OCCURRENCE OF PHAGOSOMES AND PHAGO-LYSOSOMES IN DIFFERENT SEGMENTS OF THE NEPHRON IN RELATION TO THE REABSORPTION, TRANSPORT, DIGESTION, AND EXTRUSION OF INTRAVENOUSLY INJECTED HORSERADISH PEROXIDASE

WERNER STRAUS, Ph.D.

From the Department of Physiology, University of North Carolina School of Medicine, Chapel Hill. Dr. Straus's present address is the Division of Metabolic Research, The Chicago Medical School Institute for Medical Research, Chicago

ABSTRACT

The size, number, and location of lysosomes, phagosomes, and phago-lysosomes in different segments of the proximal and distal tubules, in the collecting tubules, and in invading macrophages of the kidneys of rats were compared by staining lysosomes (acid phosphatase) red, and phagosomes (injected horseradish peroxidase) blue in separate sections, and by staining phago-lysosomes purple by successive application of the reactions for the two enzymes in the same sections. It was concluded from these observations that the absorption of the foreign protein from the lumen and its gradual digestion in large phago-lysosomes took place mainly in the cells of the proximal convoluted tubules of the outer cortex. Several segments of the proximal convoluted tubules were distinguished on the basis of differences in the size and location of the phago-lysosomes and the amounts of peroxidase ingested. The distal tubules showed, in addition to moderate numbers of phago-lysosomes, many small phagosomes in the apical and basal zones of the cells. Moderate numbers of phagosomes and phago-lysosomes were observed in the cells of the collecting tubules. Macrophages showing very large phago-lysosomes were seen in the peritubular capillaries of the medulla, after injection of peroxidase. When high doses of peroxidase were administered, enlarged phago-lysosomes, parts of which seemed to be extruded into the lumen, were formed in the terminal segments of the proximal convoluted tubules.

Horseradish peroxidase can be used as a marker protein to study the development of "phagosomes" and their relationship to lysosomes. It was observed in earlier experiments (1) that, a few minutes after the intravenous injection of horseradish peroxidase into rats, many phagosomes, in which peroxidase was segregated, appeared at the base of the brush border of the cells of the proximal convoluted tubules and close to the apical membranes of the cells of the distal tubules of the kidney. By staining peroxidase-positive granules blue, and acid phosphatase-positive granules red in the same cells (2, 3), it was shown that the newly formed phagosomes were separate from preexisting lysosomes during the first 30 minutes after treatment, approximately. Later, phagosomes and lysosomes were combined, as judged by the purple color reaction for the two enzymes in the

same granules. Peroxidase disappeared from the "phago-lysosomes" gradually over a period of 1 to 3 days, probably by being degraded by the catheptic enzymes concentrated in the granules (4).

In the present work, the properties of the phagosomes and phago-lysosomes have been investigated in the cells of different segments of the nephron 1 hour after treatment and later, when the fusion of most phagosomes with the lysosomes was completed. Characteristic differences in the size, number, and location of the phagosomes and phago-lysosomes in the cells of different renal segments indicated that certain segments were very active in the absorption of the protein from the lumen and its subsequent intracellular digestion, and that in other segments an exchange of peroxidase might occur between the cells and the peritubular capillaries. In still other segments, an extrusion of peroxidase from the cells into the lumen was observed, under certain conditions.

Since relatively simple staining procedures are now available to detect the fusion of lysosomes with phagosomes, a stricter definition of the terms is indicated. This question has also been discussed elsewhere (2). "Lysosomes" were defined by de Duve *et al.* (5, 6) as cytoplasmic granules containing certain hydrolytic enzymes. The term "phagosome" was proposed (7) to characterize the segregating ability of cytoplasmic granules. As used earlier, the term "phagosome" comprised two varieties of the granules: phagocytic or pinocytic vacuoles containing ingested materials (for example, horseradish peroxidase) alone, and these vacuoles or granules combined with lysosomes (hydrolytic enzymes). In order to distinguish the "pure" (young) lysosomes and phagosomes from the combined granules, the term "phago-lysosome" or "lyso-phagosome" was used for the combined granules in recent reports (2, 3). It should be noted that acid phosphatase-positive granules in the cells of untreated animals may contain ingested materials segregated during the normal function of the cells and that no methods may be available to detect these materials. Therefore, the acid phosphatase-positive granules in the cells of untreated animals, called "lysosomes" in the following paragraphs, may correspond to "phago-lysosomes" containing unknown ingested materials. Phagosomes can be identified, by cytochemical means, after injection of peroxidase only when they have reached a certain size (0.2 to 0.3 μ diameter).

Since the combined nature of the phago-lysosomes was detected by staining the exogenous protein and the endogenous lysosomal enzyme in contrasting colors in the same granules, color prints were required for illustration. In the present report, color reproductions of double-stained phago-lysosomes are shown mainly for cells other than those of the proximal convolutions. The combined nature of granules in the cells of the proximal convoluted tubules may be seen in the previous color illustrations (3).

