QUINTUPLE-LAYERED MEMBRANE JUNCTIONS
AT TERMINAL BARS BETWEEN ENDOTHELIAL CELLS

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METHODS

In electron micrographs, the plasma membrane at the surface of a cell is about 8 mμ thick (16) and has a triple-layered structure consisting of two dark layers separated by a light layer. Recent studies suggest that, in the majority of tissues, the plasma membranes of adjacent cells are separated by a distance of not less than 15 to 20 mμ (15), this intercellular space being distensible, e.g. by fat globules (12). In some situations, notably during the formation of a myelin sheath, this separation is lost and the adjacent plasma membranes come together to form a structure about 15 mμ thick. Robertson (17) has called this structure an "external compound membrane," while Karrer (7, 8) prefers the term "quintuple-layered cell interconnection" since, at the point of contact between the plasma membranes, the surface dark layers of the adjacent membranes appear to fuse so that only five layers are visible at the point of contact, three dark layers separated by two lighter layers.

Robertson (19) suggests that the function of these contacts may be to regulate diffusion of substances between cells, and he illustrates such a contact between the plasma membranes of an endothelial capillary cell from the spinal cord of a developing mouse. The present investigation was carried out to determine whether "quintuple-layered units" are a general feature of endothelial cell junctions.

Tissues were fixed in 1 per cent osmium tetroxide, which was adjusted to a pH of 7.4 with either veronal acetate or chromate buffers. Araldite was used as the embedding medium and the sections were stained with 1 per cent potassium permanganate, according to the method of Lawn (9). The electron microscope was an A. E. I., E. M. 6 operated at 40 kv.

OBSERVATIONS

In low-power electron micrographs of capillaries, the junction between two adjacent endothelial cells, or when the capillary wall is formed by a single cell, the junction between two areas of the plasma membrane of the same cell (Fig. 1), is apparent as a region of marked density (Fig. 1, J), the terminal bar. The density is that of a small area of cytoplasm on both sides of the junction and it is at these terminal bars that quintuple-layered contacts occur (Figs. 2, 4, and 5, Q and Q_1). At the point of contact between the adjacent triple-layered plasma membranes (Fig. 2 P), the surface dark layers of the membranes lose their separate identity and come together to form an intermediate dark layer, so that only five layers can be identified in this region (Figs. 2 and 4, Q and Q_1). The over-all dimension of such a quintuple-layered unit is about 15 mμ, and the spacing from centre to centre of the limiting dark layers of the unit is about 12 mμ.

In transverse sections of capillaries, a quintuple-layered unit always appears to be present at the junction between plasma membranes, and when the junctional region is short, as in Fig. 2, the area of contact is often confined to the luminal end of the juction. The fact that quintuple-layered units are constantly found in transverse sections can only be explained by assuming that a continuous band of line of contact extends around each endothelial cell, along the edge where its plasma membrane is involved in a junction. On the other hand, in longitudinal sections passing

through the luminal end of the junctional regions, e.g. in the plane Q-Q in Fig. 2, the quintuple-layered unit often appears to be intermittent as in Fig. 4 (Q and Q_1). This appearance is probably the result of the undulating nature of the luminal surface of the capillary so that the section passes in and out of the junctional zone.

While areas of marked density of the cytoplasm on both sides of a junction always appear to be related to the presence of quintuple-layered units (Figs. 2 and 4), such units are sometimes present where no darkening occurs (Fig. 4 c, Q_1). Such marked density of the cytoplasm has been observed by Schultz, Maynard, and Pease (20) in endothelial cells in the cortex and by Hama (6) in the blood vessels of the earthworm, while Bennett, Luft, and Hampton (1) have recorded a similar appearance in capillaries in other regions.

Areas of contact giving rise to quintuple-layered units have, in our own studies, been observed between endothelial cells in arterioles, venules, and capillaries present in skeletal muscle, cardiac muscle, optic nerve, cerebrum, and cerebellum from adult rats and mice and Xenopus tadpoles, as well as in capillaries from the optic nerve of 7-day postnatal rats. All these capillaries are non-fenestrated, but the same type of junction is also seen in the porous endothelium of the intestinal mucosa and neurohypophysis (Fig. 5).

