ELECTRON MICROSCOPY OF ABSORPTION OF TRACER MATERIALS BY TOAD URINARY BLADDER EPITHELIUM

JAE KWON CHOI, M.D.

From the Department of Biological Structure, University of Washington School of Medicine, Seattle, Washington, and the Department of Anatomy, University of California School of Medicine, San Francisco, California. Present address, Department of Anatomy, Chonnam University Medical School, Kwangju, Chonnam, Korea

ABSTRACT

The absorption of Thorotrast and saccharated iron oxide by the epithelium of the toad urinary bladder was studied by electron microscopy. Whether the toads were hydrated, dehydrated, or given Pitressin, no significant differences in transport of colloidal particles by epithelial cells were observed. This implies that these physiological factors had little effect on the transport of the tracer particles. Tracer particles were encountered in three types of epithelial cells which line the bladder lumen, but most frequently in the mitochondria-rich cells. Tracer materials were incorporated into the cytoplasm of epithelial cells after being adsorbed to the coating layer covering the luminal surface of the cells. In the intermediate stage (1 to 3 hours after introducing tracer) particles were present in small vesicles, tubules, and multivesicular bodies. In the later stages (up to 65 hours), the particles were more commonly seen to be densely packed within large membrane-bounded bodies which were often found near the Golgi region. These large bodies probably were formed by the fusion of small vesicles. Irrespective of the stages of absorption, no particles were found in the intercellular spaces or in the submucosa. Particles apparently did not penetrate the intercellular spaces of the epithelium beyond the level of the tight junction.

INTRODUCTION

The urinary bladder of the toad is of interest to biologists because of its ability to transport substances through its walls and because of the relative ease with which its structure can be related to its function. Water can be reabsorbed from urine in response to dehydration or administration of neurohypophyseal hormone (18, 49, 51). From *in vitro* experiments, it seems that water and urea move passively across the bladder wall whereas sodium appears to be driven across actively (33). Transport of these molecules and ions is enhanced by a mammalian neurohypophyseal hormone (6, 23, 25, 33, 34) or aldosterone administration (14) to the serosal side of the bladder wall.

Recently, several electron microscopic studies have dealt with this interesting tissue under normal, *in vivo* (11, 12, 46), and experimental, *in vitro* (10, 42, 46) conditions. Little is known about the mechanisms involved in the movement of ions and small molecules, although Thorotrast and saccharated iron oxide, which are easily visible in the electron microscope, have been used to study the possible mechanisms for transport of such substances. In this study, attempts were made to

TABLE I

Time Intervals of Fixation of Urinary Bladders Following Infusion of Tracers (Thorotrast or Saccharated Iron Oxide) and the Number of Toads Studied under Hydration, Dehydration, or Pitressin Injection

Time after administration of tracer	Number of Toads		
	Hydrated	Dehydrated	Pitressin-injected
10 min.		1	
40 min.		1	
1 hr.	1	1	2
3 hrs.	2	2	
14 hrs.		3	
65 hrs.	1	2	

investigate the movement of such particulate tracer substances across the bladder walls.

MATERIALS AND METHODS

The urinary bladders of the toad *Bufo marinus* were used. Thorotrast or saccharated iron oxide (Proferrin), used as supplied by the manufacturer (aqueous colloidal solutions), was the tracer employed. Animals were kept in a dry place for 2 to 5 hours before and throughout the experiments in order to dehydrate them and thereby insure the retention of tracer materials in the bladder lumen. To study the effects of Pitressin administration on the absorption of tracer materials by the bladder epithelium, two animals were injected with Pitressin (1 unit) 5 minutes prior to Thorotrast infusion. Hydrated animals were also investigated for comparison with the dehydrated or Pitressin-injected animals.

Bladder tissues were fixed at intervals of 10 minutes to 65 hours following the infusion of Thorotrast (0.2 to 0.4 cc) or saccharated iron oxide (0.4 cc) into the bladder lumen via a narrow-tipped glass pipette inserted through the cloaca (consult Table I for schedule). The tissues were fixed *in situ* with ice-cold 2 per cent OsO_4 in *s*-collidine buffer (5) by simultaneously injecting it into the lumen of the bladder and applying it to the serosal surface. Portions of the ventral wall of the bladder about 1 to 2 cm from the mid-line were removed, cut into pieces, and placed in fresh fixative for 2 hours at 0°C. The specimens were dehydrated in ethanol, embedded in Epon (35), and cut on a Porter-Blum microtome with glass knives. Stained (15) or unstained thin sections were examined in an RCA EMU 2C. All photographs were taken on Kodak Fine Grain Positive film (56).

OBSERVATIONS

The urinary bladder of the toad consists of epithelium, submucosa, and seroa, all of which are characteristic in their components.

The bladder epithelium consists of one or two cellular layers and varies in thickness from about 5 up to 15 μ. In the epithelium, four types of cells are found: granular cells, mitochondria-rich cells, mucous cells, and basal cells (12). Some of these features are illustrated in Fig. 4.

Since tracer materials were incorporated only into the granular cells, the mitochondria-rich cells, and the mucous cells, and not into the basal or migratory cells, only the first three cellular types will be discussed in this paper.

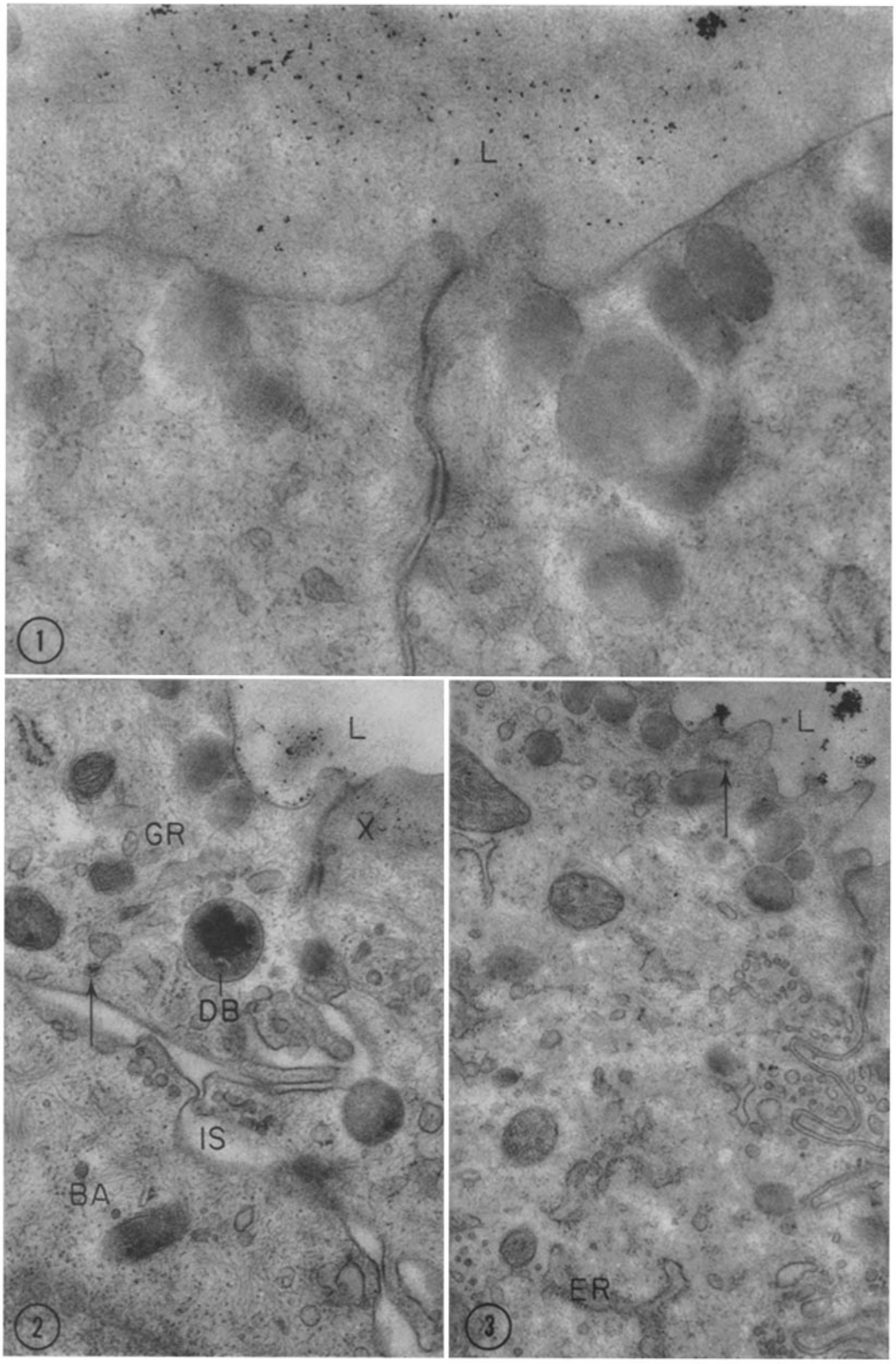
Observations after Tracer Administration

For the convenience of description, the stages which were observed are divided into early, intermediate, and late stages. Although it is difficult to

FIGURE 1 Ten minutes after Thorotrast administration under dehydration. Particles of Thorotrast are embedded in the outer portion of the extraneous luminal (*L*) coating on the epithelial cells of toad urinary bladder. In the coating layer are seen filamentous structures. No particles are present in the cytoplasm of the cells. × 38,000.

