HEMOGLOBIN ABSORPTION BY THE CELLS OF THE PROXIMAL CONVOLUTED TUBULE IN MOUSE KIDNEY

FRITZ MILLER, M.D.

From The Rockefeller Institute. Dr. Miller is a Fellow of The Rockefeller Foundation, on leave of absence from Pathologisches Institut der Universität, Innsbruck, Austria

ABSTRACT

The formation of protein absorption droplets in the cells of the proximal convolution was studied in mouse kidney. Ox hemoglobin was administered intraperitoneally and kidney specimens were collected at intervals of 30 minutes to 4 days after injection. In the lumen of the nephron, hemoglobin was concentrated to an opaque mass whose relations with the brush border and the epithelium could be easily followed. It was found that hemoglobin passes through the brush border in between the microvilli, enters the channels of tubular invaginations at the bases of the brush border, and is transported in bulk into vacuoles in the intermediate cell zone. These vacuoles increase in size and are transformed through further concentration into dense absorption droplets. Using the opaque hemoglobin content of the nephron as a tracer, functional continuity of the system of the tubular invaginations with the lumen on one side and the vacuoles on the other was demonstrated. Mitochondria lie closely apposed to vacuoles and droplets, but are not primarily involved in droplet formation. 15 hours after injection and later, ferritin and systems of layered membranes become visible in the droplets as their density decreases. These membranes are interpreted as lipoprotein membranes; similar membranes are found in the lumen of the tubuli. It is suggested that phospholipids enter into the vacuoles together with hemoglobin from the tubular lumen and form membrane systems of lipoproteins in the droplets. At 3 to 4 days the droplets contain aggregates of ferritin, and the iron reaction becomes positive in the tubule cells. No significant changes were found in the Golgi apparatus or in the microbodies during hemoglobin absorption. At all time points investigated, the terminal bars seal the intercellular spaces against penetration by hemoglobin in the proximal and distal convolutions and in the collecting ducts.

INTRODUCTION

It has been established by numerous investigations that proteins administered parenterally filter through the glomerulus and are partly reabsorbed by the cells of the proximal convoluted tubule (1–3). Protein absorption droplets (the hyaline droplets of pathologists) appear in the proximal convoluted tubule cell when the reabsorptive and digestive capacity of the cell is overtaxed (4). Homologous plasma proteins cause droplet formation only when present in great excess in the glomerular filtrate, as evidenced by the rarity of droplets in human congestive kidney disease; proteins coupled to dyes form droplets which can scarcely be metabolized by the tubule cell and persist for long periods of time (5). Thus, droplet formation indicates an intracellular accu-

Received for publication, June 2, 1960.

mulation of reabsorbed protein due to delay or failure of intracellular disposal. The droplets containing the reabsorbed protein disappear with time, rather promptly when they contain homologous proteins, slowly when foreign proteins are involved. They can be regarded as a pathological expression of the mechanism of metabolic disposal (4).

Oliver and coworkers (3, 6–11), on the basis of extensive investigations on droplet formation in the rat with histological, histochemical, and immunological methods, arrived at the conclusion that mitochondria are involved in droplet formation. Droplets were thought to represent a combination of reabsorbed protein with mitochondria or their disintegration products, and to serve as a site of intracellular digestion.

Straus (12, 13) later investigated droplet formation in rat kidneys, after injections of horse radish peroxidase. By using cell fractionation techniques he isolated the droplets and found that they contained hydrolytic enzymes and segregated peroxidase (phagosomes) but not mitochondrial enzymes. Mayersbach and Pearse (14), using fluorescein-labeled egg white did not detect a concentration of the protein in droplets, while Niemi and Pearse (15) held that fluorescent protein absorption droplets are distinct from mitochondria.

Earlier electron microscopic investigations did not resolve this conflict. Rhodin (16), experimenting with egg white, concluded that mitochondria fuse with reabsorbed protein to form the droplets. Miller and Sitte (17) also believed in the fusion of mitochondria with reabsorbed protein, but questioned Rhodin's (16) assumption that mitochondria emerge unaltered when the protein is metabolized. Gansler and Rouiller (18) stated that individual mitochondria transform into droplets by uptake and storage of reabsorbed protein, an opinion advanced already by Zollinger (19) and Rüttimann (20) on the basis of observations with the phase contrast microscope, and shared by Farquhar, Vernier, and Good (21) in an electron microscopic investigation of cases of human nephrosis. Miller (22) later found no evidence of a primary alteration of mitochondria and concluded that droplets arise either by condensation of the contents of vacuoles or by transformation of granules, both present in normal tubule cells; mitochondrial fusion with droplets was believed to be a secondary phenomenon.

