

AN INTERPRETATION OF LIVER CELL MEMBRANE AND JUNCTION STRUCTURE BASED ON OBSERVATION OF FREEZE-FRACTURE REPLICAS OF BOTH SIDES OF THE FRACTURE

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ABSTRACT

A modification of the freeze-fracturing technique to permit observation of replicas of both sides of the fracture is described. It has been used to study mouse liver cell membrane structure. Membranes break to give two faces with three-dimensional complementarity, although there is some small-scale mismatching which is discussed. Since the two distinctive sets of membrane faces are complementary sets, they cannot be the two outside surfaces. In particular, structures (such as particles) seen on these faces are within the membrane. It is not possible from this work to say precisely where the fracture plane goes with respect to a plasma membrane, only that it must be close to the interface between membrane and cytoplasm, or at that interface. Models, consistent with the appearance of the matching replicas, are derived for three regions of the plasma membrane: (a) The nonjunctional plasma membrane, which contains many scattered particles. Except for these particles, the otherwise flat fracture face is not at variance with a bimolecular leaflet structure. (b) Gap junctions. Each of the two membranes comprising a gap junction contains a close-packed array of particles. (c) Tight junctions. Here membranes have ridges within them.

INTRODUCTION

In thin sections observed with the electron microscope the plasma membrane of a cell shows the characteristic trilaminar unit membrane structure (Robertson, 1959). Gap junctions show a 20 A space between the two apposed plasma membranes, and lanthanum hydroxide applied during fixation penetrates this intercellular space to show a 90 A center-to-center array of particles in the plane of the junction (Revel and Karnovsky, 1967; Brightman and Reese, 1969). The tight junction shows either extended or punctate fusions of the outer leaflets of the plasma membranes of the two adjoining cells (Farquhar and Palade, 1963; Revel, 1968; Matter et al., 1969).

Before discussion of the results of freeze-etching

and freeze-fracturing, some terms will be defined. The term "surface" will only be used to mean the true membrane surface, bordering on either cytoplasm or extracellular space. The term "face" will be used to mean a fracture face parallel to the plane of the membrane. Freeze-fracturing of plasma membranes produces two distinct types of faces: one type has fracture faces covered with many small particles, the other has faces with fewer particles (Weinstein and Someda, 1967; Branton, 1969; Bullivant, 1969 a; Meyer and Winkelmann, 1969). These two faces are as follows:

(a) A convex face covered with many small particles, which gives the appearance, in freeze-

fracture replicas, of being seen from outside the cell. This face is the face of membrane-associated material left adhering to the cytoplasm after fracturing. In the terminology of Meyer and Winkelmann (1969), it will be referred to as a (+) face. (b) A concave face with fewer particles, which gives the appearance of being seen from inside the cell. This face is the face of membrane-associated material left adhering to the extracellular ice. It will be referred to as a (-) face.

Moor and Mühlethaler (1963) originally believed that in some cases the fracture revealed only one surface of a membrane and its complementary ice face. The more recent view of Moor's group (Moor, 1969; Mühlethaler et al., 1965) is that both surfaces of membranes are seen and that particles on fracture faces are attached to a membrane surface. On this interpretation, the (+) face is the true outer surface and the (-) face the true inner surface of the membrane. Branton (1966, 1969) has presented the view that the fracture splits the membrane, and hence that the particles are within it. In his view, the two faces seen represent complementary sets.

Workers who have studied cell junctions by freeze-fracturing and freeze-etching have generally agreed with Moor's interpretation. For example, the gap junction shows a 90 Å center-to-center array of particles on the (+) face of the membrane and a similarly spaced array of depressions visible in the (-) face; and it has been supposed that the particle array is sandwiched between the two membranes of the junction, the array of depressions being on the cytoplasmic surface of each membrane (Kreutziger, 1968 *a*; McNutt and Weinstein, 1969; Bullivant, 1969 *a*, 1969 *b*). The tight junction (+) face shows a series of interconnected concertina-like ridges, while the (-) face shows a similar pattern of furrows. The interpretation has been that the ridges are sandwiched between the membranes, with the furrows being on the cytoplasmic surface of each membrane (Kreutziger, 1968 *a*; Staehelin et al., 1969).

Bullivant (1969 *a*), adopting Branton's (1966) hypothesis, suggested that, for membranes in general, the two distinct particle-bearing and smoother surfaces may represent the complementary sets produced by fracturing. This suggestion was not specifically applied to junctions. If it did apply, previous interpretations would be inverted, for the particles and depressions, or ridges and furrows, could not be on opposite sides of a membrane if they were complementary sets. An obvious

way to decide which interpretation is correct is to freeze-fracture such junctions and look at replicas taken from both sides of the fracture. A technique for obtaining replicas of both sides by using a particular kind of freeze-etch device has already been published by Steere and Moseley (1969). Those authors showed complementary faces in myelin sheath, but made no interpretation of the membrane structure. We have used a different technique to obtain complementary replicas of the membranes of cells in mouse liver and have paid particular attention to the junctional and non-junctional plasma membranes in the region of the bile canaliculus.