FIGURE 2 One hour after Thorotrast administration under dehydration. Portions of granular cells (*GR*) and basal cells (*BA*) are shown. Thorotrast particles are close to the luminal (*L*) plasma membrane. (Those at *X* are assumed to be extracellular, but in tangential section.) A vesicle containing particles is marked by the arrow. Intercellular spaces (*IS*) and a dense body (*DB*) do not show the particles. × 27,000.

FIGURE 3 One hour after Thorotrast administration under the influence of Pitressin. Thorotrast particles, clumped and dispersed, are seen close to the luminal (*L*) plasma membrane of granular cells. Such clumping of particles is not frequently seen at this time period. A vesicle containing particles is seen at the arrow. *ER* indicates endoplasmic reticulum. × 27,000.


L
1
L
GR
X
DB
IS
BA
2
L
ER
3

make clear distinctions between these stages, there are differences in the number and distribution of intraluminal and intracytoplasmic particles.

1. Early Stage after Administration of Tracer Material (Thorotrast) to Dehydrated Animals (10 Minutes up to 1 Hour)

The tracer particles were sparse in the coating layer covering the luminal surface of the bladder epithelium. Early in the time period (10 minutes), particles were numerous in the superficial portion of the coating layer which often appeared as a coarse filamentous zone running parallel to the surface membrane, or else in irregular dense masses (Fig. 1). This coating layer often appeared to be thicker than that of normal epithelium which is usually only about 0.5 μ thick.

One hour following the administration of Thorotrast (Figs. 2 and 3), the majority of the tracer particles were found dispersed near the luminal surface of the cells. A rare instance of clumping is shown in Fig. 3. The distribution and concentration of the particles in the lumen varied considerably. Some areas showed much greater concentration than others. During the first 40 minutes after tracer administration, a few vesicles in the apical cytoplasm of an occasional cell were labeled with particles. However, 1 hour after administration of colloidal solution, more cells showed a small number of vesicles or tubules containing one or more particles. Even in this early time period, multivesicular bodies (50) containing a few particles were found in the epithelial cells. Sometimes a cloud of vesicles (Fig. 13) with or without particles, resembling mulberries, was found. These structures were similar to the multivesicular bodies but lacked the outer surrounding membrane. The slow uptake of particles by the epithelial cells of the dehydrated animal did not appear to be altered by Pitressin administration. Fig. 3 illustrates portions of granular cells after Pitressin injection; no apparent difference in particle uptake is seen.

2. Intermediate Stage of Thorotrast Absorption (2 to 3 Hours)

At the luminal surface, in the superficial portion of the coating layer, tracer particles appeared frequently to form aggregates (Figs. 4 to 6), some of which were interfused with material of moderate density (Fig. 4). On the other hand, in the inner zone of the filamentous coating layer, a larger number of sparsely distributed particles were seen close to the surface membrane (Figs. 5 and 6).

More than half of the population of cells was labeled with particles in some way. These particles were present only in membrane-limited compartments. Fig. 5 shows a portion of a granular cell in which vesicles (*V*), tubules (*T*), and multivesicular bodies (*MV*) contain various numbers of colloidal particles. In multivesicular bodies (*MV*, Figs. 5, 6, and 14) particles were present both in the small vesicles and the matrix.

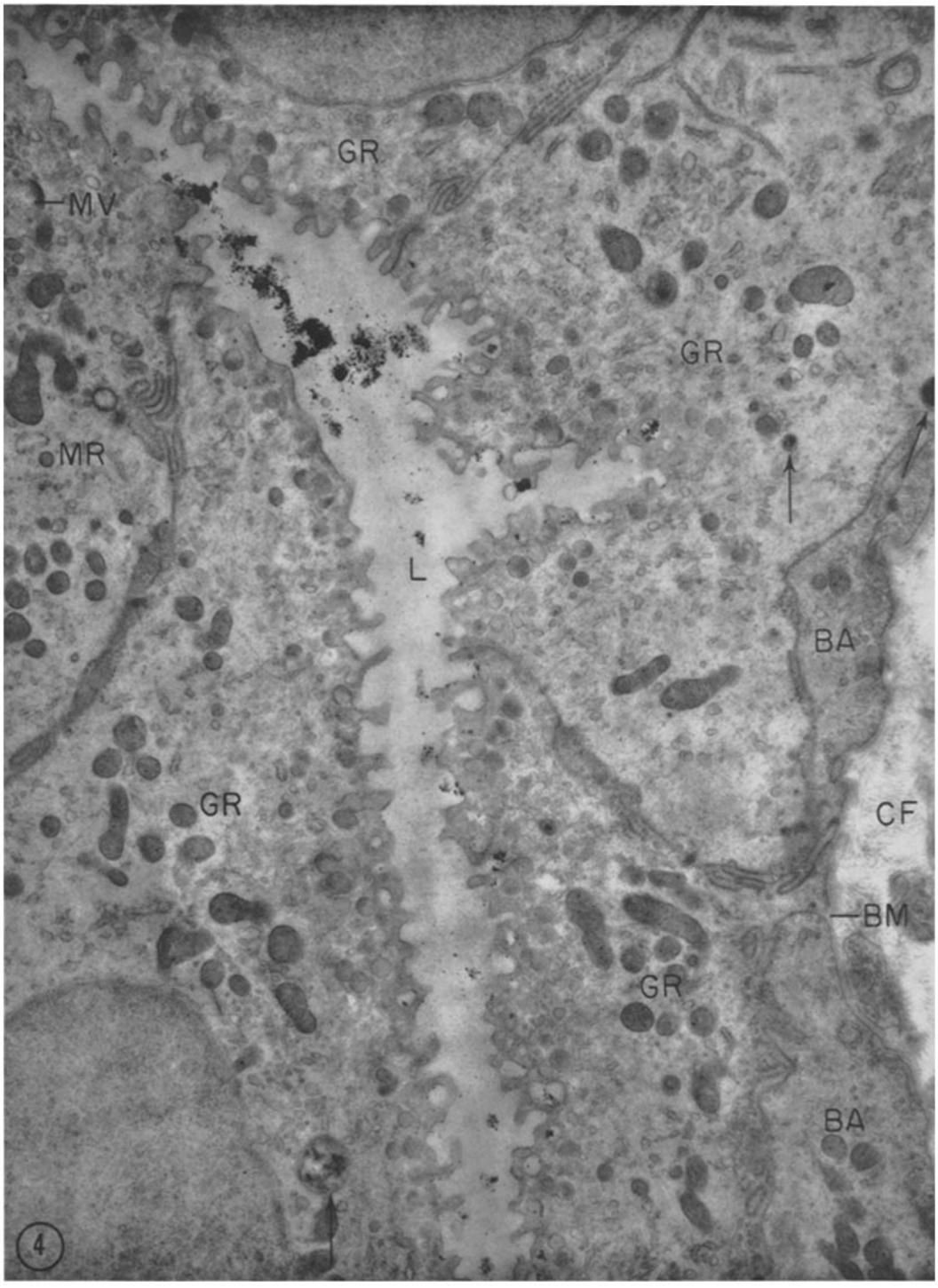
In one hydrated animal in which Thorotrast was administered, the number of particles within the cytoplasm was very small. Only in rare cases were a few vesicles and multivesicular bodies labeled in the granular cells, although many particles were present in membrane-bounded bodies of the mitochondria-rich cells, such as shown in Fig. 6.

3. Later Stage of Colloidal Particle Absorption (14 to 65 Hours)

The number of small vesicles (400 A) containing particles was drastically reduced, although the number of cytoplasmic vacuoles or membrane-bounded bodies of various types (Figs. 7, 8, 10 to 12, 15) containing widely varying amounts of

---

Figure 4 Three hours after Thorotrast administration under hydration. Low-power electron micrograph of a section through a folded portion of the epithelium and submucosa. Thorotrast particles are seen in the lumen (*L*) both as clumps and dispersed particles. Although particles are difficult to identify and reproduce at this magnification, they appear in a multivesicular body (*MV*) of a mitochondria-rich cell (*MR*). Cell boundaries with regular intercellular spaces and interdigitations are seen. Also shown are several granular cells (*GR*), portions of basal cells (*BA*), basement membrane (*BM*), and submucosa with collagenous fibers (*CF*). Arrows indicate dense bodies which do not contain Thorotrast particles. These bodies are also present frequently in the epithelial cells of bladders not exposed to markers. × 6,000.


GR
MV
GR
MR
L
BA
GR
CF
BM
GR
BA
4

tracer particles was increased. These particle-containing bodies usually were in the mid-region of the cells and often near the Golgi region (Figs. 7, 8, and 11). After Proferrin administration (Fig. 8), the particle-containing bodies were generally larger than those found after Thorotrast administration (Fig. 7). No deposits were seen within the vesicles or cisternae of the Golgi apparatus. The number of typical multivesicular bodies containing particles appeared to be decreased. In the lumen of the bladder, almost all of the particles appeared to be aggregated and to form masses of particles. A few dispersed particles were still present at the luminal surface.

