STRUCTURAL MODULATIONS OF PLASMALEMMAL VESICLES

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ABSTRACT

Structural modulations affecting a small fraction of the population of plasmalemmal vesicles of vascular endothelia are described. They include forms which are apparently produced by the fusion of the vesicular membrane with the plasmalemma and by the successive elimination of the layers of the two fused membranes. Such modulations are assumed to represent stages in the discharge process of vesicular contents. Other forms, characterized by their flask shape and elongated neck, are assumed to represent stages in the formation and loading of membrane invaginations, followed by their being pinched off to form isolated vesicles. Stages in a membrane-fusion process leading to the formation of apertured fenestrae and channels are also described in fenestrated endothelia. The visualization of these structural details is greatly facilitated by staining tissue specimens with uranyl acetate before dehydration.

According to a previously advanced hypothesis (1–4), transport of fluid in quanta across the endothelium of blood capillaries implies the following sequence of events: (*a*) appearance of a pocket by invagination of the plasmalemma on either cell front; (*b*) formation of a closed vesicle by a pinching off (membrane fission) of the pocket; (*c*) movement of the vesicle across the endothelial cytoplasm; (*d*) fusion of the vesicle membrane with the plasmalemma on the opposite cell front; and finally (*e*) discharge of the vesicular content into the extracellular medium.

Most electron micrographs so far published seem to represent either (*1*) invaginations of the cell membrane or (*2*) closed vesicles assumed to be in transit across the endothelial cell. Recently, intermediate stages have tentatively been identified in the form of vesicles provided with a narrow neck (3) or an aperture (diaphragm) (5, 6). The first are considered intermediary forms between (*a*) and (*b*), whereas the second are assumed to represent vesicles approaching the discharge stage (3, 5, 6).

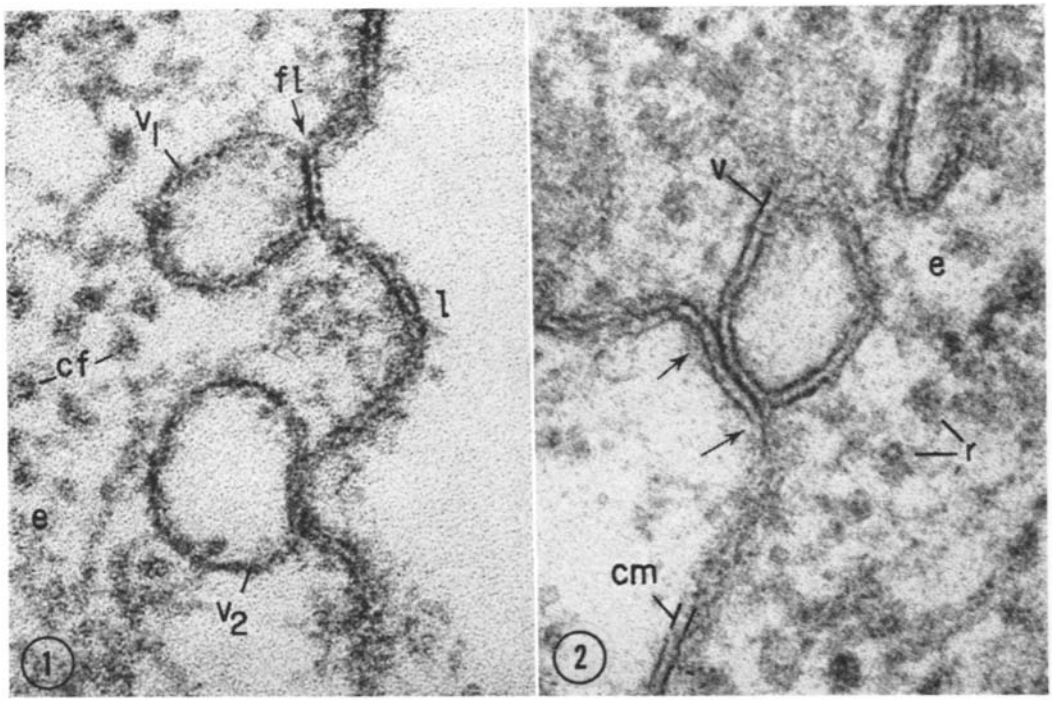
A priori, clear visualization of the stratified structure of plasmalemmal and vesicular membranes should allow the recognition of more graded intermediary forms, especially during those phases of the process which involve membrane fusion prior to vesicle opening and discharge. For instance, appearances representing contact and fusion of the two membranes, as well as subsequent progressive elimination of their layers, should be encountered with a frequency proportional to the time taken by these events in a complete traverse of the endothelium.

The present paper reports the occurrence of modulations in vesicle structure which probably represent such intermediate stages.

MATERIALS AND METHODS

Our observations were carried out on (*a*) blood capillaries of the tongue, myocardium, diaphragm, intestine, and pancreas; (*b*) lymphatic capillaries of the tongue and intestine; and (*c*) endocardium of adult rats and guinea pigs.

Tissue specimens were fixed for 2 hr at $\sim 0°$ in



All figures represent specimens fixed in OsO_4, stained in block in uranyl acetate before dehydration (14), and embedded in Epon. Unless otherwise stated, the sections were doubly stained with ethanolic uranyl acetate and lead citrate.

Abbreviations

bm, basement membrane
cm, cell membrane (plasmalemma)
ol, outer leaflet } of cell or vesicle membrane
il, inner leaflet }
e, endothelium
er, endoplasmic reticulum
l, vascular lumen
m, mitochondrion
n, nucleus
r, ribosome
v, plasmalemmal vesicle

FIGURE 1 Blood capillary; endothelium (rat tongue). The membrane of the plasmalemmal vesicle v_1 is fused with the cell membrane which, at this level, forms a shallow infundibulum. A heavy fusion line (*fl*, arrow) is clearly demonstrated, but the other dense leaflet of the vesicle membrane is hardly visible. This is an example of straight, apparently rigid diaphragm often found at sites of membrane fusion. Vesicle v_2 appears also involved in a fusion process, but the number of layers in its diaphragm cannot be ascertained on account of the obliquity of the section. Normally cut, cytoplasmic filaments are marked *cf*. × 300,000.

FIGURE 2 Blood capillary; endothelium (rat tongue). The five-layered diaphragm (arrows) formed by the fusion of the membrane of vesicle (*v*) with the cell membrane (*cm*) is clearly demonstrated. Note that the diaphragm buckles towards the lumen, that its fusion leaflet is slightly thicker and denser than the other two dense leaflets of the structure, and that the light leaflet of the vesicle membrane is thinner and less regular in the diaphragm than in the rest of the vesicle wall. × 285,000.

1% OsO_4 in acetate-Veronal, or phosphate buffer, pH 7.2–7.5, to which 10 mM $CaCl_2$ or $MgSO_4$ had been added in some cases. After fixation, the tissue blocks were treated for 1–2 hr at room temperature with 0.5% uranyl acetate in acetate-Veronal buffer (pH 5.0), with or without added NaCl, and subsequently were dehydrated in a series of graded ethanols and embedded in Epon (7). Sections were

