

# ISOLATION OF THE PLASMA MEMBRANE OF THE LUMINAL SURFACE OF RAT BLADDER EPITHELIUM, AND THE OCCURRENCE OF A HEXAGONAL LATTICE OF SUBUNITS BOTH IN NEGATIVELY STAINED WHOLE MOUNTS AND IN SECTIONED MEMBRANES

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## ABSTRACT

A method of isolating the thick luminal membrane from homogenates of bladder epithelium is described, which entails pretreatment of the epithelium with fluorescein mercuric acetate and centrifugation of the homogenate on sucrose density gradients. A hexagonal array of hexamers is illustrated by negative contrast staining in whole mounts of the isolated thick membrane. Subunits are also shown in tangential sections of this thick membrane, in fixed, embedded bladder epithelium. The significance of the subunits is discussed in the context of membrane structure and permeability.

## INTRODUCTION

The superficial cells of the rat urinary bladder epithelium are limited on their luminal face by a rigid 120 Å thick plasma membrane, which has an asymmetric unit structure (1, 2). The bladder epithelium acts as a barrier to the loss of body water to the urine but this barrier function is lost if the thick surface membrane is damaged, either physically or chemically (1, 2). This indicates that the surface membrane itself, unlike most other plasma membranes, is relatively impermeable to water. This appears to be a passive function of the membrane. Thus the excised bladder continues to resist a flow of water across it in the absence of added nutrients or oxygen (2); electron microscopy of superficial cells shows no obvious morphological relationship of mitochondria to the

membrane, as would be likely if the barrier to water flow were an active process coupled to oxidative phosphorylation; and no nucleoside phosphatase activity is associated with the surface membrane, although this enzyme is active along the thinner lateral and basal membranes of the same cells (1).

In this paper, a method is described for isolating the thick luminal membrane from homogenates of bladder epithelium. The availability of a relatively pure, thick membrane preparation has enabled the membrane structure to be reexamined in detail by comparing the images obtained in sectioned material to those of negatively stained whole mounts, and these results are reported here.

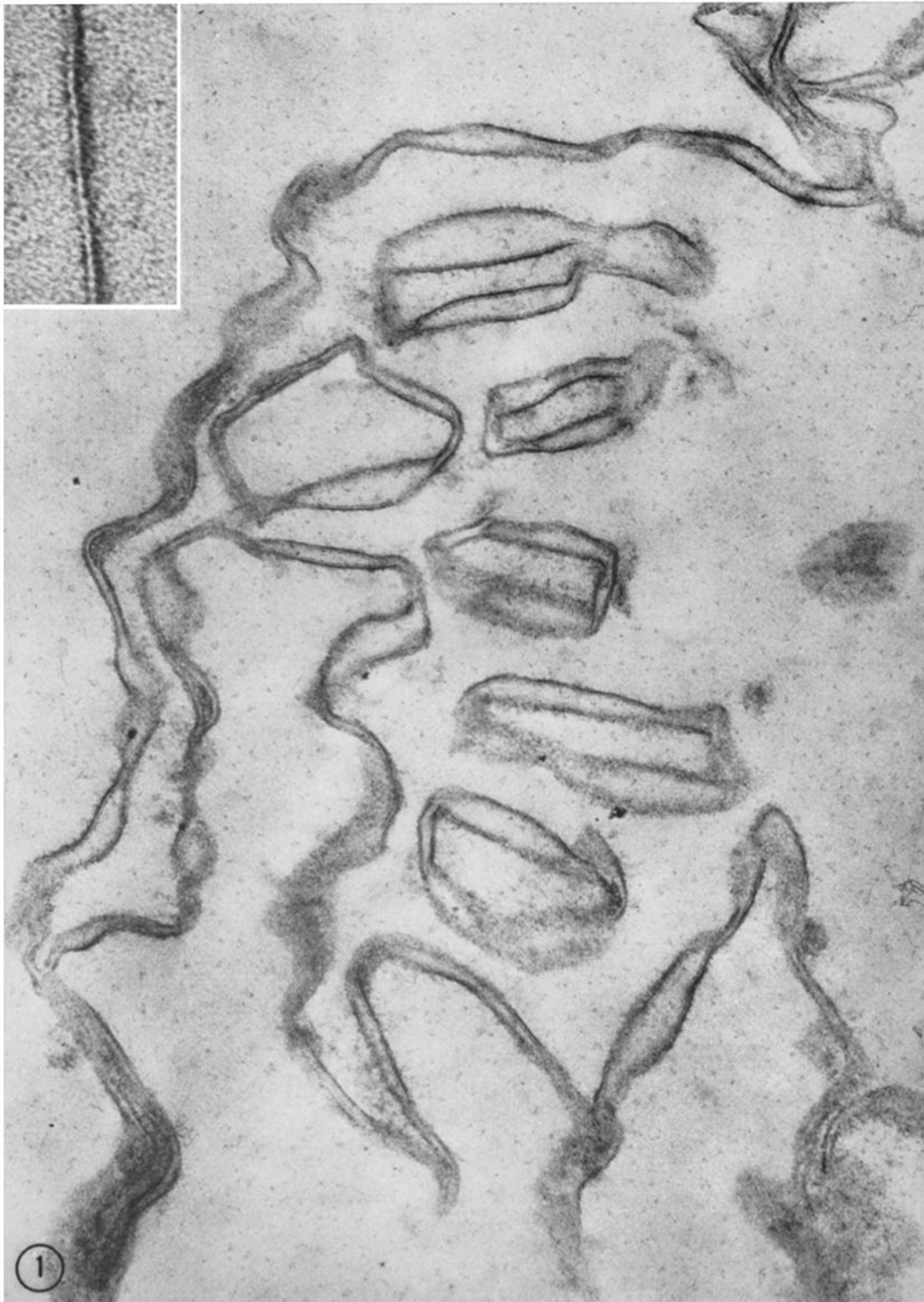


FIGURE 1 The field shows a section of the pellet prepared from the fraction  $d = 1.13$ . The pellet is composed of asymmetrically thickened membrane, shown at higher magnification in the *inset*. The membrane is in the form of large sheets which have folded back on themselves so that the thinner cytoplasmic faces are adjacent. There appears to be no cytoplasmic material adhering to the membranes, which still have an angular appearance comparable to the image they present *in situ*.  $\times 90,000$ ; *inset*,  $\times 300,000$ .

