anastomoses. Pease (6, 7) studied other portions of the nephron, and indicated that the system is most elaborately developed in the distal tubule, and is even present to a moderate extent in the collecting tubule.

Pease (6, 7) presented evidence that the osmiophilic lines, which one observes penetrating the mass of the cell, correspond in fact to the diffusion barrier of the cell surface. The narrow gap (110 A.U., Sjöstrand and Rhodin (9)) normally present between osmiophilic lines is thus a potential extracellular space.

The morphology of the system suggests that it is a device for increasing the surface area in the basal region of a cell. Here, where there are basement membranes and where epithelial cells must be anchored, there is no possibility of developing anything corresponding to a brush border. The problem seemingly is solved in this different, but perhaps equally effective manner.

It seemed important to establish whether or not the system of basal surface infolding is a common specialization, or whether it is a particular feature of the kidney. Although suggestions of it have been incidentally reported in other organs, it has not been systematically searched for, or been the principal object of study.

Since an increase in the cell surface might be particularly important in water transport mechanisms, epithelia of the choroid plexus, the ciliary body, and the submaxillary gland have been examined with the possibility of basal specialization in mind. This paper demonstrates that the basal surfaces of all these epithelia are folded in manners reminiscent of the kidney. Specific differences in the pattern of folding suggest independent specialization.

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Methods

Rat tissues were used for all parts of this study. Ice cold 2 per cent osmic acid, buffered in the manner of Palade (3), was the fixative. Tissue was ordinarily fixed for one-half hour, although some of the choroid plexus samples were exposed for as long as 4 hours.

The several tissues were obtained in somewhat different ways. The best choroid plexus material was removed from the fourth ventricle immediately after the death of the animal. The posterior skull was exposed under nembutal anesthesia. This portion of the skull was then rapidly excised as the animal bled to death from the damaged venous sinuses. The cerebellum was quickly removed, and cold fixative dripped on the exposed choroid plexus. After about 10 minutes of dripping, the plexus was cut away and placed in a vial of cold fixative.

Submaxillary tissue was obtained from glands exposed under nembutal anesthesia. The bulk of the gland was cut off and discarded, leaving the hilar region, which was then flushed with cold fixative and undercut only after about 10 minutes of dripping *in situ*.

Ciliary bodies were obtained from anterior halves of eyes fixed in generous quantities of cold osmic acid solution. These were agitated for the first few minutes. As an additional precaution to assure good preservation and to simplify orientation, half-eyes were rejected if they did not remain hemispherical. The strip representing the ciliary body was dissected and isolated during the period of dehydration.

OBSERVATIONS

The three epithelia to be considered are taken up below in the following order: submaxillary gland, choroid plexus, and ciliary body.

1. Submaxillary Gland.—The epithelium of the submaxillary gland is specialized regionally in a variety of ways. Of greatest interest for the present account are the serous alveoli and the secretory ducts. In both of these epithelial regions there are deep infoldings of the basal surface membranes (Figs. 1–3) which are quite reminiscent of the distal tubule of the kidney.

In the secretory duct (Fig. 1) most of folding is confined to the basal region of the cells, whereas mitochondria are scattered throughout all but the most apical cytoplasm. Thus it is only in the basal zone that there is close approximation of the two systems, and this may well be fortuitous.

In the serous cells, however, there is very close coincidence between the distribution of mitochondria and the folds. The basal half or two-thirds of active serous cells is ordinarily so jammed with mitochondria that there is hardly room for other components. Yet a rich system of infolded membranes extends throughout this entire zone, and the membranes lie in close approximation with the mitochondria (Fig. 3). Figs. 2 and 3 do not do justice to this usual situation, for they have been selected from cells that were more than usually hydrated and so show the membranes in relative isolation. Deep within the cell there is a rather sudden transition to the apical zone, characterized during activity by closely packed secretory granules with neither mitochondria nor membranes.

In the serous cells this relation of the infolded basal membranes to the mitochondria is quite similar to what has been observed in the proximal and distal

tubules of the kidney. But this sort of association definitely does not exist in the collecting tubule of the kidney (Pease (7)), the choroid plexus, or the ciliary body (see below).