Although electron microscope data are not well suited for quantitative estimations, it appeared that granular cells did not take up particles as readily as mitochondria-rich cells. For example, during the time period from 1 hour up to 65 hours after tracer administration, almost all of the mitochondria-rich cells (Figs. 4, 6, 10 to 12) were found to be labeled when they were sectioned through the appropriate area of cytoplasm. Often the granular cells in the same section were not labeled or showed a few labeled structures (Fig. 6). In some cases, particles were much more closely aggregated at the luminal surrace of mitochondria-rich cells (Fig. 10) than at the surface of the granular cells. Adjacent to the inner lamina of the luminal plasma membrane of mitochondria-rich cells in both normal and tracer-treated animals were often hairlike structures (insert, Fig. 10) similar to those described in some cells of other tissues (48, 55). Mucous cells (Fig. 16) were also labeled, the particles being located in membrane-limited structures such as the large vacuoles, vesicles, and multivesicular bodies in the luminal region of the cells. Examination of serial sections revealed that the large vacuoles were found to be connected to the lumen. However, the number of particles in the cytoplasm of mucous cells was very few.

In all the stages of absorption studied in this experiment, no examples were seen of particles in vesicles along the lateral membrane of the epithelial cells, or in the submucosa (Figs. 8, 9, and 17). Colloidal particles were never found in the tight junction area (21) (Fig. 6) nor were they seen between the cells below this level. However, particles were often present at the serosal surface of the mesothelial cells at all time intervals after the administration of tracer material (arrow, Fig. 18).

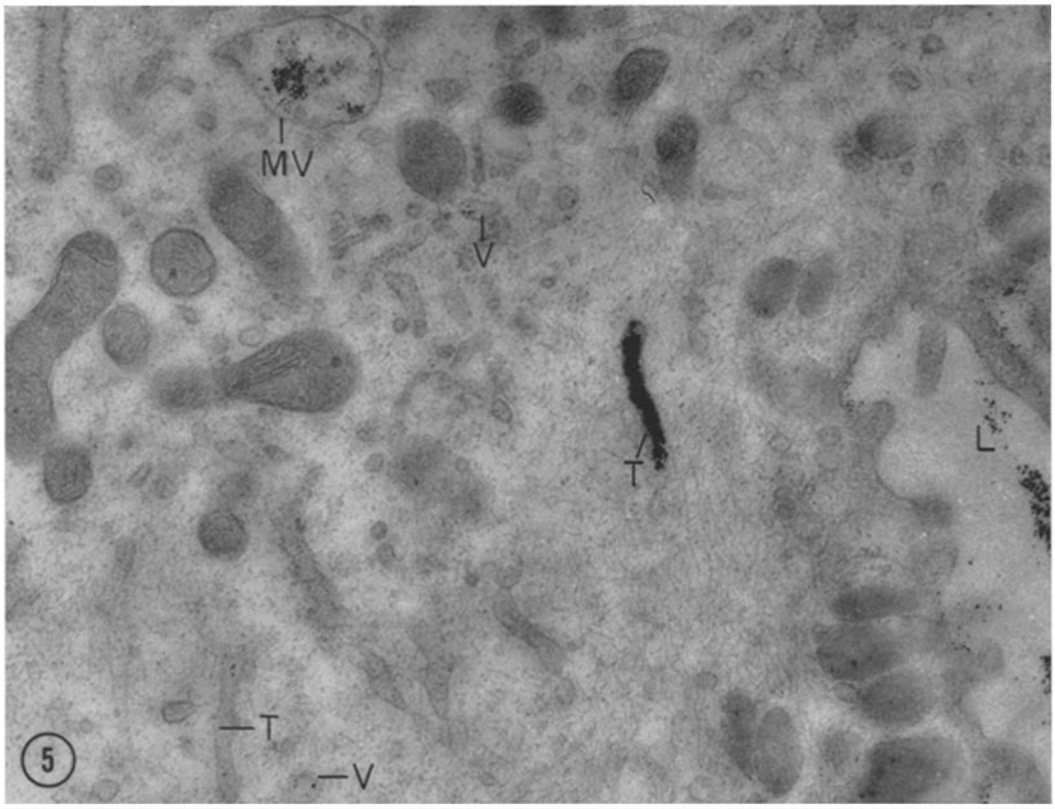
DISCUSSION

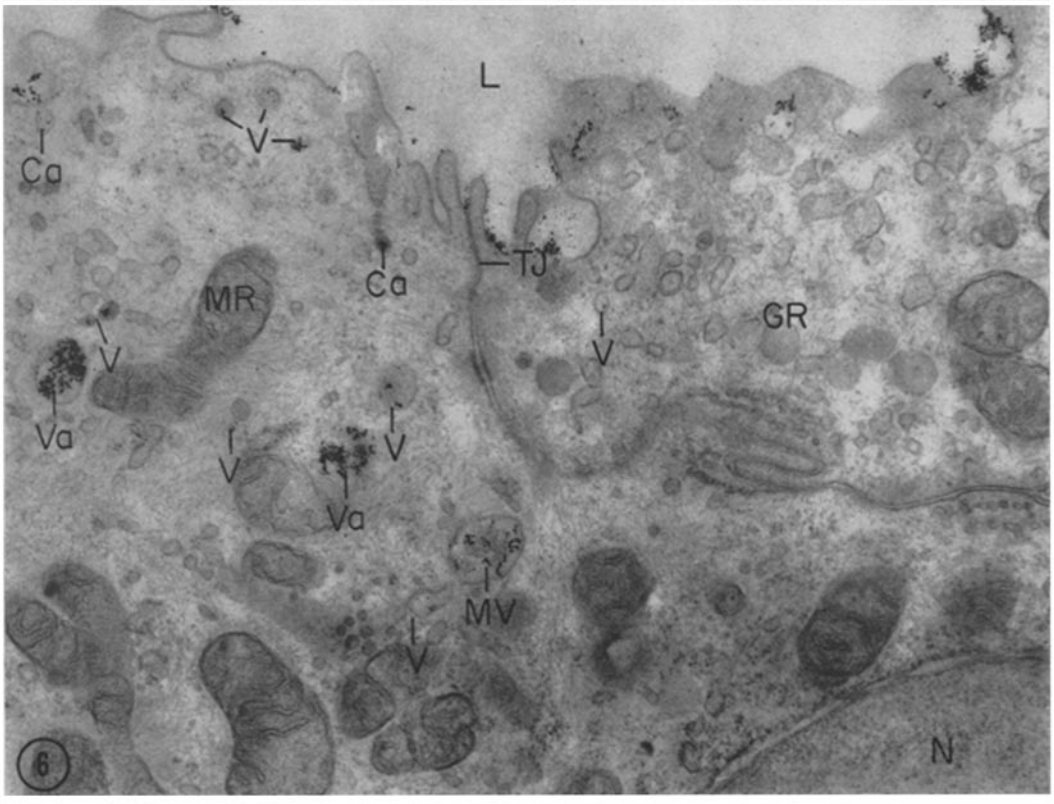
It is apparent from the data obtained in this study that the tracer substances introduced into the lumen of the toad bladder can be taken up by some of the epithelial cells lining the surface.

In the early stage of absorption, the markers are seen in the coating layer of the bladder epithelium, suggesting that this layer may have an important function as the binding site or primary barrier for certain molecules. It has been shown (12) that the substance comprising the coating layer of the normal toad bladder is morphologically filamentous (antennulae of Yamada, 57) and histochemically either a mucoprotein or a mucopolysaccharide or both. Furthermore, it was postulated that this layer can function as an ion exchange resin which can bind cations in the lumen for subsequent transport into the cell (4, 12). Filamentous extraneous (or cell) coatings have been observed on the surface of various transporting cells of other tissues (7–9, 29, 52). In these

FIGURE 5 Three hours after Thorotrast administration under dehydration. Clumped as well as dispersed particles are seen in the lumen (*L*) near the plasma membrane of granular cell. In the cytoplasm, the vesicles (*V*), tubules (*T*) (possibly smooth-surfaced endoplasmic reticulum), and multivesicular bodies (*MV*) contain considerable amounts of Thorotrast particles. × 28,000.

FIGURE 6 Three hours after Thorotrast administration under hydration. Apical portions of mitochondria-rich cell (*MR*) and granular cell (*GR*) are shown. Thorotrast particles are seen in the lumen (*L*) close to the plasma membrane both as clumps and dispersed particles. In the cytoplasm of the mitochondria-rich cell, the vesicles (*V*), caveolae (*Ca*), vacuoles (*Va*), and multivesicular bodies (*MV*) contain particles, although only one vesicle in the granular cell (*GR*) contains particles. No Thorotrast particles appear in the intercellular space below the level of the tight junction (*TJ*). *N* indicates nucleus. × 22,000.