## MATERIALS AND METHODS

### *Animals*

Male Wistar strain rats weighing approximately 200 g were used. They were killed by cervical dislocation.

### *Chemicals*

Fluorescein mercuric acetate (FMA) was obtained from Sigma Biochemical Company, London, England. Analytical grade sucrose and 2-amino-2-(hydroxymethyl) propane-1:3-diol (Tris) were obtained from B. D. H. Poole, Dorset, England. Epikote 812 was obtained from G. T. Gurr Ltd., Chadwell Heath, Essex, England.

### *Isolation of Subcellular Fractions from the Bladder Epithelium*

The bladders of 10 rats were exposed, emptied of urine by gentle pressure, and clamped at the trigone end with small bulldog clips. Each bladder was moderately distended by injection with a saturated solution of FMA in 0.02 M Tris-HCl buffer, pH 8.0, at room temperature. After 20 min, the clamps were removed and the bladders were excised and turned inside out through the cut end. The epithelium was gently scraped off each bladder with a scalpel blade into about 0.5 ml of 0.02 M cold Tris-HCl buffer, pH 8.0. The epithelium could be removed from the bladder wall as a thin, transparent sheet, uncontaminated by underlying collagenous connective tissue. The pooled scrapings from 10 bladders in a total volume of 5 ml were homogenized by five strokes of the pestle in a TenBroeck tube.

This homogenate was mixed with an equal volume of 60% (w/v) sucrose and divided into three parts, each of which was layered over 4 ml of 45% (w/v) sucrose in transparent polycarbonate tubes for the 3 × 10 ml swing-out rotor of the Superspeed 50 ultracentrifuge (Measuring & Scientific Equipment Ltd., London, England) and then centrifuged for 45 min at 4000 g (average). The supernatants (S<sub>1</sub>) were collected, pooled, and refrigerated. The interfacial regions were also collected and pooled, layered again

over 40% sucrose, and spun for 1½ hr at 5500 g (average) to give a further supernatant, S<sub>2</sub>. S<sub>1</sub> and S<sub>2</sub> were pooled, diluted to 35 ml with water, and centrifuged for 1½ hr at 100,000 g (average) in the 8 × 10 ml fixed angle rotor of the Superspeed 50. The resulting pellet was suspended in 2 ml of 17.5% sucrose. 1 ml samples were layered over discontinuous density gradients consisting of 20, 25, 30, 35, 40, and 45% (w/v) sucrose (d = 1.08, 1.10, 1.13, 1.15, 1.17, and 1.20, respectively) and spun for 6 hr at 70,000 g in a 3 × 10 ml Superspeed 50 swing-out rotor. Discrete bands of material were not obtained. There was a region of maximum turbidity in the 35% sucrose. The 30 and 40% sucrose layers were also turbid and there was slight but noticeable turbidity in the 25% sucrose zone. 1 ml fractions were cut corresponding to these zones of turbidity.

### *Electron Microscopy*

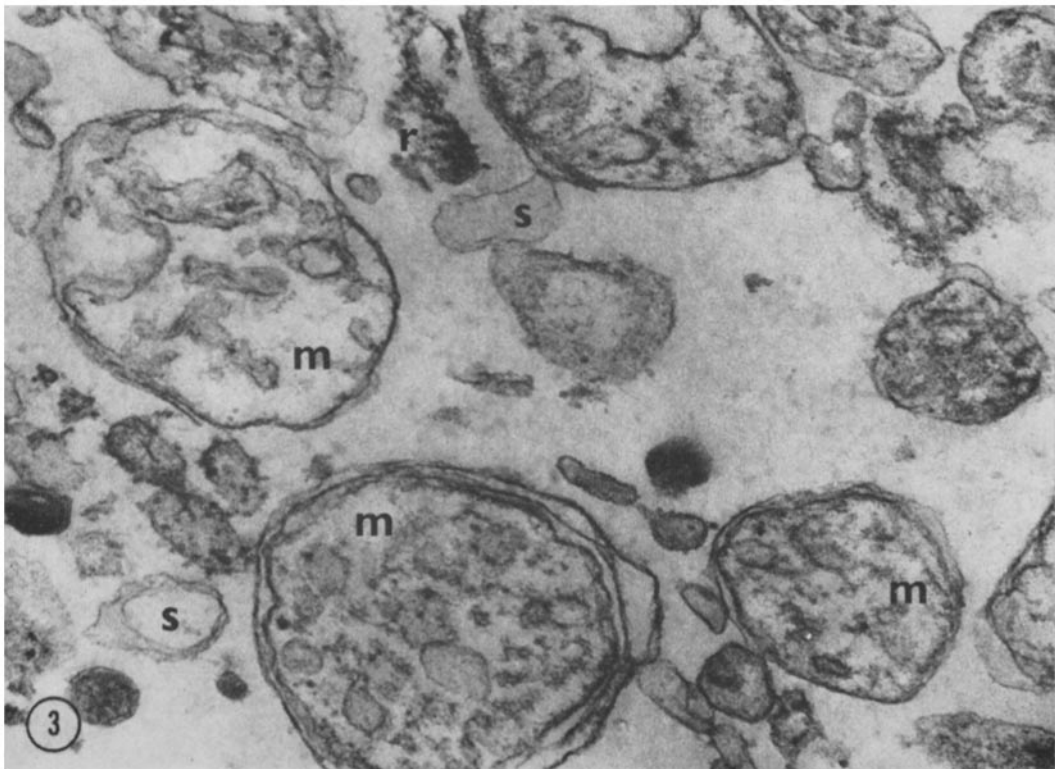
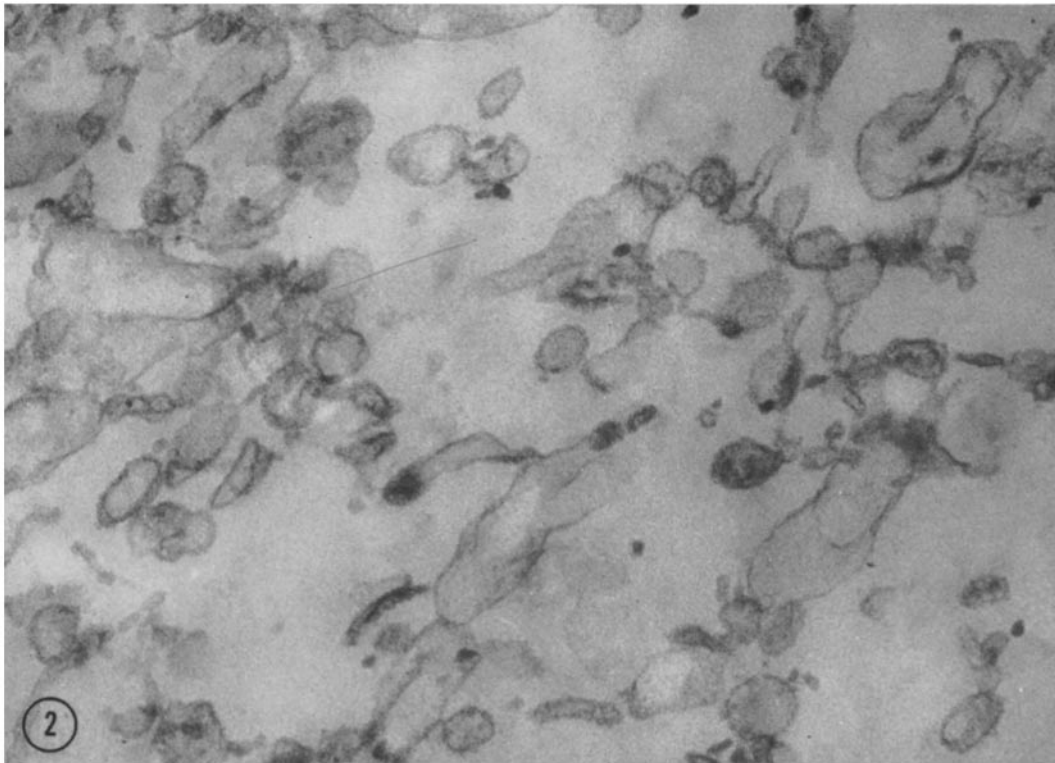
**ISOLATED MEMBRANES:** The final fractions were diluted and pelleted at 100,000 g (average) for 1½ hr in the 8 × 10 ml Superspeed 50 fixed angle rotor. The pellets were fixed for 1½ hr by immersion in cold, 1% (w/v) osmium tetroxide, buffered with phosphate (3) to pH 7.3. Large pellets were cut into pieces approximately 1 mm<sup>3</sup>, and small pellets were fixed whole. After dehydration through graded alcohols, they were embedded in Epikote 812, and thin sections were prepared for examination in a Siemens Elmiskop 1 or Philips EM200 by the methods described previously (2).

