SERUM ALBUMIN UPTAKE IN
ISOLATED PERFUSED RENAL TUBULES

Quantitative and Electron Microscope Radioautographic
Studies in Three Anatomical Segments of the Rabbit Nephron

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ABSTRACT
Proximal convoluted, proximal straight, and cortical collecting tubular segments isolated from rabbit kidney were perfused with I 125-labeled rabbit serum albumin (RSA-I 125) in ultrafiltrate of serum for up to 3 hr. After perfusion, the segments were fixed with glutaraldehyde, embedded in Epon, and either counted with a gamma spectrometer to quantitate protein accumulation or analyzed by electron microscope radioautography to sequentially localize radioactivity.

Proximal convoluted and proximal straight segments accumulate RSA-I 125 nearly linearly as a function of time whereas cortical collecting segments do not accumulate measurable amounts of protein. The rate of accumulation of RSA-I 125 in the proximal convoluted tubule is 2.6 times as great as that in the proximal straight tubule.

Electron microscope radioautography of the isolated proximal tubule demonstrated that RSA-I 125 is taken up via small apical vesicles and tubular invaginations, released into large cytoplasmic vacuoles, and finally concentrated in membrane-bounded structures, some of which are acid phosphatase positive. These results show that albumin is absorbed by proximal tubules and may be degraded intracellularly within lysosomes. In addition, less radioactivity was located at all times over the lateral intercellular and basilar labyrinthine spaces, suggesting that labeled albumin and/or its breakdown products may be transported across the peritubular cell membrane.

INTRODUCTION
Protein is absorbed from tubular fluid by endocytosis in proximal tubular epithelial cells of the mammalian nephron (1, 2, 3, 4). The absorbed protein may be degraded intracellularly by lysosomal cathepsins (5, 6, 7) or possibly may be transported intact to the peritubular space (8). Mechanisms of protein handling in cells distal to this segment are relatively unknown. Accurate quantitation of protein uptake by tubular epithelial cells of various nephron segments has not been possible because of the technical difficulties associated with handling the nephron in vivo. It was the purpose of the present study to quantitate directly, by using an in vitro isolated tubular per-
fusion technique, albumin accumulation in three anatomical segments of the rabbit renal tubule and to examine by electron microscope radiography the ultrastructural basis for this process in each segment.

MATERIALS AND METHODS

Preparation and Characterization of 125-labeled Serum Albumin

Blood was obtained by cardiac puncture from two female New Zealand White rabbits and centrifuged. The sera were combined, and 5 ml were dialyzed overnight at 2°C against barbital buffer (pH 8.65, I = 0.077) (9). The dialysis tubing had been boiled previously in 0.2 M Na₂CO₃ (10). The dialyzed serum was subjected to powder block electrophoresis using Pevikon C-870 (Mercer Chemical Corp., New York), which had been equilibrated with barbital buffer, as a supporting medium (11, 12). A voltage gradient of 4.0 V/cm was placed across the block for 19 hr at 2°C. 1 cm sections of the block (40 cm × 20 cm × 0.8 cm) were eluted by suction with 10 ml portions of bicarbonate Ringer's solution (NaCl, 115 mM; KCl, 5 mM, Na acetate, 10 mM, NaHCO₃, 25 mM, NaH₂PO₄, 1.2 mM, MgSO₄, 1.2 mM, CaCl₂, 1.0 mM), the eluant protein concentrations, as determined by the method of Lowry et al. (13), revealed a normal serum electrophoretic pattern. The three fractions corresponding to the albumin peak were combined and placed upon a 2.5 cm × 100 cm Sephadex G-100 gel filtration column equilibrated with the bicarbonate Ringer's solution. Optical density measurements at 280 μM of 9.5 ml fractions demonstrated one sharp peak; the three fractions containing the highest concentrations of albumin were pooled.

Polyacrylamide gel electrophoresis in 8.0 M urea and density gradient polyacrylamide gel electrophoresis in Tris-glycine buffer (pH 8.63) demonstrated that the albumin was greater than 98% pure by weight. Immunoelectrophoretic analysis (agar gel, barbital buffer, pH 8.62, I = 0.1) of the albumin with goat anti-whole rabbit serum (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) revealed only one precipitin line.

