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Fracture Faces in Frozen Outer Segments from the Guinea Pig Retina*

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Summary. Outer segments from the retina of the guinea pig have been examined with the freeze-etch technique. In many ways their appearance corroborates previous descriptions of their fine structure. However, the fracture faces of the disc membrane are distinctive and unlike those of any other membrane examined by freeze-etching. One face has the appearance of shallow, irregularly shaped pits surrounded by steep, interconnecting ridges. The other face has the appearance of worn cobblestone pavement. The "stones" are somewhat irregularly shaped, are tightly packed together, and have dimensions of 200–250 Å. In transverse fracture, a single disc membrane is represented by a pair of ridges and has a thickness of about 90 Å.

Evidence is presented that the disc membranes split during fracture and that the two faces seen in freeze-etched replicas are apposed in the intact membrane. This interpretation, assuming fractures split membranes, is compared with one assuming fractures occur along membrane surfaces. The inadequacies of both interpretations are discussed. Plastic deformation can occur during fracture. Such deformation may explain some interpretational difficulties and may account for the lack of perfect match between the two fracture faces in the disc membrane.

Introduction

During the 1930's SCHMIDT (1935, 1951) conducted a classic study of the vertebrate retina using polarized light. On the basis of his experiments, SCHMIDT made specific proposals about the structure of photoreceptor cell outer segments. SJÖSTRAND (1953) subsequently confirmed these proposals using the electron microscope. Since that time, the precise molecular architecture of the outer segment and its membranous discs has been the subject of much interest. Several investigators (BROWN et al., 1963; MCCONNELL, 1965; NILSSON, 1965; BLASIE et al. 1965; FERNÁNDEZ-MORÁN, 1966) have found globular or particulate substructure within disc membranes using techniques such as thin-sectioning, negative staining, and low-angle X-ray diffraction. ROBERTSON (1966a, b), on the other hand, believes that the disc membrane is a unit membrane and that the substructure seen by others is merely a representation of granulo-fibrillar material on the hydrophilic surface of the membrane.

Since the technique of freeze-etching (STEERE, 1957; MOOR et al., 1961) splits membranes and reveals faces predominantly hydophobic in nature (BRANTON 1966) we thought its use might prove relevant to the question of substructure within the disc membrane.

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Materials and Methods

Guinea pigs were used in this study since their retinas have a high ratio of rods to cones. Albino and pigmented guinea pigs of both sexes weighing 300–900 gs were anesthetized with Nembutal. After rapid enucleation, the eyes were treated in several different ways. Some were placed in a glycerol-supplemented T9 culturing solution (TROWELL, 1963) made by dissolving the salts in 20% (V/V) glycerol instead of glass-distilled water. The pH of the solution was usually 7.55, although this parameter did not seem to be critical. The retinas were gently dissected out and cut into small pieces with a scalpel. The pieces of retina were then dipped briefly in a 20% glycerol, 1% (W/V) gum arabic-supplemented T9 solution, placed on copper discs, and rapidly frozen in Freon-22. The gum arabic helped the frozen retinal tissue adhere to the copper discs. Frozen discs were stored in liquid N_2. Retinas which were treated in this fashion and then freeze-etched and replicated (MOOR et al., 1961) were the most convenient to work with.

Specimens were allowed to etch from 0 to 2 min. With the freeze-etch device, etching or the sublimation of ice from the specimen can be controlled with precision. A more complete description of the process is given by BRANTON and SOUTHWORTH (1967).

For control purposes, eyes were also treated with T9 solutions supplemented with 0–40% glycerol and then frozen as already described. In addition, eyes were placed in shallow well-slides and the retina dissected out and cut up in the aqueous and vitreous humor. The pieces of fresh retina were then frozen directly on the copper discs without further treatment and without gum arabic adhesive. Although these procedures increased the technical difficulties of freeze-etching, fracture faces from retinas treated in all the different ways were obtained; all were similar to those prepared in the glycerol-gum arabic-supplemented T9 solution. For convenience, however, retinas treated in the latter solution were used for routine work.

Retinas were also immersed in a 20% glycerol-supplemented T9 solution made with reduced amounts of salts. Experiments were performed in which the amount of NaCl was halved or eliminated completely, and retinas were also treated with solutions of glass-distilled water and 20% glycerol. All these experiments changed the appearance of the retinal discs as described below.

A few guinea pigs were allowed to die from respiratory arrest. After 30–60 min the eyes were enucleated and the retinas dissected in solutions of T9 and 20% glycerol, frozen, and freeze-etched as described above.

Several guinea pigs were dark-adapted for 2–4 hours. All the freezing procedures described above were carried out using solutions of T9 and 20% glycerol. The enucleation of the eyes and the dissection of the retinas were performed under infra-red light with the aid of a dissecting microscope, an RCA 6914 A image-converting tube, and a light source filtered by a Corning 7–69 visible absorbing infra-red transmitting filter. The pieces of retina were briefly (approximately 1 second) exposed to dim red light (Kodak 1 filter, 15 watt bulb, two feet away) during the time they were being frozen in Freon-22. Controls were pieces of the same retina which were subsequently bleached on exposure to room light and a white dissecting microscope light. No difference was found between the dark-adapted and the bleached rod fracture faces.

Retinas prepared for thin-sectioning were fixed in glutaraldehyde according to the method of KUWABARA (1965).

In collaboration with Professor R. M. EAKIN, the eyes of the snail *Helix aspersa* were dissected into a mixture of snail Ringer's solution and 20% glycerol. The Ringer's solution was adjusted so as to be at normal strength. After the eyes had been dissected they were frozen and freeze-etched in the usual fashion.

Polystyrene latex spheres, 0.26 μ in diameter, were provided by the Dow Chemical Company, Midland, Michigan. The spheres were diluted with 20% glycerol and centrifuged. The pellet was broken up into a thick slurry with 20% glycerol and then freeze-etched in the usual way.

Results

The guinea pig retina shown in Fig. 1 has been treated by conventional means of fixation and embedding for electron microscopy. Parts of both inner and outer