The unfixed fractions were also examined in the electron microscope after negatively staining with phosphotungstic acid (PTA) adjusted to pH 7.2 with 1 N NaOH. A small drop of a fraction from the final gradient was placed on a carbon-coated specimen grid, and a drop of PTA was added. After 1 min, the excess was drawn off with a filter paper, and the grid was allowed to dry at room temperature. Alternatively, a clean steel needle was inserted into the unfixed, pelleted material, and then passed through the meniscus of 1 ml of PTA contained in an embryo pot. The material which spread out over the surface of the PTA was picked up on a carbon-

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FIGURE 2 A section of the light membrane fraction d = 1.10, is shown here. This fraction is predominantly composed of vesicles of normal 75 Å thick membrane, the origin of which is unknown. × 90,000.

FIGURE 3 A section of the fraction d = 1.15 showing swollen, disrupted mitochondria (m) and rough (r) and smooth (s) microsomal vesicles. Also present in this fraction, but not illustrated here, are dense lysosomal-type bodies and some thick walled, fusiform vacuoles. × 51,000.



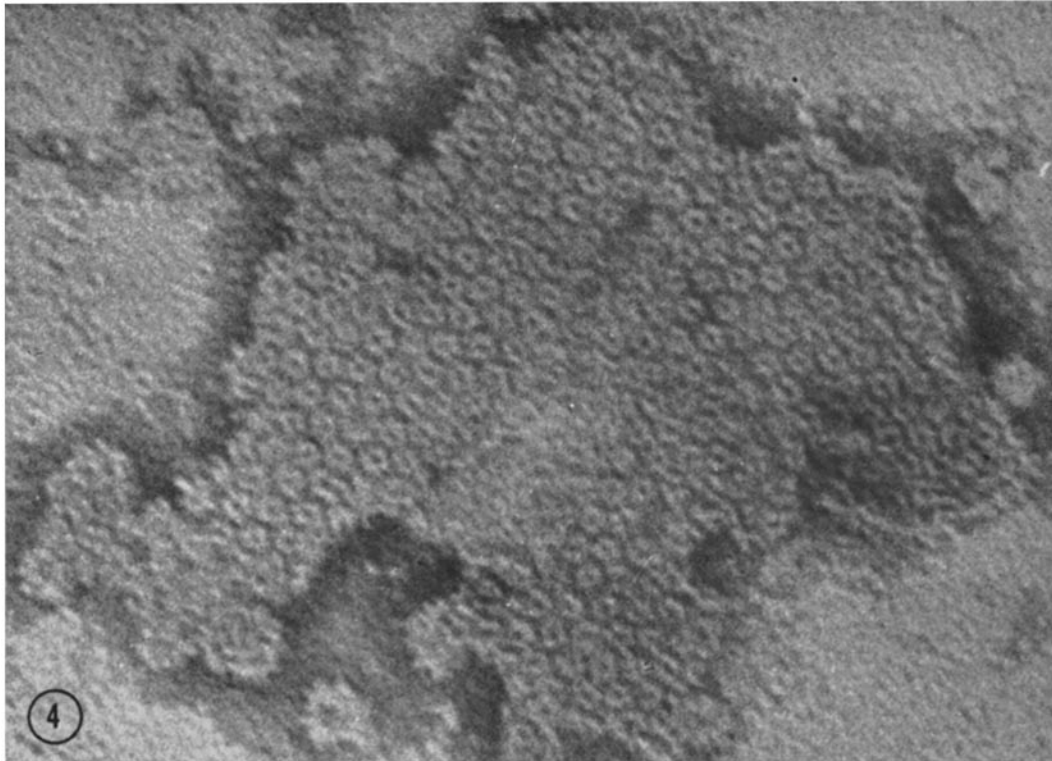


FIGURE 4 Part of the same fraction as is illustrated in Fig. 1. Here the membrane is unfixed and has been negatively stained with PTA at room temperature. The membrane appears to be a hexagonal lattice of large subunits, with a center-to-center spacing of 140–150 Å.  $\times 300,000$ .

coated grid. The material was stained either at room temperature or in an ice/water bath.