MV
V
T
L
T
V
5


L
V
Ca
Ca
TJ
MR
GR
V
V
Va
V
V
Va
MV
V
N
6

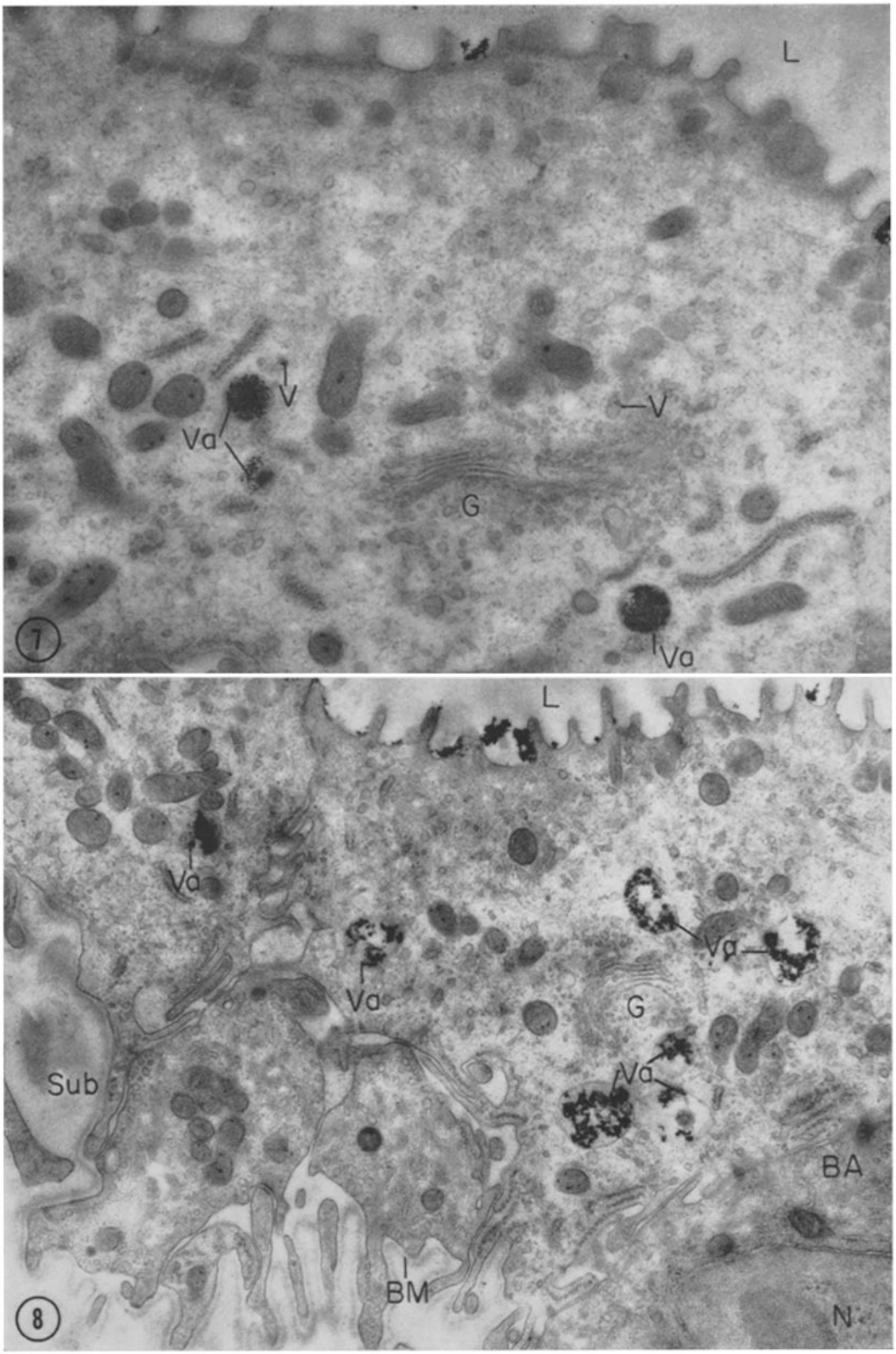
tissues also, this filamentous structure was implicated as the binding material for the function of absorption. Bennett (2) suggested that, as a mechanism for selective absorption, the exposed surface of the cell might possess binding groups which would hold suitable particles to the external surface of the membrane. Recently, Bennett (4) proposed the term "glycocalyx" for the extracellular polysaccharide-rich coating structure common to many types of cells and discussed in detail the functional significance of this structure with respect to the mechanism of molecular absorption by the cells.

The observed increase in the thickness of these coating layers in the early stage of absorption may be caused by enhanced secretion of mucous substance from mucous or granular cells, or from both, due presumably to stimulation by Thorotrast. It has also been described by Brandt and Pappas (8) in the form of a thickening of the filamentous coat of the plasmalemma of the amoeba, and by Kaye *et al.* (29) in the form of a thickening of the amorphous coat of the corneal endothelium, after exposure to thorium dioxide. In the later stage of absorption in the toad bladder, further entry of the Thorotrast particles into the cell may be prevented by the aggregation of these particles in the coating layer itself. Therefore, beside the function of adsorption, it is apparent that this layer would also have a protective function, as suggested by Rhodin *et al.* (47) for tracheal cells. It is possible that this coating layer, and especially the outer portion, may be constantly replaced, even under normal conditions of bladder epithelial cell activity, by newly secreted mucous substance.

In this study, the finding that vesicles and caveolae are labeled with particles 1 hour after infusion of tracer supports the notion that vesiculation at the plasma membrane, in general, provides a means of absorption, transport, or accumulation of substances by cells as suggested by Bennett (2), Palade (43), and Holter (27). The presence of ingested particles in the multivesicular bodies in the relatively early stage confirms the findings of Farquhar *et al.* (19, 20) and would suggest that multivesicular bodies are one of the intermediates in the development of dense bodies of complex internal structure from small, isolated vesicles. These dense bodies may grow in size at the expense of other smaller vesicles, resulting in the accumulation of a few, large, fully packed vacuoles, usually near the Golgi region. These accumulations of particles in the dense bodies confirm previous workers' results on liver (24), kidney (19, 20, 32, 52), mesothelium (41), small intestine (13) and bladder of other species (53). This process of formation of dense bodies appears to be similar to that of formation of lysosomes as suggested by Bennett (3), Novikoff (40) and de Duve (16), in that pinocytosed materials may be stored in the cytoplasm as a segregated mass in which hydrolytic enzyme activity is high. To establish that these Thorotrast-containing bodies are lysosomes requires demonstration of acid hydrolase activity within them. The remaining problem is the final disposition of the tracer particles. If the tracer particles move through the epithelium into the submucosa, the process must be very slow since no tracer particles were found in the submucosa up to 65 hours. The presence of colloidal particles on the coelomic side of the bladder is interpreted as contamination due to the urine flow from the bladder lumen over the coelomic surface of the serosal cells when the bladder is opened during fixation. The presence of such particles bears no relationship to the time of colloid administration, and successive stages of absorption by the serosal cells were not found. In any case, the mechanism

FIGURE 7 Fourteen hours after Thorotrast administration under dehydration; portion of a granular cell is shown. Aggregated particles are seen between the microvilli in the lumen (*L*), and round bodies (*Va*) packed with particles are found near the Golgi region (G). A few vesicles (*V*) containing particles are also seen. × 23,000.

FIGURE 8 Fourteen hours after saccharated iron oxide administration under dehydration. General findings are similar to those of Fig. 7, although particle-containing bodies (*Va*) are larger than those seen in Fig. 7. These bodies are close to Golgi region (*G*). No particles are seen in the submucosa (*Sub*) or intercellular spaces. A portion of a basal cell (*BA*) with its nucleus (*N*) is also shown. *L* and *BM* indicate lumen and basement membrane, respectively. × 15,000.