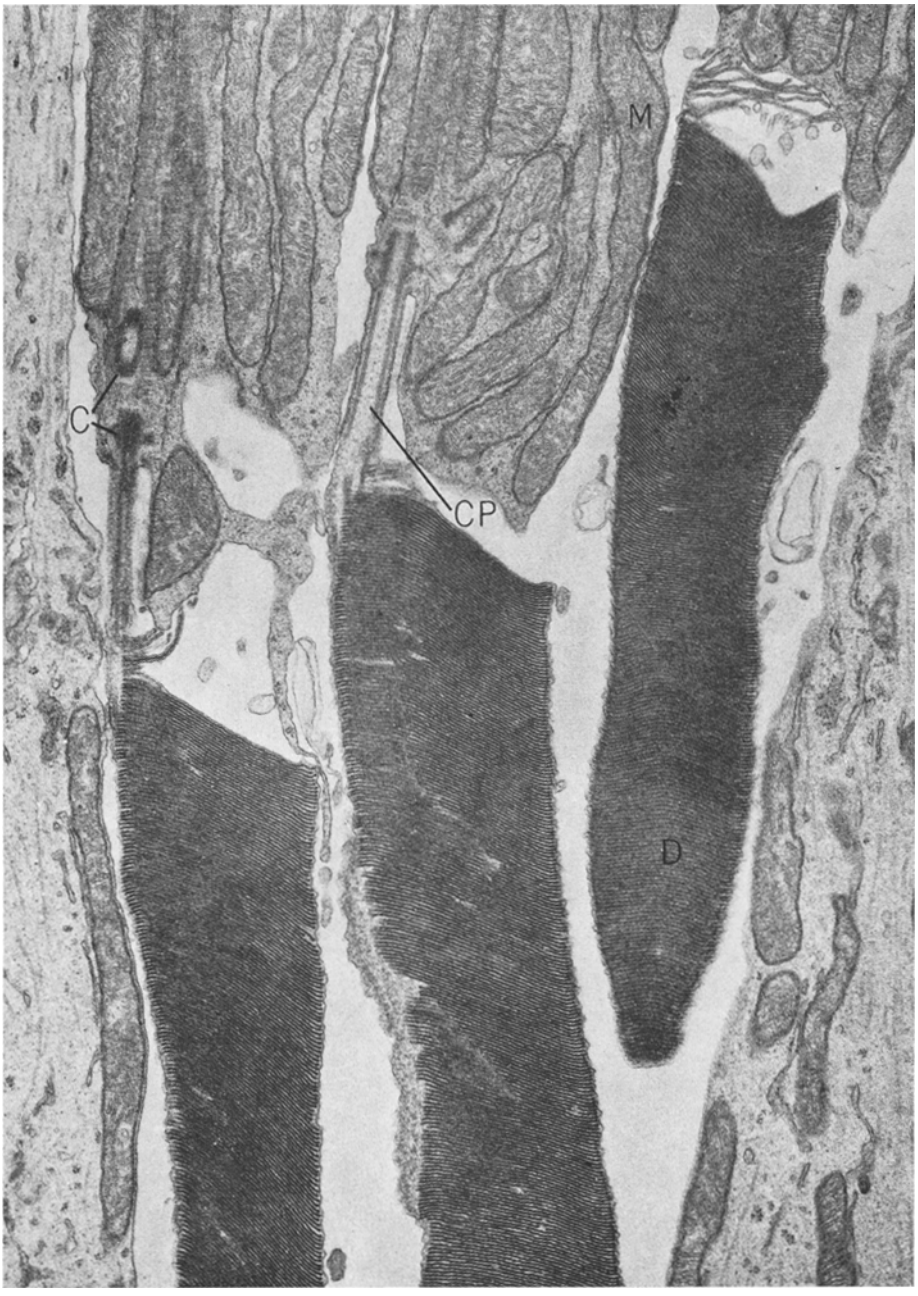


Fig. 1. Oblique thin section through a glutaraldehyde-osmium fixed guinea pig retina showing portions of several rod inner and outer segments. Each outer segment contains a large number of discs (*D*). Inner segments each contain mitochondria *(M)* and a pair of centrioles *(C)*, one of which gives rise to a highly modified cilium composed of a connecting piece *(CP)* and an outer segment. ×26,000

segments are visible. Each inner segment contains a large numer of mitochondria and a pair of centrioles. One of the latter gives rise to a highly modified cilium

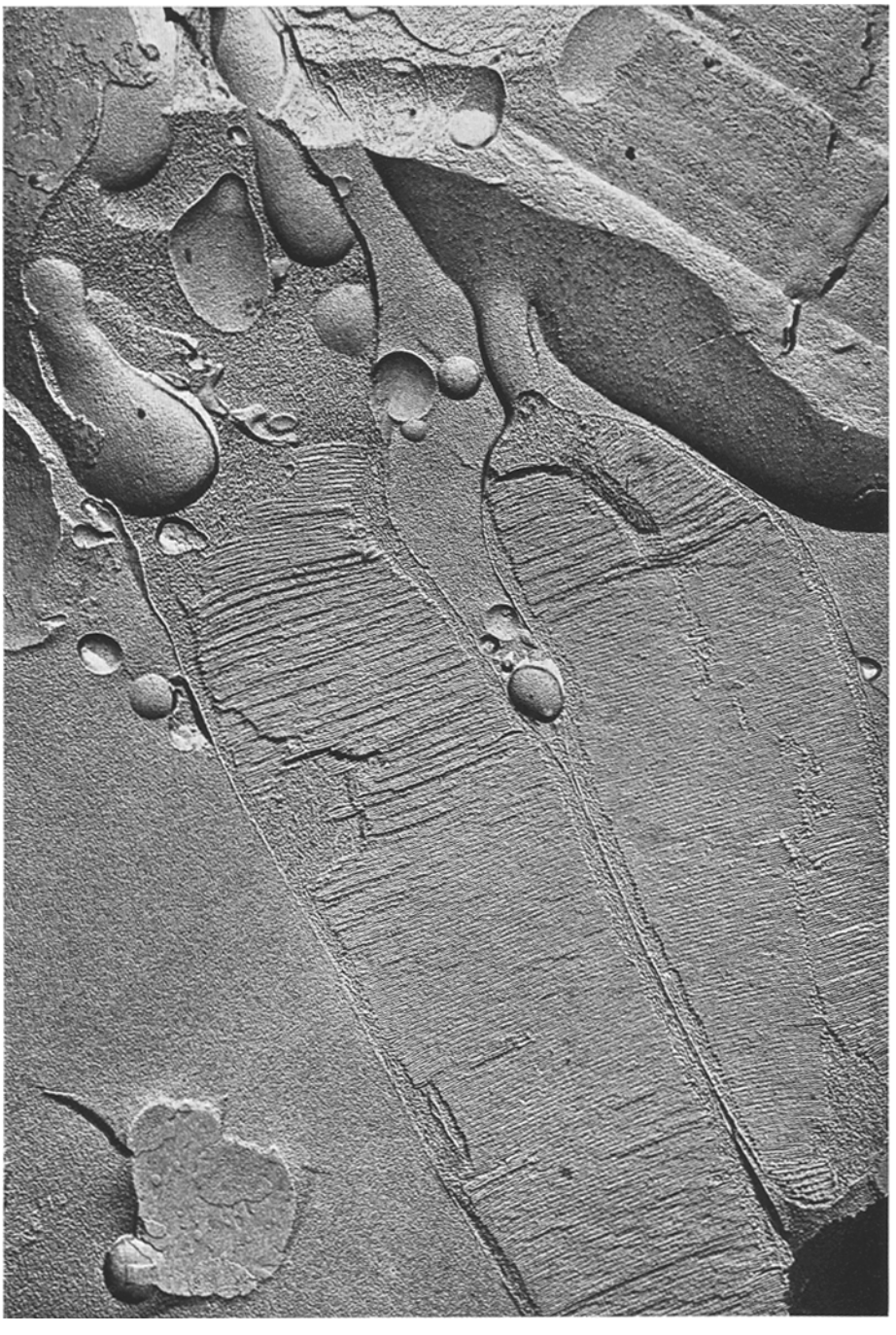


Fig. 2. Guinea pig retina prepared with the freeze-etch technique. With the exception of the centrioles, all the structures referred to in Fig. 1 are visible here. ×28,000

composed of a connecting piece and an outer segment. Each outer segment contains a large number of flattened membranous sacs or discs. With the exception of the