**BLADDER EPITHELIUM:** Bladder epithelium was fixed, embedded, and sectioned as described previously (2). Thin sections were contrast stained with uranyl and lead salts and were examined for good examples of perpendicularly and tangentially cut thick membrane.

## RESULTS

### *Isolated Membranes*

Examination of the various fractions cut from the final gradient showed the luminal membrane to be concentrated in the fraction with a density of 1.13 (Fig. 1). The membrane was in the form of large, folded sheets and complex vesicles, and the angularity of its profile and the asymmetry of its unit structure (Fig. 1, *inset*) clearly identified it as having come from the luminal surface of the superficial epithelial cells. There was very little

microsomal contamination of this fraction, and little or no identifiable cytoplasmic material adhering to the thinner, cytoplasmic lamina of the thick membrane.

The lighter fraction ( $d = 1.10$ ) above this from the gradient was also relatively homogeneous and contained other thinner membranes (Fig. 2). The two heavier fractions, of  $d = 1.15$  and  $d = 1.17$ , contained swollen and damaged mitochondria (Fig. 3) and also dense bodies of various sizes which appeared to be lysosomal in origin. These fractions also contained a number of fusiform vacuoles composed of the same type of thick asymmetric membrane as appeared in the main luminal membrane fraction.

Negative contrast staining of the unfixed, thick luminal membrane fraction showed the membrane to have a clearly defined substructure consisting of hexagonally arranged subunits, with a center-to-center spacing of 140–150 Å (Fig. 4). Most

subunits appeared to be composed of six smaller units, each of which was about 40 Å in diameter. The transforms obtained from a preliminary examination of the membrane in an optical diffractometer confirmed that the lattice is composed of hexagonally packed, widely spaced hexamers, and optical rotary diffraction has shown each hexamer to be skewed through a fixed angle with respect to its neighbors. The occasional pentamers seen in electron micrographs (Fig. 5) may well be optical interference patterns produced by superimposition of hexamers in two membrane sheets.

This fraction from the gradient was uniformly composed of membranes showing a hexagonal array, but the lattice structure was not found in the lighter membrane fraction,  $d = 1.10$ , and was present in small patches only in the heavier,  $d = 1.15$  fraction. The pattern was present when the luminal membrane was stained with PTA, either at 2°C (Fig. 6) or at room temperature (Fig. 4). This hexagonally patterned membrane was also found in negatively stained scrapings prepared from fresh bladders not treated with

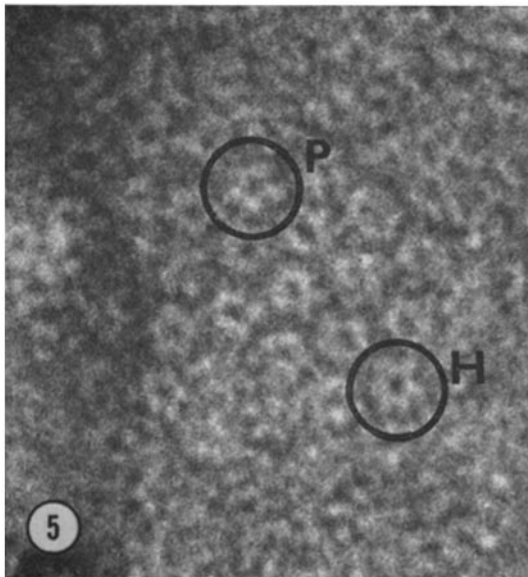


FIGURE 5 Another example of the thick membrane stained with PTA at room temperature. In this field most subunits appear to be composed of six smaller components, each of which is approximately 40 Å in diameter. In addition to these hexamers (*H*), the occasional pentamer (*P*) can be observed. However, two membranes are superimposed in this field, and the image could be artifactually produced by the superimposition of hexamers.  $\times 600,000$ .

FMA and collected into an isotonic Earle's solution (Fig. 7). The hexagonal lattice in the fraction from the gradient was thus not induced either by reaction of FMA with the membrane or by hypotonic treatment of the tissue.

Where a membrane sheet was folded (Fig. 6), the hexamers could be seen on both surfaces, i.e., they were visible from both sides of the membrane although not necessarily located on both faces.

#### *Membrane In Situ in the Bladder Epithelium*

A substructure could also be observed in favorably orientated sections of the thick membrane fixed *in situ*. Where the membrane was sectioned tangentially and a face-on view of the membrane was obtained, a substructure could clearly be seen in the plane of the membrane (Figs. 8 and 9). It appeared as light colored rings with a dark central spot, on a denser background, so that the light areas appeared raised above the dark surface. This structure was comparable to, but less distinct than, that revealed in negative contrast stained, unfixed, whole mounts (Figs. 4–7). The center-to-center distance between subunits was 120 Å. Where the membrane was sectioned perpendicularly, a pattern of dense and less dense short lines, of varying width and about 60 Å high, could be seen in the outer leaflet, arranged approximately at right angles to the plane of the membrane (Figs. 10–13). In places, the inner cytoplasmic dense lamina had a beaded appearance, and in some instances the dense beads appeared to be aligned with the densities in the outer lamina (Figs. 10–13, short arrows). The pattern of densities in both leaflets was irregular, but if hexamers, arranged in the pattern indicated by the optical diffraction studies (R. M. Hicks, R. W. Horne, R. Warren, and B. Ketterer, unpublished observations), are sectioned, the exact appearance in cross-section varies with the direction of section relative to the orientation of the lattice, and also with the thickness of the section. The images shown in Figs. 10–13 are consistent with certain preferred directions of section through such a lattice, even allowing for a section thickness of 4–5 subunits in depth.