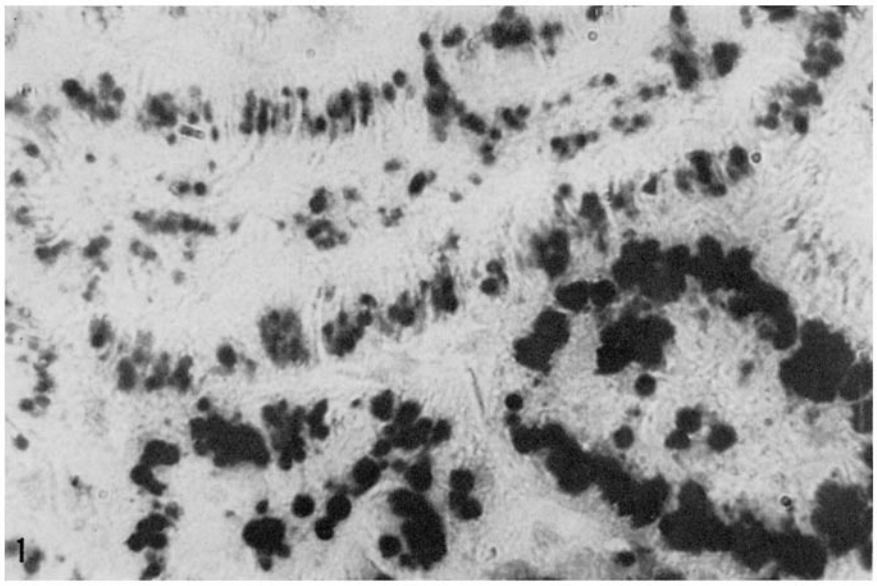
MATERIALS AND METHODS

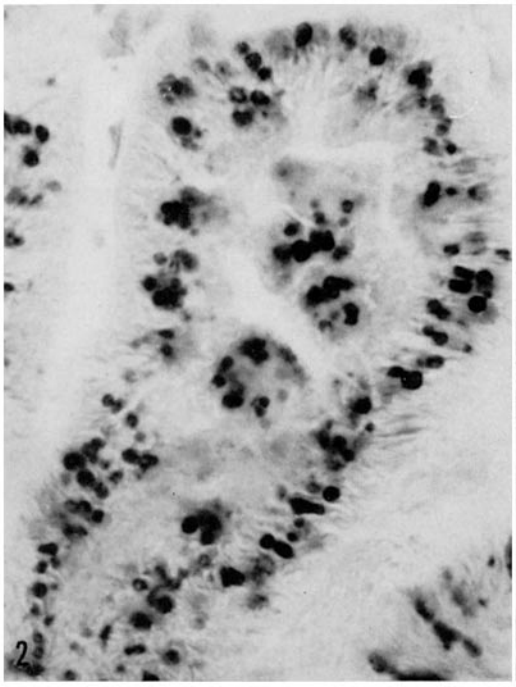
Male rats of the Sprague-Dawley strain, each weighing approximately 200 gm, were used. The animals received an intravenous injection of 12 mg/100 gm body weight of horseradish peroxidase (type II, Sigma Chemical Company, St. Louis). In some experiments, the dose was increased to 20 mg/100 gm body weight; in others, it was reduced to 5, 2, and 1 mg/100 gm, respectively. In most cases, the tissue was removed between 1 and 6 hours following in-

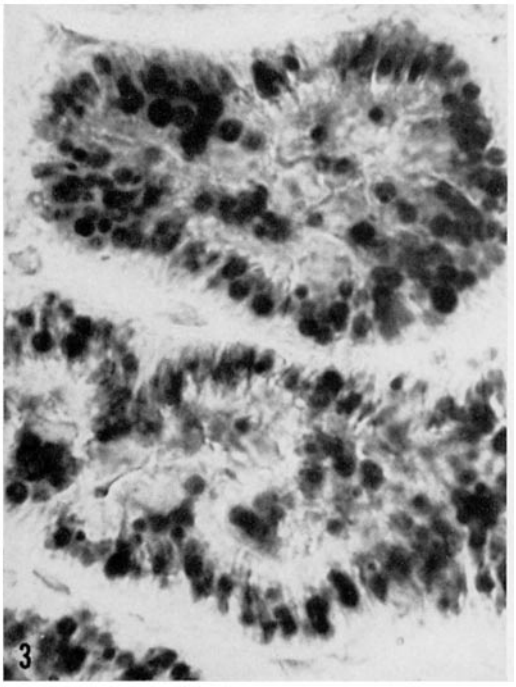
FIGURE 1 Phago-lysosomes in cells of proximal convoluted tubules of outer cortex, stained with benzidine, 3 hours after intravenous administration of the standard dose of horseradish peroxidase. Note the differences in the amounts of ingested peroxidase and the size of the granules in the cells of different segments. × 1330.

FIGURE 2 Lysosomes in cells of proximal convoluted tubules of outer cortex of *untreated* rat, stained for acid phosphatase. Note the smaller size of the granules as compared to those of the peroxidase-treated animals shown in Figs. 1 and 3. × 1330.

FIGURE 3 Phago-lysosomes in cells of proximal convoluted tubules of outer cortex, stained for acid phosphatase, 3 hours after injection of peroxidase. Note the enlarged size of many acid phosphatase-positive granules as compared to those of untreated animal (Fig. 2). × 1330.







jection and was fixed for 18 hours in a 10 per cent solution of formaldehyde containing 30 per cent sucrose (8). Some studies were made with kidneys removed at 10 or 30 minutes, or at 1, 2 or 3 days following treatment.

Cryostat sections of the fixed tissue were stained for peroxidase with benzidine as indicated elsewhere (9). For the double staining of the two enzymes, a frozen section of the formaldehyde-fixed tissue was stained for acid phosphatase with naphthol AS-TR phosphate (phosphate ester of 4'chloro-3-hydroxy-2-naphtho-o-toluidine) (Burstone, reference 10) as substrate and hexazotized pararosaniline as coupler, according to Barka and Anderson (11). Subsequently, the same section was stained for peroxidase (3, 8). Control sections, stained for acid phosphatase alone and for peroxidase alone, were always compared with the double-stained preparatons.

CYTOCHEMICAL OBSERVATIONS

Proximal Convoluted Tubules

A. CELLS WITH LARGE AND INTENSELY-STAINED PHAGO-LYSOSOMES IN THE OUTER CORTEX: After treatment with the standard dose of peroxidase, most of the phago-lysosomes in these cells were very large (3 to 5 μ diameter) and were intensely stained for peroxidase; they had the round shapes of "droplets" (Fig. 1). The majority of the granules was located in the apical and intermediate zones of the cells.

The lysosomes of *untreated* animals when stained for acid phosphatase appeared in a similar location in the cells of the outer cortex. However, they were, in general, of smaller size (1 to 2.5 μ diameter) (Fig. 2). It was possible to detect the increase of size of many granules after treatment with peroxidase, by staining for acid phosphatase (Fig. 3).

After administration of the standard dose, the peroxidase reaction decreased gradually in the large phago-lysosomes of the outer cortex and became negative after 3 to 4 days. The cytochemical reaction for acid phosphatase persisted in the same granules (Fig. 10). After complete digestion of peroxidase, the granules showed again the smaller size of the lysosomes of untreated animals.

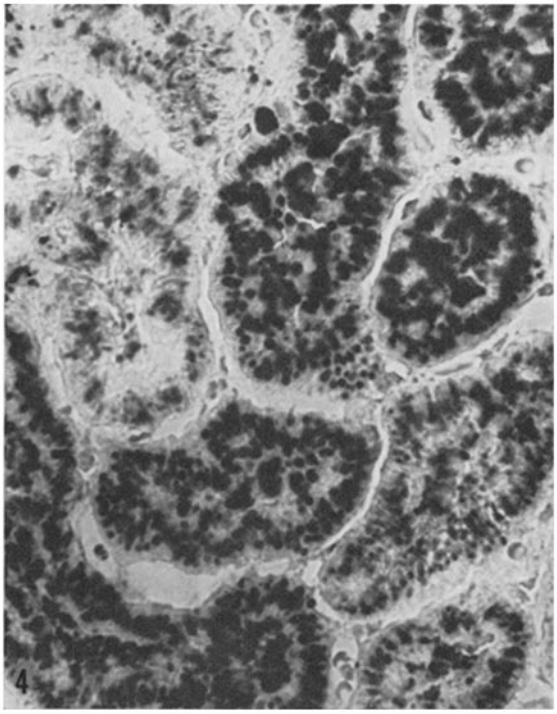
B. CELLS WITH INTERMEDIATE-SIZED AND LESS INTENSELY STAINED PHAGO-LYSOSOMES IN THE OUTER CORTEX: The peroxidase-positive granules in these cells, in general, were smaller (0.5 to 2 μ diameter), less numerous, and less intensely stained, as compared to those in the first type of cells (Fig. 1). The granules were aligned in apical-basal direction in narrow compartments with relatively straight borders and sometimes had elongated shapes (Fig. 1; see also Fig. 7, reference 1).