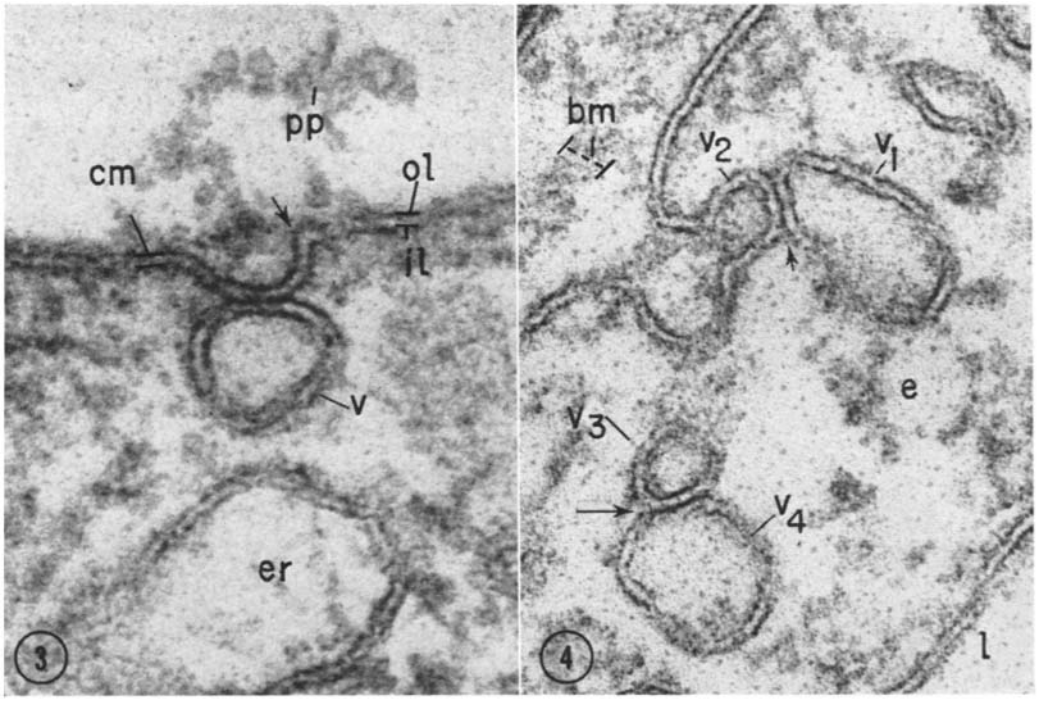


FIGURE 3 Blood capillary; endothelium (rat tongue). Five-layered diaphragm formed by fusion of the limiting membrane of a plasmalemmal vesicle (*v*) with the cell membrane (*cm*) at the level of a shallow recess which may represent a recently opened vesicle, as suggested by the sharpness of its rim (especially on the right side, arrow). Continuity of the layers of the fused membranes shows clearly throughout the fusion area. Precipitated plasma proteins (*pp*) occupy part of the lumen. × 310,000.

FIGURE 4 Blood capillary; endothelium (rat tongue). A five-layered diaphragm (short arrow) is seen between a closed vesicle (v_1) in the endothelial cytoplasm and another vesicle (v_2) opened on the tissue front of the cell. Two other fused vesicles (v_3, v_4) appear in the cytoplasm, but the number of layers in the corresponding diaphragm (long arrow) cannot be ascertained. The diaphragm has probably five layers. × 275,000.

cut with diamond knives (du Pont de Nemours & Co., Inc. or Sugg, Wilmington, Delaware) on LKB or Servall MT2 microtomes, they were then mounted on grids covered with Formvar reinforced with an evaporated carbon film, and stained with aqueous (8) or ethanolic (see reference 9) uranyl acetate followed by lead citrate (10, 11). The technique of staining tissues in block with uranyl acetate is an adaptation of a procedure originally introduced by Kellenberger et al. (12) for the fixation of bacterial cells. As described, or with slight modifications, it has already been used on fungi (13) and animal tissues, primarily frog skin (14), and rat adrenal medulla (6) and bone marrow (15). In some cases, the specimens were fixed initially in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (3 hr at ~ 0°) (16), then postfixed in 1% OsO_4 in the same buffer, and finally processed as above.

Micrographs were taken at magnifications ranging from 40,000 to 80,000 with a Siemens Elmiskop I operated with a double condenser, an objective lens of 2 mm focal length, 50-μ objective apertures, and an anticontamination device.

Preparations fixed only in OsO_4 allowed a better visualization of membrane detail on account of the extensive extraction incurred by their cytoplasmic matrix during uranyl acetate treatment. Little or no extraction occurred in glutaraldehyde + OsO_4–fixed specimens. Addition of Ca^{2+} or Mg^{2+} improved the preservation of membranes and increased their contrast; it also increased, or rendered more visible, the precipitation of proteins in the blood plasma and the cytoplasmic matrix.

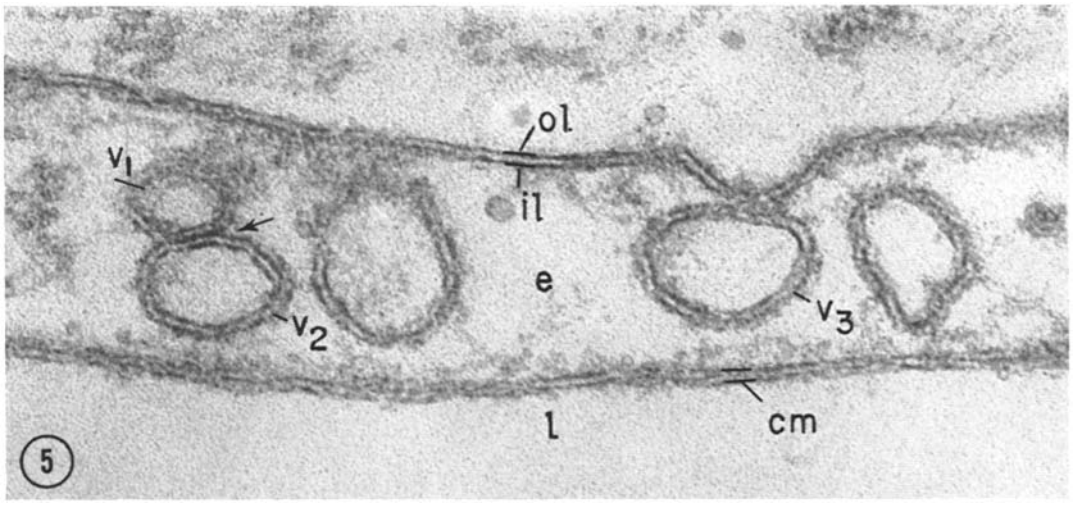


FIGURE 5 Lymphatic capillary; endothelium (rat tongue). A five-layered diaphragm is seen in between two fused vesicles (v_1, v_2), both apparently closed and located relatively deep in the cytoplasmic matrix. Another vesicle (v_3) is in close contact with the plasmalemma at the level of a shallow recess, but no fusion layer is visible in the most proximate area. Arrow indicates diaphragm. × 270,000.

RESULTS

Plasmalemmal Vesicles

These observations rely primarily on the clear visualization of the stratified structure of the membranes under consideration, i.e., the plasmalemma and the limiting membrane of the plasmalemmal vesicles. The outer leaflet of the plasma membrane and the inner leaflet of the vesicular membrane are hardly visible after usual fixation and staining procedures (3). It is only after staining in block with uranyl acetate, followed by simple (lead) or especially double (uranyl and lead) staining (3, 6) of sections, that these leaflets acquire enough density for satisfactory visualization. In such preparations, the two membranes are generally similar in terms of dimensions and density of their strata, although in some cases, the original asymmetry is reversed. Continuity layer-by-layer between the cell membrane and the vesicle membrane can be reliably ascertained whenever images, interpretable as forming or opening vesicles, are encountered (3, 5).

As already shown (3), a large proportion of plasmalemmal vesicles (~70%) are opened to the cell surface, either directly or through a neck or channel ~150–400 A in inner diameter, while ~30% of the vesicles are closed and apparently free in the endothelial cytoplasm.

In addition to these common appearances, the following forms have been encountered.

(*a*) (fu_1)[1] Vesicles whose limiting membrane is fused in part with the plasmalemma. Fusion may be limited to a small area where the plasmalemma is tangent to the vesicle membrane, or it may occur over a wider region, in which case the fused membranes form a five-layered diaphragm. The latter is often straight, apparently rigid, or under tension (Fig. 1), but occasionally it buckles convexly towards the outside (Fig. 2). Quite often the fusion layer is thicker and denser than the other leaflets of the two membranes (Figs. 1, 2), a situation reminiscent of an internal compound lamella (17) in myelin sheaths.

Similar appearances occur between a vesicle and a shallow recess of the plasmalemma (Fig. 3), between a closed vesicle and one already opened (Fig. 4) on any cell front, or between two closed vesicles located in the cytoplasmic matrix (Figs. 4, 5).

What we describe as "membrane fusion" may represent a whole spectrum of conditions from simple membrane apposition to true fusion accompanied by partial elimination of the dense leaflets involved. In our case, there is no indication that

[1] The abbreviation *fu* (fusion) is in keeping with the assumption that these appearances represent steps in a vesicle–cell membrane fusion process.