L
V
V
Va
G
Va
7
L
Va
Va
Va
G
Va
Sub
BA
BM
N
8

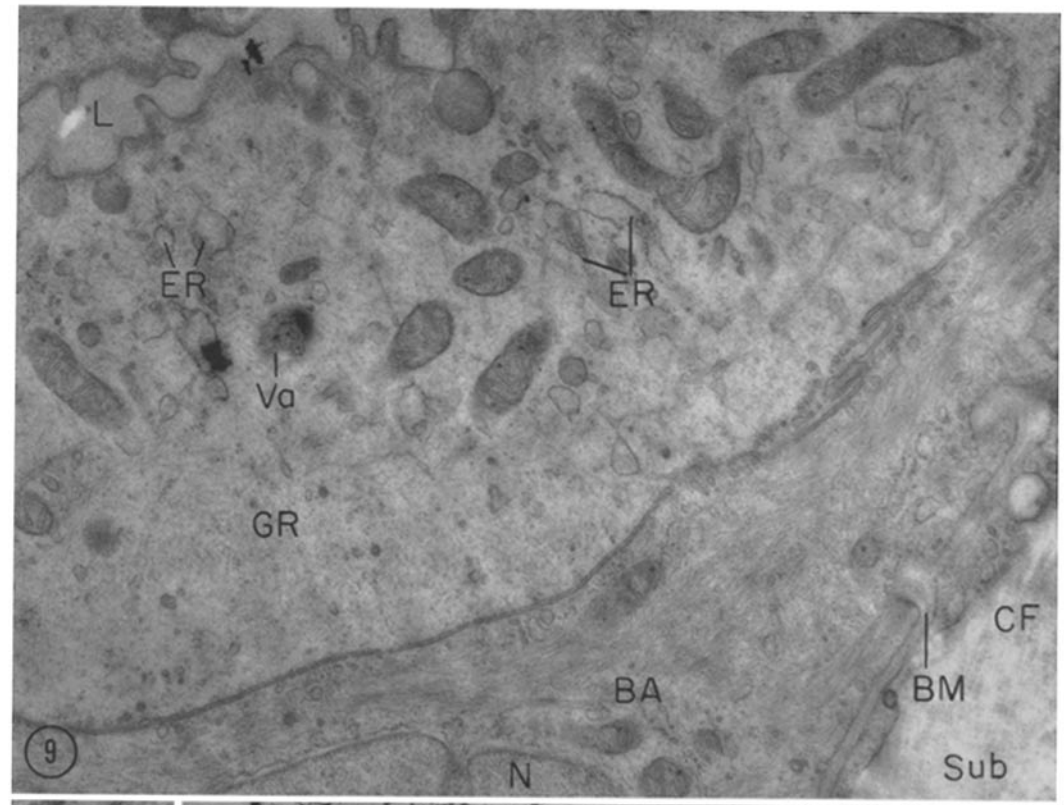
involved in the transport of these colloidal materials into the epithelium of the toad bladder does not appear to be related to water or ion transport because the tracer particles used in the present study apparently are not transported into the submucosa under dehydration or Pitressin injection, which is known to enhance the transport of water through the epithelium of the toad bladder *in vivo* (18, 49, 51) and also of sodium *in vitro* (6, 23, 25, 33, 34). Kaye *et al.* (30) reported experimental work on the normal rabbit corneal epithelium *in vitro*, showing that thorium dioxide particles may not be transported into the epithelium in spite of the fact that sodium ions are rapidly and apparently actively transported through the rabbit corneal epithelium (17).

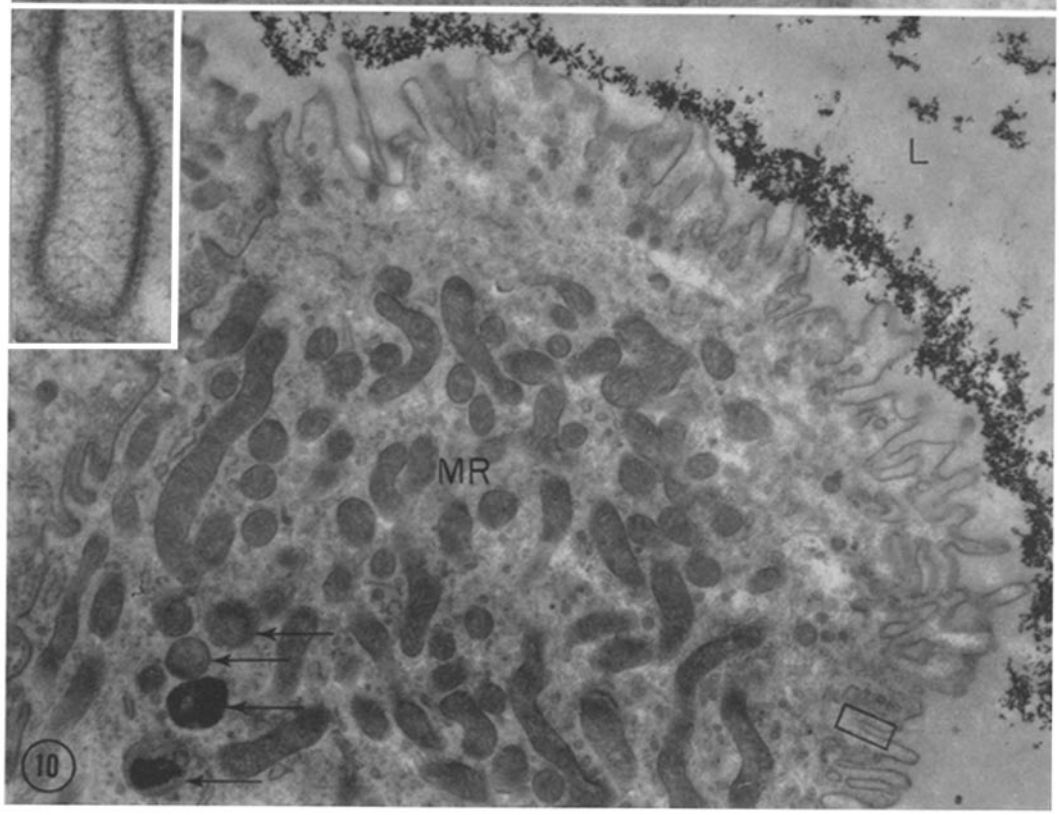
In connection with water transport in urinary bladder epithelial cells under the influence of mammalian hypophyseal hormone, pores of about 40 A in the cell membrane, as calculated by Hays and Leaf (25), have not been visualized in the electron microscope (42, 46). However, apparent widening of intercellular spaces (10, 42) and cellular swelling (10, 46) have been reported. The question arises whether the hormonal enhancement of sodium and water transport *in vitro* is accompanied by a modification of the vesicles in the cytoplasm. Different species appear to give differing results, for Pak Poy and Bentley (42) and Peachey and Rasmussen (46) found no changes in vesicles in the bladder epithelial cells in *Bufo marinus*, while Carasso *et al.* (10) described a significant increase in the number of vesicles in the bladder epithelial cells of *Bufo bufo* following hormonal stimulation. However, a more complete comparative study is required before general answers can be given.

Different cell types appear to handle colloidal tracers in different fashions. Colloidal particles are described as being transported, from one side of the cell to the other side, through endothelial cells of the blood vessels (1, 28, 36, 45, 54) and of the cornea (29, 30), and through gall bladder epithelial cells (26) and hepatic cells (24). Such transport occurs in caveolae and vesicles as well as through intercellular spaces in some cases (22, 29, 30, 45). This mechanism could be related to water or fluid transport or to the passage of protein through the cells. Nemoto's light microscope study (39) of the ingestion of India ink, corn oil, and milk by the bladder epithelial cells in the dog showed the appearance of aggregated substances in the submucosa within 48 hours after administration. Also, vegetable oil introduced into the lumen of the rabbit urinary bladder was found to be present in the vesicular components of epithelial cytoplasm (31). The latter study was limited to the early stage of absorption (30 minutes), which makes difficult a comparison with fat absorption through the epithelium of the intestinal villi described by Palay and Karlin (44). Besides effecting transcellular transport of colloidal particles, some cells store the particles in the cytoplasm for an extended period of time, as described for glomerular epithelium (19), mammalian bladder epithelium (53), and in the present study.

FIGURE 9 Sixty-five hours after Throtrast administration under dehydration. The full thickness of bladder epithelium is seen, showing portions of granular cell (*GR*) and basal cell (*BA*) with its nucleus (*N*). In the lumen (*L*), clumped particles are seen. Round body (*Va*) containing particles is present. Intercellular space is of normal width. Mitochondria and dilated endoplasmic reticulum (*ER*) are seen in the cytoplasm. No particles are seen in intercellular spaces or in the submucosa (*Sub*). *CF* and *BM* indicate collagenous fibers and basement membrane, respectively. × 14,000.

FIGURE 10 Sixty-five hours after Thorotrast administration under hydration. Apical portion of a mitochondria-rich cell (*MR*) is shown facing lumen (*L*) of bladder. Clumped particles are numerous along the luminal surface of the cell. Dense bodies (arrows) packed with Thorotrast particles are seen in the cytoplasm. Note that the luminal plasma membrane of the mitochondria-rich cells generally appears to be thicker or more dense than that of other epithelial cells. At higher magnification, characteristic hairlike structures are seen immediately subjacent to the luminal plasma membrane. This latter feature is shown in the inset which represents the area of the small rectangle in the larger figure. × 9,000. Inset, × 71,000.


L
ER
ER
Va
GR
CF
BM
BA
N
Sub
9


L
MR
10

It is apparent, therefore, that the mechanisms of transport of the same or similar substances differ according to the cell type, although the mechanism of uptake into the cell may be the same. This is supported by the fact that the morphology of Thorotrast absorption (penetration) in the early stages is similar in a wide variety of cells.

Tight Junction

The fact that no tracer particles were found in or below the tight junction (zonula occludens) indicates that these areas are not a pathway for particles of this size in the toad bladder. As indicated by others (21, 29, 37, 38), the tight junction area may well be the barrier to penetration of certain molecules. In this manner it may serve to seal off the intercellular space from the lumen. Since no separation was seen in the tight junction area after Pitressin injection or dehydration (10, 42, 46), additional indirect support is gained for the view that the tight junction may be a barrier to penetration of the water molecules and ions from the lumen.

Cellular Difference in Uptake of Particles

All three types of epithelial cells which border the lumen of the toad bladder ingested the marker substance. Although there is some variability in the uptake of particles from cell to cell in the same animal, the great abundance of ingested particles seen in mitochondria-rich cells indicates that this cell type is the more active in ingesting particles and perhaps other materials. In this regard, it is of especial interest that the mitochondria-rich cells frequently show a characteristic hirsute structure immediately beneath the luminal plasma membrane. Quite similar structures have been described recently by Wissig (55) at the apical plasmalemma of proximal convoluted tubule cells of kidney and by Roth and Porter (48) on the surface of the developing mosquito oocyte which they relate to protein uptake. The abundant mitochondria in the mitochondria-rich cells may be expected to provide energy for the pinocytotic or phagocytotic activity.

