LUMENAL PLASMA MEMBRANE OF THE
URINARY BLADDER

II. Isolation and Structure of Membrane Components

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ABSTRACT
A technique has been devised for isolation of lumenal plasma membranes from transitional
epithelial cells lining the urinary bladder in rabbits and for subsequent separation of
particle-bearing plaque regions from particle-free areas of the membranes. The success of
the procedures employed and their effects on the isolates were assessed by electron micros-
copy of conventional plastic sections, negatively stained preparations, and freeze-etch
replicas. When bladders are distended with a solution of 0.01 M thioglycolic acid, which
reduces sulfhydryl bridges, cytoplasmic filaments are disrupted, and large segments of the
lumenal membranes rupture and float free into the lumen. A centrifugation procedure was
developed for isolating a fraction enriched with the large fragments. A comparison of
membranes isolated in the presence of thioglycolate with those isolated from epithelial
cells homogenized in sucrose medium indicates that thioglycolate has little effect on their
fine structure except for the removal of filaments which are normally associated with their
cytoplasmic surface. The curved plaques of hexagonally arrayed particles and the particle-
free interplaque regions, both characteristic of membranes before exposure to thioglycolate,
are well preserved. Subsequent treatment of thioglycolate-isolated lumenal membranes
with 1% sodium deoxycholate (DOC) severs many of the interplaque regions, releasing
individual plaques in which the particles are more clearly visible than before exposure to
deoxycholate. Presumably, DOC acts by disrupting the hydrophobic bonds within the
membrane; therefore, this type of cohesive force probably is a major factor maintaining the
structural integrity of interplaque regions. This conclusion is consistent with the observation
that interplaque regions undergo freeze-cleaving like simple bilayers with a plane of hydro-
phobic bonding.

INTRODUCTION
Hicks (7, 8) postulated that the specialized lumenal plasma membranes of transitional epithelial cells
lining the urinary bladder have, as an important constituent, keratin or a keratin-like protein that is
responsible for the impermeability of the bladder to water and solutes (6, 11). Evidence for this
hypothesis came from experiments in which a solution of thioglycolate was injected into the lumen of
the bladder. This agent reduces sulphydryl bridges and was reported to attack specifically the lumenal plasmalemma, leaving the underlying cytoplasm and fibers intact (8).

Negative staining of isolated luminal membranes (9, 10, 16, 17) focused attention on a hexagonal array of particles associated with the membrane. It was implied (10) that these particles might represent the essential membrane element that determines the permeability barrier of the membrane to salts and water, since the particles were believed to cover the entire lumenal surface and to be keratin or at least keratin-like protein. However, in the companion paper (15) we have demonstrated that the particles are confined to plaques that occupy only about 73% of the membrane, and we have suggested that they might serve as attachment points where the membrane is linked to a meshwork of cytoplasmic filaments. These morphological observations suggested that the particles have chiefly a structural role rather than a physiological one.

This conflict between our interpretation and that of other workers led us to attempt isolation of the luminal membrane with the ultimate aim of analyzing it biochemically. In doing so, we were led to reexamine the previously reported effect of thiglycolate on the morphology of the luminal membrane of the urinary bladder. In interpreting our experiments, reported previously in abstract form (5), we suggest that the primary effect of thiglycolate is on the network of cytoplasmic filaments underlying the luminal membrane. Indeed, thiglycolate proves to be efficacious in the isolation of a fraction enriched with luminal membranes that are free of cytoplasmic filaments. The particle-bearing plaque regions of these isolated membranes are largely invulnerable to subsequent exposure to sodium deoxycholate (DOC) but, the interplaque regions are generally destroyed by this emulsifying agent, thereby splitting membranes into their constituent plaques.

MATERIALS AND METHODS

Isolation of Luminal Plasma Membranes

Adult, female rabbits obtained from Charles River Breeding Laboratories, Inc., Wilmington, Mass., were anesthetized with intravenous injections of sodium pentobarbital (Nembutal, Abbott Laboratories Ltd., Queenborough, Kent, England). For each preparation, the urinary bladders of three rabbits were exposed surgically and the urethral ends were clamped with hemostats to produce closed bags. The bladders were then excised and quickly chilled on ice. All operations thereafter were performed at 4°C. A 50 ml syringe with an 18 gauge needle was used to flush the lumens of the bladders by injecting and withdrawing several changes of 0.01 M sodium bicarbonate. Then the bladders were distended fully for 5 min by injecting a solution of 0.01 M thiglycolic acid (Nutritional Biochemicals Corporation, Cleveland, Ohio), adjusted to pH 7.4 with NaOH (see reference 8).