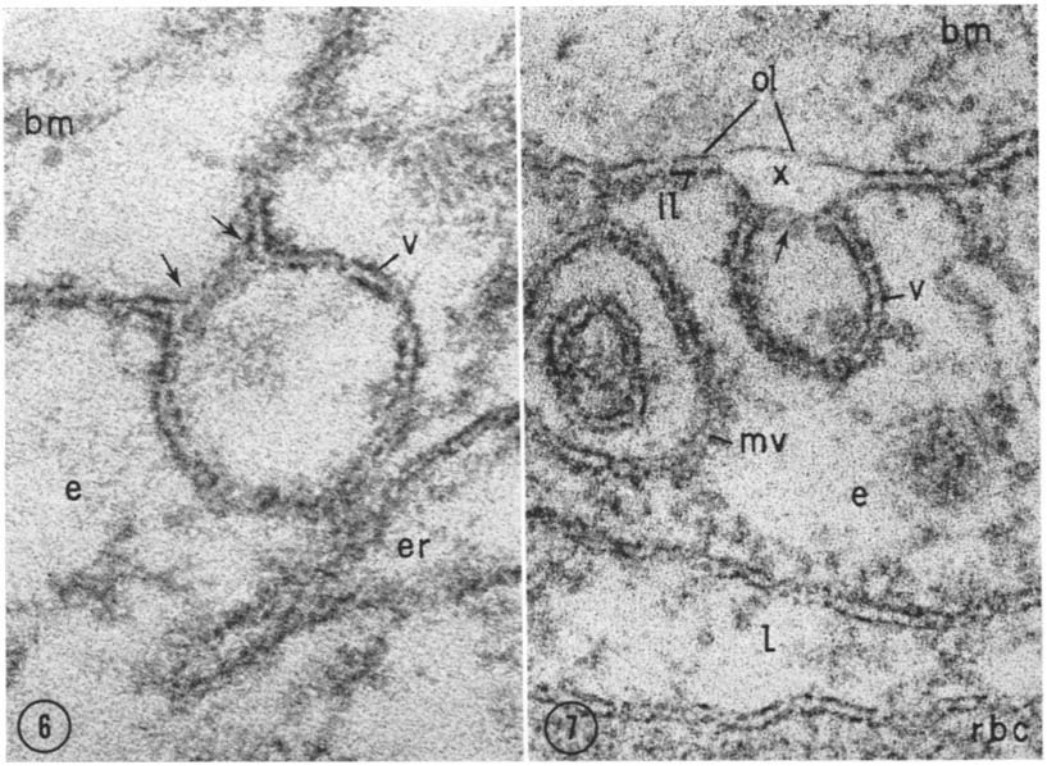


FIGURE 6 Endocardium; endothelium (rat right atrium). The content of the plasmalemmal vesicle (*v*) is separated from the external medium by a diaphragm (arrows) which seems to have only three layers. Their continuity with the corresponding dense and light layers of the cell and vesicle membrane appears more clearly at the left arrow. × 380,000.

FIGURE 7 Blood capillary; endothelium (rat tongue). The plasmalemma has been "exploded" by resin infiltration (presumably during embedding) at the level of a fusion diaphragm. The outer dense leaflet (*ol*) pulled away and is seen stretching without bending or interruption over the fusion area. The thickness of the light layer (*x*) is greatly expanded. The other dense leaflet of the diaphragm was left behind (arrow) near the rest of the vesicle. Such an appearance probably results from the explosion of a three- or five-layered diaphragm (in this case, apparently a three-layered one). It is highly unlikely that it represents the exploded rim of an open vesicle with the outer leaflet bulging far out into the stoma. Such an explanation requires that the bulging leaflet be stretched to more than double its area without breaking. A small multivesicular body is marked *mv*, and a red blood cell in the lumen *rbc*. × 290,000.

the process is reversible; hence, we assume that the membranes are fused when their adjacent dense layers merge into a single one. Our interpretation and our use of the term "fusion" are thus different from those proposed by Robertson in the case of myelin lamellae (17).

(*b*) (fu_2) Vesicles whose content appears separated from the extracellular medium by a single unit membrane located either in the plane of the plasmalemma or at the bottom of a shallow infundibulum (Fig. 6). In such cases, continuity layer-by-layer between this three-layered diaphragm, on the one hand, and the plasmalemma and the vesicle membrane, on the other hand, is seen with varying degrees of certainty but rarely is equally clear for all light layers at the two ends of the diaphragm. The view that in such cases a single unit membrane separates the content of the vesicle from the extracellular fluid is supported by occasional findings of the type illustrated in Fig. 7. In this occurrence, the dense layers of the plasmalemmal unit membrane have been spread

apart ("exploded"), presumably by an accidental accumulation of plastic monomer in its lipid phase (light layer) during embedding. The dense leaflets have retained, however, their continuity, and the outer leaflet is seen stretching uninterrupted, without bending or invagination, over the stoma of a vesicle. Such appearances are expected from a true, three-layered diaphragm and are difficult to explain as artifacts produced at the expense of other structures.

Similar three-layered diaphragms are occasionally found between two isolated vesicles in the cytoplasm (Fig. 8).

(*c*) (*fu*$_3$) Vesicles similar to those under *fu*$_2$ except that the triple-layered diaphragms appear disorganized to a varied extent. For instance, the three layers cannot be followed from one side of the diaphragm to the other (Figs. 9–11), or the light layer appears wider than a usual unit membrane, and its dense layers are thinner and apparently discontinuous (Fig. 12).

(*d*) (*fu*$_4$) Vesicles closed by a straight, dense, single-layered diaphragm of rather uneven thickness and density. The diaphragm is usually provided with a central knob (5, 6) and is located either at the bottom of a shallow recess (Fig. 13) or in the plane of the plasmalemma (Fig. 15). In either location, the plasmalemma is characteristically bent into a sharp angular rim all along the insertion line of the diaphragm which thus appears to be taut enough to overcome the surface tension of the cell membrane. Occasionally, a second sharp rim appears between the infundibulum and the rest of the plasmalemma (Fig. 14). In general, vesicles provided with a single-layered diaphragm have a large stoma whose diameter can be as large as that of the vesicle itself (see Fig. 8 in reference 3). The latter is then nearly hemispherical in shape and appears to be ready to open directly on the cell front. The stoma is smaller for vesicles opening on shallow infundibula.

In specimens in which the blood is well fixed and thereby retained in the vascular lumina, the density of the content of apertured vesicles is generally lower than that of the blood plasma (Fig. 16).

Similar one-layered diaphragms are occasionally found between vesicles fused in a chain or cluster within the endothelial cytoplasm (Fig. 17). As in the case of vesicles opening on a cell front, these intervesicular diaphragms have central knobs and pull into sharp edges, along their line of insertion, the limiting membrane of the fused vesicles.

Finally a whole, finely graded spectrum of flask-shaped vesicles is encountered which includes the following: (*a*) (*fi*$_1$)[2] vesicles with a simple elongated neck whose inner diameter may be less than 200 A (Fig. 18); (*b*) (*fi*$_2$) vesicles whose elongated neck seems to be pinching off (Fig. 19); (*c*) (*fi*$_3$) vesicles which appear connected to the plasmalemma by a strand or a linear condensation of dense material (Fig. 20) rather than an open, membrane-limited channel.

The neck of the vesicles designated *fi*$_1$ and *fi*$_2$ is usually outlined by a smoothly curving membrane (Fig. 18). Quite often, however, remnants of