The author is most grateful to Dr. John H. Luft for aid and advice during the course of this study and the preparation of the manuscript. His thanks are extended to all the members of the Department of Biological Structure, University of Washington, Seattle, for their kind help and encouragement.

This work supported by Research Grants NB-00401, H-5251, and H-4512 from the United States Public Health Service.

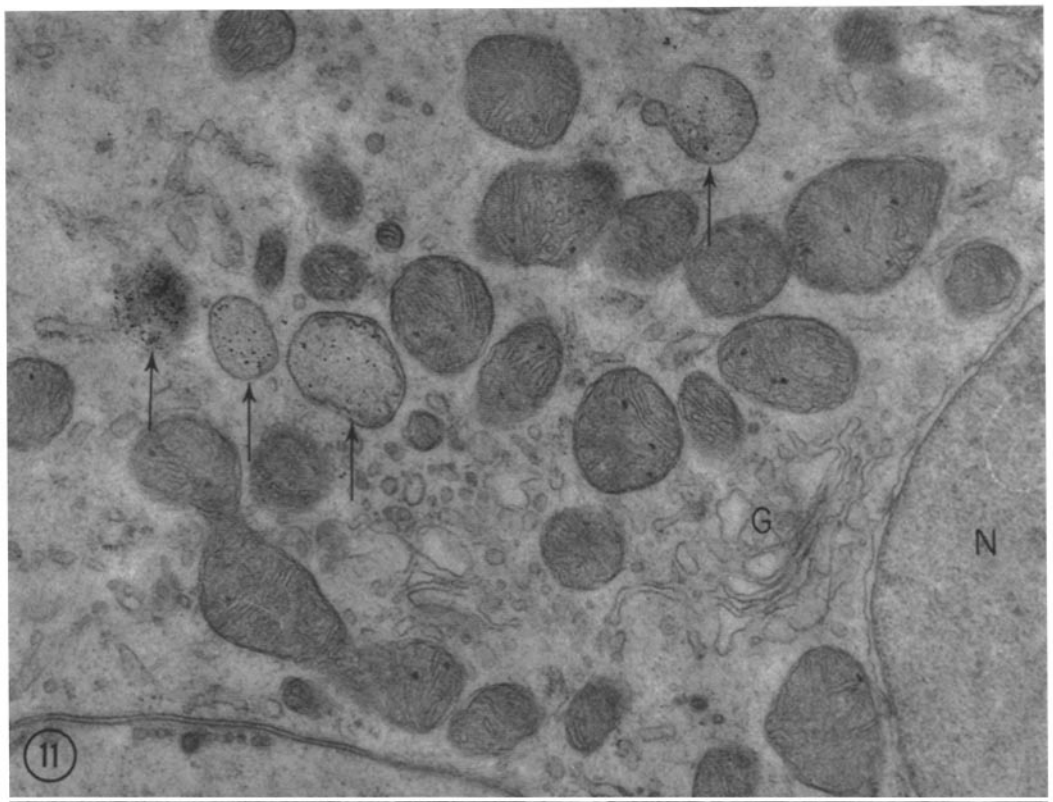
Received for publication, August 26, 1963.

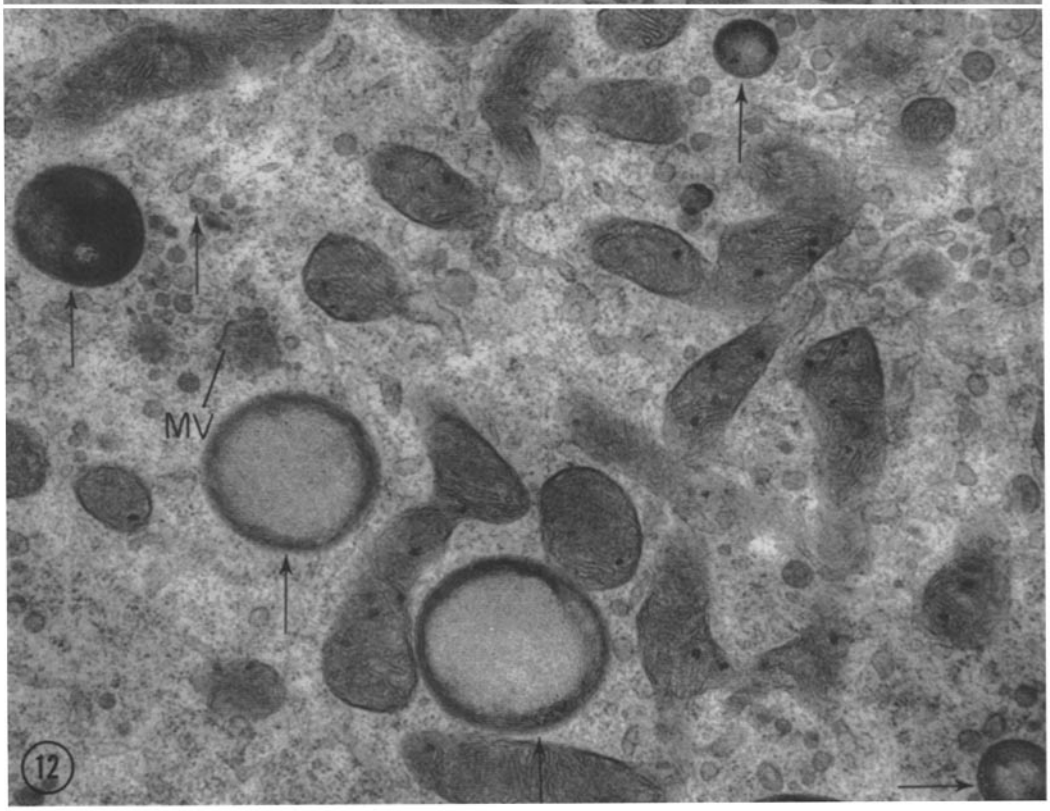
REFERENCES

1. ALKSNE, J. F., The passage of colloidal particles across the dermal capillary wall under the influence of histamine, *Quart. J. Exp. Physiol.*, 1959, **44**, 51.
2. BENNETT, H. S., The concepts of membrane flow and membrane vesiculation as mechanisms for active transport and ion pumping, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 99.
3. BENNETT, H. S., A suggestion as to the nature of the lysosome granules, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 185.
4. BENNETT, H. S., Morphological aspects of extracellular polysaccharides, *J. Histochem. and Cytochem.*, 1963, **11**, 14.
5. BENNETT, H. S., and LUFT, J. H., *s*-Collidine as a basis for buffering fixatives, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 113.
6. BENTLEY, P. J., The effects of neurohypophysial extracts on water transfer across the wall of the isolated urinary bladder of the toad, *Bufo marinus*, *J. Endocrinol.*, 1958, **17**, 201.
7. BRANDT, P. W., A study of the mechanism of pinocytosis, *Exp. Cell Research*, 1958, **15**, 300.
8. BRANDT, P. W., and PAPPAS, G. D., An electron

FIGURE 11 Sixty-five hours after Thorotrast administration under dehydration. Portion of a mitochondria-rich cell is shown. Several bodies containing particles (arrows) are seen near the Golgi structure (*G*) which is dilated. *N* indicates nucleus. × 22,000.

FIGURE 12 Sixty-five hours after Thorotrast administration under hydration. The deeper portion of a mitochondria-rich cell shows various membrane-limited bodies (arrows) with complex internal structure containing particles. Multivesicular body (*MV*) does not contain particles. × 23,000.


G
N
11


MV
12

microscopic study of pinocytosis in amoeba. 1. The surface attachment phase, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 675.