A very distinctive feature of salivary cell membranes is their tendency to form S-shaped patterns. This shows beautifully in Fig. 2 in which an infolded membrane can be followed from the cell surface, through multiple S-bends to an end of the fold, where the osmiophilic line turns back on itself.

In Fig. 3, what may appear at first glance as a stack of parallel osmiophilic lines is, in reality, S-shaped folding of a single pair. In Fig. 1 also, what seem to be multiple infoldings of the basal surface in closely spaced groups is actually a single surface fold turned back and forth on itself several times.

It is interesting to note that the intercellular surface membranes of salivary epithelium, as well as the membranes of the basal surface, have unusual tendencies to fold. Thus multiple S-folds occur between cells of the secretory duct as indicated by the arrows in Fig. 1. Fundamentally the same type of folding may be seen occasionally between mucous cells, although it is not a conspicuous feature of this cell type. Intercellular folding is rather well developed in intercalated ducts, although there is not basal folding. Very deep folding occurs between serous cells.

2. Choroid Plexus.—The choroid plexus has been studied with Mr. David Maxwell as a collaborator. It presents a variety of specializations which will be reported in detail elsewhere (Maxwell and Pease (2)). Of interest in the present connection is the basal aspect of the ependymal epithelium.

Much of the basal surface of each epithelial cell is without special folding. But laterally, near cell junctions, very elaborate infoldings occur. These show to good advantage in Fig. 6. In this micrograph the plane of section is nearly parallel to the base of the cell and passes obliquely into the basement membrane region at the lower right. Fig. 5 shows another fold in a plane vertical to the basal surface.

Besides folds from the basal surface, the intercellular membranes are highly folded in the basal zone. This is not a separate system, but a continuation of the basal folding between adjacent cells, for there are free anastomoses from one fold to the next.

The extent of the system is indicated in Fig. 4 at relatively low magnification. The plane of section is nearly parallel to the basal cell surface, obliquely sectioning the basement membrane in two places. Intercellular folds can be seen between the two nuclei and also coursing towards the upper right of the figure in the zone between the broken lines. Basal folds blend with intercellular ones after penetrating from the regions of the basement membrane.

The gap between the osmiophilic lines is approximately 110 to 170 A.U. In this it resembles the kidney and the ciliary body and probably the submaxillary gland as well. These folds were not reported in the two previous

electron microscopical studies which have dealt with the choroid plexus in a different connection (Dempsey and Wislocki (1) and van Breemen and Clemente (10)).

3. Ciliary Body.—The epithelium of the ciliary body is, of course, made up of two cell layers derived from different parts of the optic cup. The conspicuous cytoplasmic component of the outer layer is its pigment (Fig. 7), but it does not possess a specialization that will interest us here. The inner layer, on the other hand, has a rich system of folds penetrating from the surface adjacent to the aqueous humor.

The folds from the surface penetrate very deeply into the cytoplasm, as may be seen in Fig. 7. In fact, they often nearly extend through the full thickness of the cell layer. Their course is curiously haphazard, without the semblance of order or regional localization found in all the other tissues so far considered. Mitochondria are scattered in cytoplasmic areas between folds without evident relation to them. But as with the other tissues, there are extensive lateral anastomoses between the folds. These may be seen particularly in Fig. 8 which is sectioned nearly parallel to the free surface.

It is interesting to note that there is a basement membrane at the free surface of the epithelium adjacent to the aqueous humor. This side of the epithelium, of course, is embryologically basal in that it rests upon mesenchyme. The components of the basement membrane show clearly in Fig. 8. At the extreme surface there is a thin osmiophilic line. By analogy with other basement membranes, this is probably the structural component. Between this and the cell surface, there is a homogeneous layer of low density. The low density material extends into the penetrating folds and fills the gap between plasma membranes. Exactly the same relationship pertains in the kidney epithelium and has been discussed at length by Pease (5–7). Relations in the salivary gland and choroid plexus epithelia are similar, although micrographs have not been selected to demonstrate this point in this article. It is also apparent that the same sort of low density material is found between neighboring cells. Sjöstrand and Rhodin (9) and Rhodin (8) have discussed the possibility that it is lipoid in nature. Pease (5, 7) used the non-committal term "cement substance" to identify it.