Iodination was accomplished by a modification of the methods of Izzo et al. (14) and Helmkamp et al. (15) with an average of 1.00 atom of iodine being incorporated per molecule of albumin. After reaction for 20 min, the iodinated protein solution was concentrated by ultrafiltration (Amicon PM-10 membrane, Amicon Corp., Lexington, Mass.) and 1.5 ml were placed upon a 20 ml Sephadex G-25 gel filtration column to remove free iodide. The radioactivity eluted from the column in two peaks: the first was 97.5% precipitable with an equal volume of 10% trichloroacetic acid (98.5% if carrier protein was added) and the second was nonprecipitable. The first fraction was characterized by radioautographic immunoelectrophoresis, and the specific activity of the labeled albumin was determined by employing an iodine 125 standard (Amersham-Searle Corp., Amersham, England) and a Packard AutoGramma Spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Radiimmunelectrophoresis against goat anti-whole rabbit serum demonstrated one band corresponding to albumin (Fig. 1). The specific activity of the albumin prepared for the functional studies was 78.1 μC/mg whereas that

Figure 1. Immunoelectrophoresis of rabbit serum and I 125-labeled rabbit serum albumin. Fig. 1a. Above the central trough (which contained goat anti-rabbit serum) is the immunoelectrophoretic pattern of rabbit serum albumin. Below the trough is a single arc representing the I 125-labeled rabbit serum albumin. The electrophoretic mobilities of albumin in both samples are identical. Fig. 1b. Contact radioautograph of the slide shown above. The only detectable labeled protein corresponds to the albumin band.

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used for electron microscope radioautography was 886 μCi/mg.

The I 125-labeled rabbit serum albumin (RSA-I 125) was dialyzed 4 hr against a solution of 75% ultrafiltrate of rabbit serum (Microbiological Associates, Inc., Bethesda, Md.) prepared with an Amicon PM-10 membrane and 25% bicarbonate Ringer's (pH of final solution 7.38, 300 mosmols/l). The final preparation was frozen in 100 μl portions in paraffin-sealed Spinco microcentrifuge tubes (Beckman Instruments, Inc., Fullerton, Calif.) and thawed immediately before use.

Isolated Tubular Perfusion

Methods for dissection and perfusion of rabbit renal proximal and cortical collecting tubules have been described in detail (16, 17, 18). Female New Zealand White rabbits were decapitated, and the left kidney was rapidly excised. A 1–2 mm slice of tissue perpendicular to the long axis of the kidney was removed and placed in rabbit serum gassed (95% O2-5% CO2) at 2-5°C. Under a dissecting microscope, individual nephron fragments were teased with sharpened Dumont No. 5 tweezers from cortical or medullary tissue and cut with a microknife. The segments were transferred in a small portion of serum to a microperfusion chamber (containing rabbit serum at 25°C) located on the stage of an Unitron inverted bright-field microscope (Unitron Instrument Co., Newton Highlands, Mass.). One end of the isolated fragment was sucked into a holding pipette and the lumen was cannulated with a perfusion pipette. The fragment was perfused freely for approximately 30 sec before the distal end was sucked into a collection pipette. The bath was then replaced with fresh serum and the temperature was increased to 37°C. The bath was bubbled constantly with 95% O2-5% CO2. Serum in the chamber was replaced every 10 min to correct for evaporation and to eliminate contamination with RSA-I 125 which may have occurred by leaks at either the perfusion or collection end of the fragment. Observations and photographs were made periodically at high magnification (× 200–600) (Figs. 2–4).

Handling of Perfusion Fluid

Protein solution was centrifuged and then diluted to a final concentration of 21 mg/100 ml with 75% ultrafiltrate of serum plus 25% bicarbonate Ringer's solution. The fluid was loaded into the glass perfusion pipette through PE-10 tubing and covered with

Figure 2 Bright-field microscope photographs of functioning perfused rabbit renal tubules. Fig. 2 a, Proximal convoluted segment. Fig. 2 b, Proximal straight segment. Fig. 2 c, Cortical collecting segment. Note the larger size of the convoluted segment and the characteristic morphology of the collecting segment. × 220.
mineral oil. Perfusion at a constant rate of 18–20 nl/min was accomplished by using a microsyringe pump.