Bennett, Luft, and Hampton (1) have surveyed the structure of capillaries in a variety of tissues from different species and they draw attention to the attachment zone between adjacent cells, stating that no gaps, defects, openings, pores, or slits have been discerned in the many hundreds of junctions examined. Thus, these authors conclude that attachment surfaces surround each endothelial cell like a girdle, and their illustrations indicate that this girdle is present in the luminal half of the junction between two endothelial cells. Such a continuous junction around the inner cell margin resembles the terminal bar of columnar epithelium, and we agree with Fawcett (4) that terminal bars should be distinguished from desmosomes. The latter are circumscribed ovoid or circular areas of cell adhesion, with a series of alternating dark and light layers between the apposed membranes and with discernible lamination of the dense material in the adjacent cytoplasm of each cell (Fig. 3), while the girdle-like terminal bars have a simpler structure with amorphous dense material in the cytoplasm close to the apposed membranes. In capillaries from the rete mirabile of fish swim bladders, Fawcett (4) illustrates terminal bars and desmosomes between endothelial cells, but in our material only terminal bars were observed. It is at these terminal bars of endothelia and intestinal epithelium that the present observa-

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Explanation of Figures

EN, Endothelial cell nucleus
F, Fenestrae in walls of capillary
J, Junction between endothelial cell
   plasma membranes

L, Lumen of capillary
RBC, Erythrocyte
P, Triple-layered plasma membranes
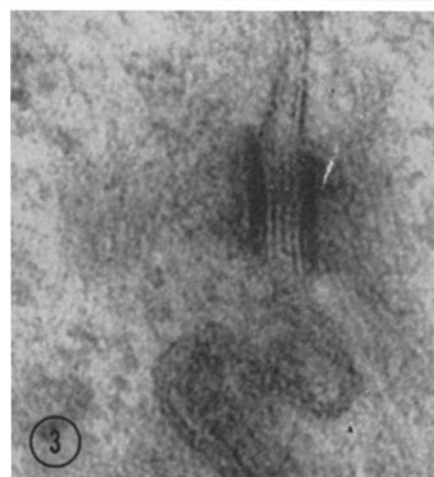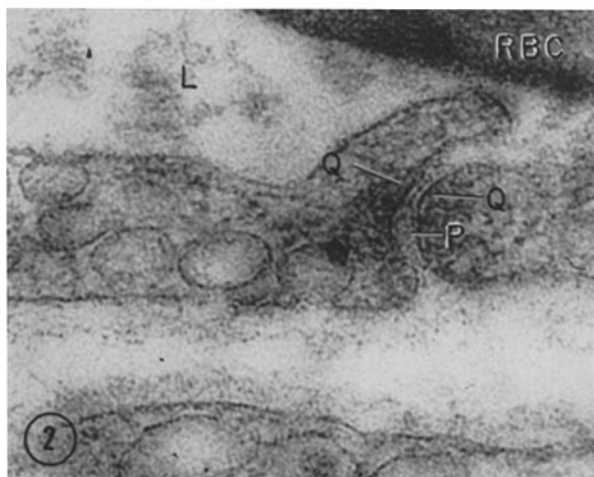Q and Q_1, Quintuple-layered unit

FIGURE 1

Transverse section of a capillary in the diaphragm of a mouse. The capillary wall is formed by a single endothelial cell so that only one junction (J) is present. X 28,000.

FIGURE 2

Transverse section through a junction between two endothelial cells in a capillary from the rat heart. The triple-layered cell membranes (P) come together to form a quintuple-layered unit (Q) at the luminal end of the junction. X 165,000.

FIGURE 3

Transverse section of a desmosome between two epithelial cells from rat jejunum. X 165,000.

tions on osmium tetroxide–fixed material, and those of Robertson (18, 19) after permanganate fixation, show the formation of a "quintuple-layered unit" by the plasma membranes.