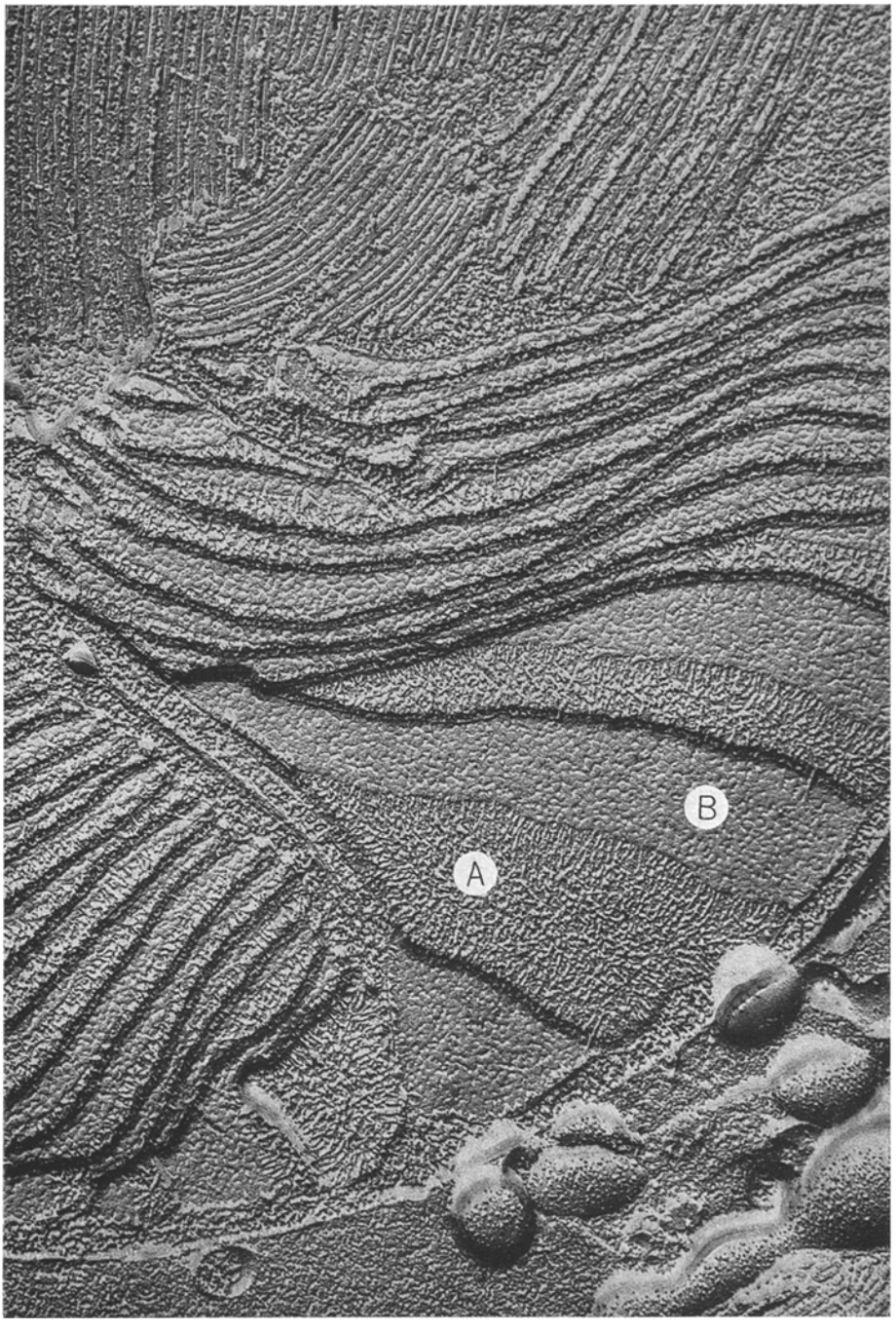


Fig. 3. Oblique fracture through a guinea pig rod outer segment showing parts of several disc membrane fracture faces. One of the faces (arbitrarily labeled "A") has the appearance of shallow, irregularly shaped pits surrounded by steep, interconnecting ridges. The other face (arbitrarily labeled "B") has the appearance of worn cobblestone pavement. The "stones" or particles are irregularly shaped, are tightly packed together, and have dimensions on the order of 200–250 Å. The retina was placed in slightly hypotonic media prior to freezing. ×63,000

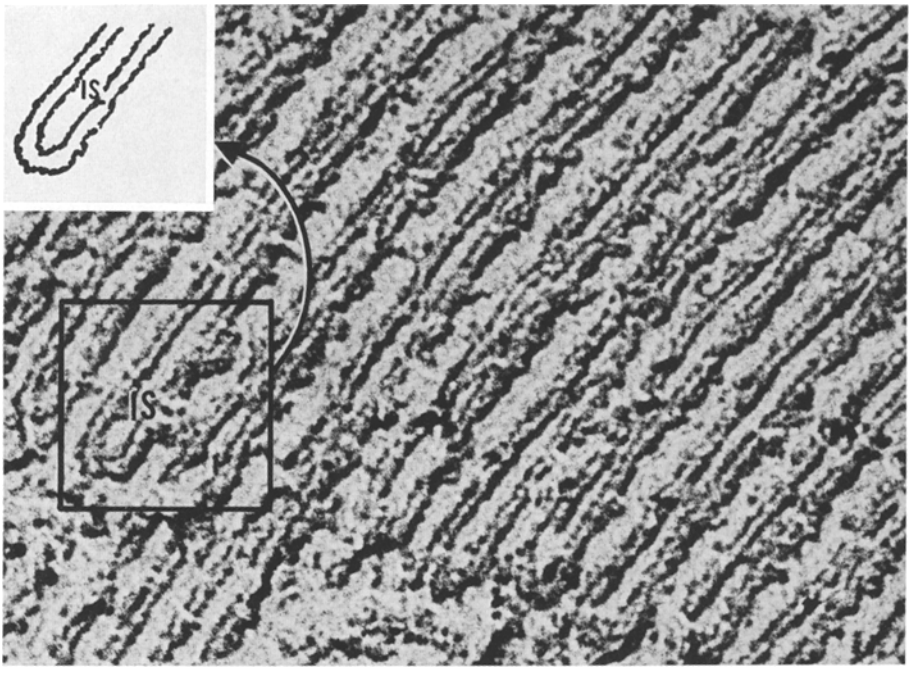


Fig. 4. Transverse fractures through several disc membranes from a guinea pig rod outer segment. A single disc membrane is represented by a double ridge, both of whose parts are continuous around the needle eye at the disc rims (see inset). Each membrane is about 90 Å thick. This retina, like the one in Fig. 3, was placed in slightly hypotonic media prior to freezing. Such treatment leads to some swelling of the intradiscal space *(IS)* ×300,000

centrioles, the structures visible in the freeze-etched guinea pig retina shown in Fig. 2 are comparable to those in the thin-sectioned preparation.