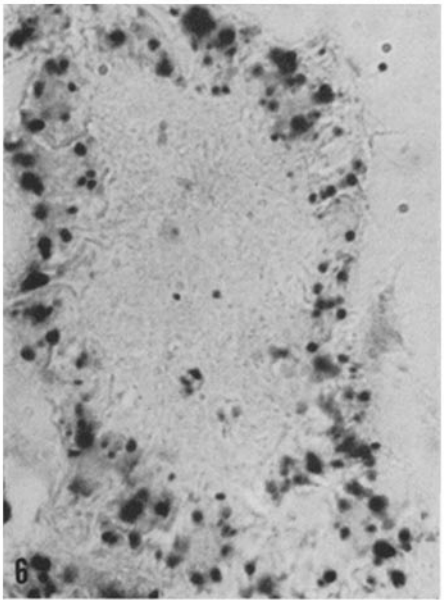
FIGURE 4 Phago-lysosomes in cells of proximal convoluted tubules of outer cortex, stained with benzidine, 3 hours after injection of peroxidase. Note that the amounts of ingested peroxidase and the size of the phago-lysosomes differ in the cells of different segments, and that the magnification is lower than in Figs. 1 to 3. × 530.

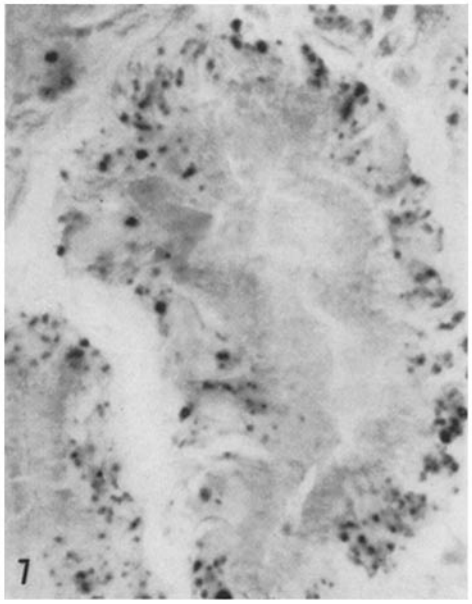
FIGURE 5 Small phagosomes in cells of proximal convoluted tubules of outer cortex, stained with benzidine, 10 minutes after intravenous injection of peroxidase. Note that phagosomes develop at the base of the brush border, and that very small phagosomes are present in the basal and intermediate zones (arrow), and that they seem to be connected with the apical phagosomes by fine membranes. (Compare with Fig. 7 in reference 1 showing the same type of cells at 30 minutes after injection.) The strong staining along the peritubular capillaries may be due to peroxidase uptake by macrophages or endothelial cells in this animal but was rarely encountered in the outer cortex of other animals. × 1330.

FIGURE 6 Phago-lysosomes in terminal portions of proximal convolution, stained with benzidine, 3 hours after injection of peroxidase. Note the smaller size of the granules as compared to those in the outer cortex (Figs. 1 and 3), and the location of phago-lysosomes close to the basement membrane. × 1330.

FIGURE 7 Lysosomes in terminal portion of proximal convolution in inner cortex of *untreated* animal, stained for acid phosphatase. Note the smaller size of lysosomes in inner cortex as compared to those in outer cortex of untreated animal (Fig. 2), and as compared to the phago-lysosomes of peroxidase-treated rat (Fig. 6). Note also the location of some lysosomes close to the basement membrane. × 1330.







The lysosomes of *untreated* animals when stained for acid phosphatase showed a location similar to that of the phago-lysosomes of peroxidase-treated animals. The increase of size of many acid phosphatase-positive granules was less pronounced and their peroxidase reaction less lasting (after the standard dose) than in the cells described in section A (Fig. 10)). The two types of cells seemed to be present in the outer cortex in approximately equal numbers (Fig. 4).

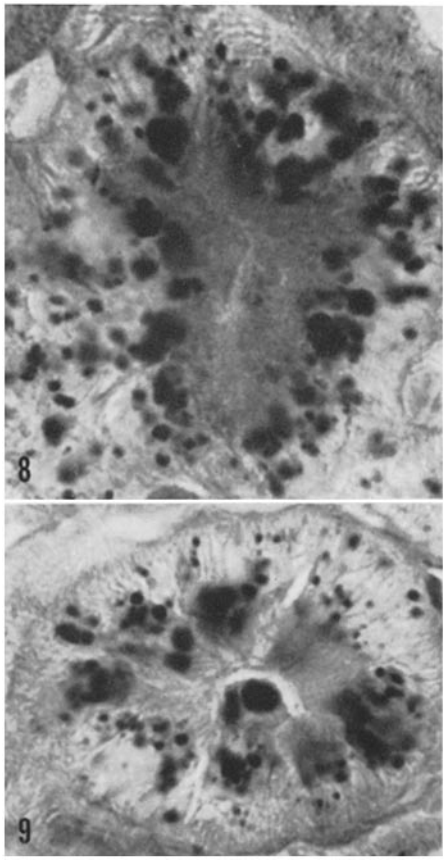
In addition to the phago-lysosomes just mentioned, much smaller peroxidase-positive granules (0.3 μ diameter or smaller) were seen in the cells of the proximal convolutions (Fig. 5). These granules were observed in the apical and basal zones of the cells. No reaction for acid phosphatase could be detected in these small phagosomes.

C. CELLS OF THE PROXIMAL CONVOLUTED TUBULES IN THE INNER CORTEX

Administration of low or average doses of peroxidase. When peroxidase was adsorbed to the brush border, the cells of the terminal portions of the proximal convolutions could be identified by the considerable width of the brush border (see Fig. 3, reference 1). In these segments of the nephron, the phago-lysosomes were smaller (0.5 to 1.5 μ diameter) and less numerous (Fig. 6) than in the cells of the outer cortex. Many phago-lysosomes in these cells were located close to the basement membranes as early as 45 minutes after injection of peroxidase. Other phago-lysosomes were present in the apical and intermediate zones of the cells.

The lysosomes in the cells of the terminal portions of the proximal convolutions of *untreated* animals when stained for acid phosphatase were relatively small (0.2 to 0.7 μ diameter) (Fig. 7). They showed a location similar to that of the phago-lysosomes of peroxidase-treated rats (Fig. 6), including their location close to the basement membranes (Fig. 7). The increase of size of many granules after treatment with peroxidase could be recognized. After administration of the standard dose, the peroxidase reaction of the phago-lysosomes became negative approximately 24 hours after treatment, whereas the reaction for acid phosphatase persisted. After complete digestion of peroxidase, the size of the granules was reduced to the size shown before treatment.