MATERIALS AND METHODS

Freeze-fracturing was carried out with a standard type II device (Bullivant and Ames, 1966; Bullivant et al., 1968; Bullivant, 1969 *a*) in which shadowing and backing were done through tunnels in a cold block, thus protecting the cold specimen surface against contamination. A modified brass specimen holder was used (Fig. 1 *a*). This consisted of two standard cylindrical holders filed lengthwise until semicylindrical halves, with the specimen holes exposed, were left. Semicircular copper wings were added to the holders to facilitate handling and orientation.

Small pieces of liver from C3H mice were fixed in 6% glutaraldehyde in phosphate buffer and stored for at least 1 hr at 4°C in 25% v/v glycerol in the buffer. (It should be noted that glutaraldehyde fixation does not significantly alter the fracturing characteristics of membranes.) Very fine strips (0.1 × 0.1 × 2 mm) of the liver were cut in the glycerol solution. Several of these strips, always moistened with glycerol solution, were laid lengthwise in the specimen holder, held in its end-to-end orientation (Fig. 1 *a-b*) with locking forceps. The holder and specimens were frozen in this orientation by plunging into melted Freon 12 at -155°C. The holder was quickly blotted dry of Freon and dropped into the liquid nitrogen bath containing the precooled blocks of the freeze-fracture device. The liver was fractured by gripping the holder with two pairs of cooled forceps and bending and pulling (Fig. 1 *c*). The two halves were then placed fracture side uppermost in the hole in the lower brass block of the freeze-fracture device so that the dividing line between halves was at right angles to the direction of platinum-carbon shadowing (Fig. 1 *d*). This orientation ensures that complementary faces are shadowed at the same angle. Previously described operating procedure for the type II device was followed (Bullivant and Ames, 1966; Bullivant et al., 1968; Bullivant, 1969 *a*). The device was assembled under liquid nitrogen and carried over to the evaporator. After a suitable vacuum

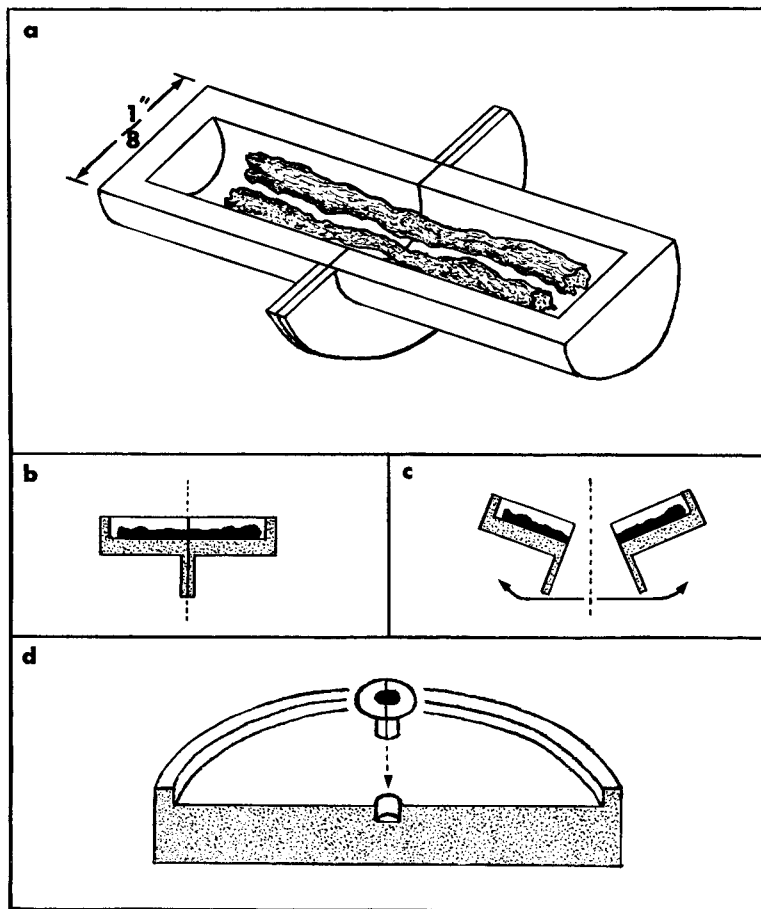


FIGURE 1 Method of breaking tissue and aligning in lower block to obtain replica of both sides of fracture. Fig. 1 *a* View of specimen holder in end-to-end position, with two thin strips of liver in place. Fig. 1 *b* and 1 *c* Longitudinal sections of holder before *b* and after *c* fracturing. Fig. 1 *d* Cutaway view of lower block showing insertion of specimen holder. After rapid freezing, steps *b-d* are carried out under liquid nitrogen. Subsequently, the two fracture surfaces are shadowed with platinum and backed with carbon, using the standard techniques for freeze-fractured material (Bullivant and Ames, 1966; Bullivant, 1969 *a*).