The guinea pig rod outer segment shown in Fig. 3 has been fractured obliquely and exposes fracture faces of the membranous discs. Two faces are revealed. There is a rough face (arbitrarily labeled "A") which has the appearance of shallow, irregularly shaped pits surrounded by steep, interconnecting ridges. The second face (arbitrarily labeled "B") has the appearance of a worn cobblestone pavement. The "stones" or particles are irregularly shaped, are tightly packed together, and have a dimension on the order of 200–250 Å. The two faces are unetchable. That is to say, the appearances of the two faces are the same in preparations exposed to very little or no etching and in preparations etched for periods of up to two minutes.

In cross fracture (Fig. 4, also Figs. 8 and 9) an individual disc membrane is a double-ridged structure of total thickness around 90 Å. Depending upon the tonicity of the surrounding medium, the interdiscal spaces vary from 150 to 200 Å and the intradiscal spaces vary from zero to 100 Å.

Occasionally outer segments are encountered which have fracture faces with an altered appearance. The fracture faces shown in Fig. 5 are typical of this alteration. The cobblestones of the B face may occur as isolated patches and are usually larger (400–900 Å in Fig. 5). At the same time, the ridges of the A face are less

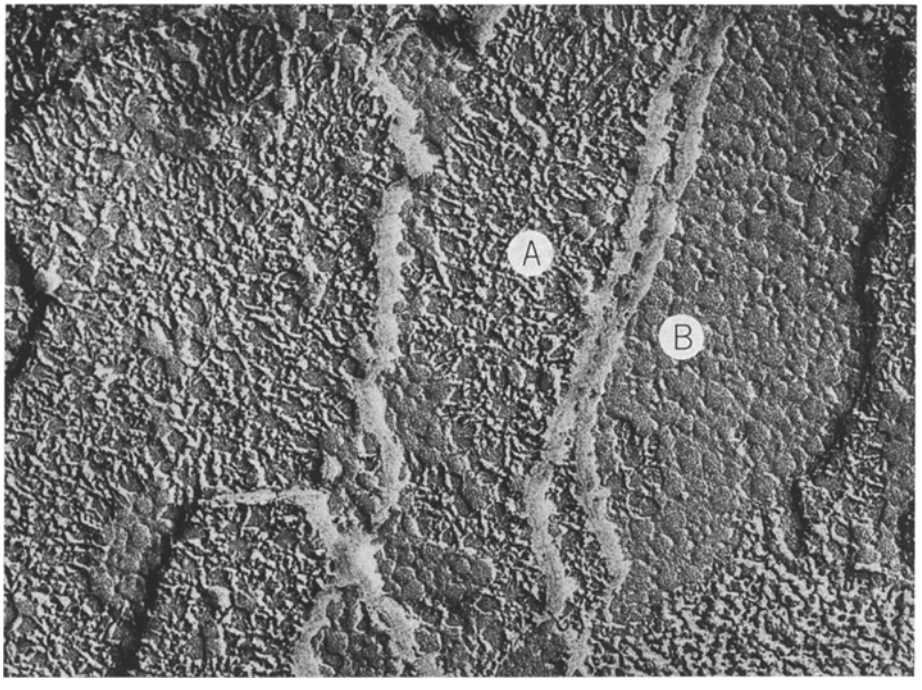


Fig. 5. Several altered fracture faces from guinea pig rod outer segment. Compared with Fig. 3, the ridges of the A face are fewer in number, surround larger smooth areas, and the cobblestones themselves are larger (400–900 Å). ×42,000

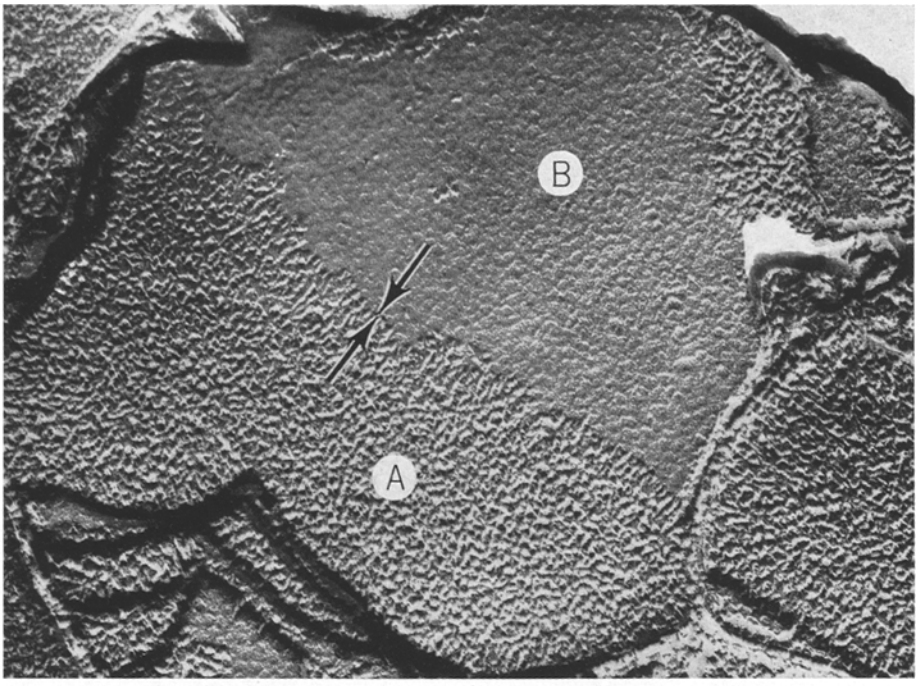
numerous and surround relatively wide smooth areas. Very rarely, A faces are seen in which the ridges have almost entirely disappeared and only scattered particles remain. The B faces in this type of outer segment are much smoother and the cobblestones are almost impossible to detect.

Efforts have been made to determine the nature of these altered faces. They were first encountered in dark-adapted retinas and then subsequently in bleached controls. They are seen with greater frequency in retinas from animals that have been dead for as long as an hour before dissection and in retinas purposely kept in the glycerol and gum arabic-supplemented T9 solution for 30–60 min prior to freezing. It is our impression that these altered fracture faces represent a post-mortem change in the disc membrane. There is also the possibility that some are fractures faces of cone disc membranes.

Alteration in the appearance of the fracture faces also occurs when retinas are placed in extremely hypotonic or salt-free media prior to freezing (Fig. 7). As before, the cobblestones on the B face are hard to see, but when measurable they still are 200–250 Å in diameter. The B face also has a pockmarked appearance.