After this interval, the thiglycolate solutions were withdrawn from the bladders and pooled. Portions of the bladders were fixed immediately for electron microscopy. Meanwhile, the pooled solution was centrifuged at 1500 g for 15 min in a Sorvall HB-4 swinging bucket rotor to yield a pellet, which was resuspended in 0.01 M sodium bicarbonate and washed once by a similar centrifugation. The washed pellet was resuspended in 1.8 M sucrose and layered carefully under 4 ml of a continuous sucrose gradient, extending from 1.3 M to 1.8 M. The preparation was centrifuged for 1.5 hr in a 50L Spinco swinging bucket rotor at 200,000 g. The nuclei formed a pellet and were discarded. The membranes, which floated up to form a layer in the upper third of the gradient, were removed with a pipette, suspended in 0.01 M sodium bicarbonate, and washed twice by centrifugation at 1500 g for 15 min. This preparation, which was enriched in luminal membranes, was fixed for routine electron microscopy, frozen before freeze-etching, or treated with DOC to isolate the individual plaques composing the membranes.

Isolation of Plaques

Isolated luminal membranes were resuspended in 2 ml of 0.01 M sodium bicarbonate. This suspension was mixed with an equal volume of 2% sodium deoxycholate (DOC) (Fisher Scientific Company Fairlawn, N. J.) in 0.01 M sodium bicarbonate (see reference 3) and allowed to stand for 1 hr at room temperature. Then it was centrifuged at 20,000 g for 15 min in a Spinco 50L rotor. The pellet was resuspended and washed by a similar centrifugation in 0.01 M sodium bicarbonate. The final pellet, which was enriched with plaques, was fixed for routine electron microscopy or frozen before freeze-etching.

Fixation, negative staining, and freeze-etching of tissues and membrane fractions were carried out as described in the preceding paper (15).

RESULTS

The normal configuration of the free surface of rabbit transitional epithelial cells is illustrated in
Figure 1  An electron micrograph of the free surface of an epithelial cell lining the rabbit urinary bladder. The lumenal plasma membrane has a scalloped profile, composed of curved plaques (PL) (regions where the thick unit membrane displays a periodic structure) and thinner, particle-free, interplaque regions (IN), which appear as protuberant ridges. A dense population of cytoplasmic filaments (F) is seen in cross- and oblique section. Discoidal vesicles, which appear fusiform (FV) when sectioned transversely, and small cytoplasmic vesicles penetrate the dense web of filaments. Mitochondria (M) and ribosome-like particles are also present. X 65,000.

Figure 2  A section through the lumenal surface of a transitional epithelial cell of rabbit urinary bladder, showing effects of exposure of the cell surface to 0.01 M thioglycolate (pH 7.4) for 5 min in vitro. The membrane is ruptured; X's mark its broken ends. Plaques (PL) and interplaque (IN) regions may be identified. The dense feltwork of cytoplasmic filaments is no longer seen. Instead, many short pieces of lightly stained material are seen in the cytoplasmic matrix. Some small filaments still seem to be attached to the membrane (arrows). Fusiform vesicles (FV) are occasionally distended; but the structure of their limiting unit membrane remains normal. Mitochondria (M) appear to be quite disrupted. L, lumen. X 42,000.

Figure 3  A specimen similar to that shown in Fig. 2, but presented at lower magnification. It can be seen that large pieces of lumenal plasma membrane remain intact after exposure to thioglycolate solution and apparently can float free (arrows). In one of the cells (C1), the lumenal plasma membrane remains intact in the field of view. In the adjacent cell (C2), segments of the plasmalemma have come detached, allowing the cytoplasmic contents to escape into the lumen, while the lateral and basal plasma membranes remain in place. X 11,000.
Fig. 1. The detailed structure of the plasma membrane limiting this surface and its relationship to the underlying cytoplasmic filaments have been described in the previous paper (15); only those features which will be useful for comparison with experimentally treated tissue are emphasized in the present report.