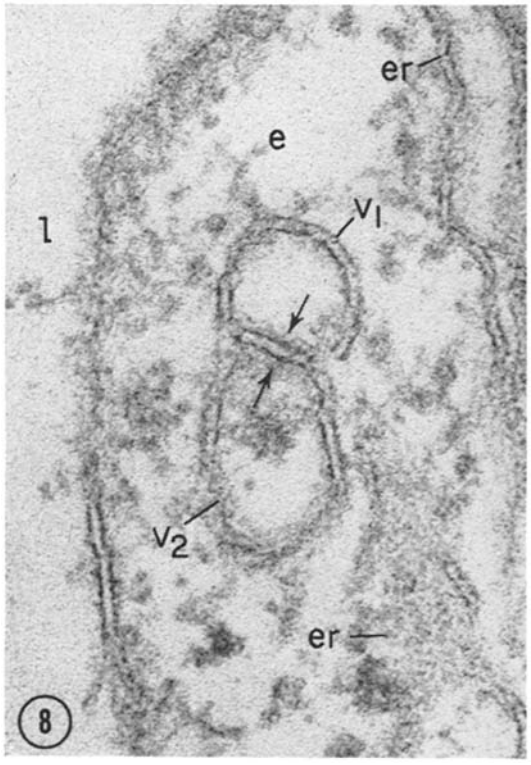
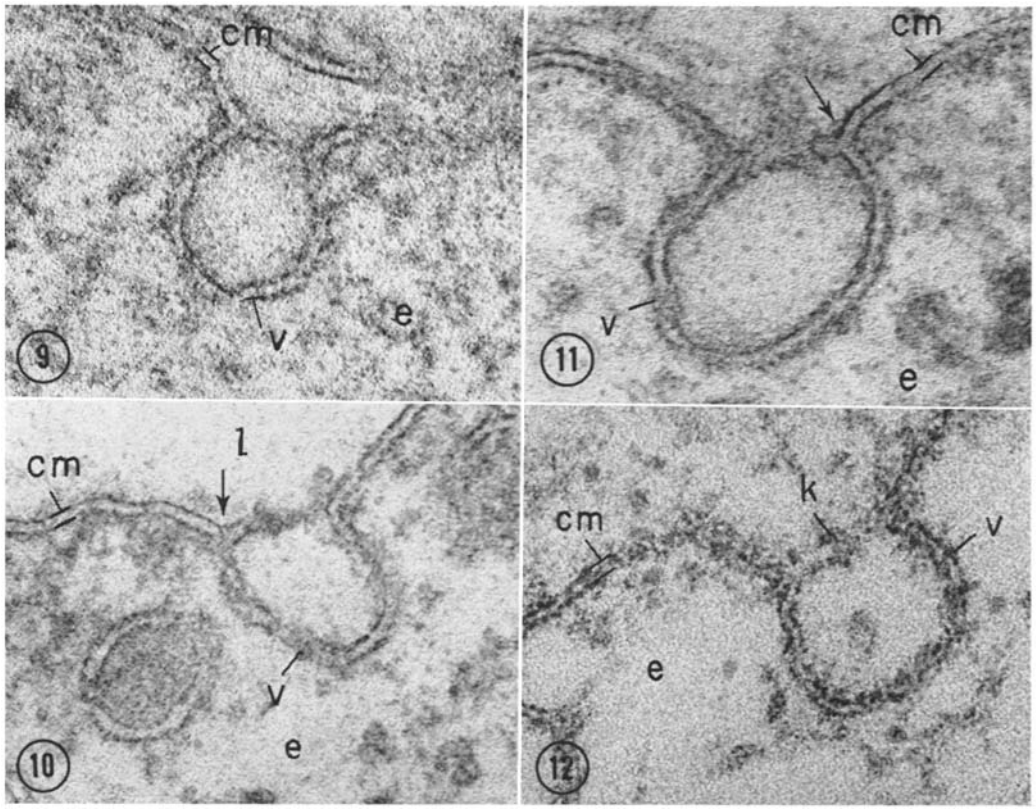


FIGURE 8 Blood capillary; endothelium (rat tongue). A three-layered diaphragm (arrows perpendicular to its plane) is seen between two fused vesicles (v_1, v_2) within the cytoplasm of an endothelial cell. × 245,000.

[2] The abbreviation *fi* (fission) is in keeping with the assumption that such appearances represent steps in a fission process by which an invaginating pocket or an open vesicle separates from the rest of the membrane to become a closed vesicle.



FIGS. 9–12 show three-layered diaphragms in varied degrees of disorganization.

FIGURES 9–11 Blood capillaries; endothelium (rat tongue). Full (Figs. 9, 11) or partial (Fig. 10) continuity with the corresponding dense and light layers of the cell and vesicle membrane is visible at arrows. The rest of the diaphragm seems to be either missing (Fig. 9) or replaced by a single-layered structure (Figs. 10, 11) in which a central knob is apparent. Fig. 9, × 310,000; Fig. 10, ×245,000; Fig. 11, × 320,000.

FIGURE 12 Endocardium; endothelium (rat right atrium). The light layer of the diaphragm is apparently expanded, the dense layers seem discontinuous, and a small central knob (*k*) is visible. × 295,000.

sharply bent rims can be recognized on one or both sides of the vesicular profiles (18, 19).[3]

Most of the appearances described are rare; those designated fu_1–fu_4 may represent less than 1% of the total vesicle population. The modulations described were found in the endothelial cells of all capillary beds examined and, in addition, were encountered in the endothelial cells of lymphatic capillaries, lacteals, large vessels (arterioles and venules), and endocardium. Hence, the events with which such appearances are connected seem to be of widespread occurrence in vascular endothelia. The forms designated fu_1–fu_4 were seen, however, more frequently in the generally fenestrated endothelium of visceral capillaries, in the partially fenestrated endothelium of capillaries of the tongue lamina propria, and in the endothelium of venules and lymphatics.

[3] Adinolfi, L. and G. E. Palade. Unpublished observations.

Coated Vesicles

Practically the same spectrum of structural modulations has been observed for the small population of coated vesicles of the endothelium (3). Figs. 21 and 22 illustrate such vesicles whose content is separated from the extracellular medium by a five-layered and a single-layered aperture, respectively. Coated vesicles seem to lose their

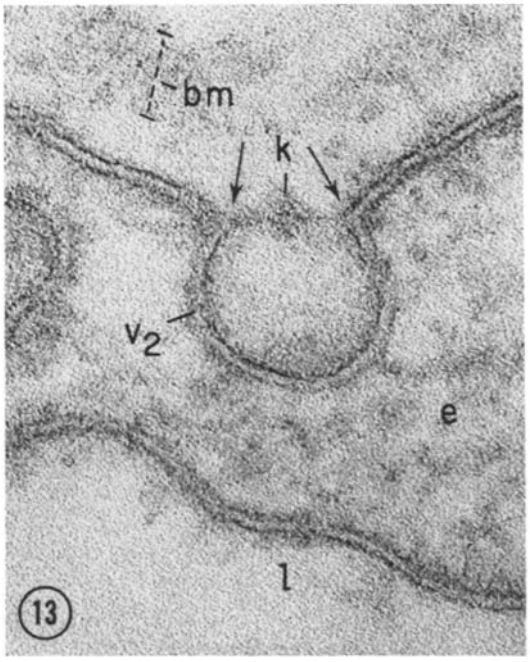


FIGURE 13 See legend under Fig. 15.

filamentous corona where they approach the cell membrane (Fig. 23). Flask-shaped, coated vesicles, apparently pinching off from the plasmalemma, are also relatively frequently encountered (Fig. 24).

Endothelial Fenestrae

The endothelium of visceral capillaries is known to be interrupted by fenestrae (18–23) generally closed by apertures or diaphragms (18, 21–23). Similar fenestrations are found in the endothelium of tongue capillaries especially in the lamina propria of the lingual mucosa (3).

In micrographs taken at sufficiently high resolution, the diaphragms appear single-layered (5, 6) like those which close the stomata of vesicles designated fu_4 in the preceding description. Moreover, in both cases, the insertion of the diaphragm exerts enough tension on the plasmalemma to keep it bent into a sharp edge. Hence, the questions arise whether these apertures also result from membrane fusion and whether appearances expected from earlier stages in a fusion process can be detected in fenestrated endothelia.

Five-layered diaphragms are occasionally found closing the endothelial fenestrae of visceral and lingual capillaries (Fig. 25), and in such cases continuity layer-by-layer shows clearly that the diaphragm is the result of a local fusion of the plasmalemma on the blood front of the cell with that on the tissue front.

Although suggestive appearances have occasionally been encountered, convincing images of intermediate stages between the five-layered and single-layered diaphragms have not yet been recorded.

In addition to apertured fenestrae, channels which connect the two fronts of the cell and are closed at each end by a single-layered diaphragm (Fig. 26) are quite often found and have already been described in the capillaries of the intestine and adrenal medulla by Luft (5) and Elfvin (6), respectively. A probable stage in the formation of such channels is represented by forms closed at one end by a five-layered diaphragm and at the other by a single-layered aperture. A rarer variant appears in Fig. 27; the channel is closed at each end by a single-layered diaphragm and is interrupted in the middle by a five-layered aperture.