Administration of high doses of peroxidase. When very high doses of peroxidase were injected, or when certain commercial preparations which seemed to be toxic were given, the cells of the terminal portions of the proximal convolutions contained, in addition to the smaller phago-lysosomes mentioned in the preceding paragraph, much enlarged phago-lysosomes with irregular shapes (Fig. 8). The same treatment caused the formation of large vacuoles, containing acid phosphatase and injected peroxidase, in the parenchymal cells of the liver (2, 3), especially in the neighborhood of the portal veins. The enlarged granules in kidney cells and the large vacuoles in liver cells seemed to arise by the fusion of several lysosomes and several phagosomes. They probably were related to or identical with the "composite



FIGURES 8 and 9 Enlarged phago-lysosomes, stained with benzidine, in terminal portions of proximal convolution, 1 hour after administration of a high dose of peroxidase. Note the large size and irregular shape of the bodies, their location in the apical zones of the cells (Fig. 8), and their apparent extrusion into the lumen (Fig. 9). × 1330.

bodies" described previously (see Figs. 6 to 8, reference 12). One hour after treatment, many of the enlarged bodies were located in the apical regions of the cells (Figs. 8 and 11). A part of the bodies seemed to be extruded into the lumen (Fig. 9). The extruded material often included nuclei and apical fragments of the cells. At later periods after treatment, the enlarged bodies were also seen in the intermediate and basal zones of the cells. Whereas the peroxidase reaction became negative in the normal-sized phago-lysosomes 24 hours after treatment, approximately (see the preceding paragraph), the enlarged bodies still contained much peroxidase 48 hours after injection.

Distal Tubules

A. THICK ASCENDING LIMBS OF HENLE'S LOOP: Only a moderate number of lysosomes in perinuclear location could be detected in the thick ascending limbs of *untreated* animals, by staining for acid phosphatase. Early after injection of the foreign protein, peroxidase-positive granules appeared in the apical zones of the cells, separate from preexisting lysosomes (Fig. 12). At 1 hour after treatment and later, the phagosomes had merged with the lysosomes in the apical regions of the cells (Fig. 13). The peroxidase reaction became negative in these phago-lysosomes approximately 24 hours after treatment.

It was characteristic for these cells to show the presence of small phagosomes (0.5 μ diameter and smaller) in the basal zones of the cells and close to the basement membrane (Figs. 14 and 21). These small granules often appeared to be aligned along fine membranes. No reaction for acid phosphatase could be detected in these small phagosomes.

B. CELLS OF THE DISTAL TUBULES IN THE OUTER CORTEX: The lysosomes, phagosomes, and phago-lysosomes in these segments of the distal tubules were respectively similar in properties to those in the thick ascending limbs. The lysosomes of *untreated* rats surrounded the nuclei (Fig. 15). One hour after injection of peroxidase and later, the lysosomes were combined with phagosomes (Fig. 16). Small phagosomes were also seen in the basal zones of these cells (Fig. 16).

Collecting Tubules

A moderate number of intermediate-sized phago-lysosomes (0.5 to 1.0 μ diameter) were observed in the cells of the collecting tubules of the inner medulla (Figs. 17 and 22) and of the collecting tubules of the outer cortex (Fig. 18). Peroxidase-positive granules appeared in these cells approximately 30 minutes after administration of the standard dose. The reaction disappeared approximately 12 hours later. Two types of cells were often distinguished in the collecting tubules of the inner medulla: one type contained much more peroxidase than the other. Since this concentration of peroxidase often was in a diffuse form, there was no certainty whether or not it was due to an artifact of the treatment.

Glomerulus

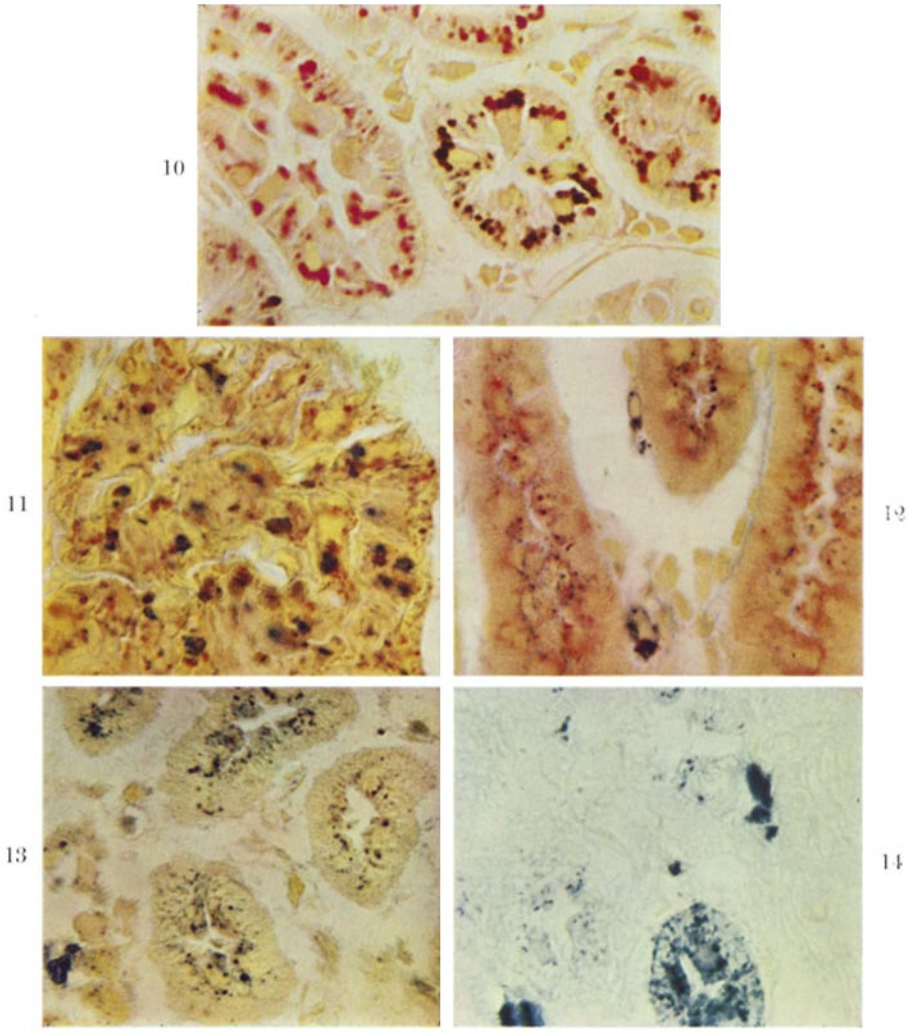
After treatment with low or average doses of peroxidase, certain cells of the glomerulus contained small, peroxidase-positive granules (2) (Fig. 23). These granules did not show the reaction for acid phosphatase (Fig. 19). The peroxidase-positive cells resembled leucocytes stained for endogenous peroxidase. However, they may also be the phagocytic endothelial cells which have been investigated by Farquhar and Palade (13) by electron microscopy. A few cells with combined phago-lysosomes were seen in the glomerulus (Fig. 19).

Macrophages

After treatment with peroxidase, many macrophages appeared along the capillaries or spaces surrounding the tubules of the outer and inner medulla (Figs. 12, 14, 17 and 24). Their number was larger after the administration of low doses than after high doses of the foreign protein. Phagosomes, containing peroxidase, first were seen on the surface of the macrophages (Fig. 12). Later, they were located in the interior of the cells and reached very large size (3 to 6 μ diameter) (Figs. 14 and 17). The reaction for acid phosphatase seemed to decrease in the phago-lysosomes of the macrophages during the main uptake of peroxidase (2).