Fig. 6. Fracture faces from a guinea pig retina placed in isotonic media prior to freezing. The step between the A face and the B face can easily be seen (*arrows*), but is abbreviated. ×80,000

Fig. 7. Fracture faces from a guinea pig retina placed in very hypotonic media prior to freezing. The appearance of the A face is altered in the region where the two faces join. Long obvious



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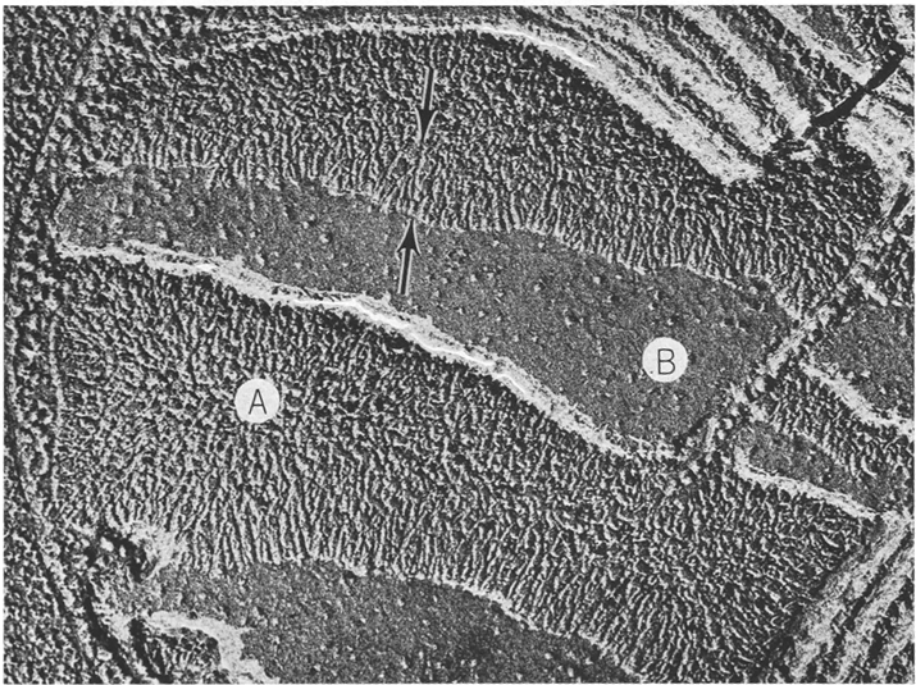

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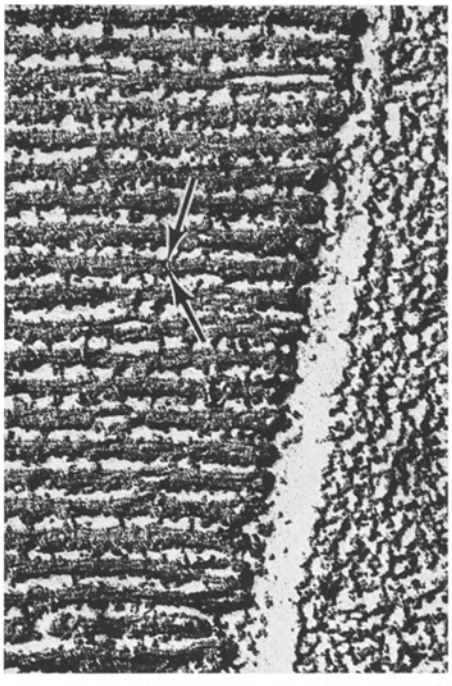
Figs. 6 and 7

strands of material lead up to the B face *(arrows)* and correspond to the increased intradiscal space produced in hypotonic media (see Fig. 9). The B face has a pockmarked appearance. Although difficult to distinguish in this particular figure, the cobblestones still have dimensions on the order of 200–250 Å. ×80,000



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Figs. 8—10

Fig. 8. Transverse fractures in a guinea pig retina placed in isotonic media prior to freezing (as in Fig. 6). The inner surfaces of each disc membrane are apposed *(arrows)* and no intradiscal space appears except for the needle eye at the disc rim. ×126,000

The most obvious change in the A face of hypotonically treated retinas occurs in the region where the two faces join (Fig. 7, arrows). Long strands of material lead from the A face up to the B face. On the other hand, when retinas are placed in isotonic media prior to freezing, the step between the two faces can easily be seen but is comparatively reduced, the strands being much shorter or absent (Fig. 6, arrows). The retina in Fig. 3 has been placed in slightly hypotonic medium prior to freezing, and the alteration of the A face at its junction with the B face can also be seen. A series of decreasing tonicity and increasing alteration of the junction between the A face and the B face is shown by the sequence: Figs. 6, 3, 7.

The alterations seen in face views of the A face-B face junctions are accompanied by changes in the intradiscal space which can easily be seen in transverse fractures. When retinas are placed in isotonic or hypertonic media prior to freezing, the inner surfaces of the disc membrane are apposed except at the disc rim where the membrane loops back upon itself. Retinas treated in this fashion therefore have no intradiscal space except for the needle eye at the disc rim (Fig. 8). In contrast, retinas placed in hypotonic media prior to freezing exhibit a dilation of the intradiscal space (Fig. 9). It is apparent in Fig. 9 that the inner surfaces of the disc membranes are no longer apposed. A series of decreasing tonicity and increasing intradiscal space is shown by the sequence: Figs. 8, 4, 9.

Areas that clearly show the relationship between face views of membranes and transverse fractures are occasionally found. One such area of transition is shown in Fig. 10. Two of these transitions have been marked with arrows. Our interpretation of the relationship between the faces and the transverse fractures is shown diagrammatically in Fig. 11. The interpretation assumes that the A and B faces are within the membrane matrix and that the step from face A to face B is a fracture across the intradiscal space.

Although freeze-etching results have been termed artifact free (Moor, 1966) we could not reconcile all of our results without involving the possibility that plastic deformation occurred during the fracture process. Our attention was therefore drawn to some remarkable results obtained with polymer-containing structures. For example, in collaboration with Professor R. M. Eakin, eyes from the snail *Helix aspersa* were freeze-etched. The eye is surrounded by a collagenous (polymeric) sheath, and a part of the sheath near the exit of the optic nerve is shown in Fig. 12. Several collagen fibers have been torn out of the extracellular matrix and they project above the rest of the replica surface. These fibers cast long shadows that reveal their bent shapes. One of the fibers has flopped back down onto the fracture face of a nerve and is shown at higher magnification in the inset.

Fig. 9. Transverse fractures from a guinea pig retina placed in hypotonic media prior to freezing. The inner surfaces of each disc membrane are no longer apposed and there is a considerable intradiscal space *(arrows)*. ×126,000

Fig. 10. Guinea pig rod outer segment showing transverse views of disc membranes becoming face views. Beginning at the left of the figure, in two places the double ridge representing a single disc membrane has been bracketed with arrows. Moving to the right, it is apparent that the double ridge splits. One of the ridges becomes a fracture face (A face in the upper example, B face in the lower), whereas the other one continues on as a narrow ridge at the base of the exposed membrane face. ×180,000