(10^{-5} mm Hg) was obtained, the lid was lifted and the specimen was shadowed with platinum-carbon at an angle of 45° and backed with carbon through the tunnels in the central block above it. The shadowing source consisted of 0.1 mm diameter, pure platinum wire wound on 1 mm diameter carbon points. Shadowing and backing were done when the specimen temperature was about -150°C . After admitting air, the specimens were allowed to thaw and matching pairs were selected. These were floated, replica side uppermost, on buffered glycerol, then on a 50% solution of Janola¹ (a household hypochlorite bleach) for removing the tissue, and finally on several changes

¹ Manufactured by Reckitt and Colman (NZ) Ltd., Auckland, New Zealand.

of distilled water. Care was taken to keep the replicas afloat throughout. Replicas were picked up on formvar-coated 2×1 mm single-hole grids and examined with a Phillips EM200 electron microscope operating at 80 kv.

OBSERVATIONS

For illustrating features of membrane and junction structure seen by freeze-fracturing, two micrographs from replicas which were not members of matched pairs will be used (Figs. 2 and 3). These replicas are of better quality than those in the matched pairs.

The pair of replicas, from which the two pairs of matched micrographs used to illustrate this paper



FIGURES 2-5 Freeze-fracture replicas from the region of the bile canaliculus in mouse liver, showing membrane faces revealed by fracturing. The line *L-L'* on Figs. 2-4 refers to the models of Fig. 6. The magnifications are all the same ($\times 110,000$), and the shadowing direction is from below. These are indicated in each figure, with a scale line for magnification and an encircled arrow for shadowing direction. The micrographs are printed as positives.

FIGURE 2 A replica which is relatively free from contamination and thus shows to advantage the difference between the smoother (-) face of the plasma membrane of one cell and the more particulate (+) face of the plasma membrane of the adjoining cell. Depressions in the smoother (-) surface are ringed. The array of depressions of one gap junction (G_1) and the array of particles (G_2) of another are seen. The fracture plane of G_1 steps to the other face (arrowhead), and this shows that the face with depressions is superimposed on top of the particle array within a particular junction.

were taken, were the best we have so far obtained, and they showed correspondence over an area covering about 10 cells. Examination of these two pairs of matched micrographs (Figs. 4 *a-b* and 5 *a-b*) shows that the main features of one have their three-dimensional complements in the other. Large-scale contaminants are easily detected for they appear on one member of a pair only. Three main areas of membrane face will be described with respect to the single replicas and the matching pairs. These areas are the general plasma membrane, the gap junction, and the tight junction. They will be described only in terms of fracture faces; no judgment will be passed on which surfaces the faces represent until the Discussion.

(*a*) The general plasma membrane of a cell shows a (+) face (Fig. 2) covered with many small 100–150 Å particles. The (–) face is smoother but may bear a few particles and a few depressions. Several authors have commented on the asymmetry of distribution of these particles (Weinstein and Smeda, 1967; Branton, 1969; Bullivant, 1969 *a*; Meyer and Winkelmann, 1969).

The general plasma membrane breaks so that the smoother (–) face (*A* in Figs. 4 *a*, 5 *b*) is the complement of the particle-covered (+) face (*A'* in Figs. 4 *b*, 5 *a*).

(*b*) Gap junctions are found as differentiated patches on the membrane faces of adjoining cells in the region of the bile canaliculus, but only rarely immediately adjacent to it. The gap junction is seen as a close-packed array of particles (G_2 , Fig. 2) on the (+) face of one cell membrane and a close-packed array of depressions (G_1) on the (–) face of the adjoining cell membrane. The depressions often appear as a 90 Å center-to-center hexagonal array, but this regular packing is not so apparent for the particles.

The gap junction membrane breaks so that the depression-covered (–) face (*B*, *C*; Figs. 4 *a-b* and 5 *a-b*) is the complement of the particle-covered (+) face (*B'*, *C'*). The large-scale matching of these two surfaces is striking. One point deserves comment here. The arrays of depressions (*B*, *C*) may give the impression of being arrays of particles. Where the array is less ordered, it is obvious that the array really does consist of depressions, when one takes into account the direction of shadow (Fig. 2). Where there is close packing and a low shadowing angle, the shadow side of one depression may be associated with the platinum-covered side of its next neighbor, thus giving the impression that one is seeing a particle. From a close study of these and other gap junction repli-