It was determined that adsorption of RSA-125 to the glass of the tapered portion of the perfusion pipette (volume = 500 nl) substantially decreased the concentration of albumin delivered to the tubule (up to 12.5% in 1 hr). To eliminate adsorption, perfusion pipettes were soaked overnight with a 500 mg/100 ml solution of rabbit albumin (Pentex Biochemicals, Miles Laboratories, Inc., Kankakee, Ill.) in bicarbonate Ringer's solution. Before an experiment a pipette was washed with deionized water and air dried. No decrease in RSA-125 concentration was detectable in pipettes so treated.

Determination of RSA-125 Accumulation Rates

After perfusion (2–90 mm for proximal tubules and 9–130 mm for collecting tubules), the tubular frag-

FIGURE 8 Electron micrograph of a proximal convoluted tubule perfused 78.3 min with 21 mg/100 ml I 125-labeled serum albumin at 37°C. Apical cytoplasmic vacuoles (V) are enlarged with respect to those seen when tubules are perfused without protein. × 13,300.
Figure 4  Electron micrograph of a cortical collecting tubule perfused 45 min with 21 mg/100 ml I
125-labeled serum albumin at 37°C. No apical vacuoles are evident. L, tubular lumen. × 14,400.

...ment was fixed by replacement of the serum in the microperfusion chamber with a solution of 3% gluta-
taraldehyde in 0.1 M sodium cacodylate (pH 7.4, 500 mosmols/1) at room temperature. The per-
fusion pump was turned off immediately and the perfusion pipette was retracted. 15 ml of fixative were
flowed over the fragment for 15 min thereafter. No changes in cell volume or morphology, with the ex-
ception of slight dilatation of the intercellular spaces, were observed. The fragment was cut free from the
pipettes, photographed for length, osmicated, dehydrated in a graded series of ethanol, rinsed in pro-
pylene oxide, and embedded in Epon 812. After hardening at 60°C for 24 hr, the block containing the
fragment was counted for I 125 radioactivity in a Packard AutoGamma Spectrometer (efficiency was
57.4% in Epon or in 6 25 g/100 ml bovine serum al-
bumin in normal saline). Because the radioactivity of
the tubular fragments was low, each block was
counted at least 12 hr in 50 min intervals. Background
was 50 cpm.

To ascertain loss of radioactivity from the tissue
during fixation and embedding, two proximal straight
fragments were perfused 1 hr with a 21 mg/100 ml
solution of RSA-I 125, after which the pump was
turned off and the tubules were allowed to collapse.
Each segment was bisected with a microknife and
photographed. One half of each fragment was fixed
and embedded as usual. The other half was rinsed
three times in noncontaminated rabbit serum and
placed directly in a counting vial. There was no loss of
radioactivity in the embedded fragments as compared
to the unfixed fragments per unit volume of tissue.
**Electron Microscope Radioautography**

Experimental procedures were similar to those described above. Seven proximal convoluted segments were perfused for from 10.0 to 85.3 min, three proximal straight segments for from 45.0 to 87.2 min, and one collecting tubule for 45.0 min. Thin sections were cut with a diamond knife on a Porter-Blum MT-2 Ultratome and placed on 200-mesh, carbon-coated nickel grids. After staining with saturated uranyl acetate, the sections were covered with a 60–100 Å carbon layer by vacuum evaporation and coated with Ilford L4 nuclear emulsion (2). After 5 days’ to 6 weeks’ exposure at 2°C, the grids were developed 90 sec in Kodak D19 developer and fixed 60 sec in one-half strength Kodak acid fixer. Sections were examined with a Philips EM 300 electron microscope with an accelerating voltage of 60 or 80 Kv. Approximately 570 micrographs were taken of the radioautographic specimens at magnifications of 3600–71,000.