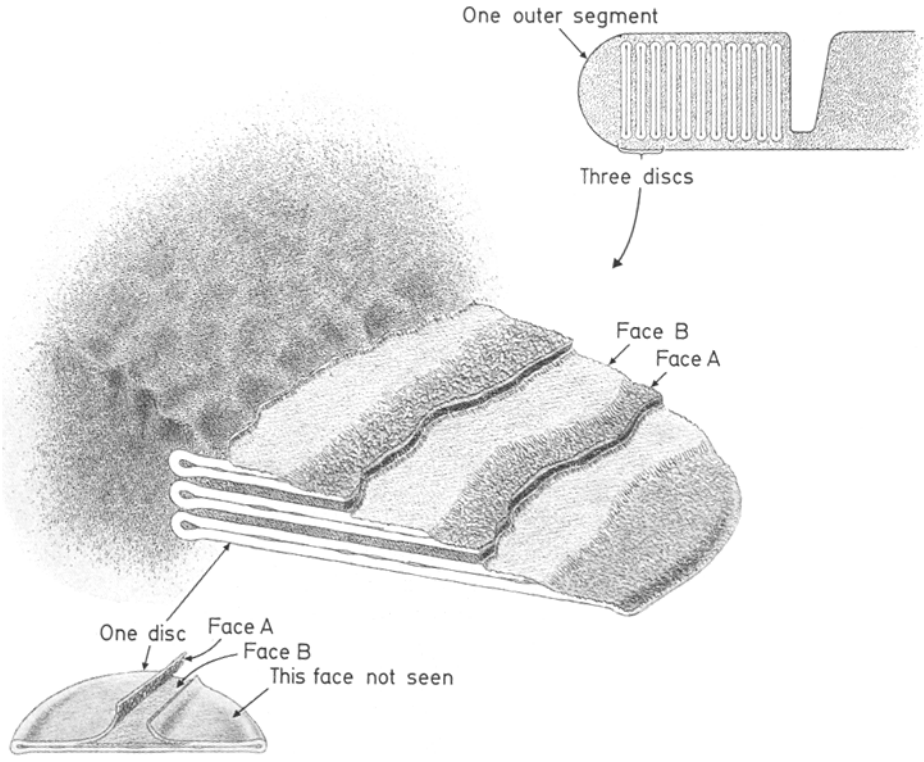
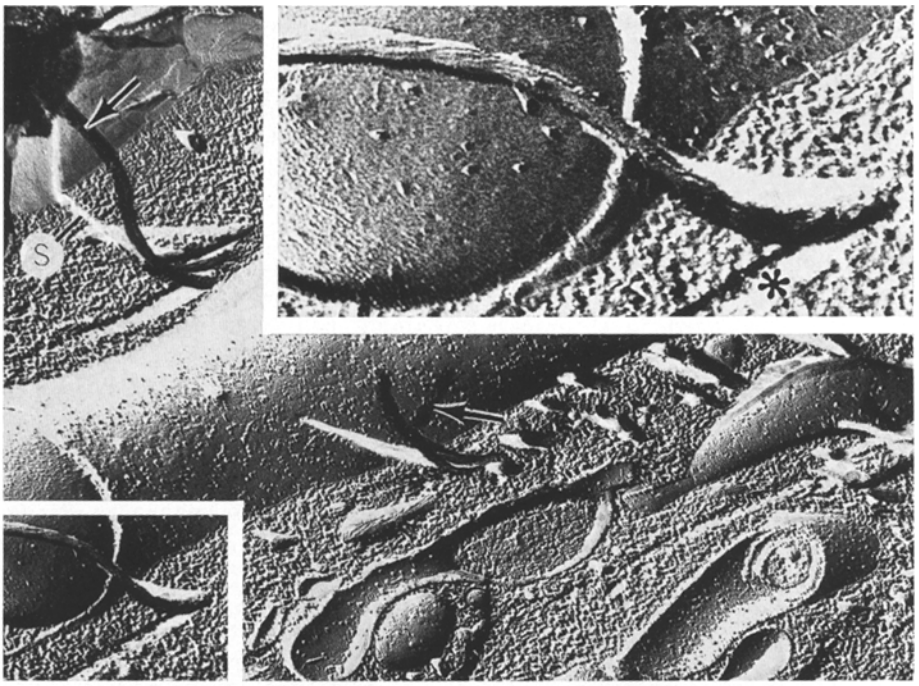


Fig. 11. Interpretation of freeze-etching in guinea pig rod outer segments. The middle drawing shows the fracture faces of three rod discs in their frozen matrix and postulates the location of the fractures by relating the faces to disc transections. This hypothetical relationship implies that a membrane is split during fracture, as shown schematically in the lower drawing

In an attempt to elucidate the nature of the deformation of the collagen fibers, we repeated observations presented by Moor before the Royal Microscopical Society Symposium on Fine Structure, Leeds, 1967. Polystyrene latex spheres 0,26 μ in diameter were prepared with the freeze-etch technique. Fig. 13 is a typical field through such a preparation. Depressions represent places where spheres have been scooped completely away from the underlying ice. The other structures represent spheres that have remained in the ice and have been pulled out like taffy during the fracturing process. All the spheres have not been deformed in the same direction. Instead, they appear to have been pulled straight up and then to have flopped back down onto the ice in a random fashion. The surface of the deformed spheres has the appearance of long parallel strands (arrows).

Fig. 12. A portion of the eye of the snail *Helix aspersa* freeze-etched in collaboration with Professor R. M. Eakin. The eye is surrounded by a collagenous sheath. A part of the sheath near the exit of the optic nerve is shown. Several collagen fibers *(arrows)* have been ripped out of the extracellular matrix. These fibers stand out above the replica and cast long shadows *(S)*. One of the collagen fibers has flopped back down onto the fracture face of a nerve *(box)* and is seen at higher magnification in the inset. Notice the strandedness of the fiber. The trough in the ice (*) out of which the fiber was ripped can also be seen in the inset. ×52,000, inset ×140,000



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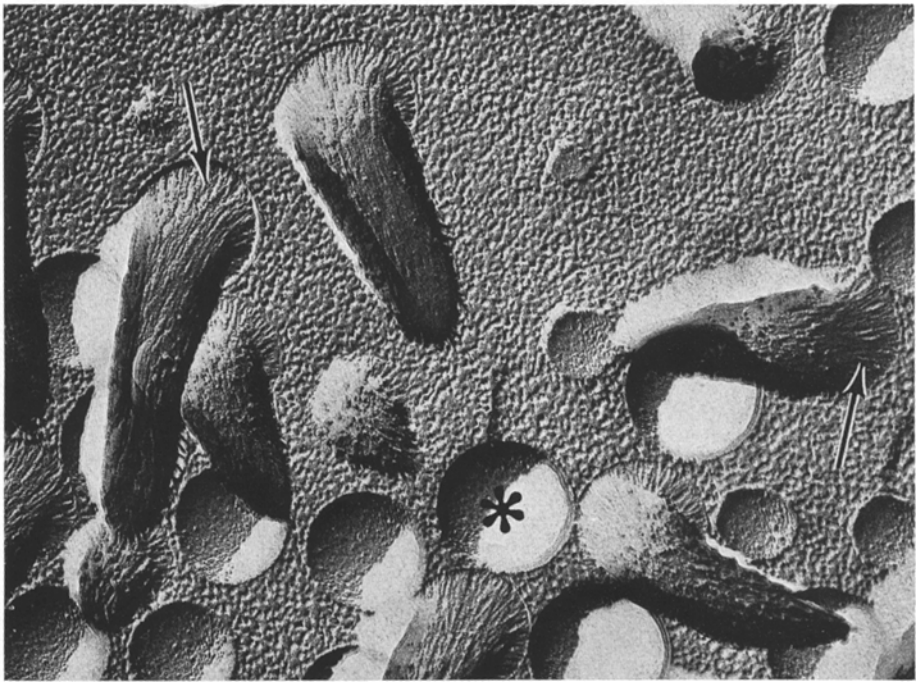