DISCUSSION

The experiments have shown that most of the injected protein was taken up in the kidney by the cells of the proximal convolutions. However, the cells of the distal tubules and the collecting tubules, and the macrophages along peritubular capillaries of the medulla also contained phagosomes and phago-lysosomes in which peroxidase was segregated.



FIGS. 10 to 20, × 660.

FIGURE 10 Combined staining for acid phosphatase and peroxidase in the cells of the proximal convoluted tubules of rats treated with peroxidase 48 hours previously. Note that the amounts of peroxidase remaining after 48 hours in the phago-lysosomes are similar in each tubule section, and that peroxidase disappeared from some segments earlier than from others.

FIGURE 11 Combined staining for acid phosphatase and peroxidase in animal injected with a high dose of peroxidase one hour previously. Note much enlarged phago-lysosomes ("composite bodies," see reference 12) with irregular shapes in cells of the terminal segment of the proximal convolution, and the relatively small size of the "normal" phago-lysosomes in the same cells.

FIGURE 12 Combined staining for acid phosphatase and peroxidase in cells of thick ascending limbs of outer medulla in animal treated with peroxidase 15 minutes previously. Note the separate location of phagosomes and lysosomes in the cells of the thick ascending limbs; note also the adsorption of peroxidase to the surface membranes of macrophages, located in the peritubular capillaries or spaces, and the development of phagosomes close to these membranes.

FIGURE 13 Combined staining for acid phosphatase and peroxidase in cells of thick ascending limbs, 3 hours after injection of peroxidase. Note that phagosomes and lysosomes are combined as shown by the intermediate color; compare this color with that of a preparation stained for peroxidase alone (Fig. 14).

FIGURE 14 Staining for peroxidase alone in the thick ascending limbs of the outer medulla, 3 hours after injection of the standard dose. Note the presence of phagosomes in all zones of the cells, including the basal zones. Note also the very large phagosomes in macrophages present in the peritubular capillaries or spaces.

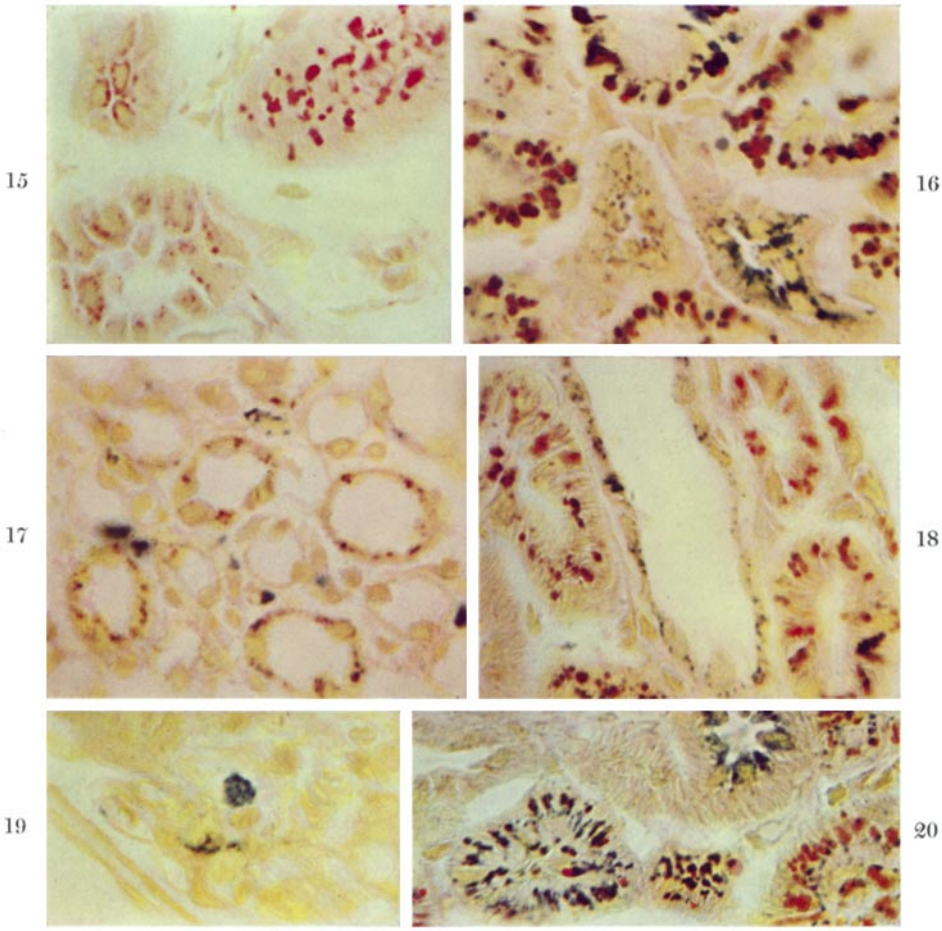


FIGURE 15 Staining for acid phosphatase alone of lysosomes in distal tubules of outer cortex of untreated rat. Note the perinuclear location of lysosomes in distal tubules, and the difference in the size of the lysosomes in the proximal and distal tubules of the outer cortex.

FIGURE 16 Combined staining for acid phosphatase and peroxidase in phago-lysosomes of distal tubules in outer cortex, 1 hour after injection of peroxidase. Note the presence of small phagosomes in the basal zones of the distal tubule cells, and the difference of size among the phago-lysosomes in the distal and proximal tubules in the outer cortex.

FIGURE 17 Combined staining for acid phosphatase and peroxidase in phago-lysosomes in cells of collecting tubules of inner medulla, 6 hours after the injection of peroxidase. Note also that macrophages, located in the peritubular capillaries or spaces, contain phago-lysosomes (not in sharp focus).

FIGURE 18 Combined staining for acid phosphatase and peroxidase in phago-lysosomes of collecting tubules in outer cortex, 24 hours after treatment with peroxidase. In other cases, peroxidase did not persist in these cells as long as it did in this animal.

FIGURE 19 Combined staining for acid phosphatase and peroxidase in glomerulus, 3 hours after injection of peroxidase. Note peroxidase-positive granules in cell of glomerulus, the absence of acid phosphatase reaction there, and the presence of a few phago-lysosomes in another cell of the glomerulus.

FIGURE 20 Combined staining for acid phosphatase and peroxidase in cells of proximal convolution, 30 minutes after injection of peroxidase. Note the location of a phagosome adjacent to a lysosome, probably preceding their fusion.