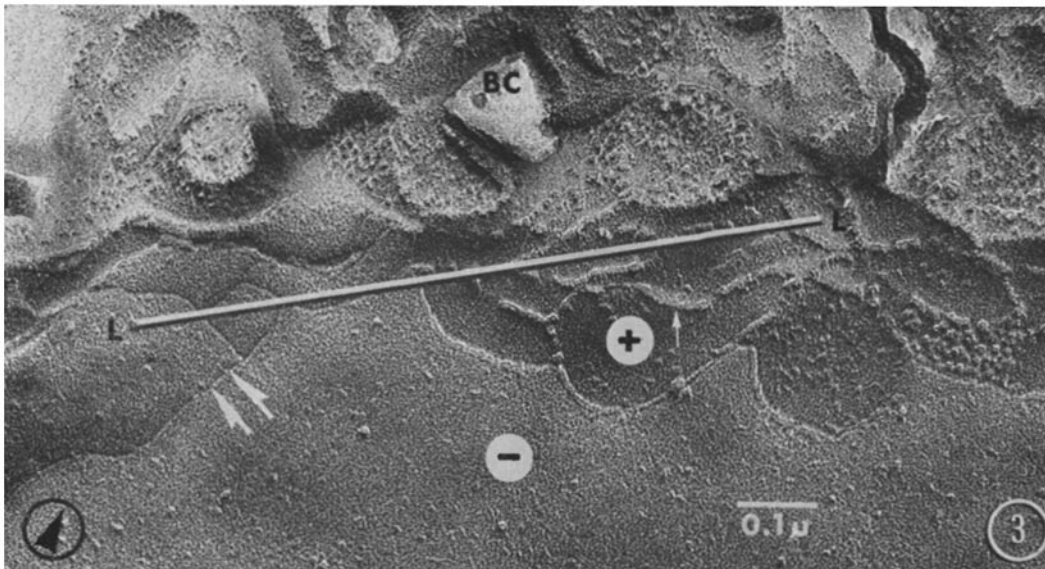


FIGURE 3 A tight junction in which the fracture passes from the (–) face of one plasma membrane to the (+) face of the next. Furrows (double arrow) are present on the (–) face, and ridges (arrowhead) on the (+) face. The fracture face of the plasma membrane shows gentle hills between furrows and gentle valleys between ridges. Cross-fractures of microvilli appear in the bile canaliculus (*BC*).

cas, we are convinced that the arrays on the (-) faces of membranes in these paired replicas are actually arrays of depressions.

(c) Tight junctions form continuous seals between adjoining cells and are immediately adjacent to the bile canaliculus. Within the tight junction, the (+) face (Fig. 3) of one cell membrane is covered with an interconnected series of concertina-like ridges (about 70 Å high). The (-) face of the adjoining cell membrane shows a similar interconnected array of furrows. If the break jumps from the (+) face of one membrane to the (-) face of the other, it is seen that the ridges and furrows show continuity in direction and it is, therefore, presumed that the furrows on a (-) face are in register with the ridges on the (+) face below it. Between the ridges the (+) face shows gentle valleys, and between the furrows the (-) face shows gentle hills, giving the tight junction area a quilted appearance.

The tight junction breaks so that the (-) face (with furrows) (*D*, Fig. 4 *a*) is the complement of the (+) face (with ridges) (*D'*, Fig. 4 *b*). The pattern of furrows is reflected exactly in the pattern of ridges. In addition, the valleys between the ridges find their exact counterpart in the hills between furrows.

DISCUSSION

The three main areas of membranes referred to earlier will now be discussed.

General Plasma Membrane

The paired replicas show some contamination of the faces possibly due to condensation of oil or water vapor on the cold fracture faces before replication. Even on relatively clean faces, such as those seen in Fig. 2, there is an excess of particles on the (+) face compared with depressions out of which similar particles might have pulled from the (-) face.

If the fracture had proceeded without distortion of the faces, and there had been no subsequent alteration prior to or during replication, then the two faces produced would have been exactly complementary. In particular, there would have been sufficient depressions to accommodate all the particles. This is not so. In our opinion, the most likely explanation is that the particles are real structures, and not artifacts of preparation, although they may be deformed plastically to some

extent during fracturing (Clark and Branton, 1968). Our reasoning is as follows: (*a*) It is noticeable that depressions are seen in general plasma membrane fracture faces only when there is little evidence of contamination; therefore, slight contamination could account for the disappearance of many depressions. (*b*) There could be slight etching or melting of the sharp edges of holes, making them more difficult to see. Heating during platinum evaporation could cause this, even though the bulk specimen temperature is about -150°C . (*c*) All other things being equal, isolated holes are always more difficult to see than projections in a shadowed preparation.

In the next section it will be shown that, in the gap junction, the fracture goes either near to or at the interface between membrane and cytoplasm, revealing particles within the membrane. There is no step on the fracture face between the plane bearing the depressions of the gap junction (G_1 , Fig. 2) and the smoother general plasma membrane (-) face; nor is there a step between the plane on which the particles of the gap junction sit (G_2) and the surrounding particle-bearing plane of the general plasma membrane (+) face. Hence the fracture plane probably proceeds at the same level within both gap junction membrane and general plasma membrane. Accordingly we can construct a model of the two plasma membranes of adjoining cells and the probable path of the fracture (Fig. 6 *a*). Each membrane contains particles within it. This diagram is related to a particular line on Fig. 2 (see legend to Fig. 6 *a*). The fracture goes around particles within the membrane, but we do not know whether it goes within the membrane or at the interface between membrane and cytoplasm when it is not in the region of particles. This will be discussed in the next section. We have, so far, ignored particles which appear on the smoother (-) face. There are always some present even in the best preparations. These particles are less prominent than the particles of the (+) face. A likely explanation is that they are revealed when the fracture plane goes around the other side of the particle, leaving it attached to and slightly protruding above the (-) face. This kind of fracture would appear to be less common than that which leaves particles attached to the (+) face. The particles observed on the (-) face may differ structurally from those of the (+) face, and this difference may be reflected in the way they fracture.