Palade (4), studying macrophages, observed deep clefts into the cells reminiscent of the surface folds described here. He further observed that the clefts were frequently continuous in the cytoplasm with rows of circular and oval profiles, suggesting that droplets had been pinched off. He suggested that this might be a route for pinocytosis and phagocytosis. Comparable rows of droplets, looking as though they were once connected to a nearby cleft, have been seen fairly often in the ciliary body epithelium, although none is illustrated here. This relationship has not been seen, however, in the other epithelia examined.

Wislocki and Ladman (11), in studying the barrier between blood and aqueous humor, observed that there were clefts in the epithelium of the ciliary body. However, they did not study the epithelium in detail.

DISCUSSION

Four different epithelia including that of the kidney tubules are now known to be analogously specialized in having a complexly folded basal plasma membrane. Certainly the potential surface area of the basal side of each epithelium is vastly increased by this mechanism. It seems significant that all four of these epithelia are noted for their water transport, and the specialization is presumably related to this function.

One might well ask whether the infolded basal plasma membranes of the different epithelia are homologous, or whether they represent independent and unrelated specializations. This writer favors the latter view, for the morphological pattern is highly distinctive in the four different epithelia. It is fully as distinctive as specializations at the apical ends of cells of unrelated epithelia. (Consider and compare, for example, the "brush border" of the kidney, the "stereocilia" of the epididymis, and the "striated border" of the intestine.) Functionally, too, there are differences in the direction of fluid movement across these epithelia, so they are not related to one another in a simple manner at the physiological level. It seems best to regard the specializations as analogous but not homologous.

In electron microscopy one inevitably studies static pictures. It is not likely, though, that these basal folds are entirely static structures. It is quite within the realm of possibility that water movement might be controlled by altering the surface area, and it will be pertinent to try to correlate the degree of folding with functional activity.

SUMMARY

Epithelia noted for their water transport have been studied by electron microscopy with particular emphasis upon basal specializations. Epithelia of the submaxillary gland, choroid plexus, and ciliary body are described in this article, and compared with previous observations on the kidney.

The basal surface of all these epithelia is tremendously expanded by folds which penetrate deeply into the cytoplasm. In the submaxillary gland this is particularly notable in cells of the serous alveoli and in the secretory ducts. In these instances the folds have a fairly regular distribution and have a marked tendency to turn back upon themselves and so form repeating S-shaped patterns. In the choroid plexus the penetrating basal folds are limited to the lateral regions of each ependymal cell where they blend with the intercellular membranes that are also folded. In the epithelium of the ciliary body it is the inner layer that is specialized. The surface adjacent to the cavity of

the eye penetrates irregularly, nearly through the full depth of the cell layer. The exposed surface is, in a fundamental sense, the basal surface of this epithelial layer.

It is apparent that the pattern of folding is quite distinctive in the different epithelia. Therefore, the specializations should be regarded as analogous rather than homologous.

Topographic considerations presumably limit the manner in which basal cell surfaces might be expanded. Penetrating folds would seem to represent almost the only possible solution.