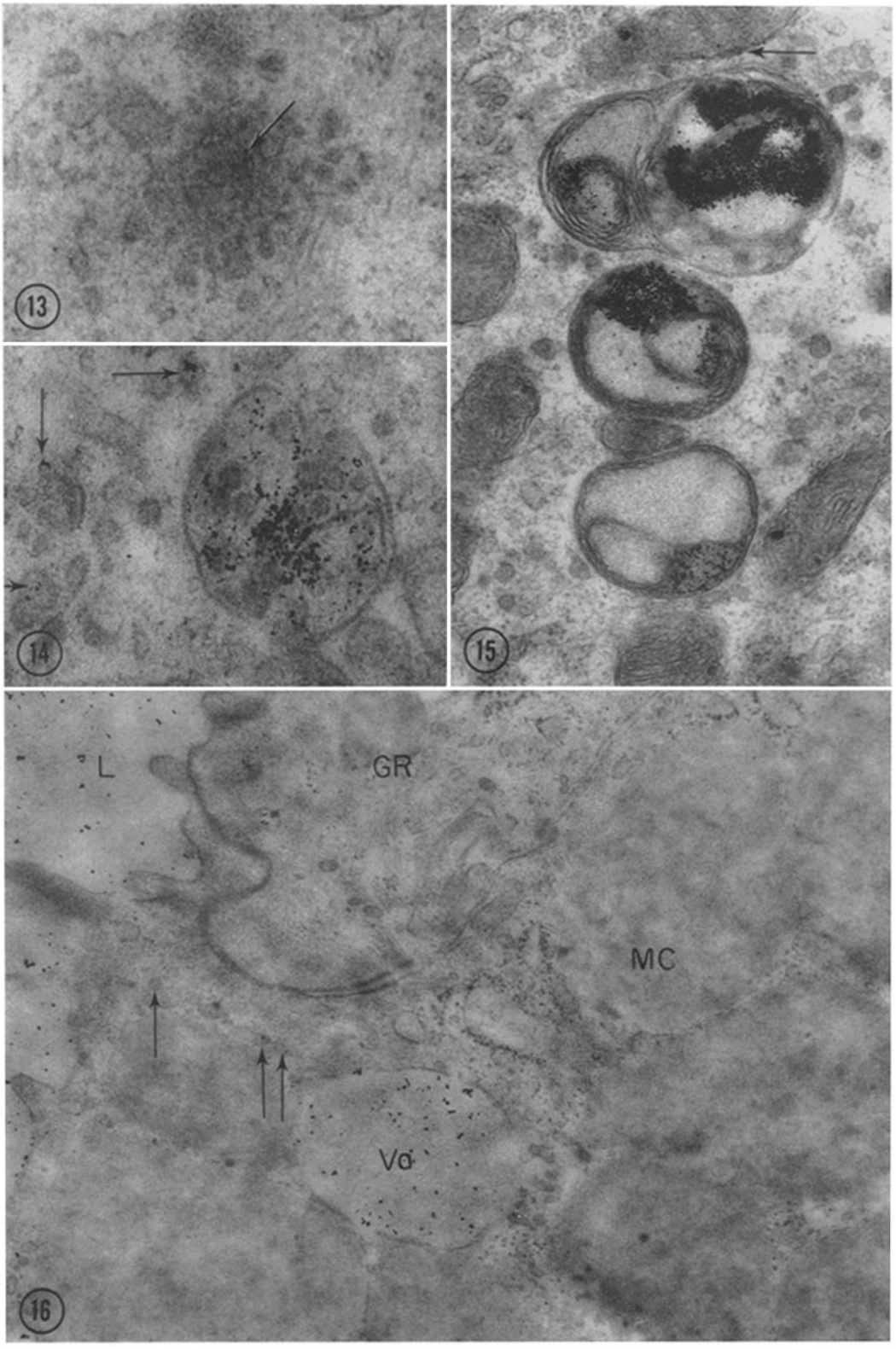
9. BURGOS, M. A., The role of amorphous cellular coatings in active transport, *Anat. Rec.*, 1960, **137**, 171.
10. CARASSO, N. P., FAVARD, P., and VALÉRIEN, J., Variations des ultrastructures dans les cellules épithéliales de la vessie du Crapaud après stimulation par l'hormone neurohypophysaire, *J. Micr.*, 1962, **1**, 143.
11. CHOI, J. K., Electron microscopic study on the transitional epithelium of the toad bladder, *J. Appl. Phys.* 1960, **31**, 1832.
12. CHOI, J. K., The fine structure of the urinary bladder of the toad, *Bufo marinus*, *J. Cell Biol.*, 1963, **16**, 53.
13. CLARK, S. L., JR., The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 41.
14. CRABBÉ, J., Stimulation of active sodium transport by the isolated toad bladder with aldosterone *in vitro*, *J. Clin. Invest.*, 1961, **40**, 2103.
15. DALTON, A. J., and ZEIGEL, R. F., A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 409.
16. DE DUVE, C., Lysosomes. A new group of cytoplasmic particles, *in* Subcellular Particles, (T. Hayashi, editor), New York, The Ronald Press, 1959, 128.
17. DONN, A., MAURICE, D. M., and MILLS, N. L., Studies on the living cornea *in vitro*. II. The active transport of sodium across the epithelium, *Arch. Ophth.*, 1959, **62**, 748.
18. EWER, R. F., The effect of pituitrin on fluid distribution in *Bufo regularis* Reuss, *J. Exp. Biol.*, 1952, **29**, 173.
19. FARQUHAR, M. G., and PALADE, G. E., Functional evidence for the existence of a third cell type in the renal glomerulus. Phagocytosis of filtration residues by a distinctive "third" cell, *J. Cell Biol.*, 1962, **13**, 55.
20. FARQUHAR, M. G., WISSIG, S. L., and PALADE, G. E., Glomerular permeability. 1. Ferritin transfer across the normal glomerular capillary wall, *J. Exp. Med.*, 1961, **113**, 47.
21. FARQUHAR, M. G., and PALADE, G. E., Junctional complexes in various epithelia, *J. Cell Biol.*, 1963, **17**, 375.
22. FAWCETT, D. W., The fine structure of capillaries, arterioles and small arteries, *in* The Microcirculation, (S. R. M. Reynolds and B. W. Zweifach, editors), Urbana, University of Illinois Press, 1959, 1.
23. FRAZIER, H. S., DEMPSEY, E. F., and LEAF, A., Movement of sodium across the mucosal surface of the isolated toad bladder and its modification by vasopressin, *J. Gen. Physiol.*, 1962, **45**, 529.
24. HAMPTON, J. C., An electron microscope study of

FIGURE 13 One hour after Thorotrast administration under Pitressin influence. One of the vesicular components of the apical cytoplasm of granular cell is shown. A cloud of small vesicles (200A) gives the appearance of a mulberry. Note that few particles (arrow) are seen inside this structure. × 54,000.

FIGURE 14 Three hours after Thorotrast administration under dehydration. Typical multivesicular body shows many particles. Most of the tracer particles are seen in the matrix rather than the small vesicles within the multivesicular body. Near the multivesicular body, small vesicles (arrows) in the cytoplasm also contain particles. The limiting membrane of the multivesicular body shows unit membrane structure which is characteristic of this membrane. Unstained. × 53,000.

FIGURE 15 Sixty-five hours after Thorotrast administration under hydration. Deeper portion of mitochondria-rich cell shows three dense bodies (inclusions). These bodies consist of membranous components at the periphery and pale homogeneous substance in the center. Segregated particles are seen inside these bodies. In the mitochondrion, several particles seen at the arrow may be artefacts (presumably displaced during sectioning). × 33,000.

FIGURE 16 Three hours after Thorotrast administration under dehydration. Apical portions of granular (*GR*) and mucous (*MC*) cells. In the cytoplasm of the mucous cell, small vesicles (arrows) contain few particles and a large vacuole (*Va*) contains many particles. Examination of serial sections reveals that the vacuoles are continuous with the lumen (*L*) of the bladder. Unstained. × 30,000.