The plasma membrane facing the lumen of the bladder possesses curved plaques whose concave surfaces face the lumen (Fig. 1). The plaques appear rigid, in that their curvature is concave whether the bladder is fixed in a distended or in a collapsed state. Plaques are interconnected by short segments of membrane that, in transverse sections, look like crests. These interplaque regions are areas where the membrane is often seen bent at sharp angles. The membrane as a whole is attached to an extensive system of cytoplasmic filaments (15), which constitute a major cytoplasmic component, one that is particularly abundant in the apical cytoplasm of the lumenal cells (Fig. 1). Intermingled in this web of cortical filaments are discoidal vesicles with their characteristically thickened membrane, small vesicles that lack the thickened membrane, mitochondria, and particles resembling ribosomes.

After distention of the urinary bladder with 0.01 M thioglycolic acid for 5 min, marked changes are observed in the structure of the epithelial cells lining the lumen. As illustrated in Fig. 2, occasional small ruptures are seen in the luminal membranes of some cells. These breaks expose the cytoplasm directly to the luminal contents, allowing cytoplasmic constituents to be extruded. In many cells the plasma membrane is detached from the luminal surface (C2, Fig. 3). It appears that the released luminal membranes float into the lumen, while most of the lateral and basal membranes remain behind, apparently held back by their desmosomal attachments to each other and to the underlying cell layer. However, in other cells, no breaks in the plasma membrane are visible in the plane of section (C1, Fig. 3). Thus, although many luminal membranes are released into the thioglycolate solution, the effect of the treatment varies on a cell-to-cell basis.

Aside from the ruptures, thioglycolate-treated tissue does not suffer any obvious damage to the unit membrane structure of the plasma membrane. In examining sectioned material, we have been unable to detect any reduction in thickness of the membrane or noticeable change in its staining properties. With respect to the size and curvature of the plaques and also to the bending of the membrane at the interplaque areas, the treated membrane resembles the untreated one very closely. Exposure to thioglycolate does have an effect on membrane structure, however, as one can deduce from freeze-etched preparations (see below), but this effect is less drastic than that observed on other cellular components.

In contrast to luminal membranes, cytoplasmic filaments are affected by the treatment. This network, as such, may no longer be identified. Instead, a matrix of short, lightly stained, thread-like fragments is observed (Fig. 2). These entities might represent remnants of disaggregated filaments. The destruction of filaments is seen both in cells where breaks in the luminal membrane are visible and in those where no ruptures are apparent in the plane of section.

Other cytoplasmic components display effects of

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**Figure 4** A piece of luminal plasma membrane isolated from the bladder epithelium after its exposure to thioglycolate for 5 min. The scalloped profile, consisting of plaques (PL) and interplaque regions (IN), resembles untreated membrane (Fig. 1) in its unit membrane structure and staining properties. Treatment has apparently removed the small filaments that normally attach the longer cytoplasmic filaments to the membrane. Fusiform vesicles (FV) and other membranous material of uncertain identity cling to the membrane. × 40,000.

**Figure 5** A plaque on a luminal plasma membrane, isolated as in Fig. 4. As in intact cells, the asymmetrical unit membrane consists of a relatively thick outer lamella (arrow), within which densely staining particles may be detected, and a thinner inner lamella. No attached filaments are seen. × 200,000.

**Figure 6** Tangential section through a preparation similar to that shown in Fig. 5. A barely discernible striated pattern (100 Å periodicity) indicates the presence of a regular array of particles similar to that existing in untreated membranes. Note again the absence of attached short filaments or associated long cytoplasmic filaments. × 100,000.
the treatment to varying degrees. Discoidal vesicles
seem little affected, but mitochondria are severely
changed in appearance. The outer limiting mem-
branes of mitochondria are refractive to stains and
are discontinuous. The cristae appear swollen, and
the dense inner mitochondrial matrix is no longer
seen (Fig. 2). The ribosome-like particles are in-
tact, although they seem to stain less intensely.
When treatment is continued beyond 5 min, the
superficial cells become totally disrupted, and the
underlying layer of epithelial cells begins to be
disrupted also (see reference 8). For this reason,
only 5-min treatments were used in these experi-
ments.