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Figs. 12 and 13

Fig. 13. Freeze-etched polystyrene latex spheres, 0.26 μ in diameter. Some of the spheres have torn completely away from the ice (*) whereas the ones that remained have been pulled out like taffy. It should be noted that the spheres have not all been distorted in one direction. Instead, they appear to have been pulled straight up and then to have flopped back down at random onto the ice. Arrows indicate the stranded appearance of the distorted spheres. ×74,000

Discussion

Unlike any other technique, freeze-etching reveals that membranes with complex functions, such as those of chloroplasts (Mühlethaler et al., 1965; Branton and Park, 1967), nucleated erythrocytes (Koehler, 1968), and plant vacuoles (Branton, 1966) all have complex and individually unique structural features. Conversely, freeze-etching reveals that membranes with little functional complexity are simple in their structure. Artificial membranes (Deamer and Branton, unpublished results) and the membranes of the myelin sheath (Branton, 1967) have extremely smooth fracture faces and are difficult to distinguish from each other. It has been assumed that the disc membranes of the retinal rod outer segments are the site of the transduction of light (Wald et al., 1963). Whether or not this assumption is correct, freeze-etching reveals fracture faces in guinea pig outer segment discs with complex structural features unlike those of any other membrane so far examined with this technique.

Since freeze-etching avoids the use of chemical fixatives or the necessity for marked dehydration, it is useful in correlating observations made with other techniques. De Robertis and Lasansky (1961), working with thin sectioned toad retinas, found that rod discs were very sensitive to hypotonic fixatives. The swelling of the rod discs they observed coincides exactly with the response of retinas placed in hypotonic media prior to freeze-etching. The sensitivity of rod discs to hypotonic media suggests that these structures are discrete discs and not open to the extracellular space. Several investigators have found continuities between the disc membrane and the plasmalemma, but such patent discs are few in number and always confined to the base of the rod outer segment (see Cohen, 1963, for review).

The experiments using hypotonic media also show that the step height from the A fracture face to the B fracture face increases as the disc swells (Figs. 6, 7). Since the interdiscal spaces remain the same or decrease slightly as the intradiscal spaces swell (Figs. 8, 9), it follows that the step from the A face to the B face lies across the intradiscal space. The drawing in Fig. 11 shows this relationship more clearly.

The question of what is actually being seen in the disc fracture faces is a difficult one. We believe that during freeze-etching disc membranes split and reveal faces predominantly hydrophobic in nature. There are several reasons for this view.

The most direct is exemplified by Fig. 10. This view of faces becoming transverse fractures resembles exactly the situation found in other membrane types (Branton, 1966). The double ridge between the arrows on the left of Fig. 10 represents a single, transversely fractured disc membrane with a total thickness around 90 Å and certainly less than 100 Å. As the transition to face views is followed, the two ridges separate. If the two ridges together constitute the rod disc membrane, then it follows that freeze-etching exposes an inner membrane face and not the membrane surface.

Alternatively, an argument can be made that only one of the ridges seen in transverse fracture represents the disc membrane and that the other ridge is a eutectic layer apposed to the surface of the membrane. This alternative does not seem reasonable because it implies that the thickness of the membrane is less

than 50 Å. Even after osmium fixation and dehydration, thin sections reveal a membrane not less than 70 Å thick (SJÖSTRAND, 1961).

Other reasons for believing the frozen discs split along hydrophobic interiors have been extensively discussed in reference to endoplasmic reticulum, vacuolar membranes, Golgi membranes (BRANTON, 1966), chloroplast lamellae (BRANTON and PARK, 1967), myelin (BRANTON, 1967) and artificial membranes (DEAMER and BRANTON, 1967). Among this group, artificial membranes and chloroplast lamellae may represent the opposite extremes of compositional and functional complexity. DEAMER and BRANTON (1967) used radioactive tracers to show that hydrophobic bonds provided a preferred fracture plane in frozen stearic acid bilayers. At the other extreme, BRANTON and PARK (1967) found that lipid extraction of chloroplast membranes eliminated their natural fracture planes. again suggesting the determinate role of hydrophobic bonding in the fracturing process. Finally, PARK and SHUMWAY (1968) have deep-etched chloroplasts after freezing and fracturing them in distilled water. The true membrane surfaces of the chloroplast lamellae appeared only after the ice concealing them had been sublimed away. In disc membranes, the appreciable number of hydrophobic lipid and protein groups (EICHBERG and HESS, 1967) must be embedded within the interior of the membrane. Regardless of their structural peculiarities, it seems reasonable that in these membranes as in others, weak hydrophobic bonding could provide preferred fracture planes in the frozen tissue.

Fig. 14a demonstrates what might ideally result if disc membranes split. Two faces should arise from this kind of fracture, and one of them should be the exact negative of the other. In fact, two faces (A and B) are seen in freeze-etched disc membranes, but they do not complement each other perfectly. Although the thin irregularly shaped ridges of the A face could fit in between the cobblestones of the B face like mortar, and although the increased size of the pits in the altered faces shown in Fig. 5 matches the greater size of the cobblestones, in neither case can a perfect match be realized unless some plastic deformation has occurred during fracture. Fig. 14c suggests how plastic deformation might account for the faces which are actually seen.

The idea of membrane faces undergoing plastic deformation at temperatures of —100°C and below may seem unlikely. Nevertheless it is clear from Figs. 12 and 13 that collagen fibers and polystyrene latex spheres are deformed at such temperatures. No claim is made for these substances as membrane models. However, both are long-chain polymers which have undergone plastic deformation during freeze-etching and long-chain polymers in the form of protein make up about 60% of the total dry weight of the retinal rod outer segment (EICHBERG and HESS, 1967). It may be that the forces generated as the fracture plane breaks weak bonds in the disc membrane are enough to cause deformation wherever these weak bonds are interrupted by stronger bonds. If this hypothesis is correct, fracture faces may be reproducible and characteristic expressions of physical-chemical domains within the living membrane. The cobblestones and ridges may not represent the *in vivo* structure but its freeze-etch equivalent.

An alternative interpretation of the fracture process has been proposed to account for the absence of matching faces. According to this view, fractures occur along membrane surfaces and the two non-matching faces are taken to