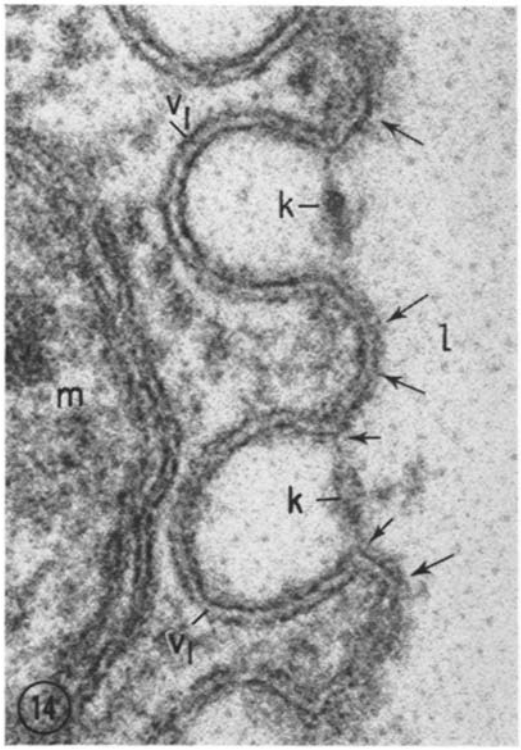
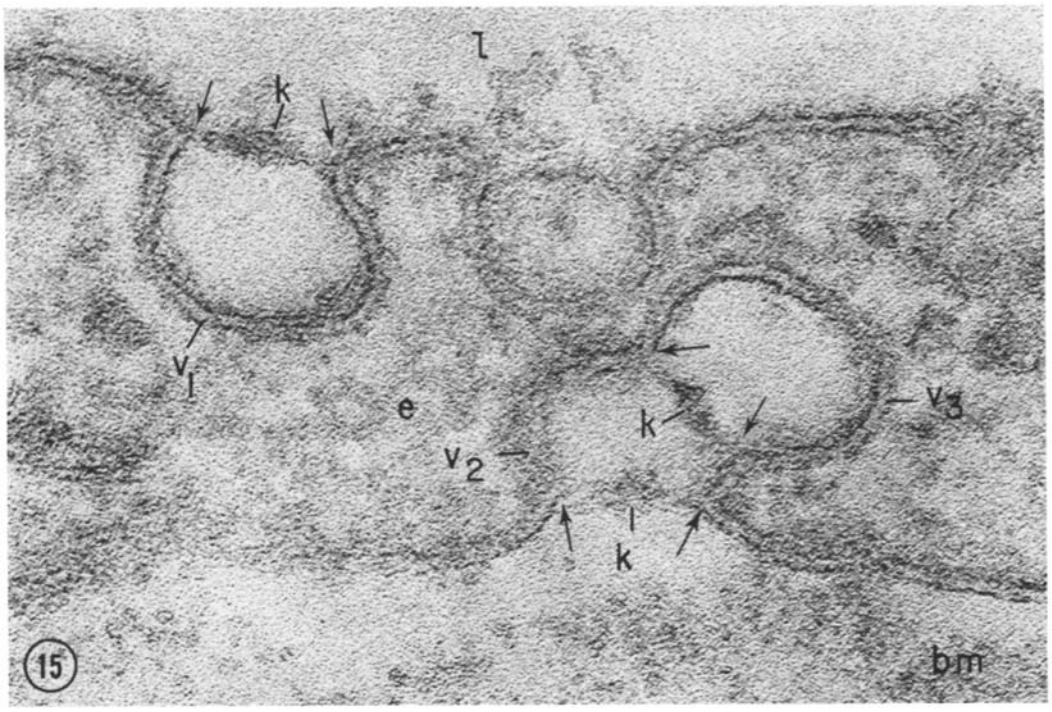


FIGURE 14 See legend under Fig. 15.



FIGURES 13–15 Blood capillaries; endothelium (rat tongue). All micrographs show vesicles provided with single-layered diaphragms (short arrows) located on either the blood (v_1) or tissue front (v_2) of the endothelial cells. A similar diaphragm appears in Fig. 15 between v_2 and v_3, i.e., two vesicles opening in tandem on the tissue front of the cell. Note that in all cases the diaphragms are provided with a central knob (k) and that the cell membrane is bent into sharp rims at the sites of diaphragm insertion. Note in Fig. 14 the existence of secondary rims (long arrows) often associated with this form of plasmalemmal vesicles. The sections for Figs. 13 and 15 were stained with uranyl acetate only. Fig. 13, × 320,000; Fig. 14, × 385,000; Fig. 15, × 300,000.

DISCUSSION

Technical Aspects

The average diameter of plasmalemmal vesicles (~700 A) is only slightly larger than the thickness (500–600 A) usually ascribed to the tissue sections in which they are examined. Hence, it can be argued that images like those we have described could be produced by the inclusion of large sectors of the rims of vesicular stomata within the thickness of our sections. We assume that this is not the case for the following reasons.

(*a*) All the sections used in this study were gray or dark gray, i.e., thinner than ~500 A (24).

(*b*) Gray sections already mounted on copper grids were embedded in Epon and perpendicularly recut. Measurements of the thickness of the first sections made on electron micrographs of the second sections gave values of 200–300 A.[3] Sections of such thickness could cut only through a diaphragm and hence allow the visualization of its structure with little or no interference from the rim of the stoma, or parts thereof, included in the depth of the sections and running nearly parallel to the plane of the latter (see Fig. 28).

(*c*) Many details in our micrographs, for instance the continuity of membrane layers around profiles of sectioned vesicles or around sharp rims of vesicular stomata, indicate that the sections are thin enough to give images unaffected by membrane curvatures having radii of ~350 A.

Structural Aspects

All the observed modulations in vesicular structure and relationship could be explained by assuming that they represent intermediate stages

in a process of fusion with, or fission from, the plasma membrane. For instance, the first step in the fusion of a vesicle with the plasmalemma is expected to give the condition described under fu_1 in which the vesicular content is isolated from plasma or interstitial fluid by a five-layered diaphragm which represents the membrane of the vesicle fused to that of the cell. The fusion area may spread and be followed by a progressive elimination of layers from the two fused membranes. Such operations would lead to the condition designated fu_2 in which the vesicular content is apparently separated from the external medium of the cell by a three-layered diaphragm only, i.e., a single unit membrane. Further elimination (fu_3) would result in the formation of vesicles closed by a single-layered diaphragm (fu_4). According to current interpretations of membrane architecture (25–28), this step would amount to the elimination of the bimolecular lipid leaflet(s) of the fused membranes and should result in major changes in the permeability of the aperture. The single-layered diaphragm is probably a stretched protein film which could consist of either residual proteins of the two dense leaflets still present in the preceding stage, or an adsorbed layer of plasma proteins comparable to the endocapillary layer of Chambers and Zweifach (29) or endothelial layer of fibrin postulated by Copley et al. (30). According to Luft (5, 31), the endocapillary layer can be demonstrated by staining with ruthenium red, a reaction which suggests that the layer consists of, or contains, acid mucopolysaccharides. The observation that in practically all cases single-layered diaphragms appear to be firmly bound to the membrane, as evidenced by the sharp rim produced by their insertion, favors the first alternative according to which the apertures are films of residual membrane proteins, rather than parts of an extraneous coat of the endothelial cell. This assumption may imply a large turnover of membrane proteins, since vesicle fusion may be a frequent event and since the diaphragm material may be eventually lost by the plasmalemma.[4] The next step in the process is probably the rupture of the diaphragm which removes any diffusion barrier between vesicular content and external medium. At this point, the opened vesicle could flatten out to become an integral part of the surface membrane, or it could reverse the direction of its movement and reinvaginate. As it moves away from the plasmalemma, the reinvaginating vesicle is expected to acquire a narrowing stoma and a progressively elongating neck, i.e. forms we described as fi_1–fi_3, while occasionally retaining, at least in part, the sharp rim characteristic of the preceding (fu_4) stage. The same transformations would be undergone by vesicles which form *de novo* by invagination of the cell membrane. The final pinching off of the neck would result in a closed vesicle apparently free to move in the cytoplasmic matrix. The process of vesicle fusion and

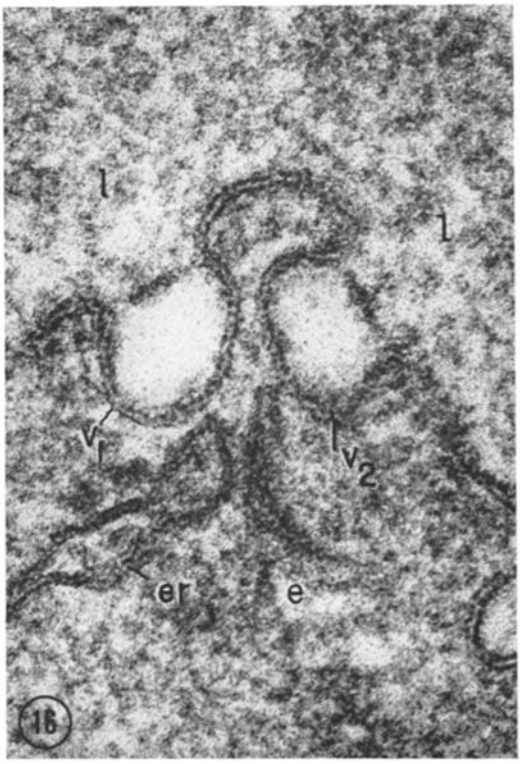


FIGURE 16 Blood capillary; endothelium (rat intestinal mucosa). Two vesicles (v_1, v_2) provided with single-layered diaphragms can be seen on the blood front of the cell. Note that the density of their content is considerably lower than that of the blood plasma in the capillary lumen. In this case, the plasma was retained and fixed in the lumen by ligating an intestinal loop and its mesentery at the time of fixation. × 205,000.