#### DISCUSSION

FMA was used in the isolation of cell membranes by Warren, Glick, and Nass (4). They isolated surface membranes from suspensions of mouse fibroblasts and claimed that FMA stabilized and

strengthened the membranes, thus enabling them to withstand the isolation procedures. We find that after pretreating the bladder epithelium with FMA, it can be scraped off as a thin transparent sheet into a hypotonic medium. Unless the epithelium is pretreated in this way, it is difficult to separate it from the underlying lamina propria. How FMA produces this effect is not known, but possibly it modifies the permeability of the membrane to water, thereby enabling the hypotonic buffer to enter and "float off" the epithelium. FMA may react chemically with membrane components (4), but it has no apparent effect on the membrane ultrastructure (see Figs. 1, 4, and 7).

Epithelium treated in this way gives rise to homogenates containing osmotically damaged subcellular components. The luminal membrane, however, remains intact, and can be separated on density gradients from other cytoplasmic components. The final product of this separation is a very small amount of thick membrane in a concentrated sucrose solution. Electron microscopy of pelleted material showed the membrane to be isolated as folded sheets and single fusiform vacuoles, comparable to its form in superficial cells *in situ* (1, 5).

We have already reported briefly that hexagonally packed subunits exist in negatively stained preparations of this membrane (6). Hexagonal lattice patterns have been observed in other biological membrane systems, for example, in sectioned Mauthner cell synapses (7), in gap junctions in smooth muscle (8), in intercellular junctions of mouse heart and liver (9), and also in negatively stained tight junctions between rat liver cells (10, 11).

Doubt has frequently been expressed as to the significance of such subunits as are revealed only by negative contrast staining (12), for similar sub-

units cannot usually be seen in the same material when it is sectioned after fixation and embedding. There always existed the possibility that deformation of molecular structure might occur as the unfixed preparation dried down on the grid; as the critical degree of hydration is reached, the configuration of the lipid moiety could change from a smectic mesophase into a micellar form (13). However, the results reported here show that a substructure of repeating units is present in the thick membrane both in conventionally fixed and sectioned bladder epithelium (Figs. 8 and 9) and in negatively stained preparations (Figs. 4-7).

In cell junctions and synapses, the center-to-center spacing between subunits is between 80 and 95 Å in both sectioned (7-9) and negatively stained material (10, 11). In the thick bladder membrane the size of subunits is larger; the repeat in sectioned material is 120 Å, and in negatively stained whole mounts it is 140-150 Å. In view of the tissue shrinkage which is well known to accompany dehydration and embedding of osmium tetroxide-fixed material (14), the sizes of the subunit correspond well for the two methods of preparation. The parallel dense lines, spaced at approximately 130 Å, observed by Porter, Kenyon, and Badenhaupt (15) in sectioned bladder membrane, may relate to this lattice, for parallel lines are one of the theoretically possible images obtainable by *oblique* section of a hexagonal lattice of hexamers.

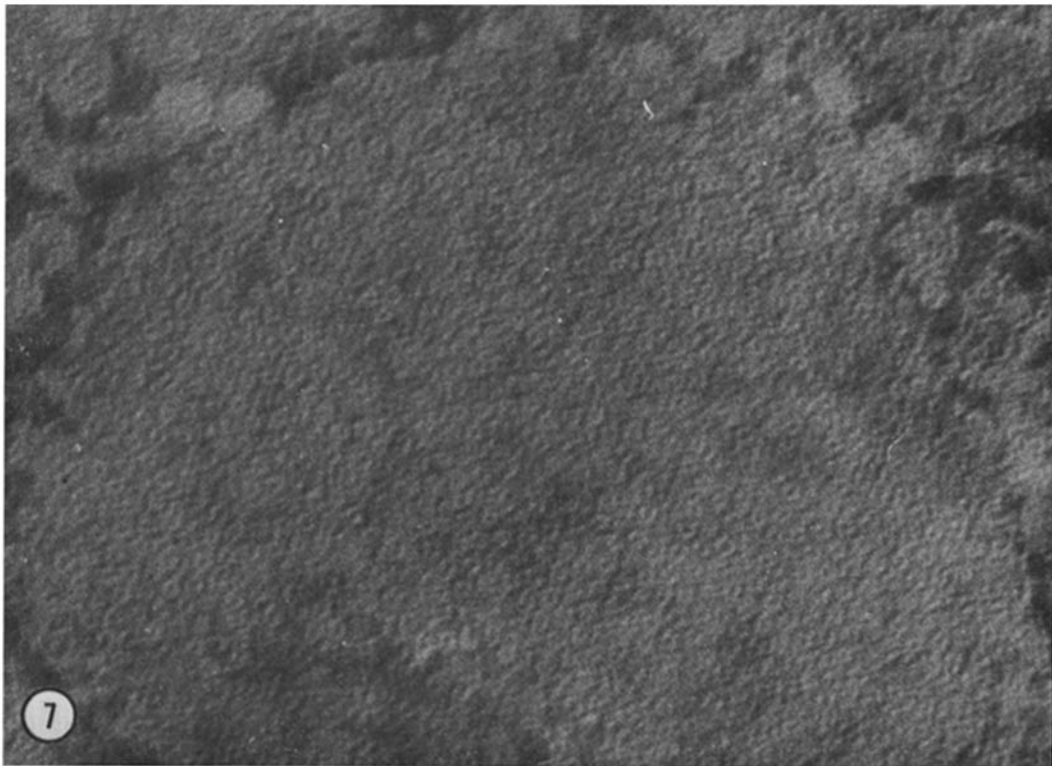
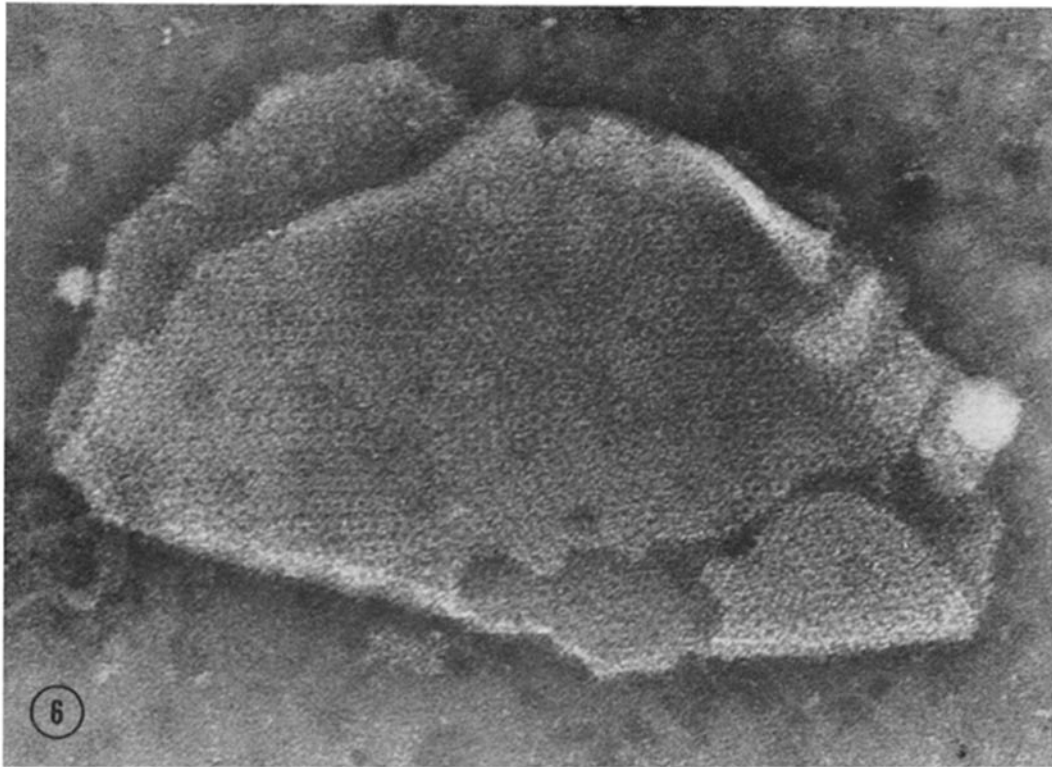
The bladder membrane is thus unique so far among animal membranes in that the hexagonal lattice is found over the total area of the membrane. In other plasma membranes, a hexagonal lattice is confined to tight junctions and is apparently found only in the outer leaflets of the apposing membranes (7, 8, 16-18). Evidence from negatively stained, folded bladder membrane, in which the subunits are visible from both sides (Fig. 6), suggests that the hexagonal pattern may