The present investigation was undertaken in the hope of gaining further insight into the protein pathway through the brush border into the tubule cell, and into the origin of droplets and their relation to mitochondria. Hemoglobin was chosen because it had been shown to form droplets easily in rats (3, 6, 23). Moreover, the site of its breakdown is marked when iron is released from its molecule and synthetized into ferritin (24). For studies in the light and phase microscope, hemoglobin has the advantage of possessing a natural colored prosthetic group. The mouse was chosen as experimental animal because the fine structure of the cells of the proximal convolution had been repeatedly investigated (16, 25–28, 41).

MATERIAL AND METHODS

19 female white Swiss mice of the Rockefeller Institute stock, kept on Purina mouse pellet diet and weighing 15 to 20 grams, were injected intraperitoneally with 1 ml. of a 10 per cent solution of 2 times crystallized ox hemoglobin (obtained from Pentex Inc., Kankakee, Illinois) in physiological saline and were killed at intervals of 30 minutes, 1½, 2½, 4, 6, 15, 18, 24, and 48 hours, and 3 and 4 days after injection. One animal received 3 injections of 1 ml. each time, 49, 18, and 1¼ hours before death. Three animals served as controls. The kidneys were exposed under light ether anaesthesia, and small pieces of the cortex were fixed by immersion into ice-cold 1 per cent osmium tetroxide solution buffered at pH 7.4 (29) containing 49 mg./ml. sucrose (30) for periods between 2 and 2½ hours. The tissues were rapidly dehydrated in increasing concentrations of ethanol and acetone, or acetone alone, starting at 70 per cent, embedded in a mixture of butyl- and methylmethacrylate (80:20) with 1 or 2 per cent Luperco and polymerized under UV light. The rest of the kidneys was fixed in 10 per cent neutral formalin or formalin-calcium. For each animal sections were cut from 2 to 10 blocks with glass or diamond (48°, Sorvall) knives on a Porter-Blum microtome. A 0.5 μ section was usually cut from a block area as large as possible and observed under a phase contrast microscope. Regions containing proximal convolutions were marked on the block by looking at the cut surface through a microscope with reflected light (oblique brightfield) illumination. The block was then trimmed down under a stereo microscope to a size suitable for thin sectioning. Silver sections were spread with xylene vapor (31), picked up on grids covered with a formvar film, and blanketed with a thin layer of evaporated carbon (32) after staining part of them with lead hydroxide (33, 34) or 2 per cent uranyl acetate (35).

A Siemens Elmiskop I operated at 80 KV with 50 μ molybdenum apertures in the objective and single or double condensor was used for electron microscopy. The study is based on the examination of 1500 micrographs taken at magnifications between 2400 and 150,000. The instrumental magnification was calibrated with a grating replica.

Frozen and paraffin-embedded sections were stained with the following methods: Hematoxylin-eosin, azan, PAS, eosin 3×10^{-5} M and methylene blue 2×10^{-4} M at pH 7.8, 6.0, 5.0, 4.0, and 3.0, Sudan black B, and the acid hematein test (Baker) controlled by pyridine extraction for the demonstration of phospholipids, the Berlin blue reaction for the demonstration of iron, and the benzidine-nitroprusside method of Lepehne for the demonstration of hemoglobin.

OBSERVATIONS

The mice tolerated well the injection of 1 ml. of the hemoglobin solution, but it was not possible, in general, to inject more than this amount either in a single or in several injections without loss of animals. About 4 hours after the injection, dark brown urine was excreted which contained hemoglobin demonstrable qualitatively with the benzidine reaction. The peritoneal cavity was usually cleared of the hemoglobin solution 15 hours after injection. At this time the kidneys were swollen and dark brown. At 24 hours and later, the kidneys appeared normal again.

1. *Controls*

Since droplet formation begins in the apical and intermediate zone of the cells lining the middle portion of the proximal convolution (3), a short description of the structures in these regions is presented.