These observations led to development of the
procedure for isolation of a fraction enriched with
luminal plasma membranes from the thioglycolate
solution which had been injected into the bladder.
When viewed at low magnification, isolated lu-
menal membranes are readily identifiable by the
presence of characteristic plaques and interplaque
regions (Fig. 4). Essentially only long profiles of

![Figure 7](image1.jpg)

**Figure 7** An area of a plasma membrane isolated from homogenized transitional epithelial cells without
thioglycolate treatment (15), washed in distilled water, freeze-cleaved, and etched for 90 sec before replica-
tion. The hexagonal arrays of particles constituting plaques can be visualized protruding out of the
luminal surface (PO face) of the membrane. Accumulation of metal along the edge of a plaque (arrow)
demonstrates that particles protrude above the smooth-surfaced interplaque region (IN). Subunits of
the particles are barely discernible in some areas, with their central depressions appearing as white dots.
The PA face represents the split inner face of the cytoplasmic leaflet. Deep-etching has obscured the
hexagonal pattern of holes that are normally observed on PA faces (see reference 15). In this micrograph
and all others of freeze-etch replicas, the encircled arrow in the lower right-hand corner indicates the
direction of shadowing. I, ice. X 186,000.

![Figure 8](image2.jpg)

**Figure 8** A freeze-etch replica of luminal membrane isolated from bladder epithelium with thiogly-
colate, freeze-cleaved and etched for 90 sec. The hexagonal arrays of particles protruding out of the
luminal surface of the membrane (PO face) appear in sharper relief than in membranes isolated from
homogenized cells (see Fig. 7). Within the plaque at the top, the subunits of some particles are seen. The
plaque on the left exhibits a fine pattern, which arises when the shadowing angle is such that the subunits
of the particles and not the particles themselves are emphasized. PA, split inner membrane face. I, ice.
X 111,000.
apical membranes are regularly found, indicating that the treatment causes but few interruptions in the membrane exclusive of those initial breaks which allow it to float free. When cut in cross-section (Fig. 5) or tangentially (Fig. 6), the isolated membranes resemble those seen in situ. The particulate component of the membrane therefore remains intact, and its organization seems unaffected.

The most striking point about the isolated membranes is their freedom from filaments (Figs. 4, 5, and 6), which are always observed in association with the cytoplasmic surface of membranes isolated from epithelial cells homogenized in buffered sucrose (see Fig. 19 in reference 15). The principal elements present in addition to lumenal membranes are vesicles clinging to their cytoplasmic surfaces, most of the discoidal type and others that cannot be identified positively. Thus, isolation by this method produces a purer lumenal membrane fraction than is possible from homogenized epithelial cells (15).

After freeze-etching, lumenal membranes isolated by thioglycolate treatment were compared with those recovered from homogenized epithelial cells in order to determine whether thioglycolate has any effect on their substructure. It previously was shown that the asymmetry imparted to the membrane by the position of hexagonal particles within it makes it possible to identify the four different surfaces (an inner, an outer, and two split membrane faces) obtained by freeze-cleaving and deep-etching of isolated membranes (15). Figs. 7 and 8 are images of freeze-etch replicas each illustrating both the deep-etched lumenal surfaces (PO faces) of outer leaflets and the split, inner PA faces of cytoplasmic leaflets obtained from homogenized cells and thioglycolate-treated tissue, respectively. In both cases the center-to-center spacing of the plaque particles (located on the lumenal surface) is similar (~160 A), but the ~40 A subunits composing the particles are more clearly visible after thioglycolate treatment. In confirmation of observations from fixed and sec-

![Figure 9](image-url)

**Figure 9** A freeze-etch specimen of lumenal plasma membrane isolated by the thioglycolate method, freeze-cleaved and etched for 90 sec. The cytoplasmic surface (PI face) of the plasma membrane appears smooth; the cytoplasmic filaments normally associated with it have been removed by the treatment. The split inner surface (PB face) of the lumenal leaflet of the membrane clearly shows the hexagonal arrays of particles that make up the plaques, as well as the smooth interplaque regions (IN) between them. X 26,000.
tioned membrane isolates (Figs. 4, 5, and 6), the cytoplasmic surfaces (PI faces) of plaques revealed by deep-etching are smooth (Fig. 9), lacking the attached short filaments observed on PI faces of membranes isolated from homogenized epithelial cells (see Fig. 18 in reference 15). Split, inner membrane faces—PA (Fig. 8) and PB (Fig. 9)—do not show any changes after thioglycolate treatment. Similarly, interplaque regions appear to be unaffected, displaying a smooth surface, whether the deep-etched lumenal surface (Fig. 8) or the cleaved, inner PB face of the lumenal leaflet (Fig. 9) is observed.