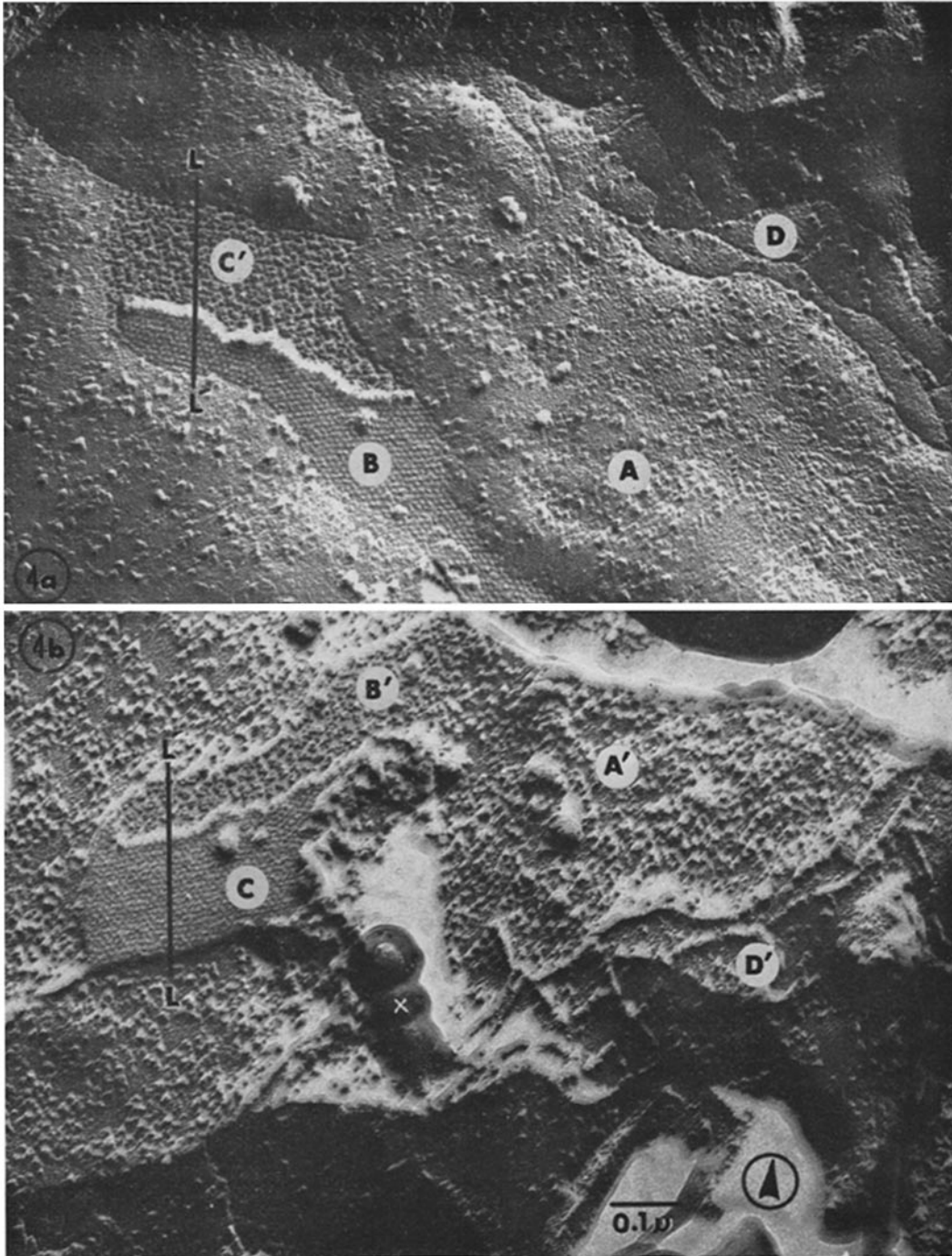


FIGURE 4 *a-b* Complementary replicas from either side of a fracture. The features of one match those on the other. In the general plasma membrane, the smoother (-) face (*A*) is the complement of the more particulate (+) face (*A'*). In the gap junction, the hexagonal array of depressions (*B* and *C*) is the complement of the array of particles (*B'* and *C'*). In the tight junction, the interconnected furrows (*D*) are the complement of the ridges (*D'*). There are gentle hills on the face between furrows, and these are reflected as gentle valleys between ridges on the complementary face. Ice crystals (*X*), which have fallen on the face after fracture, appear only on one side (Fig. 4 *b*).