[4] Once formed, the sharp insertion rims for a while seem to survive the disappearance of their corresponding diaphragms, as suggested by the presence of secondary rims in connection with some apertured vesicles (fu_4, Fig. 14) and by that of rim remnants in the neck region of some flask-shaped vesicles (Figs. 18, 19, 25).

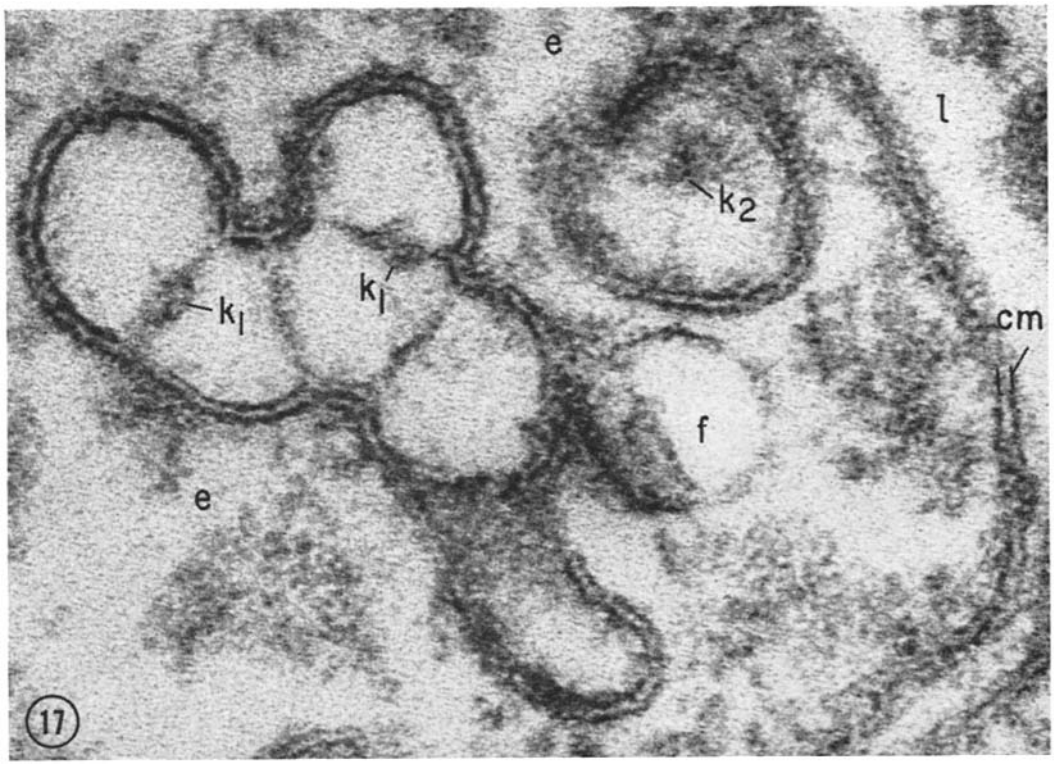


FIGURE 17 Blood capillary; endothelium (rat tongue). Cluster of vesicles located in the cytoplasmic matrix (the cell membrane appears at *cm*). Four single-layered diaphragms separate the vesicles in the cluster from one another. Two of these diaphragms have central dense knobs (k_1). Note the sharply bent rims of the vesicle membranes at the sites of diaphragm insertion. The central knob of another diaphragm parallel to the plane of the section appears at k_2. Part of a grazingly sectioned vesicle (*f*) has fallen off from the section, presumably during microtomy. × 360,000.

fission could involve either flat or slightly recessed areas of the cell membrane; the latter case would explain the diaphragms and necks found at the bottom of small infundibula.

The relative frequency of the different forms described suggests that the fusion process is extremely rapid for the usual plasmalemmal vesicles of the capillary endothelium, whereas the pinching off of invaginating pockets is slow. The higher frequency of fusion forms in the other vascular endothelia examined probably implies that the fusion process is considerably slower therein.

An identical series of modulations, apparently corresponding to a similar sequence of events, has been observed for coated vesicles. In this case, the frequency of fusion forms seems to be higher in all endothelia.

A comparable process of membrane fusion followed by a progressive elimination of membrane layers could explain the findings made on fenestrated endothelia. Fusion of the plasmalemma on the blood front with the plasmalemma on the tissue front of the cell would result in a simple apertured fenestra. Simultaneous fusion of one or two vesicles with the plasmalemma on both cell fronts would produce appearances described as doubly or trebly apertured channels, irrespective of the extent of layering of the corresponding diaphragms.

What we are proposing is simply a rationalization of structural modulations of plasmalemmal vesicles in terms of their involvement in transport in quanta across the endothelium. The sequence of events we postulate is compatible with all appearances so far described; yet it should be clear

that no direct proof exists at present that these events occur and especially that they occur in the sequence indicated.

Membrane fusion has already been envisaged by Karrer and Cox (21) and discussed more recently by Luft (5) and Elfvin (6) as a possible explanation for the occurrence of apertured fenestrae and vesicles. The last two authors have described and clearly illustrated forms designated in the present paper as fu_4 and channels closed by single-layered apertures on both endothelial fronts. Both have also discussed the possibility of elimination of layers from the fused membranes. Our work now establishes the existence of a spectrum of forms probably involved in this process.

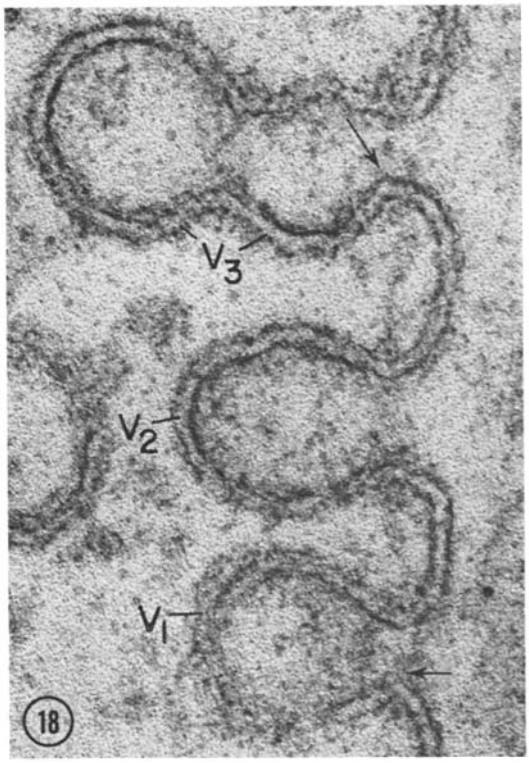


FIGURE 18 Blood capillary (rat tongue). Two simple, flask-shaped vesicles (v_1, v_2) are connected to the vascular lumen by open stomata which measure 160 A (v_1) and 320 A (v_2). A chain of two vesicles (v_3) opens through a similar stoma or channel (inner diameter $\simeq$170A). Note the distinct, layer by layer continuity of the plasmalemma with vesicular membranes; note also the sharp bends seen at arrows in the otherwise smoothly curving membranes of the stoma regions. × 380,000.