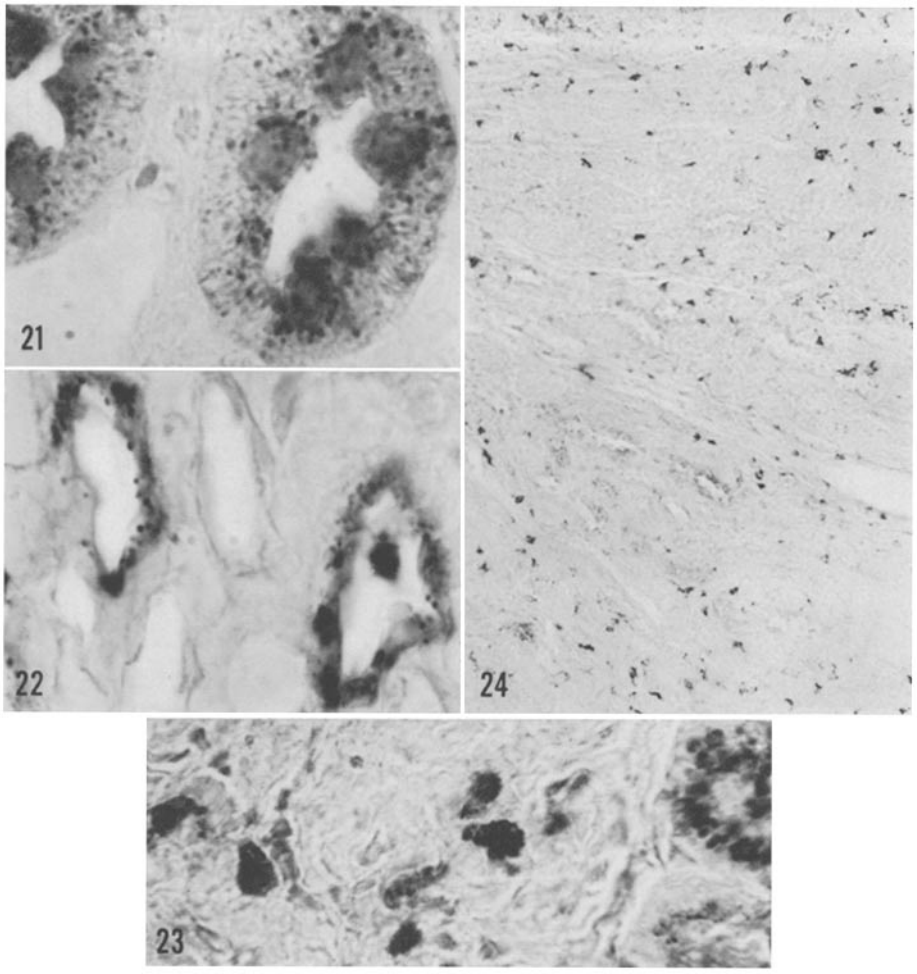


FIGURE 21 Phago-lysosomes and small phagosomes in cells of thick ascending limbs of outer medulla, stained with benzidine, 3 hours after injection of peroxidase. Note that small phagosomes are present in the basal regions of the cells. The staining of the nuclei probably is an artifact. × 1330.

FIGURE 22 Phago-lysosomes in cells of collecting tubules of inner medulla, stained with benzidine, 3 hours after injection of peroxidase. × 1330.

FIGURE 23 Small peroxidase-positive granules in cells of glomerulus, stained with benzidine, 1 hour after injection of peroxidase. × 1330.

FIGURE 24 Macrophages in medulla, stained with benzidine, 1 hour after injection of peroxidase. × 130.

As has been discussed in an earlier report (1), the appearance of peroxidase in large phagosomes close to the luminal surface of the tubule cells, a few minutes after injection, may be taken to indicate that the protein was absorbed from the lumen by a process related to pinocytosis. This is in agreement with observations made by Miller (14) by electron microscopy on the absorption of hemoglobin in the convoluted tubules. It also agrees with the opinion of other investigators (15–18) that proteins with a molecular weight below 70,000 pass the glomerulus and are reabsorbed by the cells of the proximal convoluted tubules.

At least two kinds of cells, in approximately equal numbers, were observed in the *outer cortex*. The phago-lysosomes of one type are large and

intensely stained; those of the other type are smaller and less intensely stained (Fig. 1). The two types of cells probably belong to different segments of the proximal convolution. The different size and staining intensity of the phago-lysosomes in these cells was related to differences in the amounts of ingested peroxidase, the size of the phago-lysosomes increasing with the amounts of peroxidase reabsorbed.

The third type of cell in the *inner cortex*[1] could be identified readily by its wide brush border (20, 21) as belonging to the terminal portions of the proximal convoluted tubules. After injection of low and average doses of peroxidase, the phago-lysosomes in these cells were smaller and less numerous (Fig. 6) than in the cells of the outer cortex. This difference may be related to the smaller size of the preexisting lysosomes (Fig. 7) as well as to the smaller amounts of ingested peroxidase. The location of many phago-lysosomes close to the basement membranes distinguished these cells of the inner cortex from those in the proximal convolutions of the outer cortex where relatively few granules extended to the basement membranes, early after treatment. It is of interest that Sjöstrand (22) distinguished 4 segments of the proximal convolutions and that he described in these cells cytoplasmic granules with characteristic properties of fluorescence. Since Tappel (23) found the fluorescence spectrum of flavin compounds in highly purified lysosomes, a part of the fluorescent granules seen by Sjöstrand may have been lysosomes.

It was discussed previously (2, 3) that the presence of peroxidase and acid phosphatase in the same granules probably resulted from the fusion of newly formed phagosomes with preexisting lysosomes. Since Miller (2) and Novikoff (2) seemed to suggest a different interpretation, *i.e.* the transport of acid phosphatase to the phagosomes in submicroscopic packets, the reasons for our interpretation may be restated briefly. Thirty minutes after injection of peroxidase, blue-stained phagosomes were seen to lie adjacent to red-stained lysosomes (Fig. 20; and Fig. 3, reference 3). One hour after injection of peroxidase, and later, most of the acid phosphatase-positive granules in the proximal convolutions of the outer cortex contained peroxidase (Figs. 4 and 6, reference 3). If the acid phosphatase had been transported in submicroscopic packets to the newly formed phagosomes, the preexisting lysosomes probably would have remained free of peroxidase. Since this was not the case, it was concluded that they fused with phagosomes.

In addition to the fusion of relatively large phagosomes with preexisting lysosomes, a variation of this process seems to occur frequently: the fusion of very small phagosomes (micropinocytic vesicles, 0.3 μ or smaller) with the lysosomes. It was mentioned that lysosomes in untreated animals (Fig. 7) and phago-lysosomes in peroxidase-injected animals (Fig. 6) in the cells of the terminal portions of the proximal convoluted tubules were located close to the basement membranes. Since peroxidase first entered these cells at the base of the brush border (1–3), a transport of the protein from one pole of the cell to the other must have taken place within a relatively short time. It is suggested that this intracellular transport of peroxidase to the lysosomes is mediated by small phagosomes (micropinocytic vesicles) originating at the base of the brush border or from the larger phagocytic vacuoles themselves (Fig. 5). This latter process may explain why some of the peroxidase, originally located at the base of the brush border, seemed to be displaced toward a more basal location, later. The derivation of small phagosomes from larger phagocytic vacuoles may be similar to the formation of small pinocytic vesicles from large food vacuoles in protozoa, as observed in the electron microscope by Mercer (24), Roth (25), and Jurand (26). The formation of micropinocytic vesicles from the surface membranes of kidney cells, and the transport, by these vesicles, of ingested proteins to large granules was demonstrated in electron micrographs by Farquhar and Palade (27) and by Miller (14).