Because the interplaque regions fracture in a manner similar to that of simple bimolecular layers of hydrophobically bonded molecules (15), sodium deoxycholate (DOC), which disrupts hydrophobic interactions (3), was used in an attempt to separate the plaques. After exposure to 1% DOC, many lumenal membranes are severed along interplaque areas to yield a preparation containing individual plaques (Fig. 10). The profile of the unit membrane of the plaques retains the thickness and periodic structure observed in situ. However, the particles composing the plaques are more obvious (compare Fig. 10 to Fig. 1) than in untreated membranes.

These results are confirmed by observations on negatively stained preparations of DOC-treated membranes (Fig. 11). Within the small pieces of membrane composing one or a few plaques, subunits of the hexagonally arrayed particles show more clearly (circled area, Fig. 11) than in untreated membrane preparations (see Fig. 16 in reference 15). Freeze-etch replicas of DOC-treated membranes (Fig. 12) also reveal only small pieces of membrane. In these, too, the regular array of particles and their subunits stand out more clearly than after thioglycolate treatment alone (compare Figs. 8 and 12). The cleaved, inner faces of the

![Figure 10](image-url)  
**Figure 10** An electron micrograph of a section through a pellet of lumenal membranes isolated from bladder epithelium by the thioglycolate method and subsequently treated with DOC. Most of the interplaque areas of the membrane are missing. Individual plaques retain the diameter and staining properties displayed by the membrane in situ. The striated pattern due to the periodicity of the particles is clearly visible in tangential and oblique sections. × 54,000.
FIGURE 11 A negatively stained preparation of lumenal membranes isolated by the thioglycolate method and subjected to subsequent DOC treatment. Five plaques can be identified, due to the different orientations of their particulate arrays. Individual hexagonal units stand out clearly, and some indication of their subunits can be seen (see encircled area). Interplaque areas cannot be detected. × 111,000.

FIGURE 12 A preparation similar to that described in Fig. 11, except that the membranes were freeze-cleaved and etched for 90 sec. The clarity of the subunits of particles on the lumenal surface (PO face) of the plaques is greater than that seen after thioglycolate treatment (see Fig. 8). Split, inner membrane faces (PA and PB faces) can be seen. What might be an interplaque area (IN) is demonstrated. × 79,000.

plaques appear unchanged. Thus, although DOC severs interplaque regions, it has little effect on the particulate constituents of the plaques. A simplified drawing (Fig. 13) summarizes our interpretation of these results.

DISCUSSION

The primary effect of thioglycolate, as used in these experiments, appears to be destruction of the extensive system of fine cytoplasmic filaments that is especially prominent in the cortex beneath the free surface of the superficial epithelial cells lining the urinary bladder. Treatment with thioglycolate allows detachment of the superficial membrane and its isolation in a relatively pure fraction. Unlike lumenal membranes isolated from homogenates in sucrose medium (15), these membranes are free of attached filaments. This result is consis-
FIGURE 13  A simplified diagram illustrating the successive steps in the isolation of lumenal plasma membranes from transitional epithelial cells by thioglycolate treatment and the subsequent isolation of plaques from the membranes by emulsification of interplaque regions with DOC. N, nucleus.

Our results and the interpretation they suggested were unexpected, in light of the report by Hicks (8), which indicated that thioglycolate, used under similar conditions, attacked lumenal membranes, but had little effect on cytoplasmic filaments. In the present experiments, although many of the membranes are ruptured and their connections to cytoplasmic filaments severed, the released membrane fragments retain their normal construction in both the plaque and interplaque regions (Figs. 4, 5, and 6). We have obtained similar results with rat bladders (unpublished observations), and thus the differences between our results and those of Hicks (8) are not attributable to variation in experimental animals.

Resolution of this conflict will come when the chemical structure of the plaque particles and the cytoplasmic filaments is known. Experiments of this sort are being undertaken, aimed at obtaining larger yields of membrane, in amounts sufficient for analysis, by an improved isolation method and discovering conditions sufficient for solubilizing the resistant particle proteins. Meanwhile, however, it seems reasonable to presume that the cytoplasmic filaments possess disulfide bonds that render them vulnerable to the reducing activity of thioglycolate. This belief has been encouraged by observations on the bladder epithelium stained to reveal sulfhydryl and disulfide groups by the method of Barnett and Seligman (1). After fixation with acetic alcohol (13), staining of the luminal layer did not show any extreme tendency toward margination as previously described by Hicks (8) after aldehyde fixation, but appeared as a dense, homogeneous network throughout the cell, with a moderate increase at the luminal side. This pattern of staining is consistent with the distribution of cytoplasmic filaments observed in electron micrographs.