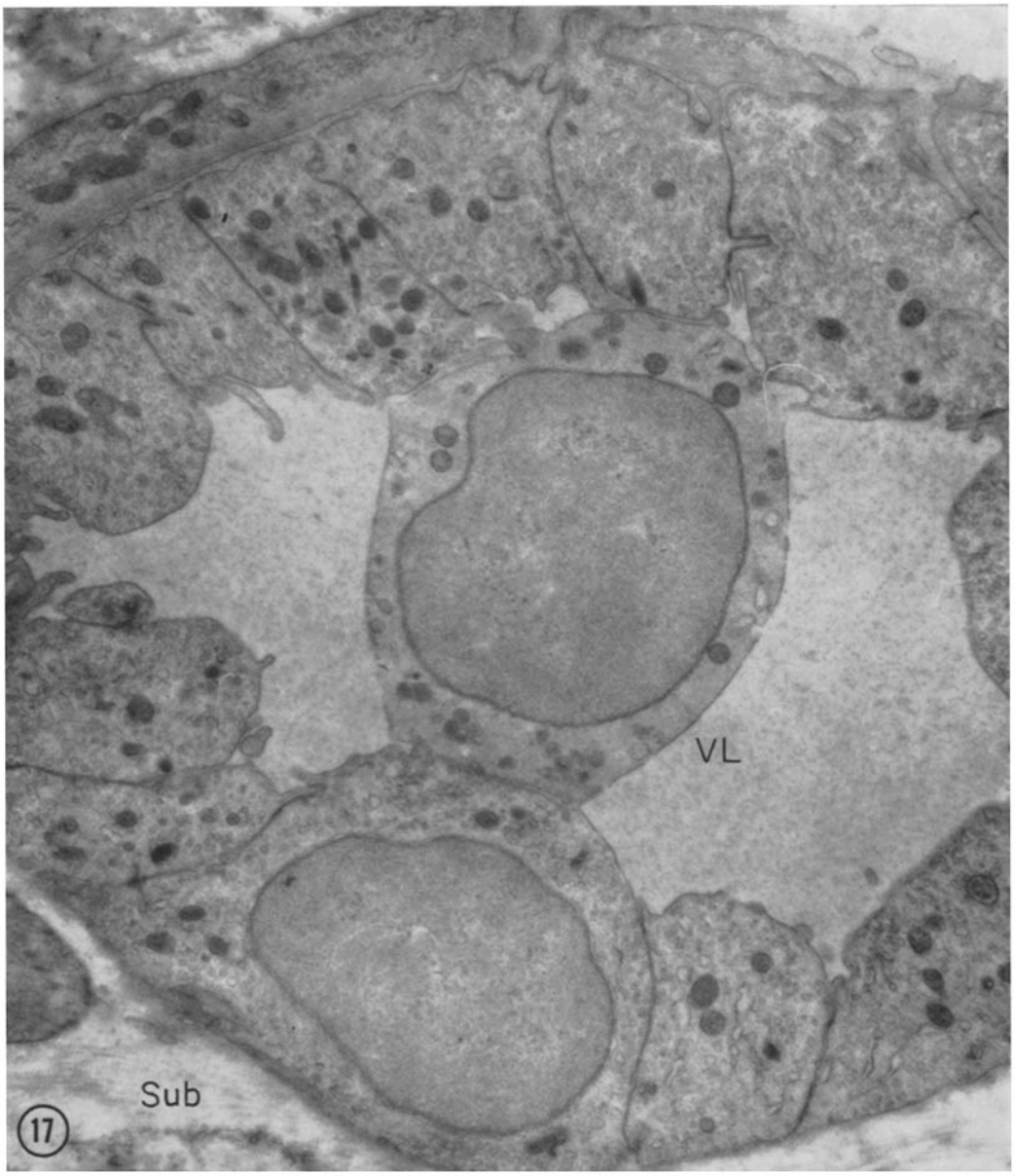

13
14
15
L
GR
MC
Vo
16

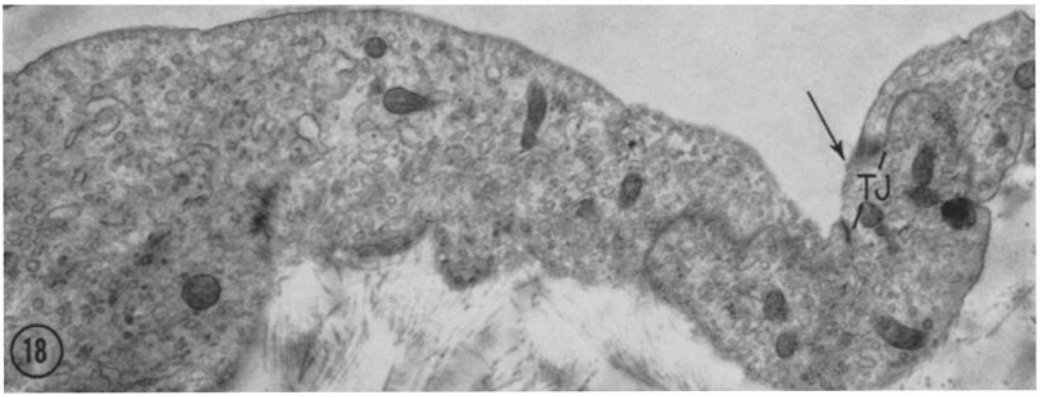
the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the bile duct, *Acta Anat.*, 1958, **32**, 262.

25. HAYS, R. M., and LEAF, A., Studies on the movement of water through the isolated toad bladder and its modification by vasopressin, *J. Gen. Physiol.*, 1962, **45**, 905.
26. HAYWARD, A. F., Electron microscopic observations on absorption in the epithelium of the guinea pig gall bladder, *Z. Zellforsch.*, 1962, **56**, 197.
27. HOLTER, H., Pinocytosis, *Internat. Rev. Cytol.*, 1959, **8**, 481.
28. JENNINGS, M. A., MARCHESI, V. T., and FLOREY, SIR H., The transport of particles across the walls of small blood vessels, *Proc. Roy. Soc. London, Series B*, 1962, **156**, 14.
29. KAYE, G. I., and PAPPAS, G. D., Studies on the cornea. I. The fine structure of the rabbit cornea and the uptake and transport of colloidal particles by the cornea *in vivo*, *J. Cell Biol.*, 1962, **12**, 457.
30. KAYE, G. I., PAPPAS, G. D., DONN, A., and MALLETT, N., Studies on the cornea. II. The uptake and transport of colloidal particles by the living rabbit cornea *in vitro*, *J. Cell Biol.*, 1962, **12**, 481.
31. KUROSUMI, K., YAMAGISHI, M., and YAMAMOTO, T., The fine structure of the transitional epithelium of urinary bladder and its functional significance as disclosed by electron microscopy, *Arch. histolog. jap.*, 1961, **21**, 155.
32. LATTA, H., and MAUNSBACH, A. B., Relations of the centrolobular region of the glomerulus to the juxtaglomerular apparatus, *J. Ultrastruct. Research*, 1962, **6**, 562.
33. LEAF, A., ANDERSON, J., and PAGE, L. B., Active sodium transport by the isolated toad bladder, *J. Gen. Physiol.*, 1958, **41**, 657.
34. LEAF, A., and HAYS, R. M., Permeability of the isolated toad bladder to solutes and its modification by vasopressin, *J. Gen. Physiol.*, 1962, **45**, 921.
35. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, 409.
36. MAJNO, G., and PALADE, G. E., Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: An electron microscopic study, *J. Biophysic. and Biochem. Cytol.*, 1961, **11**, 571.
37. MILLER, F., Hemoglobin absorption by the cells of the proximal convoluted tubule in mouse kidney, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 689.
38. MUIR, A. R., and PETERS, A., Quintuple-layered membrane junctions at terminal bars between endothelial cells, *J. Cell Biol.*, 1962, **12**, 443.
39. NEMOTO, M., Experimental and cytological studies on the function of the urinary bladder epithelium. II. On the absorption activity of the transitional epithelium of the urinary bladder and the ureter, *Arch. histolog. jap.*, 1953, **5**, 355.
40. NOVIKOFF, A. B., Lysosomes and related particles, *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York and London, Academic Press, Inc., 1961, **2**, 423.
41. ODOR, D. L., Uptake and transfer of particulate matter from the peritoneal cavity of the rat, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 105.
42. PAK POY, R. F. K., and BENTLEY, P. J., Fine structure of the epithelial cells of the toad urinary bladder, *Exp. Cell Research*, 1960, **20**, 235.
43. PALADE, G. E., Fine structure of blood capillaries, *J. Appl. Phys.*, 1953, **24**, 1424.
44. PALAY, S. L., and KARLIN, L. J., An electron microscopic study of the intestinal villus. II. The pathway of fat absorption, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 373.
45. PAPPAS, G. D., and TENNYSON, V. M., An electron microscopic study of the passage of col-

FIGURE 17 Sixty-five hours after administration of Thorotrast. A blood vessel in the submucosa, showing several endothelial cells lining the lumen (*VL*) of the vessel. No particles are seen anywhere in the submucosa (*Sub*), but the usual components with cellular and extracellular structures are evident. × 9,000.

FIGURE 18 Sixty-five hours after administration of Thorotrast. Portions of serosal cells show terminal bars (or tight junction, *TJ*), many vesicles, and other cytoplasmic components. No particles are seen within the cytoplasm nor in the submucosa. A few particles are often seen on the coelomic surface of the serosal cell (arrow). The presence of such particles (which bears no relationship to the time of colloid administration) is interpreted as indicating leakage of urine from the bladder lumen into the coelomic cavity when the bladder is opened during fixation. × 9,000.


VL
Sub
17


TJ
18

loidal particles from the blood vessels of the ciliary processes and chloroid plexus of the rabbit, *J. Cell Biol.*, 1962, **15**, 227.

46. PEACHEY, L. D., and RASMUSSEN, H., Structure of the toad's urinary bladder as related to its physiology, *J. Biophysic. and Biochem. Cytol.*, 1961, **10**, 529.
47. RHODIN, J. and T. DALHAMN, Electron microscopy of the tracheal ciliated mucosa in rat, *Z. Zellforsch.*, 1956, **44**, 345.
48. ROTH, T. F., and PORTER, K. R., Specialized sites on the cell surface for protein uptake, Fifth International Congress for Electron Microscopy, (Sydney S. Breese, Jr., editor), Philadelphia, New York and London, Academic Press, Inc., 1962, **2**, LL-4.
49. SAWYER, W. H., and SCHISGALL, R. M., Increased permeability of the frog bladder to water in response to dehydration and neurohypophysial extracts, *Am. J. Physiol.*, 1956, **187**, 312.
50. SOTELO, J. R., and PORTER, K. R., An electron microscope study of the rat ovum, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 327.
51. STEEN, W. B., On the permeability of the frog's bladder to water, *Anat. Rec.*, 1929, **43**, 215.
52. TRUMP, B. F., An electron microscope study of the uptake, transport and storage of colloidal materials by the cells of the vertebrate nephron, *J. Ultrastruct. Research*, 1961, **5**, 291.
53. WALKER, B. E., Electron microscopic observations on transitional epithelium of the mouse urinary bladder, *J. Ultrastruct. Research*, 1960, **3**, 345.
54. WISSIG, S. L., An electron microscopic study of the permeability of capillaries in muscle, *Anat. Rec.*, 1958, **130**, 467.
55. WISSIG, S. L., Structural differentiations in the plasmalemma and cytoplasmic vesicles of selected epithelial cells, *Anat. Rec.*, 1962, **142**, 292.
56. WOOD, R. L., and HOWARD, C. C., Use of fine grain positive sheet film for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 181.
57. YAMADA, E., The fine structure of the gall bladder epithelium of the mouse, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 445.