**Localization of Acid Phosphatase Activity**

Two proximal tubular fragments (one convoluted and one straight) were each perfused for 1 hr with 21 mg/100 ml RSA-I 125 and fixed 2 hr with 3% glutaraldehyde in 0.1 M sodium cacodylate. The fragments were rinsed with a cacodylate-sucrose solution, incubated 75 min at 37°C in a modified Gomori’s medium (19), rinsed with acetate buffer, and processed for electron microscopy without osmium. Control tubules were incubated with 0.01 M NaF in the Gomori medium. The unstained tubules were cut and prepared for radioautography as described above.

**Quantitative Analysis of Grain Distribution**

Distribution of silver grains over vesicles and cytoplasm of proximal convoluted tubular cells after 10 min perfusion with RSA-I 125 was analyzed in a series of 18 nonoverlapping micrographs from several grids containing sections from different portions of the same tubule. Grains were counted over the following structures (exclusive of brush border and basement membrane) (a) large apical vacuoles (diameter ≥ 1.0 μ), (b) small apical vesicles and apical tubular invaginations, (c) cytoplasm in the apical third of the cell (exclusive of regions a and b), and (d) remaining cytoplasm. In general, the location of a grain was defined as the center of the smallest circle which circumscribed the grain. Relative surface areas of the above-mentioned structures were determined by a regular point lattice placed over the enlarged micrographs (final magnification 24,000) (20) Background grains over Epon situated adjacent to the basement membrane or in the tubular lumen were negligible (<1 grain/500 μ²).

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**RESULTS**

**Rates of Accumulation of I 125-labeled Serum Albumin**

Proximal convoluted and proximal straight tubules accumulated radiiodinated homologous

**Table I**

| Tubular segment       | Perfusion duration | Tubular length | Tubular radioactivity |
|-----------------------|--------------------|----------------|-----------------------|
|                       | (min)              | (mm)           | (nCi / mm²)           |
| Proximal convoluted   | 2                  | 0.99           | 5                     |
|                       | 15                 | 1.65           | 74                    |
|                       | 45                 | 2.24           | 186                   |
|                       | 90                 | 1.15           | 325                   |
| Proximal straight      | 2                  | 2.12           | 18                    |
|                       | 15                 | 2.56           | 103                   |
|                       | 45                 | 1.59           | 88                    |
|                       | 90                 | 2.08           | 250                   |
| Thick ascending limb   | 135                | 1.80           | 42                    |
| Cortical collecting    | 2                  | 1.30           | 12                    |
|                       | 90                 | 1.41           | 10                    |
|                       | 135                | 0.54           | 2                     |
|                       | 180                | 2.10           | 10                    |

*Corrected for background and decay of I 125.

**Figure 5** The accumulation of iodinated albumin as a function of time in proximal convoluted (PCT), proximal straight (PST), and cortical collecting (CCT) segments of the rabbit nephron. All tubules were perfused with a 21 mg/100 ml solution of RSA-I 125 at 18–90 nl/min at 37°C.
Figure 6  Electron microscope radioautograph of a proximal convoluted tubule perfused 10 min with RSA-1 125. Grains are located at the base of the brush border, in small apical vesicles, and in apical vacuoles. The numerous light spots over the entire micrograph represent unexposed silver halide crystals which were dissolved during fixation, leaving a thinner portion of gelatin over the tissue. × 33,300.
serum albumin nearly linearly as a function of time whereas cortical collecting tubules did not accumulate measurable amounts of protein (Table I and Fig 5). The rate of accumulation of albumin in the proximal convoluted tubule was \(3.2 \times 10^{-3}\) ng/mm min \((r = 0.9939, p < 0.005)\) which is 2.6 times as great \((p < 0.005\) by covariance analysis) as that in the proximal straight tubule \((1.2 \times 10^{-3}\) ng/mm min, \(r = 0.9811, p < 0.025)\) when compared per unit length of tubule. One thick ascending limb was perfused 135 min with 21 mg/100 ml RSA-I 125; this segment accumulated albumin at a rate which is 4.2% that of the proximal convoluted tubule.

**Light Microscope Observations**

Perfusion of proximal tubules with 21 mg/100 ml RSA-I 125 was associated with the formation of 3-5 μ diameter vesicles in the epithelial cells. Such vesicles were observable with the bright-field microscope during the experiment and were prominent after 45 min of perfusion. By phase microscopy of Epon sections these vesicles were observed to be situated usually in the middle third of the cell. There was no change in the morphology of cortical collecting tubules perfused with albumin.