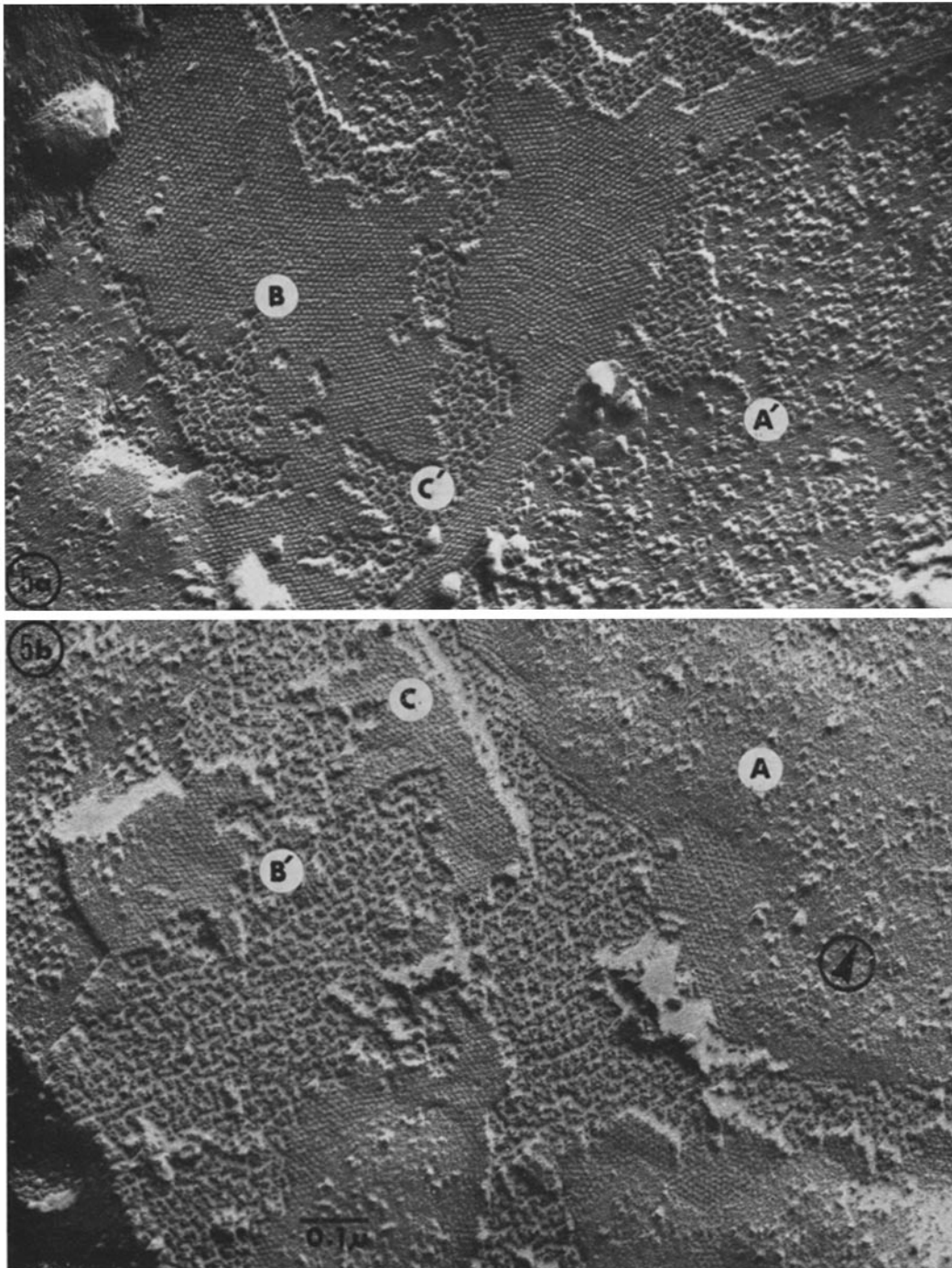


FIGURE 5 *a-b* A complementary pair to show the near-perfect matching obtained within an extensive gap junction, where the fracture plane goes from one membrane to the other a number of times. Regions with arrays of depressions are matched by regions with arrays of particles. The matching of the area of particles (*C'*) in Fig. 5 *a* and the area of depressions (*C*) in Fig. 5 *b* is particularly noticeable. There is similar matching between other areas (*B* and *B'*). The smoother (–) face of the plasma membrane (*A*, Fig. 5 *b*) is the complement of the particulate (+) face (*A'*, Fig. 5 *a*). Although all the main features, such as *C*, *C'*, match, there are some dimensional differences, possibly due to distortion of the replicas during drying on the grids.