Other published illustrations of endothelial cell junctions (1, 2, 4, 10) lack the resolution necessary to show a quintuple-layered unit, but it is noteworthy that it is impossible to trace a continuous intercellular separation of 15 to 20 mμ from lumen to extracapillary space in these illustrations. Favourable orientation and high resolution are necessary to demonstrate the contact between the external surfaces of the plasma membranes, but in none of our preparations is it possible to trace a separation between endothelial cells through the whole thickness of the capillary wall. These observations suggest that this quintuple-layered form of contact is a universal feature of endothelial cells which must have some functional significance.

Considerable evidence supports the view that the triple unit plasma membrane seen in electron micrographs can be equated with the lipoid permeability barrier at the cell boundary, as originally proposed by Davson and Danielli (3). Components of the cell boundary external to this plasma membrane may be responsible for maintaining the 15 to 20 mμ gap between cells in most tissues (15), but it is unlikely that these extracellular components can, by themselves, control the permeability to ions and small molecules. The side walls and bases of cells which transport large volumes of water, i.e. kidney tubular cells (13) and choroid plexus epithelium (21), show complex infoldings which increase their surface area, but these infoldings are separated from each other by the 15 to 20 mμ gap, and this specialisation would have no value unless water and ions could diffuse through this space. These arguments lead to the

hypothesis that a sheet of cells which separates two zones of differing constitution will have a quintuple-layered fusion of their adjacent plasma membranes to prevent intercellular diffusion of ions and small molecules.

Other systems which test this hypothesis include the gut lumen, the external surface epithelium, the "blood-brain barrier," and the cavity of the nephron. In all these sites, quintuple-layered units are demonstrated, by Robertson between the inner borders of intestinal epithelial cells (18), by Karrer between stratified squamous epithelium of the cervix uteri (7), and by Peters (14) and Gray (5) between glial cells around cerebral blood vessels and at the base of the brush border in the proximal convoluted tubule (11). It is of interest that terminal bars with quintuple-layered units are present between fenestrated endothelial cells, where there would not appear to be any function for intercellular seals; they may be ontogenetic vestiges in these specialised porous endothelia or may be serving purely as intercellular bonds.

A different function is assigned to this form of intercellular junction by Karrer (8) who demonstrated their presence between adjacent cardiac muscle cells. He considers that they may facilitate the intercellular transmission of an impulse. Careful observation is required to demonstrate these "quintuple-layered units" and it is possible that they are widely, even randomly, distributed. The function of this type of junction cannot be determined until there is more information on their occurrence, development, and molecular structure, but it is hoped that this preliminary report and hypothesis will stimulate such studies.

Figures 4 *a* to *c*

Longitudinal section through a junction between two endothelial cells from the pia mater of a mouse. These micrographs are from a single junction and are continuous with each other at X and Y. The plasma membranes from the internal surface at I form quintuple-layered units in four areas (Q and Q_1) before they diverge towards the external surface at E. $\times$ 170,000.