According to our interpretation, a vesicle closed by a single-layered diaphragm is in a predischarge phase, while a vesicle provided with an open stoma is in a loading phase. This interpretation is supported by the finding that, on the blood front of the endothelium, the content of the first type of vesicles is of considerably lighter density than plasma, while that of the second type is equal in density to the plasma. The same applies when the density of the latter is experimentally increased by the intravenous injection of serum albumin or myoglobin. These observations suggest that the single-layered diaphragm restricts, at least to some extent, the diffusion of large molecules like those of the plasma proteins.

General Aspects

Our findings provide no answer to the question whether the limiting membrane of the vesicles is chemically and functionally distinct from the plasmalemma (see reference 32). The sequence of events and the corresponding structural modulations could be the same for a distinct population of vesicles shuttling between the two fronts of the cell and for a population of vesicles produced by random invaginations of the plasmalemma.

Finally our observations suggest that the process of membrane fusion followed by the progressive elimination of the layers of the fused membranes is of wide occurrence and can be used by an endothelial cell for at least three operations: (*a*) discharge a plasmalemmal vesicle, (*b*) fusion of two or more vesicles, and (*c*) production of a fenestra. In the latter version, in which the two membranes involved are both parts of the plasmalemma, the process appears restricted so far to the vascular endothelium; but in the first and second versions it is expected to operate in intracellular transport in bulk in, as well as in uptake and discharge of matter in bulk from, all types of cells. Preliminary observations indicate that many of the functional modulations described in endothelial cells can be recognized in goblet cells during the fusion of mucus droplets and the discharge of their content into the intestinal lumen.

Fusion and fission of vesicles may also be involved in the turnover of the plasmalemma, a process found to occur at relatively high rate in the few cases so far investigated (33, 34). This assumption implies that the cell membrane is assembled and degraded in the cytoplasm and transported to and from the cell surface in vesicular units. A

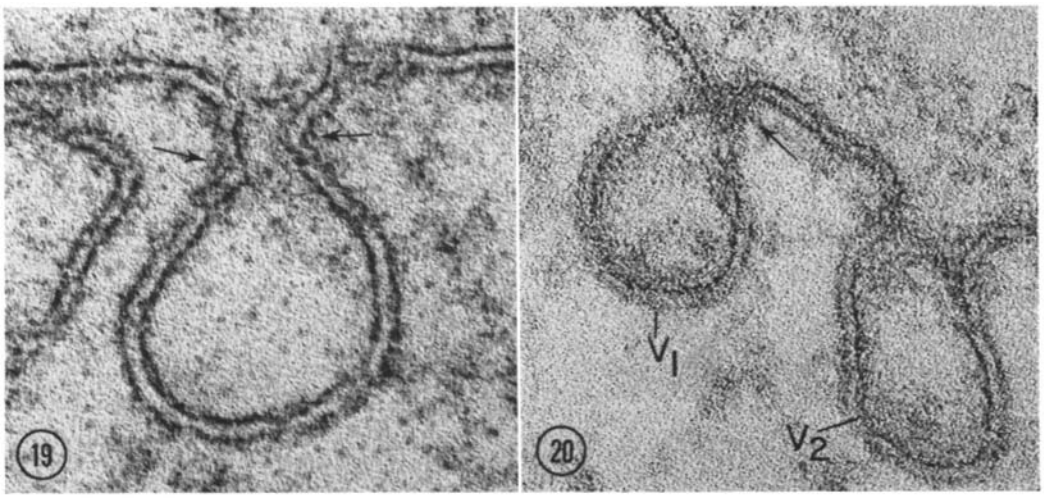
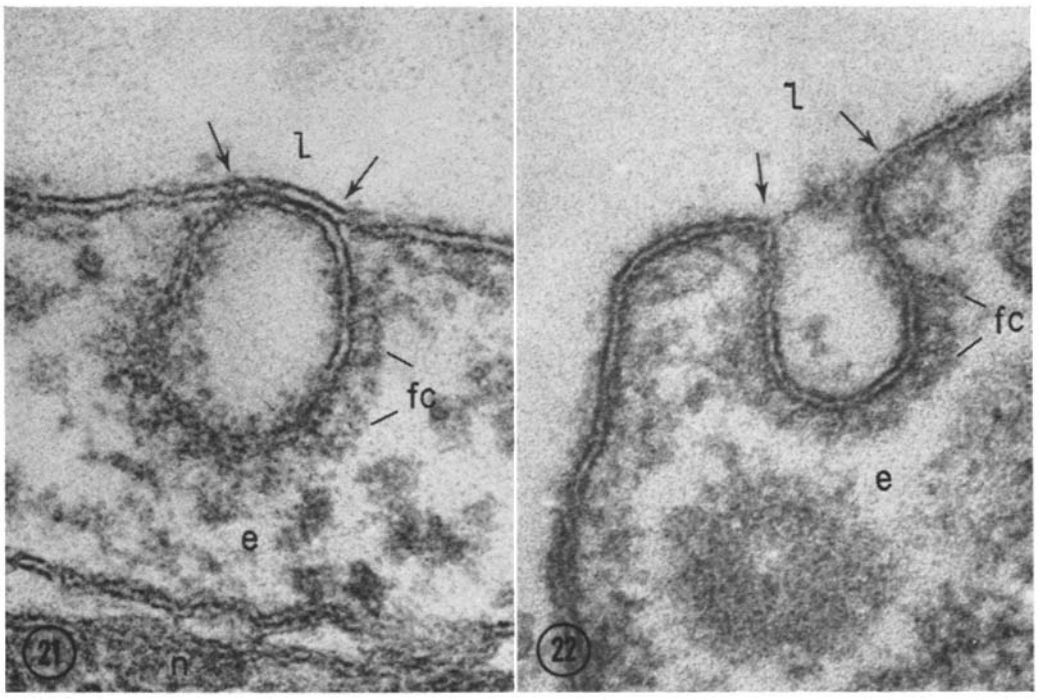


FIGURE 19 Blood capillary (rat tongue). Flask-shaped vesicle connected to the lumen by a relatively long, narrow, but still open channel, the inner diameter of which measures ~150 A. The image suggests that the channel is undergoing a pinching-off process. Note the sharp bends in its limiting membrane (arrows). × 400,000.

FIGURE 20 Blood capillary (rat tongue). Two flask-shaped vesicles of which one (v_1) is connected with the plasmalemma by an apparently solid strand of dense material (arrow), while the other (v_2) is still open to the cell surface by a large stoma. × 300,000.



FIGURES 21 and 22 Blood capillaries; endothelium (rat tongue). Coated vesicles in the process of membrane fusion with the plasmalemma. In Fig. 21, the fusion has produced a typical five-layered diaphragm (arrows). Note that the light layer of the vesicle membrane is thinner and less regular within the area of fusion than in the rest of the vesicle, a detail possibly connected with the progressive elimination of the layers of this membrane after fusion. The left side of the vesicle profile is blurred on account of the obliquity of the section. In Fig. 22, the diaphragm (arrows) is single-layered in its center and possibly three-layered at its left end. In both cases, the filamentous corona of the vesicles is marked *fc*. Fig. 21, × 260,000; Fig. 22, × 245,000.