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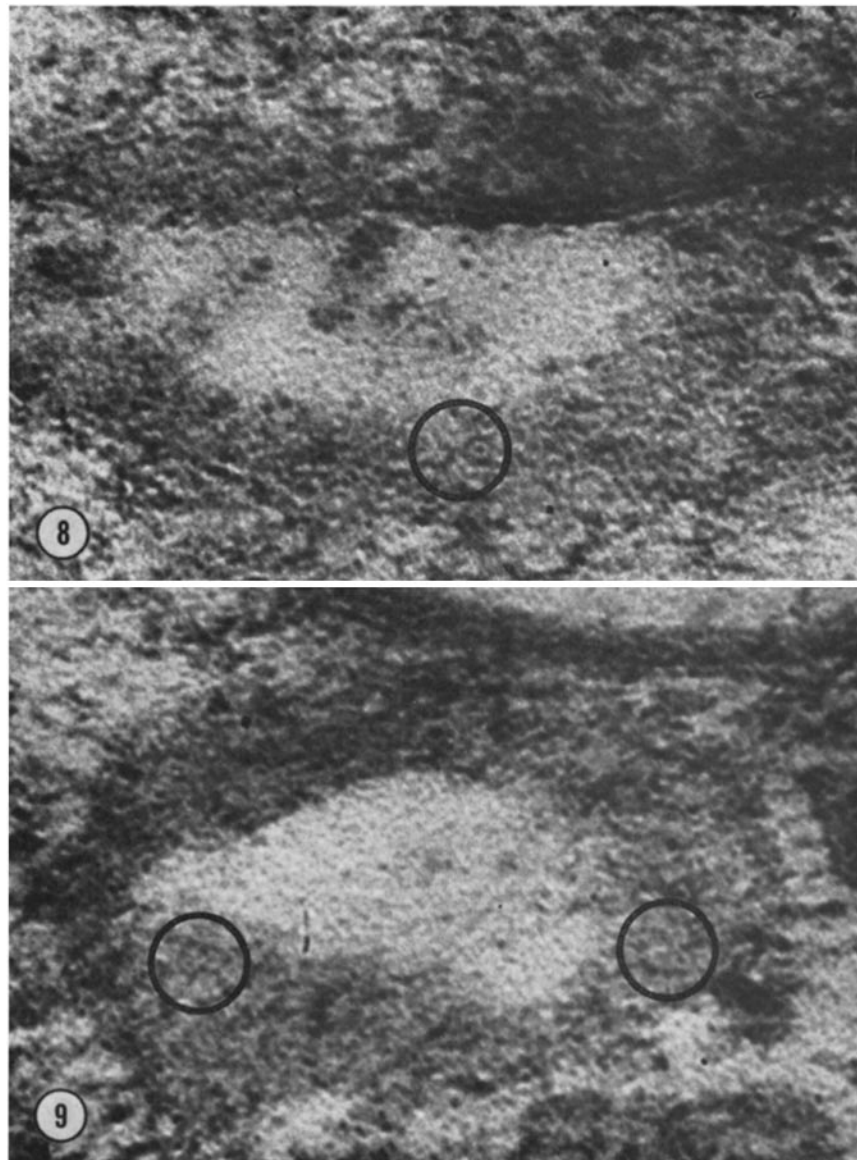
FIGURE 6 Part of the thick membrane fraction, negatively stained with PTA at approximately 2°C after both it and the stain had been incubated for 1 hr in an ice/water bath. As in membranes at room temperature, the hexagonal lattice pattern is still clearly present. Where the membrane is folded, the subunits are visible on both surfaces of the membrane. This phenomenon may also be observed at room temperature.  $\times 210,000$ .