**Electron Microscope Observations**

Uptake and concentration of RSA-I 125 in proximal tubular cells was followed sequentially. After 10 min perfusion, grains were located over the base of the brush border, in apical tubular invaginations and vesicles, and in medium-sized (1 μ diameter) apical vacuoles (Fig. 6). The medium-sized apical vacuoles were bounded by a single membrane, the inner side of which was coated by a uniformly thick amorphous layer very similar in appearance to the surface glycocalyx of the cell (Fig 7). Fusion between small apical vesicles and the larger vacuoles was suggested by some micrographs. The grains were randomly distributed over the vacuoles and their membranes. At 10 min, nearly all grains were present in the apical third of the cell (Fig 8). Quantitative analysis of grain distribution after 10 min perfusion revealed that densities were essentially equal over large cytoplasmic vacuoles (diameter ≥ 10 μ) and small apical vesicles and tubular invaginations (Table II). The remaining grains were located primarily in the apical cytoplasm, and...
TABLE II

| Region of cell | Grains | Area | Grain density (Grains/ % Area) |
|----------------|--------|------|------------------------------|
|                 | Number | Total | % | % |
| Small apical vesicles | 134 | 43.6 | 4.4 | 30.5 |
| Large apical vacuoles | 115 | 37.5 | 4.1 | 26.0 |
| Cytoplasm | Apical 1/3 | 49 | 16.0 | 26.9 | 1.82 |
|             | Basal 2/3 | 9 | 2.9 | 64.6 | 0.14 |

...none of these were located over cytoplasmic dense bodies. A very few grains were located in the remaining cytoplasm.

By 45 min, cytoplasmic vacuoles containing grains were enlarged and had shifted to the middle third of the cell (Fig. 9). In some cells grains were concentrated in membrane-bounded structures resembling lysosomes (Fig. 10). At 90 min, the number of cytoplasmic structures in which radioactivity had been concentrated was increased (Fig. 11). Combined electron microscope radioautography and histochemistry showed that some radioactivity was concentrated in acid phosphatase-positive bodies—presumably lysosomes (Fig. 12).

Discussion

Absorption of I 125-labeled homologous albumin by proximal tubule cells was demonstrated by Maunsbach in the rat by the use of micropuncture techniques (2). Labeled protein was injected into proximal tubules for several minutes, after which various periods of flow were permitted before the same tubule was fixed by micropuncture with glutaraldehyde. Sequential electron microscope radioautography combined with studies to localize acid phosphatase demonstrated that by 1 hr after perfusion, iodinated albumin was intimately associated with lysosomes. In other studies, Maunsbach isolated rat kidney lysosomes by density gradient centrifugation, ruptured them by repeated freezing and thawing, and incubated iodinated albumin this preparation (6, 7). At pH 4.8 the lysosomal contents hydrolyzed the iodinated albumin to moniodotyrosine linearly as a function of time Maunsbach found no radioautographic or morphological evidence that the labeled albumin traversed the proximal tubule either by passing between cells or by vesicular transport through cells to the peritubular space, nor did he obtain any quantitative data on the absorption process. Strauss injected rats intravenously with horseradish peroxidase and found by light microscope histochemistry that absorption of this foreign protein from the lumen occurred primarily in the convoluted portion of proximal tubules in the outer cortex (1). On the basis of differences in the size of phagolysosomes and the amount of peroxidase taken up within them, he distinguished several segments of the proximal tubule.

It is apparent, on the basis of the present studies, that quantitative differences in serum albumin accumulation rate exist between different anatomical segments of the rabbit renal tubule. Albumin accumulation is primarily a proximal tubular function accumulation rate is greatest in the convoluted segment and is significantly lower in the straight segment. Rates of fluid transport, p-aminohippurate secretion, and glucose transport are also different in the two segments (17, 21, 22).