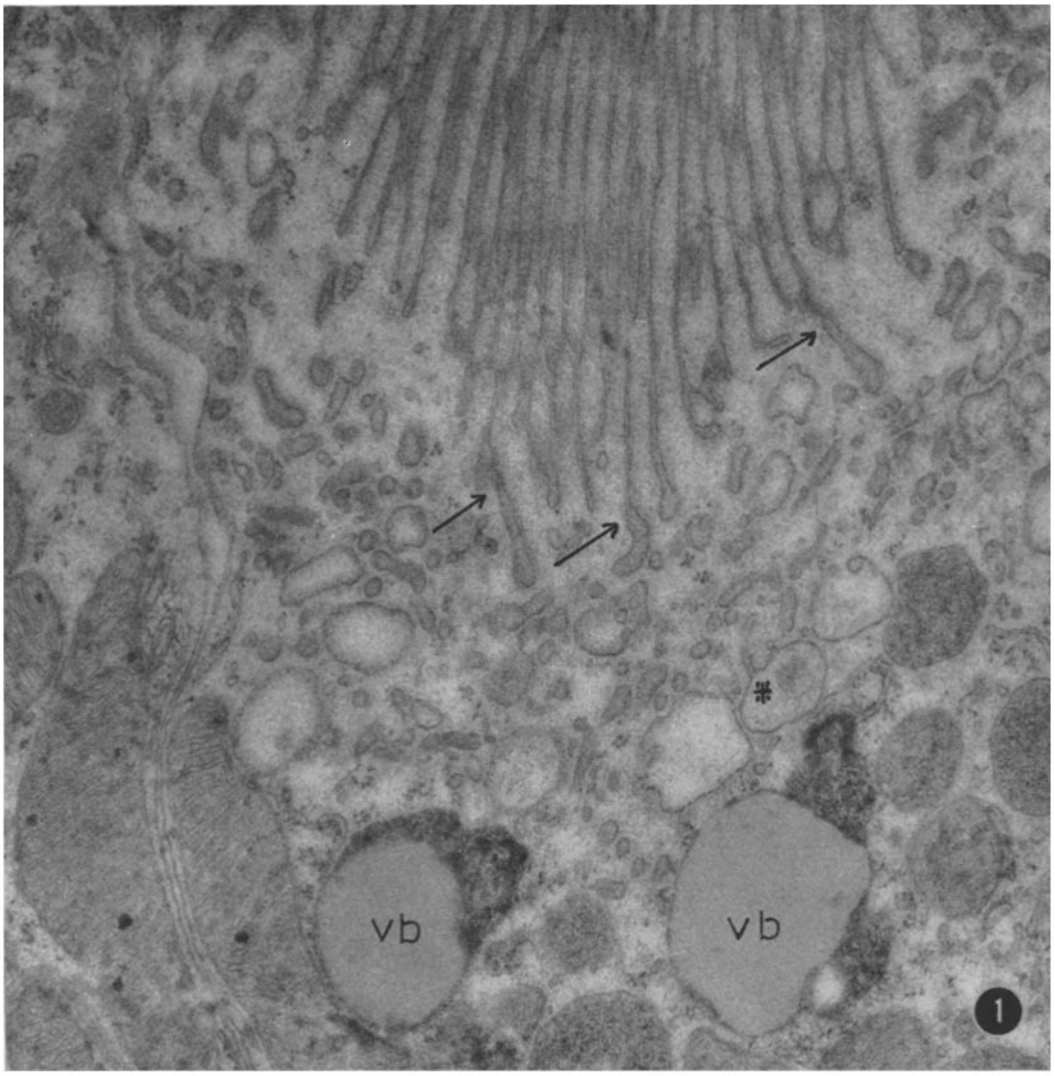
The lumen of the proximal convoluted tubule is always closed under the conditions of our fixation in the proximal and middle portions. Under phase contrast optics the brush border appears as a gray fringe and the lumen is hardly noticeable as a slightly darker line. A few minute PAS- or Sudan black B–positive granules can be seen in the intermediate cell zone below the brush border. In the electron microscope the brush border microvilli of the opposing sides of the tubule wall leave only a cleft of 100 to 200 A between them. The cell membrane around the microvilli is represented by a single line about 40 to 60 A thick; a separation into three layers was not observed.[1] In cross-sections through the brush border of the proximal and middle portion of the proximal convolution the microvilli present an imperfect close hexagonal packing (Fig. 2); the space between the microvilli measures between 50 and 100 A and is filled with a homogeneous substance the density of which is distinctly higher than that of the cytoplasm which fills the microvilli. In the distal part of the proximal convolution, towards the descending loop of Henle, the microvilli are further apart. The brush border reacts strongly with the PAS procedure, and it was suggested (36) that the dense substance between the microvilli, probably a neutral mucopolysaccharide or a mucoprotein, is responsible for this result.

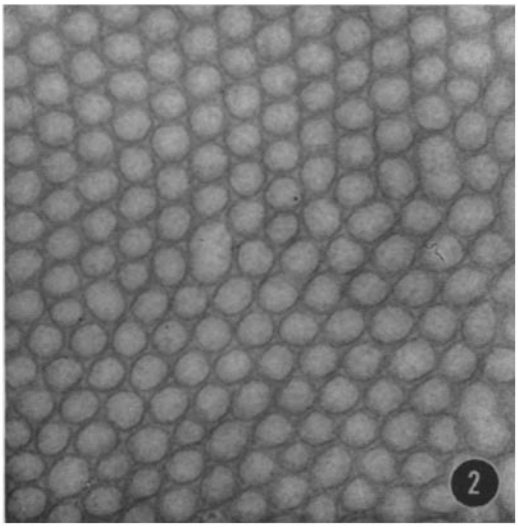
Below the brush border one finds round, elongated, or tortuous profiles bounded by a single membrane which is sometimes continuous with the cell membrane at the basis of the brush border (Fig. 1). The density inside these profiles is higher than that of the cytoplasm and corresponds to the one seen in between the microvilli. These profiles have been described as tubular invaginations by Rhodin (16), and it is generally agreed that they represent sectioned, tortuous ducts formed by deep infoldings of the cell membrane at the bases of the brush border.