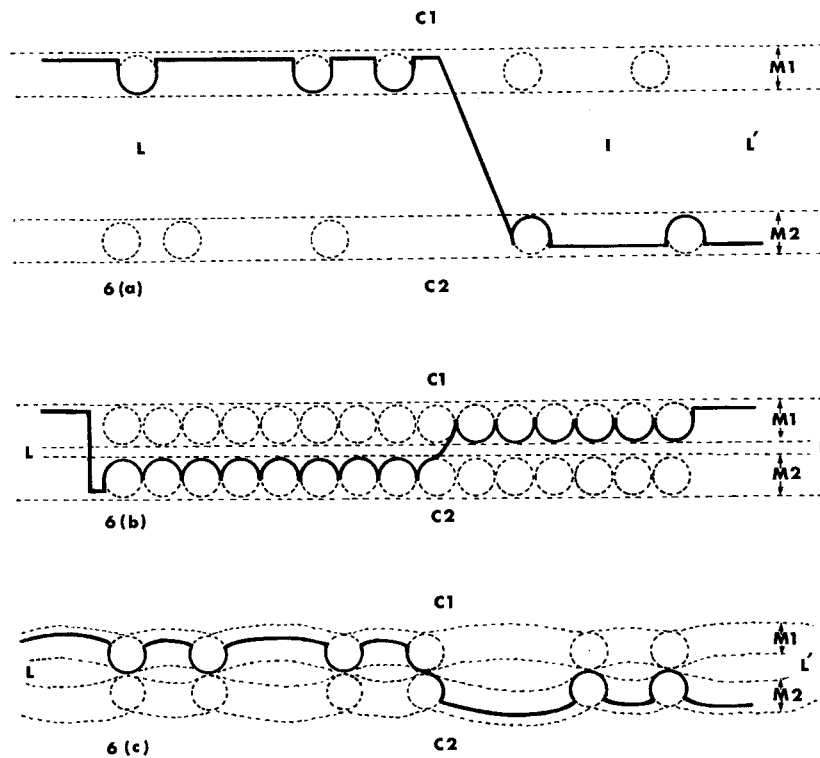


FIGURE 6 Models of membranes and junctions based on freeze-fracturing evidence. In all cases, the diagram represents a cross-sectional view of the membranes. The diagrams are not drawn to the same scale as the micrographs which they represent. C_1 and C_2 denote the cytoplasm and M_1 and M_2 the plasma membranes of adjoining cells. I is the intercellular space.

FIGURE 6 *a* Model of plasma membranes of adjoining cells in nonjunctional regions. The circles represent sections of the randomly distributed particles within the membrane. The fracture (thick line) is drawn so that, if observed from above, it represents the faces seen when going along the line $L-L'$ on Fig. 2, passing from the (-) face (with some depressions) of one plasma membrane, across the intercellular space, and then along the (+) face (with particles) of the plasma membrane fracture of the adjoining cell. Although the fracture plane between particles is drawn within the membrane, we do not have unequivocal evidence for this (see text).

FIGURE 6 *b* Model of gap junction. The circles represent a cross-section of the particle array. The fracture (thick line) is drawn so that, if observed from above, it represents the faces seen when going along the line $L-L'$ on Fig. 4 *a*; and, if observed from below, it represents the complementary faces seen when going along the mirror-image line $L-L'$ on Fig. 4 *b*. The fracture plane steps from one membrane to the other within the junction. The narrow gap between the two membranes represents the 20 Å gap seen in thin sections. In the model we have shown the particles separated by this gap, but we cannot be certain whether this is so, or whether the particles touch.

FIGURE 6 *c* Model of tight junction. The circles represent cross-sections of the ridges. The fracture (thick line) is drawn so that, if viewed from above, it represents the features seen when going along the line $L-L'$ on Fig. 3, passing from an inner face showing furrows with intervening gentle hills to an outer face of the other membrane showing ridges with intervening gentle valleys. If viewed from above, the left hand side of the diagram represents the area of furrows (D) on Fig. 4 *a*; and if viewed from below, the complementary ridges (D') in Fig. 4 *b*.

Gap Junction

It is notable that depressions in an array of the gap junction are more readily found than the scattered depressions of the (-) face of the general plasma membrane. Kreuziger (1968 *b*) has observed that these arrayed depressions are obscured under contaminating conditions, and we confirm that this can happen with both the arrayed and the scattered depressions. Arrays are obviously more easily seen than scattered depressions, but it is also possible that the array region is less susceptible to the effects of contamination.

We have shown that the surfaces are the complementary ones produced by fracturing; yet the particles on one face are not in such a regular array as the depressions on the other. It seems unlikely that the depressions could have become arrayed during fracturing or subsequently. We must, therefore, assume that the particles were originally in an array which fitted the depressions. On average, these particles have the same 90 Å center-to-center spacing as the depressions and do have some appearance of ordered array. The departure from order may be due to deformation of the particles, resulting from energy produced during fracturing.