On the basis of the segments studied, there appears to be a progressive decrease in albumin accumulation capacity along the nephron. The accumulation rate of albumin in the thick ascen-
FIGURE 9 Electron microscope radioautograph of a proximal straight tubule perfused 45.0 mm with RSA-I 135. Cytoplasmic vacuoles (V) are markedly enlarged and have extended from apical to mid and basilar portions of the cell. X 17,500.
 handling of labeled albumin in the isolated rabbit proximal tubule appears to be identical to that described in the rat. I 125-labeled homologous serum albumin enters the cell by endocytosis, is deposited in large cytoplasmic vacuoles, becomes concentrated, and finally is associated with lysosomal enzymes and presumably degraded.

The radioautographic findings of the present experiments which suggest that intact radioiodinated albumin and/or its breakdown products may be transported into the lateral intercellular or basilar labyrinthine spaces must be interpreted cautiously. In this study radioactivity was localized to these structures at all time intervals from 10.0 to 87.2 min of perfusion. Conceptually this radioactivity might represent free iodide, monoiodotyrosine, an iodinated peptide, or iodinated albumin. That free iodide was present in the intercellular spaces is considered unlikely because Maunsbach has shown that there is no detectable radioactivity in tissue blocks of rat proximal tubules microinjected in vivo with free I 125, fixed with glutaraldehyde, and processed for electron microscopy (2). For periods of perfusion longer than 10 min it is quite possible that some radioactivity was in monoiodotyrosine or an iodinated peptide because radioactivity was present in lysosomes intracellularly. Absence of grains over lysosomes in the presence of grains over the lateral intercellular spaces at 10 min suggests that this radioactivity might be in intact albumin molecules.

That I 125-labeled albumin entered the intercellular spaces via the tight junctions is considered unlikely because these structures have been shown to be impermeable to horseradish peroxidase (mol wt 40,000) (24). Abnormal leakiness of the tight junctions in isolated perfused nephron segments has not been detected by comparison of specific transepithelial electrical resistances of tubular segments in vivo (25) and in vitro (26). Finally, it is considered unlikely that grains in the intercellular spaces were the result of contamination from the bath. The bath volume was 6250 times as large as the volume of fluid delivered to the tubule in 10 min, most of which was collected at the distal end of the segment in the collection pipette, so that any leaked RSA-I 125 would be greatly diluted. Isolated tubular segments have been shown to transport fluid from lumen to bath (17), and the presumed flow in the intercellular spaces would be expected to wash out any I 125-
Figure 11  Electron microscope radioautograph of radioactively-labeled dense bodies (arrows) in cells of the proximal convoluted tubule after 85.3 min perfusion with RSA-1 125. X 10,000.
FIGURE 12 Electron micrographs of lysosomes in tubules incubated for localization of acid phosphatase. Fig. 12a, Tubule was not perfused with labeled albumin and was not osmicated. Dense reaction product is well circumscribed but is not homogeneous throughout the matrix of the organelle. × 116,000. Fig. 12b, Tubule was perfused 60 min with RSA-I 125 and processed for electron microscope radioautography but without osmication. A silver grain (arrow) is located directly over the reaction product. × 61,700.

labeled albumin which had diffused from the bath through the basement membrane into this compartment. Maunsbach may not have detected radioactivity in intercellular spaces in his experiments because microperfusion of labeled albumin was followed by 6–60 min of normal tubular flow. Nonspecific adsorption of labeled albumin to antiluminal cell membranes may have occurred and cannot be excluded as a possible explanation for our findings without further experimentation. However, the solution of a small volume of leaked RSA-I 125 in rabbit serum (the unlabeled albumin of which would be expected to occupy all adsorption binding sites) and the lack of evidence

FIGURE 13 Electron microscope radioautograph of the basilar labyrinth of a proximal convoluted tubule perfused 10 min with RSA-I 125. Three grains are present. The number of grains in this portion of the tubule did not increase with increasing periods of perfusion. × 32,500.

FIGURE 14 Electron microscope radioautograph of a lateral intercellular space (arrow) over which is located a single silver grain from a proximal convoluted tubule perfused 23.5 min with RSA-I 125. × 28,000.
for such tracer protein adsorption in vivo argue against this artifact.