FIGURE 7 A fragment of thick membrane showing a hexagonal lattice structure, found in negatively stained scrapings from the luminal surface of a fresh, untreated rat bladder. The scrapings were collected in Earle's solution and stained at room temperature.  $\times 210,000$ .









FIGURES 8 and 9 These two fields illustrate parts of thin sections of osmium tetroxide-fixed, Epon-embedded, bladder epithelium. The thick asymmetric membrane of the superficial cells has been cut tangentially, and, in the ringed areas, subunits can clearly be seen. Each subunit appears as a light colored annulus, with a dark, pitted center, on a dark background.  $\times 300,000$ .

not necessarily be confined to the luminal face of this membrane. Its appearance in these negatively stained whole mounts does not distinguish between luminal and cytoplasmic faces, or locate the position of the hexamers, which may occur at any depth in the membrane or indeed extend throughout its whole thickness. In perpendicular sections

of fixed material, there is clear evidence for a substructure in the outer, densely staining layer of the membrane. The beaded appearance of parts of the inner dense layer (Figs. 10-13) indicates the possible presence of a substructure here too. After lanthanum staining, the inner lamina of this membrane has been reported to be composed of

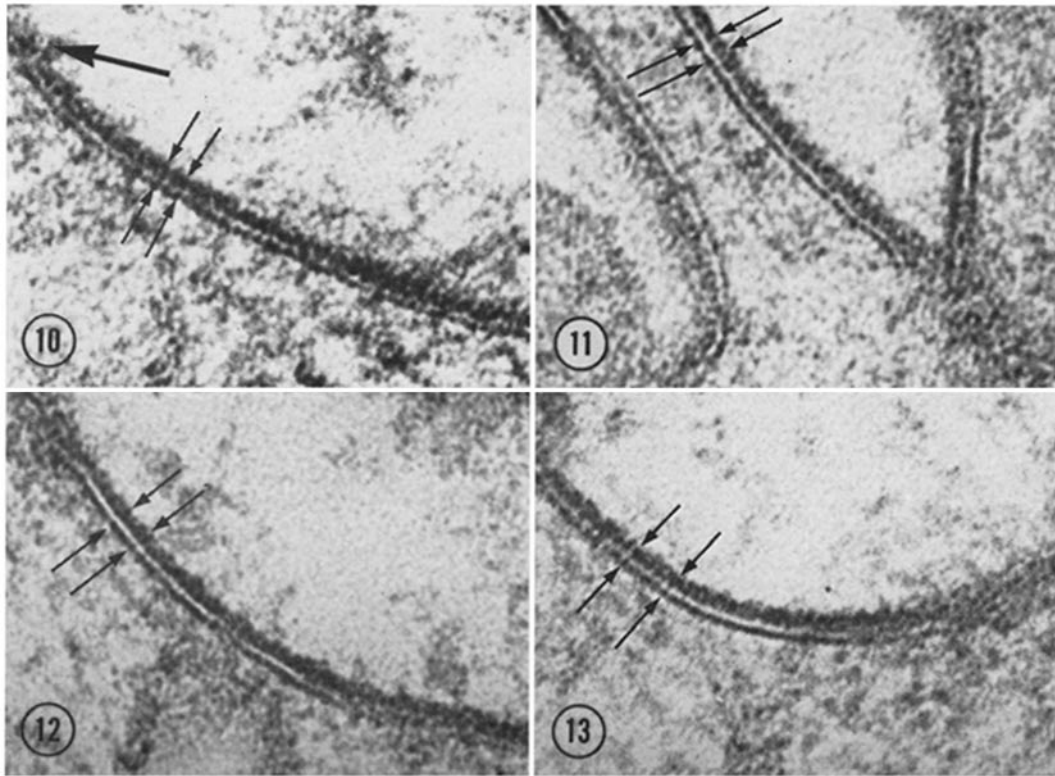


FIGURE 10-13 These figures show perpendicularly sectioned thick membrane in fixed, embedded bladder epithelium. In each, the thicker dense leaflet of the membrane is composed of irregularly spaced, dense lines or granules. The thinner, cytoplasmic leaflet of the membrane has a beaded appearance, and the individual 'beads' sometimes coincide with densities in the outer lamina (short arrows). At the top left of Fig. 10, where the direction of the membrane has changed so that it is sectioned tangentially, a single subunit may be seen in the plane of the membrane (long arrow). Like the subunits illustrated in Figs. 8 and 9, it appears to be a raised light colored annulus, with a dark, pitted center, on a dark background.  $\times 300,000$ .

granules 30-40 A in diameter (19). Structure could be present in the light, central region of the sectioned membrane, but this hydrophobic zone is not stained unequivocally by the heavy metal contrast stains used. From the appearance of both sectioned and negatively stained preparations, it is possible that the subunits may be present on both faces of the membrane, or they may even extend through its depth, and not be confined to the outer, densely staining lamina as in the cell junctions mentioned above.

A similar suggestion has recently been applied to unit membranes in general by Robertson (20) after consideration of the densities sometimes observed in sections. He maintains that the appearance of these densities is consistent with the occurrence of a hexagonal lattice either on the

inside, or on the outside, or on both sides of unit membranes. It would thus appear that the luminal membrane in the bladder could have a hexagonal lattice on both faces or be composed entirely of hexagonally arranged subunits.

It has been suggested that the hexagonal pattern in cell junctions is functionally related to increased intercellular permeability at these sites (9, 11, 18, 21). The bladder membrane, by contrast, is unusually impermeable to water. It is also unusually angular and rigid, as can be seen not only *in situ* (1, 2, 5), but also in the isolated membranes which retain their angular profiles. It is possible that the hexagonal mode of packing of these large subunits is instrumental in maintaining this rigid structure. The fluidity of the membrane lipid in turn may be limited by this rigidity and

thus maintained in a densely packed, highly organized, and relatively impermeable configuration. The membrane may also provide a stable surface which can organize the packing of water molecules into a highly impermeable structured layer, for it is known that water in contact with certain solid surfaces has considerably restricted movement by comparison with free water (22, 23) and may be organized by proteins into an icelike, crystal lattice (24, 25). Ordered water has also been detected in skeletal muscle and brain (26, 27) where it has been attributed to interaction of water molecules with macromolecular systems.