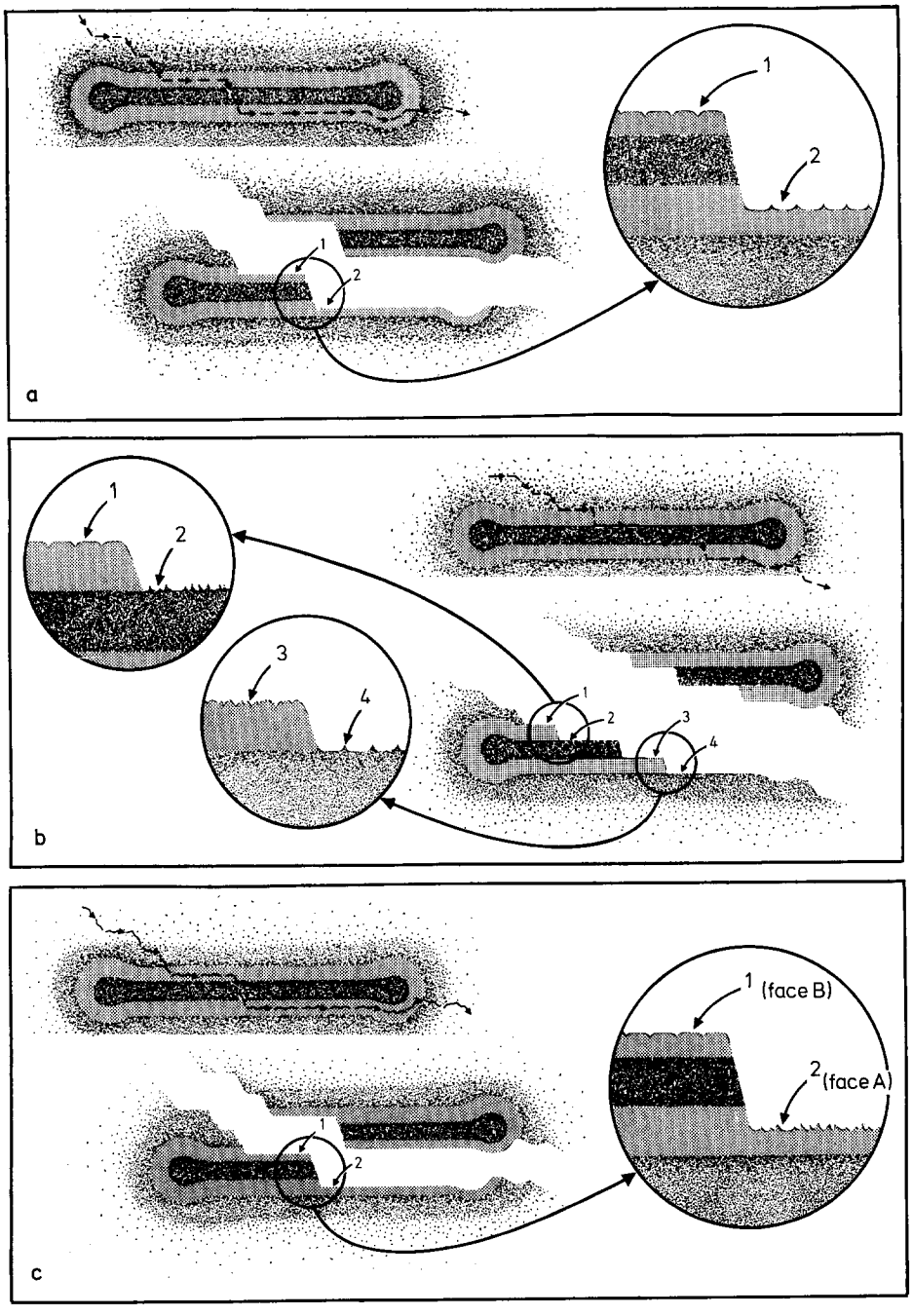

Fig. 14a–c. Alternative fracture modes and their consequences. In Fig. 14a it is presumed that the fractures tend to run along the inside of the membranes, splitting them. As a result of this split the rod disc should give rise to two perfectly matching faces. In Fig. 14b it is presumed that the fractures run along membrane surfaces. As a result, 4 surfaces with two perfectly matching pairs (1–4 and 2–3) should be produced. Neither the results predicted by Fig. 14a nor the results predicted in Fig. 14b are seen. Fig. 14c assumes that plastic deformation may occur during the fracture which splits the membrane. As a result rod discs should form two non-matching faces. Fracture faces of frozen outer segments show that the rod disc membranes do produce two non-matching faces

represent respectively the surface of the disc membrane apposed to the cytoplasm and the surface apposed to the intradiscal space. The idea that membrane surfaces are exposed during fracture has been put forth by MOOR (1966) and most recently by KOEHLER (1968) in relation to other membranes systems. A diagram showing fracture along membrane surfaces is shown in Fig. 14b. As can be seen in this diagram, surface fracture should produce four different surfaces. However, even in unetched material, only two different fracture faces, and no more than two fracture faces, are associated with a single disc. We know of no theoretical explanation or experimental evidence that can satisfactorily account for the total absence of two out of the four faces to be expected if fractures occur along membrane surfaces.

Thus neither membrane-splitting nor surface fracture accounts completely for the number of faces seen. Membrane-splitting demands that we have two, perfectly matching faces. Surface fracture demands that there should be four surfaces with two, perfectly matching pairs (Fig. 14). In disc membranes as well as in several other membrane systems already studied, only two faces are seen. They do not match. Thus, arguments based on the number of fracture faces fail to distinguish membrane splitting from surface fracture. There are, however, independent arguments for the idea that membranes split during freeze-etching (BRANTON, 1966, 1967; BRANTON and PARK, 1967; DEAMER and BRANTON, 1967), and a theoretical basis for this concept is provided by the weakness of hydrophobic bonds. The observed plasticity of collagen and polystyrene latex spheres can provide an experimental basis for speculations as to why the two faces do not match.

The cobblestones of the B face and the ridges of the A face suggest that the disc membrane has a complex internal structure. Using more familiar techniques, a number of investigators have detected particulate or globular structure in these membranes. BROWN et al. (1963) observed densities approximately 300 Å in diameter in face views of thin-sectioned mudpuppy rod discs. Since the densities could not be seen in transverse views of the disc membrane, they concluded that the densities were micelles within the membrane itself. On this basis, the dimensions of the micelles would be 50×300 Å. NILSSON (1965) observed similar densities in face views of thin-sectioned frog rod outer segment discs and interpreted them as image-overlap artifacts. A second class of particles with diameters of 50–70 Å has been observed in thin-sectioned disc membranes (NILSSON, 1965; FERNÁNDEZ-MORÁN, 1966), in negatively stained preparations of outer segments (MCCONNELL, 1965; BLAISE et al., 1965; FERNÁNDEZ-MORÁN, 1966) and in specimens examined with low-angle X-ray diffraction (BLAISE et al., 1965). Unfortunately we do not as yet have any evidence relating these particles or globules to the structural features seen in freeze-etched disc membranes.

ROBERTSON (1966a, b) has reviewed the evidence for particulate or globular substructure in disc membranes and has concluded that most of it is artifact. According to his view, the particles seen in thin-sectioned outer segments are the result of image-overlap. The granules observed in negatively stained preparations may be produced during the formation of the salt lake. Finally ROBERTSON has been unable to obtain low-angle X-ray diffraction patterns indicative of regular arrays within the plane of the membrane from preparations which he believes to be closer to the native state than those used by BLAISE et al. ROBERTSON concludes that the fundamental disc membrane backbone is a lipid bilayer.

It is fitting that the unit membrane model with a lipid bilayer backbone (Robertson, 1959) can account for the appearance of the fracture faces of artificial membranes and myelin since the model was based on evidence gathered primarily from these two systems. The faces are very smooth, as the inner faces of a bilayer, might be expected to appear. If all membranes have as a backbone a simple lipid bilayer, then it is reasonable to suppose that all membrane faces would be similar. In fact, membranes examined with freeze-etching each present unique and reproducible structural features within the plane of the membrane. The unique features in disc membranes are cobblestones and interconnecting ridges. Future research must determine the composition and function of these structural peculiarities so that their significance in the context of membrane architecture in general and light transduction in particular can be understood.

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