Because of the infrequency with which radioactivity was located over lateral intercellular spaces as compared to intracellular organelles, it is relevant to question the importance of this finding. After 10 min perfusion of the rat proximal tubule Maunsbach found 5% of radioactivity located over cytoplasm not associated with membrane-bounded organelles: specifically, grains were not located over the lateral intercellular and basilar labyrinthine spaces. In the present study, grains observed outside the cytoplasmic organelles were located over lateral intercellular and basilar labyrinthine spaces in addition to remaining cytoplasm. One possible reason for the discrepancy between the findings of Maunsbach and those of the present study is that labeled material from the intercellular spaces may have been washed out during postinjection tubular flow in the rat tubule, whereas in the present study tubules were fixed in the presence of perfusion with labeled albumin, thereby reducing the possibility of washout. The paucity of grains may reflect movement of labeled material out of the intercellular space by hydrodynamic flow, relatively insignificant release of labeled material from the cell to the antiluminal space, or may indicate leaching of radioactivity from the intercellular space during ultramicrotomy and collection of sections in water. Because labeled albumin or smaller fragments thereof which were initially located in the intercellular spaces presumably would diffuse from the tissue more readily than material within vacuoles or lysosomes, it is possible that the actual proportion of I-125-labeled albumin and/or its labeled breakdown products in the intercellular spaces was underestimated by the present technique. In summary, the significance of the electron microscope localization of grains to the lateral intercellular and basilar labyrinthine spaces cannot be evaluated until direct measurements of monoiodotyrosine, labeled albumin fragments, and RSA-I-125 are made in

**Figure 15** Electron microscope radioautograph of a lateral intercellular space (IS) over which are located two silver grains from a proximal convoluted tubule perfused 28.5 min with RSA-I-125. The apposed cell membranes diverge at the level of the arrow. X 48,000.

**Figure 16** Electron microscope radioautograph of an intercellular space (arrow) in the basilar labyrinth of a proximal straight tubule perfused 87.2 min with RSA-I-125. Two silver grains are located directly over this channel. X 51,000.
transport of intact protein molecules in the renal tubules of flounder (27). After injection into the intact fish, lysozyme (mol wt 14,000) was rapidly accumulated in renal tubular cells. By incubation of viable tubules in vitro for up to 36 hr, over 50% of the enzyme was released intact to the medium. It was concluded that intact protein was transported from the cell across the basement membrane into the fluid bath. More recently, Maack et al. have presented evidence to support the hypothesis that I 125–labeled lysozyme may be transported intact across mouse kidney tubular epithelial cells (8). By differential centrifugation of kidney cortex homogenates from mice injected intraperitoneally with I 125–labeled lysozyme, the protein was found to be distributed mostly in two intracellular compartments, phagosomes and cytosol, and that the proportion of protein in each compartment was dose dependent. Proportionally more lysozyme was located in phagosomes when the dose of injected protein was increased. Phase microscope radioautography revealed that protein uptake was confined primarily to proximal tubular cells. Maack et al. concluded that these results support their hypothesis that at normal protein loads transcellular transport predominates over intracellular catabolism whereas with protein overload catabolism within phagolysosomes becomes significant. In light of the findings of Oken et al. that proximal tubular fluid albumin concentration in the normal female rat averages 0.6 mg/100 ml (28), the concentration of 21 mg/100 ml RSA–I 125 used in the present study may be considered a substantial protein overload. Although no proof is offered, it may be stated that the results of the present study are not inconsistent with the hypothesis of Maack et al.

It has been demonstrated that reverse pinocytosis is the basis for antibody transport in the small intestine of the neonatal rat (29). Ferritin-conjugated γ-globulin is absorbed from the gut lumen by endocytosis and transferred to the extracellular spaces between adjacent epithelial cells by fusion of vesicles with the lateral intercellular membrane. Although fusion of cytoplasmic vesicles with the basilar and lateral cell membranes was observed in the present study, no radioautographic evidence was present to suggest that this mechanism is involved in albumin release from the renal tubular cell.

The results of the present study suggest that the isolated perfused rabbit renal tubule is a valid model for the study of renal tubular protein transport.
handling. With further work it may be possible to determine directly the existence or absence of transcellular albumin transport in the mammalian nephron.

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