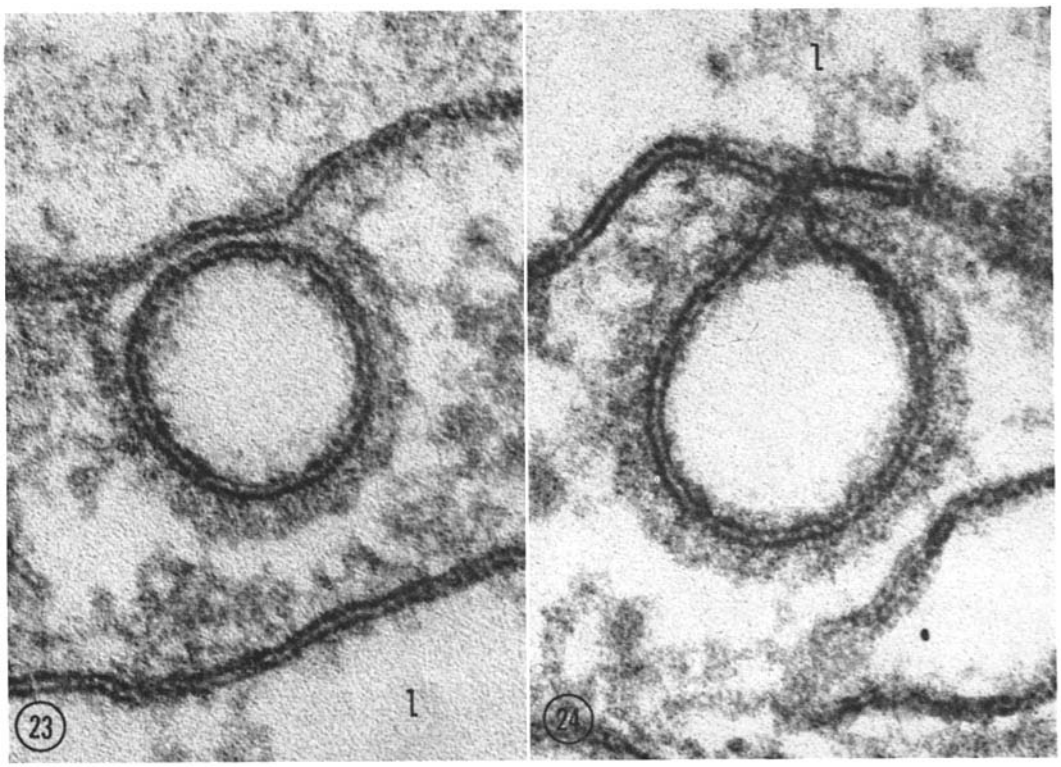


FIGURE 23 Blood capillaries; endothelium (rat pancreas). Coated vesicle located near the cell surface and free of its fibrillar coating in the area of close approximation to the plasmalemma. This area might be involved in the subsequent fusion of the two membranes. × 290,000.

FIGURE 24 Coated vesicle connected with the plasmalemma by a closed neck or short solid stalk. The appearance probably represents a late stage in the detachment (fission) of a coated vesicle from the cell membrane. × 290,000.

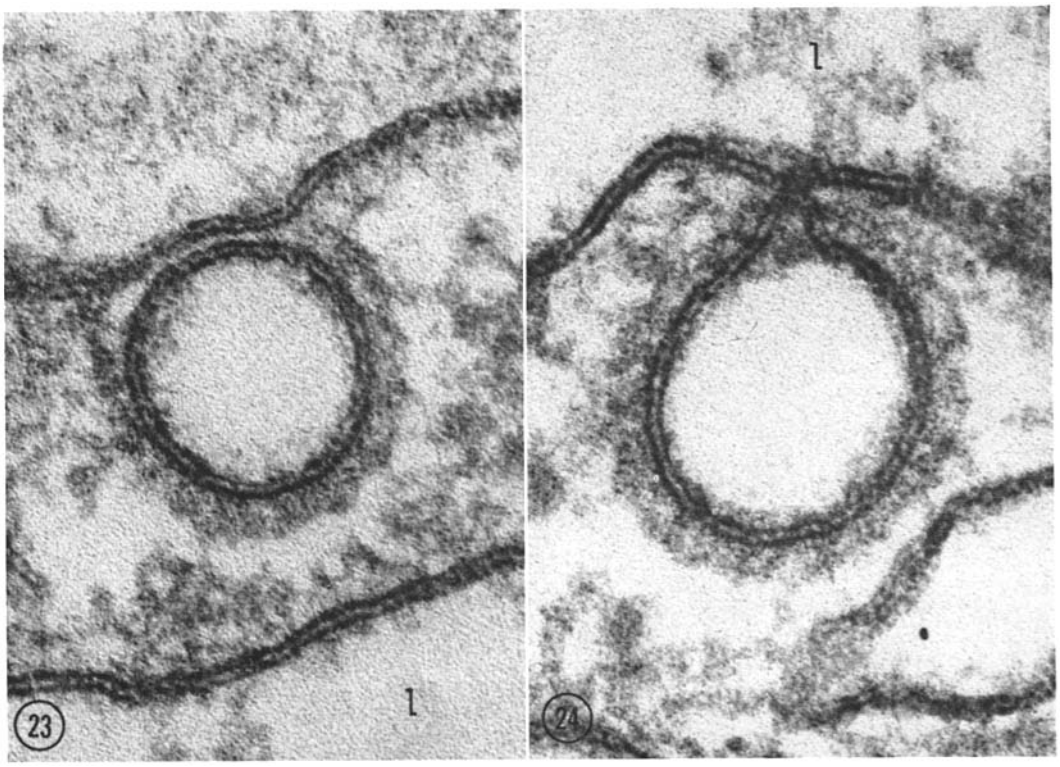


FIGURE 25 Blood capillary; endothelium (rat tongue). Endothelial fenestra closed by a five-layered diaphragm (arrows). At the right end, continuity layer by layer with the plasmalemma on each cell front is clearly visible. The membrane on the tissue front may belong to a plasmalemmal vesicle already open to the external medium as suggested by the linear density (d) which may be the residue of a single-layered diaphragm. × 365,000.

FIGURE 26 Blood capillary; endothelium (rat tongue). Channel closed by two single-layered apertures (d_1, d_2), each provided with a central knob and each inserted on a sharp rim of the plasmalemma. × 310,000.

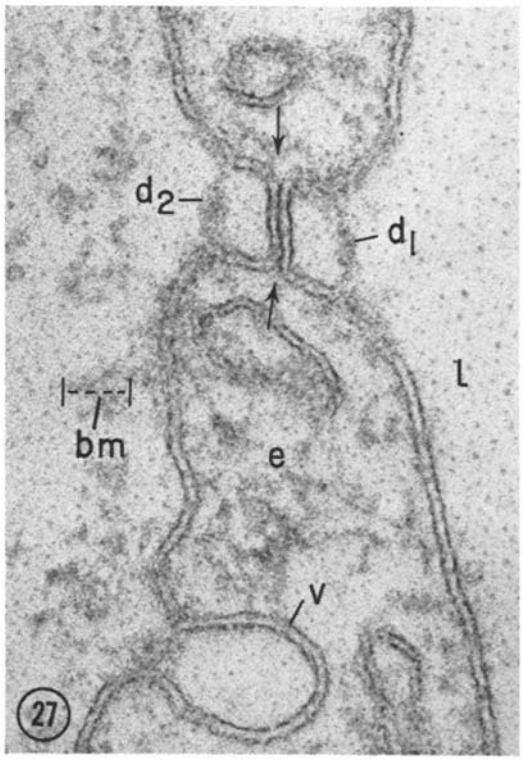


FIGURE 27 Blood capillary; endothelium (rat tongue). Complex channel provided with a single-layered diaphragm (d_1, d_2) at each end and interrupted in the middle by a five-layered diaphragm (arrows indicate the plane of the fusion layer). The layers of the diaphragm are continuous with the layers of the membrane bounding the channel (presumably the membrane of two former vesicles) and, beyond it, with the layers of the plasmalemma proper. × 280,000.

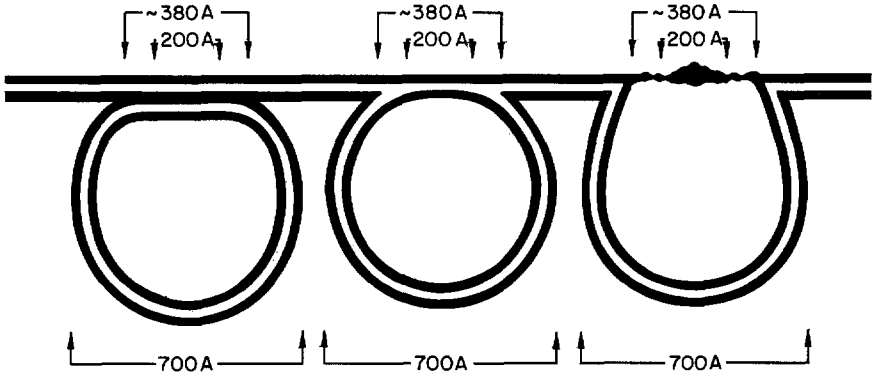


FIGURE 28 The diagram shows plasmalemmal vesicles of type fu_1, fu_2, and fu_4 (in this order, from left to right). Their relation to the plasmalemma is described in the text. The diagram indicates that sections ~200 A thick can be expected to cut exclusively through the diaphragm of such vesicles.

caveolar or vesicular unit is probably involved in the assembly of the transverse tube system during the differentiation of embryonic muscle in tissue culture (35).

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