The question whether or not a part of the ingested peroxidase is transported by small phagosomes (micropinocytic vesicles) out of the tubule cells across the basal membranes of the cells is still under investigation. The possibility of a transport of peroxidase through the endoplasmic reticulum and the infoldings of the basal membranes of the cells to the peritubular capillaries or spaces has been discussed previously (1, 12, 28).

[1] The terminology used by different authors for the zones of the kidney is not uniform. In the present text, the terms outer and inner cortex and outer and inner medulla correspond to those used by Sternberg *et al.* (19) in their excellent study on the histochemical differentiation of renal segments by staining for oxidative enzymes.

If such a transport takes place, it may occur mainly in the cells of the distal tubules where many small phagosomes were seen in the basal zones of the cells and where many macrophages containing peroxidase were located along the surrounding capillaries. Since the cells of the distal tubules contained only a few perinuclear lysosomes prior to the treatment with peroxidase, relatively few phago-lysosomes were formed early after the absorption of peroxidase from the lumen. Most of the peroxidase taken into the cells at one pole may have been transported by small phagosomes across and out of the cells at the other pole. A new formation of lysosomes and lysosomal enzymes may take place in the cells of the distal tubules at later periods after injection of peroxidase.

An extrusion of peroxidase into the lumen seemed to occur in the terminal portions of the proximal convoluted tubules (Figs. 8 and 9), when the dose of peroxidase was very high, or when certain commercial preparations which seemed to be toxic were used. Under these conditions, increased numbers of phagosomes were formed, and several phagosomes fused with several lysosomes. These "composite bodies," which may have included also cell fragments (nuclei, mitochondria, brush border material) (12), probably belong to the same type of enlarged lysosomes observed, in the electron microscope, by several investigators (29–34) in the kidney and in other cells after various treatments. The extrusion of enlarged lysosomes, nuclei, and material of the brush border from cells of the proximal convoluted tubules, after ligation of the urether, was reported by Novikoff (30).

It was mentioned briefly in previous reports (2, 3) that large vacuoles or spheres containing acid phosphatase and injected peroxidase were formed from merging lysosomes and phagosomes in liver cells after injury. The vacuolar changes in liver cells and the extrusion of enlarged phago-lysosomes and cell fragments from kidney cells occurred in the same animals. It was discussed (2) that the large vacuoles in liver cells may have been responsible for the increased amounts of lysosomal enzymes detected in the supernatant fluids of liver homogenates after injury (35, 36). A shift of lysosomal enzymes from the granular fractions to the supernatant fluids was also noted in the case of the kidney after administration of a massive dose of egg white (37). It may be suggested, from all these observations, that during the (pathological) fusion of several lysosomes and several phagosomes a partial release of lysosomal enzymes into the cytoplasm may occur *in vivo*. The cells might react against this irritation by segregating from the rest of the cytoplasm the areas most affected and, in some cases, by extruding these fragments.

As was shown by Parker, Swann, and Sinclair (38), Hanssen (39), and Longley and Burstone (40), a rapid exchange of materials between the cells of the proximal convoluted tubules and the lumen takes place *postmortem* if this is not prevented by immediate fixation. Since similar changes probably also occurred *postmortem* during the present experiments, they may be confused with changes *in vivo*. However, the extrusion of phago-lysosomes and cell fragments, which was observed in the proximal convolutions of the *inner cortex*, probably occurred *in vivo*. Such changes were not seen in preparations, stained for acid phosphatase, from untreated animals, or from animals which had received low or average doses of peroxidase. The continued excretion of low amounts of peroxidase in the urine for several days after the disappearance of peroxidase from the blood, as long as phagosomes were present in the cells of the proximal convoluted tubules (41), can best be understood by the extrusion of peroxidase from the tubule cells.

It is now possible to correlate previous data (41) on the concentration of injected peroxidase in isolated fractions with the data from the present observations. A colorimetric analysis had shown that there was a relatively slow decrease, over a period of several days, of injected horseradish peroxidase in the large granule fraction (1 to 5 μ diameter), a more rapid decrease of peroxidase in the fraction containing intermediate-sized phago-lysosomes (0.5 to 1.5 μ diameter), and an even more rapid decrease of peroxidase in the microsomal fractions (41). These observations can now be understood, in part, by the relatively long persistence of peroxidase in the large phago-lysosomes in the outer cortex, and by the faster disappearance of peroxidase from the intermediate-sized phago-lysosomes in the other segments of the nephron. The rapid decrease of peroxidase in the microsomal fractions (41) may be related to the transport of peroxidase, by small phagosomes of microsomal size, to preexisting lysosomes or to the transport of peroxidase out of the cells.

After double staining for acid phosphatase and peroxidase in the same granules at different in-

tervals following treatment, the digestion of peroxidase in individual phago-lysosomes, probably by catheptic enzymes (see footnote 3, reference 3), could be estimated from the shades of color between blue and red. In general, the staining intensity for peroxidase at different intervals after administration was similar in many phago-lysosomes of the *same* cells, and the foreign protein disappeared from many granules of the same cells at approximately the same intervals following treatment (Fig. 10; see also Fig. 7, reference 3). Only one exception was noted. Peroxidase persisted much longer in the "composite bodies" of the cells of the terminal portions of the proximal convolutions than in the "normal" phago-lysosomes of smaller size in the same cells. The persistence of peroxidase in the phago-lysosomes was very unequal, however, in the cells of different segments. This can be readily understood by the unequal amounts of peroxidase originally taken up in different segments. It could not yet be determined whether or not different amounts of peroxidase passed the glomeruli of different nephrons and thus contributed to the unequal uptake and disposal of peroxidase in different segments. It is also not known whether or not the concentration of cathepsin differed in the lysosomes and phago-lysosomes of the cells of different segments. The relatively slow disposal of peroxidase in the "composite bodies," mentioned above, may be due to a loss of cathepsin from these particles, as discussed in a preceding paragraph.

It was attempted to reach a better understanding of the cytochemical properties of the phagosomes and phago-lysosomes under physiological conditions before considering pathological effects. Unexpectedly, the formation of "composite bodies" in the terminal segments of the proximal convolutions of the kidney and the formation of large vacuoles in liver cells (2, 3) have already raised questions with implications for pathology. Since the "hyaline droplets" of kidney cells have often been related to pathological changes, the application of the peroxidase procedure to pathological tissues may be of interest.

This investigation was supported by an Established Investigatorship and a research grant from the American Heart Association.

Received for publication, July 15, 1963.