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EXPLANATION OF PLATES

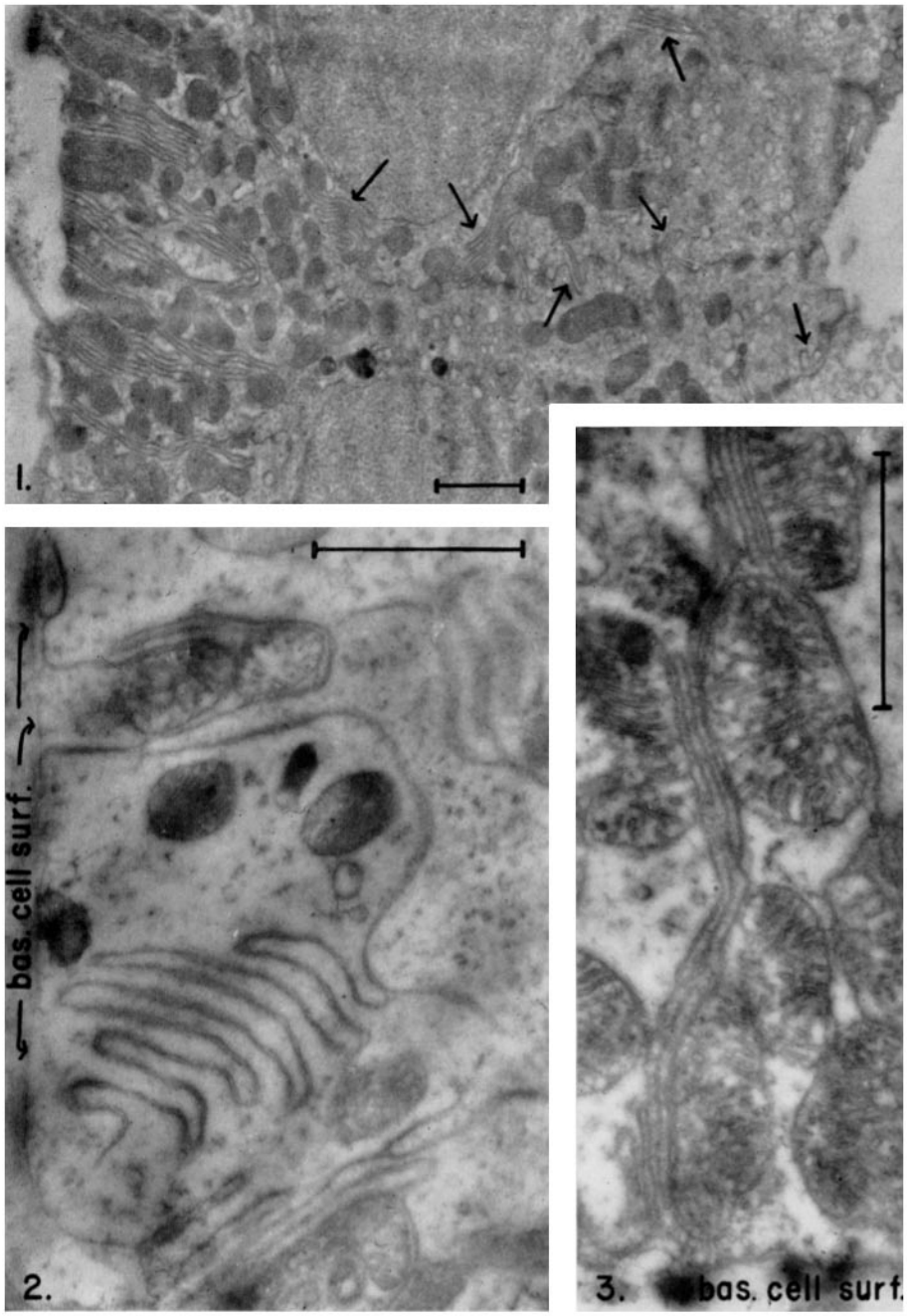
PLATE 67

Submaxillary Gland

FIG. 1. Secretory duct of the submaxillary gland. The basal margin of the cells is to the left, the apical to the right. The basal region contains penetrating folds of the surface membrane between the mitochondria. Arrows indicate where intercellular membranes are more or less elaborately folded. Micron marks accompany all figures. × 11,500.

FIG. 2. The basal region of a serous cell. A fold can be followed in from the basal cell surface (*bas. cell surf.*) to where it branches, and then one branch is folded back and forth in S-shaped configurations until it terminates abruptly. The cytoplasm of this cell is atypical in that there are relatively few mitochondria. × 30,000.

FIG. 3. The basal region of a serous cell. A single surface fold penetrates the cytoplasm and is then doubled back and forth on itself to make what appear to be multiple parallel bands. Note the close association of folds with mitochondria. × 33,500.