This membrane system clearly has unusual morphological and functional properties which are not directly comparable with those of tight

junctions. Preliminary investigations suggest that its chemistry is also unusual (28). It is richer in proline than other membranes (29, 30), and this may relate to its rigidity. Like the highly impermeable myelin sheath (31), it also contains comparatively large amounts of cerebroside (28). Ultimately, it may be possible to explain the conformation and permeability of this membrane in terms of defined interactions between characterized protein and lipid molecules.

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#### REFERENCES

- HICKS, R. M. 1965. The fine structure of the transitional epithelium of rat ureter. *J. Cell Biol.* 26:25.
- HICKS, R. M. 1966. The permeability of rat transitional epithelium. Keratinisation and the barrier to water. *J. Cell Biol.* 28:21.
- MILLONIG, G. 1961. Advantages of a phosphate buffer for OsO<sub>4</sub> solution in fixation. *J. Appl. Phys.* 32:1637.
- WARREN, L., M. C. GLICK, and M. K. NASS. Membranes of animal cells. I. Methods of isolation of the surface membrane. *J. Cell. Physiol.* 68:269.
- HICKS, R. M. 1966. The function of the Golgi complex in transitional epithelium. Synthesis of the thick cell membrane. *J. Cell Biol.* 30:623.
- HICKS, R. M., and B. KETTERER. 1969. Ultrastructure of an asymmetrically thickened plasma membrane. *Proc. Roy. Microsc. Soc.* 4: 118.
- ROBERTSON, J. D. 1963. The occurrence of a sub-unit pattern in the unit membranes of club endings in Mauthner cell synapses in goldfish brains. *J. Cell Biol.* 19:201.
- REVEL, J. P., W. OLSEN, and M. J. KARNOVSKY. 1967. A twenty-Angstrom gap junction with a hexagonal array of sub-units in smooth muscle. *J. Cell Biol.* 35:112A.
- REVEL, J. P., and M. J. KARNOVSKY. 1967. Hexagonal array of sub-units in intercellular junctions of the mouse heart and liver. *J. Cell Biol.* 33:C7.
- BENEDETTI, E. L., and P. EMMELOT. 1965. Electron microscopic observations on negatively stained plasma membranes isolated from rat liver. *J. Cell Biol.* 26:299.
- BENEDETTI, E. L., and P. EMMELOT. 1968. Hexagonal array of sub-units in tight junctions separated from isolated rat liver plasma membranes. *J. Cell Biol.* 38:15.
- FINEAN, J. B., and M. G. RUMSBY. 1963. Negatively stained lipoprotein membranes: The validity of the image. *Nature (London)*. 197: 1326.
- LUZZATI, V., and F. HUDSON. 1962. The structure of the liquid-crystalline phases of lipid-water systems. *J. Cell Biol.* 12:207.
- BAKER, J. R. 1958. In Principles of Biological Microtechnique. A Study of Fixation and Dying. John Wiley & Sons Inc., New York. 36.
- PORTER, K. R., K. KENYON, and S. BADENHAUSEN. 1967. Specializations of the unit membrane. *Protoplasma*. 63:262.
- ROBERTSON, J. D. 1966. Granulo-fibrillar and globular substructure in unit membranes. *Ann. N.Y. Acad. Sci.* 137:421.
- ROBERTSON, J. D. 1966. Design principles of the unit membrane. In Principles of biomolecular Organisation. G. E. W. Wolstenholme and M. O'Connor, editors. Churchill Ltd., London. 357.
- STOECKENIUS, W., and D. E. ENGELMAN. 1969. Current models for the structure of biological membranes. *J. Cell Biol.* 42:613.
- KOSS, L. G. 1969. The asymmetric unit membranes of the epithelium of the urinary bladder of the rat. *Lab. Invest.* 21:154.
- ROBERTSON, J. D. 1969. Molecular structure of biological membranes. In Handbook of Molecular Cytology. A. Lima-de-Faria, editor. North Holland Publishing Co., Amsterdam. 1403.

21. BENEDETTI, E. L., and P. EMMELOT. 1968. Structure and function of plasma membranes isolated from liver. In *The Membranes*. A. J. Dalton and F. Haguenu, editors. Academic Press Inc., New York. 33.
22. DERJAGUIN, B. V. 1965. Recent research into the properties of water in thin films and in microcapillaries. *Symp. Soc. Exp. Biol.* **19**:55.
23. BANGHAM, A. D., and D. R. BANGHAM. 1968. Very long-range structuring of liquids, including water, at solid surfaces. *Nature (London)*. **219**:1151.
24. SZENT-GYORGYI, A. 1957. *Bioenergetics*. Academic Press Inc., New York.
25. KLOTZ, I. M. 1958. Protein hydration and behaviour. *Science (Washington)*. **123**:815.
26. HAZLEWOOD, C. F., B. L. NICHOLS, and N. F. CHAMBERLAIN. 1969. Evidence for the existence of a minimum of two phases of ordered water in skeletal muscle. *Nature (London)*. **222**:747.
27. COPE, F. W. 1969. Nuclear magnetic resonance evidence using D<sub>2</sub>O for structured water in muscle and brain. *Biophys. J.* **9**:303.
28. HICKS, R. M., B. KETTERER, and D. BEALE. 1968. The isolation and analysis of the plasma membrane lining the lumen of the rat bladder. *Biochem. J.* **109**:41P.
29. WALLACH, D. F. H., and P. H. ZAHLER. 1968. Infra-red spectra of plasma membrane and endoplasmic reticulum of Ehrlich's ascites carcinoma. *Biochim. Biophys. Acta.* **150**:186.
30. BLUMENFIELD, O. O. 1968. The proteins of the erythrocyte membrane obtained by solubilization with aqueous pyridine solution. *Biochem. Biophys. Res. Commun.* **30**:200.
31. EICHBERG, J., V. P. WHITTAKER, and R. M. C. DAWSON. 1964. Distribution of lipids in subcellular particles of guinea-pig brain. *Biochem. J.* **92**:91.