An interpretation of gap junction fracture pairs is crucial in deciding the location of not only gap junction particles, but also general plasma membrane particles and tight junction ridges. In Fig. 4 *a*, there is a step up from the top of the particles *C'* to the depression-covered face *B*. It can also be seen that the layer of particles (*B'*) in Fig. 4 *b* is complementary to *B* and was thus originally above it before fracture. Similar relationships between the faces are also seen in Fig. 5 *a-b*. There must, therefore, be two layers of particles situated at different levels and separated by a layer which has depressions (out of which the particles came) in each side. Because the particles have a height of about 70 Å and we know from thin sectioning that the two membranes approach within 20 Å, the only type of model which fits is one shown as a diagram in Fig. 6 *b*. This diagram shows a fracture plane related to particular mirror-image lines drawn on Fig. 4 *a-b*. Each membrane contains an array of particles which, in all probability, are in register with each other. (This seems likely because we have observed in other gap junctions that the array of depressions on the (-) face can be seen to be continuous and in line with the array of particles where the fracture jumps to the (+) face of the adjoining membrane.) Sandwiched be-

tween the two arrays of particles is the layer of material which shows depressions when the particles are fractured out of it. If this central layer were permeable to water, our model would be compatible with the micrographs of similar junctions produced both by Revel and Karnovsky (1967), using lanthanum staining, and by Benedetti and Emmelot (1968), using negative staining. The particles would be outlined in negative contrast by the stain. Our model is quite similar to the one constructed by Benedetti and Emmelot.

We are certain that the particles are within the membrane, but uncertain about two other aspects of the model. Firstly, step heights are difficult to measure accurately and thus we cannot determine whether the particles are in contact across the gap or whether there is a 20 Å space. Secondly, since we know that the particles are about 70 Å high, the fracture face on which they sit must be near or even at the interface between membrane and cytoplasm. It is not possible to be more definite. Branton's original (Branton, 1966) work on the appearance of ridges on cross-fractured and face-fractured membranes provided good evidence that the fracture plane goes within the membrane. We have thus placed the plane within the membrane in our models, but we do not believe the membrane is split into equal halves.

Tight Junction

We have not obtained a matching pair of replicas in which the fracture jumps from one face to the other within the tight junction, as it does within the gap junction in Figs. 4 *a-b* and 5 *a-b*. However, by considering the two faces of the tight junction break relative to one membrane in the matched pair (Fig. 4 *a-b*) and the two faces seen when a fracture crosses from face to face in a single replica (Fig. 3), it is possible to construct a model of the tight junction (Fig. 6 *c*); and this is related to the fracture plane along a particular line in Fig. 3. The model shows a set of ridges within each membrane, and the two sets of ridges are in register. Where it is not going over a ridge, the fracture plane is again placed in the membrane near the interface between membrane and cytoplasm. This positioning of the fracture plane relies only on the fact that the fracture face is continuous and without steps from a gap junction, where the situation is known, to the tight junction. It is not possible to place the fracture plane purely by examination of replicas of the tight junctions.

In the tight junction model, the two membranes come together only at the line where two ridges meet. In between, they are bowed away towards the cytoplasm. This is a consequence of the matching gentle valleys and hills. This aspect of the model is supported by micrographs of thin sections published by other workers. Farquhar and Palade (1963) showed some micrographs in which there were "several focal splittings of the fusion line within the junction, followed by their re-fusion." Revel (1968) and Brightman and Reese (1969) showed punctate pentalaminar junctions. In a study of the same junction as we are describing, that surrounding the bile canaliculus, Matter et al. (1969) showed the two membranes looping from one punctate fusion point to the next. Sections at right angles to the line of a number of approximately parallel ridges in our model would show punctate fusions, while sections parallel to ridges and containing them would show extended fusions of the type more typically associated with tight junctions. This latter picture would be more usual, for a 500 Å thick section would generally include relatively long pieces of ridge, as can be seen by examining the disposition of the ridges (Figs. 3, 4 b)

CONCLUSIONS

To sum up, the scattered particles of the general plasma membrane, the packed particles of the gap junction, and the ridges of the tight junction are all seen on the (+) face of the fracture plane. We have shown this to be complementary with the (-) face, which shows occasional depressions in the general plasma membrane, arrayed depressions in the gap junction, and furrows in the tight junction. Models for these three types of membranes all have particles or ridges within the space which would be occupied by the membrane in thin sections. In the models and in the discussion, we have related our observations to the full thickness of the membrane rather than to the component layers put forward in the "unit membrane" hypothesis (Robertson, 1959). While our results show that there are particles within membranes, there are extensive areas in which the fracture surface is smooth and would not be at variance with a bimolecular leaflet structure, both between particles in the general plasma membrane and between the ridges of the tight junction.

The particles seen in array in the gap junction can be related to those seen either in thin sections of lanthanum-treated material (Revel and

Karnovsky, 1967) or in negatively stained material (Benedetti and Emmelot, 1968). Nothing comparable either to the scattered particles of the general plasma membrane or to the ridges of the tight junction has been visualized by such techniques. The fact that these structures do not show the order found in the gap junction may be a partial explanation. In addition, denaturation during fixation and negative staining may lead to their alteration and incorporation into the generally observed triple-layered structure. The particles in the gap junction membranes are presumably involved in cell-to-cell communication. The function of the particles in other types of membrane is not yet known. Although this paper has been concerned with the plasma membrane and its specializations, intracellular membranes fracture in the same way, the (+) face with more particles being the complement of the smoother (-) face. We have observed that this applies to membranes of the endoplasmic reticulum, the nuclear envelope, and to both inner and outer mitochondrial membranes. Thus, the particles concerned must also be located within the respective membranes.

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