(Pease: Infolded basal plasma membranes)

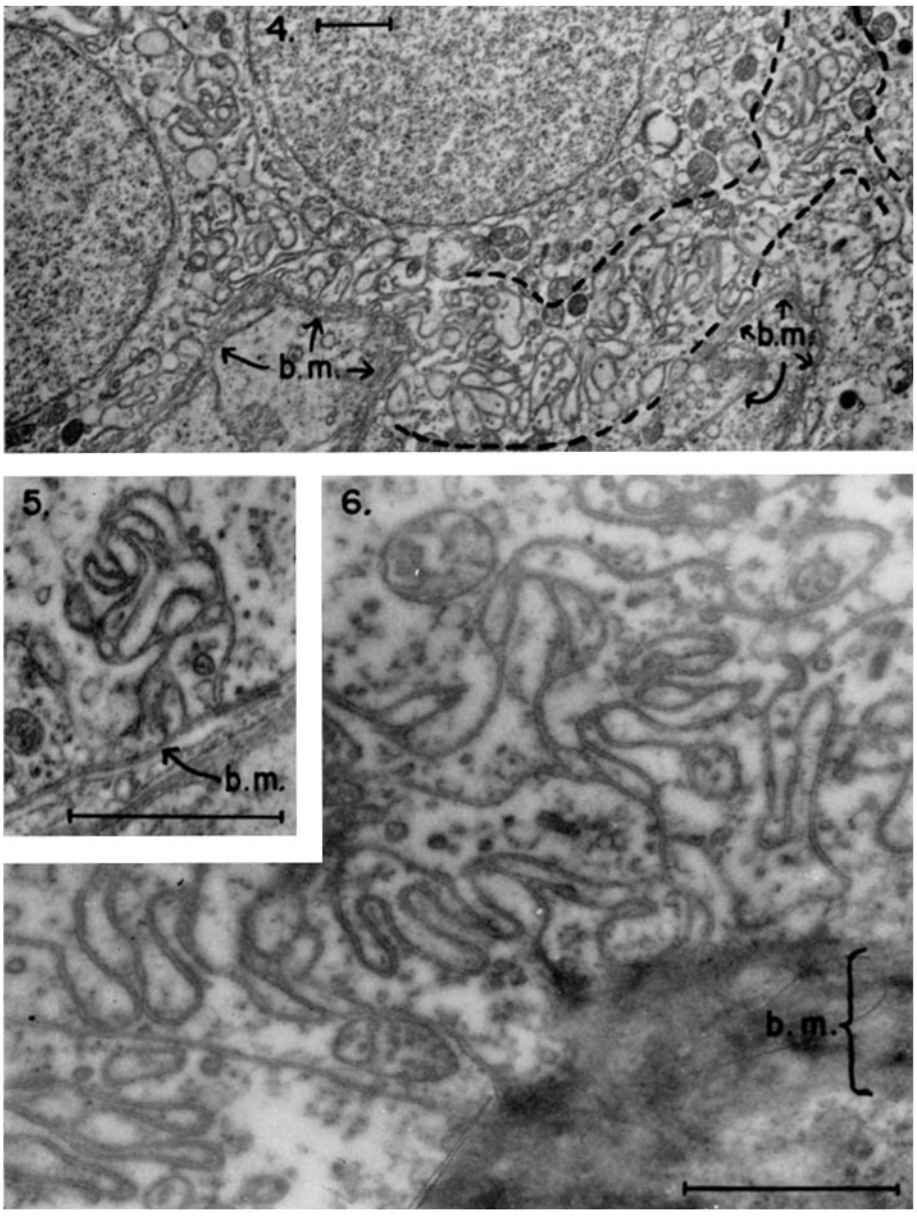
PLATE 68

Choroid Plexus

FIG. 4. Ependymal epithelium, sectioned nearly parallel to the surface and through the basal region of several cells. Obliquely sectioned basement membrane (*b.m.*) appears in two places. Highly folded intercellular membranes can be seen between the two adjacent nuclei and between the broken lines. × 9,700.

FIG. 5. A complex fold penetrating ependymal cytoplasm from the basal surface of the cell. × 28,000.

FIG. 6. A slightly oblique section, nearly parallel to the base of ependymal cells. Greatly tortuous, penetrating folds of the basal and intercellular surfaces are apparent. × 28,000.