Thus, the cytoplasmic filaments could well be similar to fine filaments found in many epithelial cells (14) and to epidermal tonofilaments (4), which have been designated as the site of sulfhydryl and/or disulfide groups (1, 2).

Although thioglycolate leaves large segments of lumenal membrane intact, it is not completely without action on the membrane. When freeze-etched preparations of untreated and treated specimens are compared (Figs. 7 and 8), it is apparent that after exposure to thioglycolate the six subunits of the hexagonally arrayed particles protruding from the luminal face stand out more clearly, as if some material associated with the subunit is removed by the procedure. Since the

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1 Zakrzewski, T. J., and M. A. Bonneville. 1970. Unpublished observations.
chief structural change observable in the membrane after thioglycolate treatment is its detachment from the filaments, it is possible that the removal of those components by which the filaments are supposedly attached to the particles is somehow responsible for the increased clarity of the subunits.

Hicks and Ketterer (10) have reported that pretreatment of rat transitional epithelium with fluorescein–mercuric acetate (FMA) aids in removal of the epithelium from the urinary bladder before homogenization of the epithelial cells and isolation of luminal plasma membranes. In our experience (15), prolonged homogenization of rabbit transitional epithelial cell scrapings, without FMA pretreatment, not only allows intact luminal membranes to be isolated, but also preserves their attachments to cytoplasmic filaments. FMA is an oxidizing agent that presumably replaces the hydrogen of sulfhydryl groups at or near a neutral pH (12). In keeping with the data presented in this report, we would expect its action to be primarily on the cytoplasmic filaments, which seem to be sulfur-rich. Indeed, the preparations shown by Hicks and Ketterer (10) do lack attached cytoplasmic filaments. Therefore, it is possible that FMA removed cytoplasmic filaments from luminal membranes in their experiments. The mechanism by which it acts remains a matter for speculation. Perhaps the attachment of the relatively large FMA molecule (mol wt ≅ 849) in place of the smallest element (mol wt of hydrogen ≅ 1) on every sulfhydryl group of the tenuously thin (~75 A) filaments leads to their disruption. If so, its mechanism of action would be quite different from that of thioglycolate, which apparently exerts its effects by the breaking of covalent bonds (i.e., S—S bonds).

The factors responsible for the impermeability of the bladder to water remain a mystery. This property cannot, we feel, be ascribed to the hexagonal particles present in the luminal plasma membrane, since the particles are confined to the plaque regions, which cover only about 73% of the free surface of the cell (15). Thus, it does not seem likely that this particulate component itself or the plaque regions as a whole can account for the impermeability of the epithelium. Rather, for reasons already outlined in the preceding paper (15), we tend to consider that the particles perform a mechanical function, serving as attachment sites for the cytoplasmic filaments.

This conclusion has caused us to examine the role that membrane components other than the particles play in maintaining the structural integrity of the luminal plasma membrane and in accounting for its permeability properties. Treatment of thioglycolate-isolated membranes with DOC emulsifies most of the interplaque regions. Since DOC is probably active in disrupting lipid–lipid, lipid–protein, and protein–protein hydrophobic interactions, it is likely that these types of bonds are important in any barrier to permeability in luminal membranes.

Although the plaques remain intact, they are affected by DOC treatment, inasmuch as freeze-etching and negative staining reveal the subunits of the particles protruding from the luminal surface with more clarity than after thioglycolate treatment alone. Thus, DOC treatment apparently solubilizes lipids and other hydrophobically bonded substances surrounding the subunits of the particles. The arrays of hexagons, on the other hand, must be held together laterally by forces other than the weak bonds which are emulsified by DOC. Since the particles are always associated with the luminal leaflet after freeze-cleaving, such nonhydrophobic bonds are probably located there (15). The fact that the plaque membranes isolated by DOC treatment are still capable of undergoing cleavage when frozen seems to indicate that a sufficient quantity of hydrophobically bonded molecules has been retained in the plaques in the form of a bilayer.

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