Figure 5

Transverse section through a junction between two endothelial cells from the neurohypophysis of a rat. The quintuple-layered unit is seen at Q, with the right hand cell containing at least two fenestrae (F). $\times$ 140,000.
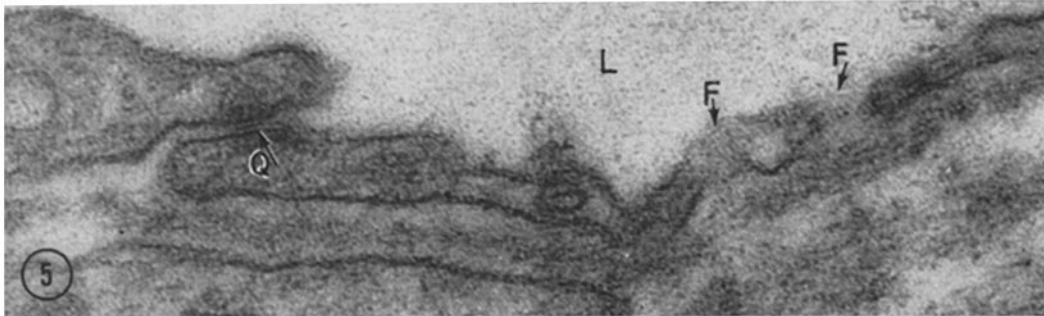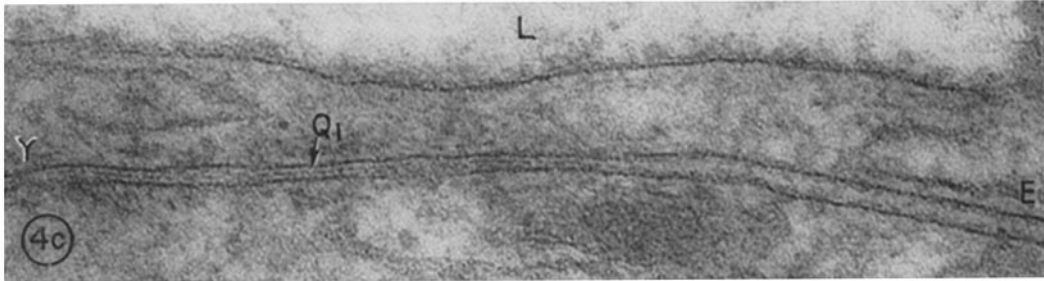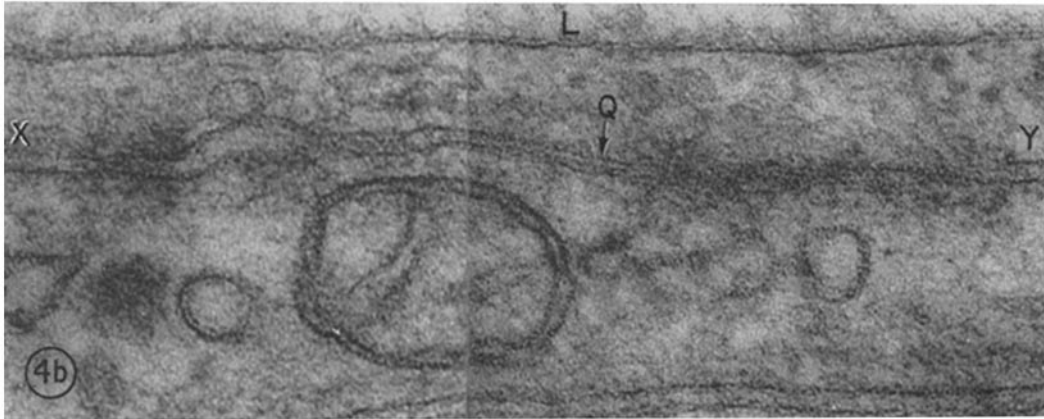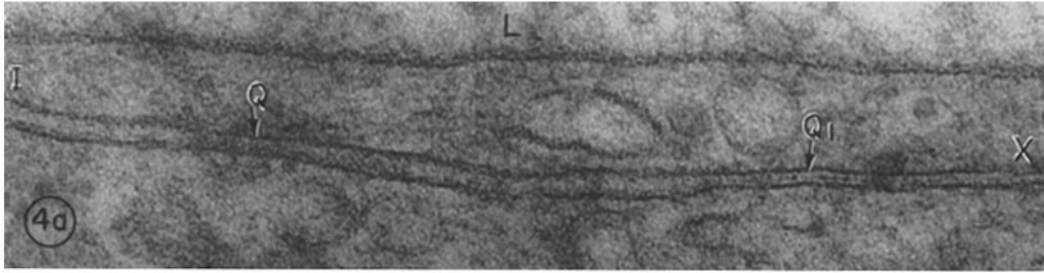
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