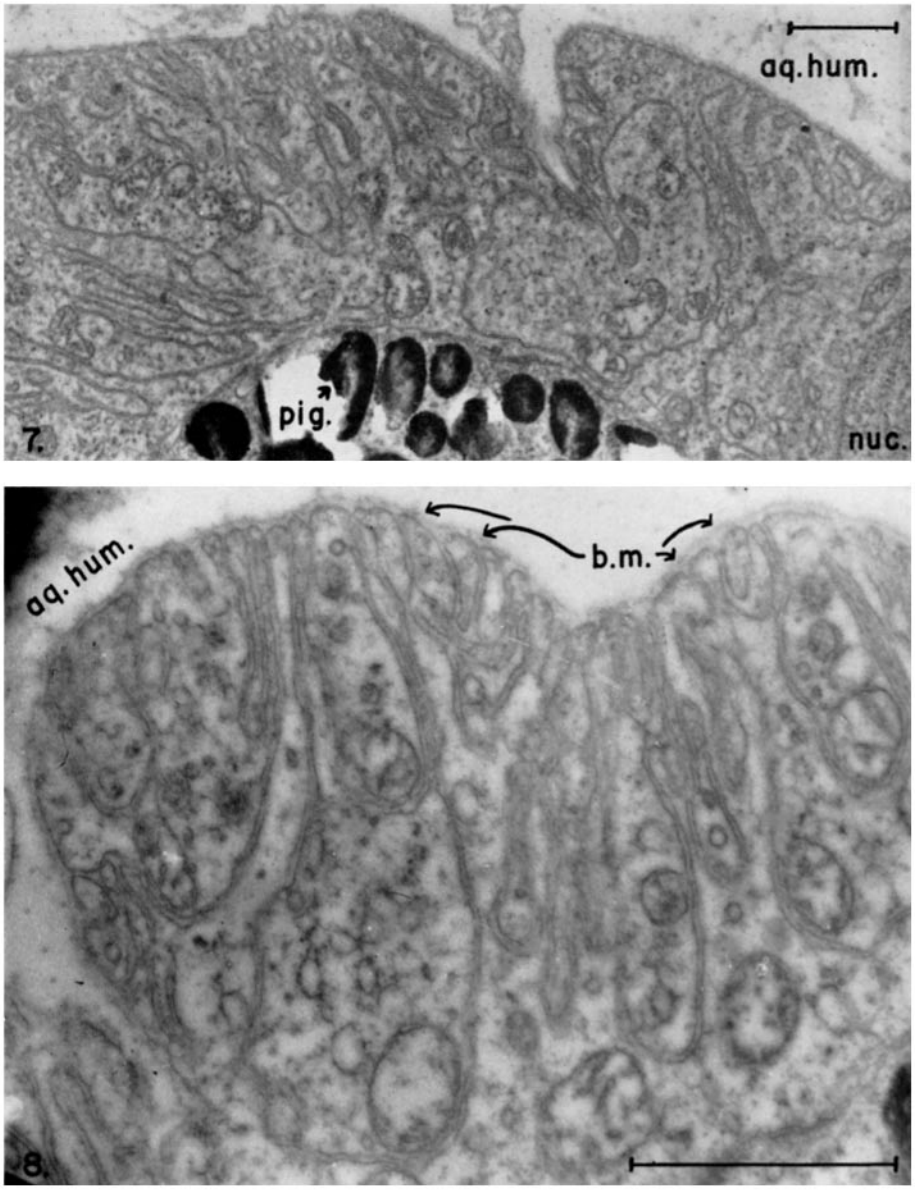
(Pease: Infolded basal plasma membranes)

PLATE 69

Ciliary Body

FIG. 7. Relatively low power section of the inner epithelial layer of the ciliary body. Parts of the lumen of the eye with its aqueous humor (*aq. hum.*) and the outer epithelial layer with pigmented cells (*pig.*) can be seen for orientation. Also, an edge of a nucleus (*nuc.*) is visible. It can be seen that folds penetrate nearly through the full thickness of the layer from the luminal side. × 14,000.

FIG. 8. The relation of the folds to the lumen of the eye shows in this section taken obliquely, nearly parallel to the surface. A basement membrane (*b.m.*) covers these cells, and it can be seen to have a thin, osmiophilic margin adjacent to a layer of low density. The low density material extends between the surface folds and fills the gap between surface membranes. Identical material is found between neighboring cells. × 35,000.



(Pease: Infolded basal plasma membranes)