REFERENCES

1. STRAUS, W., Cytochemical investigation of phagosomes and related structures in cryostat sections of the kidney and liver of rats after intravenous administration of horseradish peroxidase, *Exp. Cell Research*, 1962, **27**, 80.
2. STRAUS, W., Comparative observations on lysosomes and phagosomes in kidney and liver of rats after administration of horseradish peroxidase, *Ciba Found. Symp. Lysosomes*, 1963, in press.
3. STRAUS, W., Cytochemical observations on the relationship between lysosomes and phagosomes in kidney and liver by combined staining for acid phosphatase and intravenously injected horseradish peroxidase, *J. Cell Biol.*, 1964, **20**, 497.
4. STRAUS, W., Concentration of acid phosphatase, ribonuclease, desoxyribonuclease, β-glucuronidase, and cathepsin in "droplets" isolated from the kidney cells of normal rats, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 513.
5. DE DUVE, C., PRESSMAN, B. C., GIANETTO, R., WATTIAUX, R., and APPELMANS, F., Tissue fractionation studies. 6. Intracellular distribution patterns of enzymes in rat-liver tissue, *Biochem. J.*, 1955, **60**, 604.
6. DE DUVE, C., The lysosome concept, *Ciba Found. Symp. Lysosomes*, 1963, in press.
7. STRAUS, W., Colorimetric analysis with N,N-dimethyl-p-phenylene-diamine of the uptake of intravenously injected horseradish peroxidase by various tissues of the rat, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 541.
8. STRAUS, W., Factors affecting the state of injected horseradish peroxidase in animal tissues and procedures for the study of phagosomes and phago-lysosomes, *J. Histochem. and Cytochem.*, in press.
9. STRAUS, W., Factors affecting the cytochemical reaction of peroxidase with benzidine and the stability of the blue reaction product, *J. Histochem. and Cytochem.*, in press.
10. BURSTONE, M. S., Histochemical demonstration of acid phosphatase with naphthol-AS-phosphates, *J. Nat. Cancer Inst.*, 1958, **21**, 523.
11. BARKA, T., and ANDERSON, P. J., Histochemical methods using hexazonium pararosaniline as coupler, *J. Histochem. and Cytochem.*, 1962, **10**, 741.
12. STRAUS, W., Cytochemical observations on the transport of intravenously injected horseradish peroxidase and the development of

phagosomes in the cells of the kidney of the rat, *Exp. Cell Research*, 1961, **22,** 282.

13. FARQUHAR, M. G., and PALADE, G. E., Functional evidence for the existence of a third type of cell in the renal glomerulus, *J. Cell Biol.*, 1962, **13,** 55.
14. MILLER, F., Hemoglobin absorption by the cells of the proximal convoluted tubule in the mouse kidney, *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 689.
15. GÉRARD, P., and CORDIER, R., Esquisse d'une histophysiologie comparée du rein des vertébrés, *Biol. Rev.*, 1934, **9,** 110.
16. LAMBERT, P. P., Contribution à l'histophysiologie rénale chez les urodèles, *Arch. Internat. Biol.*, 1936, **47,** 125.
17. RATHER, L. J., Filtration, resorption, and excretion of protein by the kidney, *Medicine*, 1952, **31,** 357.
18. OLIVER, J., MACDOWELL, M., and LEE, Y. C., The structural aspects of proteinuria; tubular absorption, droplet formation, and the disposal of proteins, *J. Exp. Med.*, 1954, **99,** 589.
19. STERNBERG, W. H., FARBER, E., and DUNLAP, C. E., Histochemical localization of specific oxidative enzymes: II. Localization of diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphorase and the succindehydrogenase system in the kidney, *J. Histochem. and Cytochem.*, 1956, **4,** 266.
20. LONGLEY, J. B., and FISHER, E. R., Alkaline phosphatase and periodic acid Schiff reaction in proximal tubule of vertebrate kidney; a study in segmental differentiation, *Anat. Rec.*, 1954, **120,** 1.
21. RHODIN, J., Anatomy of kidney tubules, *Internat. Rev. Cytol.*, 1958, **7,** 485.
22. SJÖSTRAND, F. S., Über die Eigenfluoreszenz tierischer Gewebe mit besonderer Berücksichtigung der Säugetierniere, *Acta Anat.*, suppl. **1,** 1944.
23. TAPPEL, A. L., Distribution in animals and hydrolytic capacity and other properties of isolated lysosomes, *Ciba Found. Symp. Lysosomes*, 1963, in press.
24. MERCER, E. H., An electron microscope study of Amoeba Proteus, *Proc. Roy. Soc. London, Series B*, 1959, **150,** 216.
25. ROTH, L. E., Electron microscopy of pinocytosis and food vacuoles in Polymyxa, *J. Protozool.*, 1960, **7,** 176.
26. JURAND, A., An electron microscope study of food vacuoles in Paramecium aurelia, *J. Protozool.*, 1961, **8,** 125.
27. FARQUHAR, M. G., and PALADE, G. E., Segregation of ferritin in glomerular protein absorption droplets, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 297.
28. STRAUS, W., Localization of intravenously injected horseradish peroxidase in the cells of the convoluted tubules of rat kidney, *Exp. Cell Research*, 1960, **20,** 600.
29. CLARK, S. L., JR., Cellular differentiation in the kidneys of newborn mice studied with the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 933.
30. NOVIKOFF, A. B., The proximal tubule cells in experimental hydronephrosis, *J. Biophysic. and Biochem. Cytol.*, 1959, **6,** 136.
31. NOVIKOFF, A. B., and ESSNER, E., Cytolysomes and mitochondrial degeneration, *J. Cell Biol.*, 1962, **15,** 140.
32. ASHFORD, T. P., and PORTER, K. R., Cytoplasmic components in hepatic cell lysosomes, *J. Cell Biol.*, 1962, **13,** 168.
33. MOE, H., and BEHNKE, O., Cytoplasmic bodies containing mitochondria, ribosomes, and rough surfaced endoplasmic membranes in the epithelium of the small intestine of newborn rats, *J. Cell Biol.*, 1962, **13,** 168.
34. HRUBAN, Z., SPARGO, B., SWIFT, H., WISSLER, R. W., and KLEINFELD, R. G., Focal cytoplasmic degradation, *Am. J. Path.*, 1963, **42,** 657.
35. DE DUVE, C., and BEAUFAY, H., Tissue fractionation studies. 10. Influence of ischemia on the state of some bound enzymes in rat liver, *Biochem. J.*, 1959, **73,** 610.
36. BEAUFAY, H., VAN CAMPENHOUT, E., and DE DUVE, C., Tissue fractionation studies. 11. Influence of various hepatotoxic treatments on the state of some bound enzymes in rat liver. *Biochem. J.*, 1959, **73,** 617.
37. STRAUS, W., Changes in "droplet" fractions from rat kidney cells after intraperitoneal injection of egg white, *J. Biophysic. and Biochem. Cytol.*, 1957, **3,** 933.
38. PARKER, M. V., SWANN, H. G., and SINCLAIR, J. G., The functional morphology of the kidney, *Texas Rep. Biol. and Med.*, 1962, **20,** 425.
39. HANSSEN, O. E., Early post mortem renal changes in mice with one kidney exteriorized. 2. The functional and early post mortem morphology of the kidney, *Acta path. et microbiol. Scand.*, 1960, **49,** 297.
40. LONGLEY, J. B., and BURSTONE, M. S., Intraluminal nuclei and other inclusions as agonal artifacts of the renal proximal tubules, *Am. J. Path.*, 1963, **42,** 643.
41. STRAUS, W., Colorimetric investigation of the uptake of an intravenously injected protein (horseradish peroxidase) by rat kidney and effects of competition by egg white, *J. Cell Biol.*, 1962, **12,** 231.