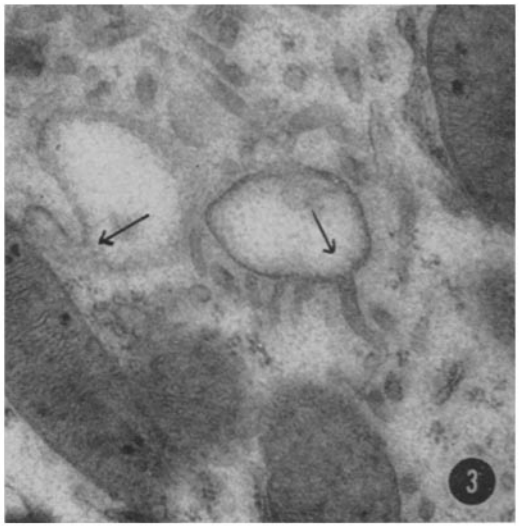
Vacuoles are always present in the intermediate cell zone between the brush border and the nucleus (16, 17, 26, 27). They measure 0.5 to 1.5 μ in diameter and are surrounded by a single membrane, 60 to 80 A thick (Figs. 1, 3, 4). Their interior is clear, with finely distributed strands of precipitated material sometimes condensed along parts of the membrane. Profiles of tubular invaginations are always seen in the vicinity, sometimes closely applied to the membrane of the vacuole or making direct connection with it (Figs. 1, 3, 4). Rarely, ring-shaped structures lie inside the vacuoles which then resemble multivesicular bodies as described by Palade (37). Such an appearance could be caused by a tangential section through several infoldings of the vacuole wall. Direct connections from the intervillous space via tubular invaginations to vacuoles were not observed.

Another component, not described previously,

[1] *Note added in proof*: In material fixed in potassium or calcium permanganate and embedded in epoxy resins the cell membrane around the microvilli appeared, however, triple-layered and of unit membrane (38) dimensions.


vb
vb
1


2


3

and here called simply "vacuolated body" (*vb*) for descriptive purposes, consists of a vacuole with an excentric accumulation of dense, coarsely granular material forming a crescent or bulge (Figs. 1, 4). This body is surrounded by a membrane appearing triple-layered after lead staining in parts and measuring *ca.* 80 A across. The vacuole is filled with a homogeneous substance of a density higher than that of the cytoplasmic matrix. The dark material extends sometimes as a small rim around the inner face of the membrane, and no membrane is seen between the vacuolated and the dense part of the body. Occasionally 3 to 4 short, dense, parallel membranes 100 to 120 A thick lie inside the dense part. One also finds membrane-bounded granules with a coarsely granular content which have similar dimensions as the crescent of the vacuolated body, and one sees occasionally a light core in them (Fig. 5). Dimensions and section geometry permit one to consider both profiles as obtained by cutting at various angles and levels through the same structure, and we assume that the granules (Fig. 5) represent sections through the crescents of the vacuolated bodies (Fig. 4). Profiles of tubular invaginations were not seen to connect with the vacuolated bodies; intermediate structures or connections between vacuoles and vacuolated bodies were not found.

A third component in the intermediate zone is represented by dense granules (Fig. 6) described as big or opaque granules by Rhodin (16). We found them surrounded by a single membrane *ca.* 60 A thick, whereas Rhodin (16) described a double membrane 100 A across. Light and dense bands with a repeating period of 80 to 90 A are visible in their interior (Fig. 6). These bands form regular concentric layers within the granule. Rhodin (16) described in addition granules containing an inner granule with layered membranes 30 A thick; such a structure was not seen in our animals. Very rarely, a few concentric rings of

Explanation of Figures

Figs. 15 and 16 are phase contrast micrographs; the rest are electron micrographs of osmium-fixed (29, 30) methacrylate-embedded sections. Figs. 10 and 29 are from unstained sections; Figs. 7, 9, 12 to 14, 21, and 22 are from sections stained with uranyl acetate (35); the rest are from sections stained with lead hydroxide (33, 34). All sections were coated with a thin layer of carbon (32). Unless indicated otherwise, all sections are from cells of the proximal convolution. Intermediate cell zone refers to the region between brush border and nucleus; basal zone, to the region between nucleus and basement membrane (16).

FIGURE 1

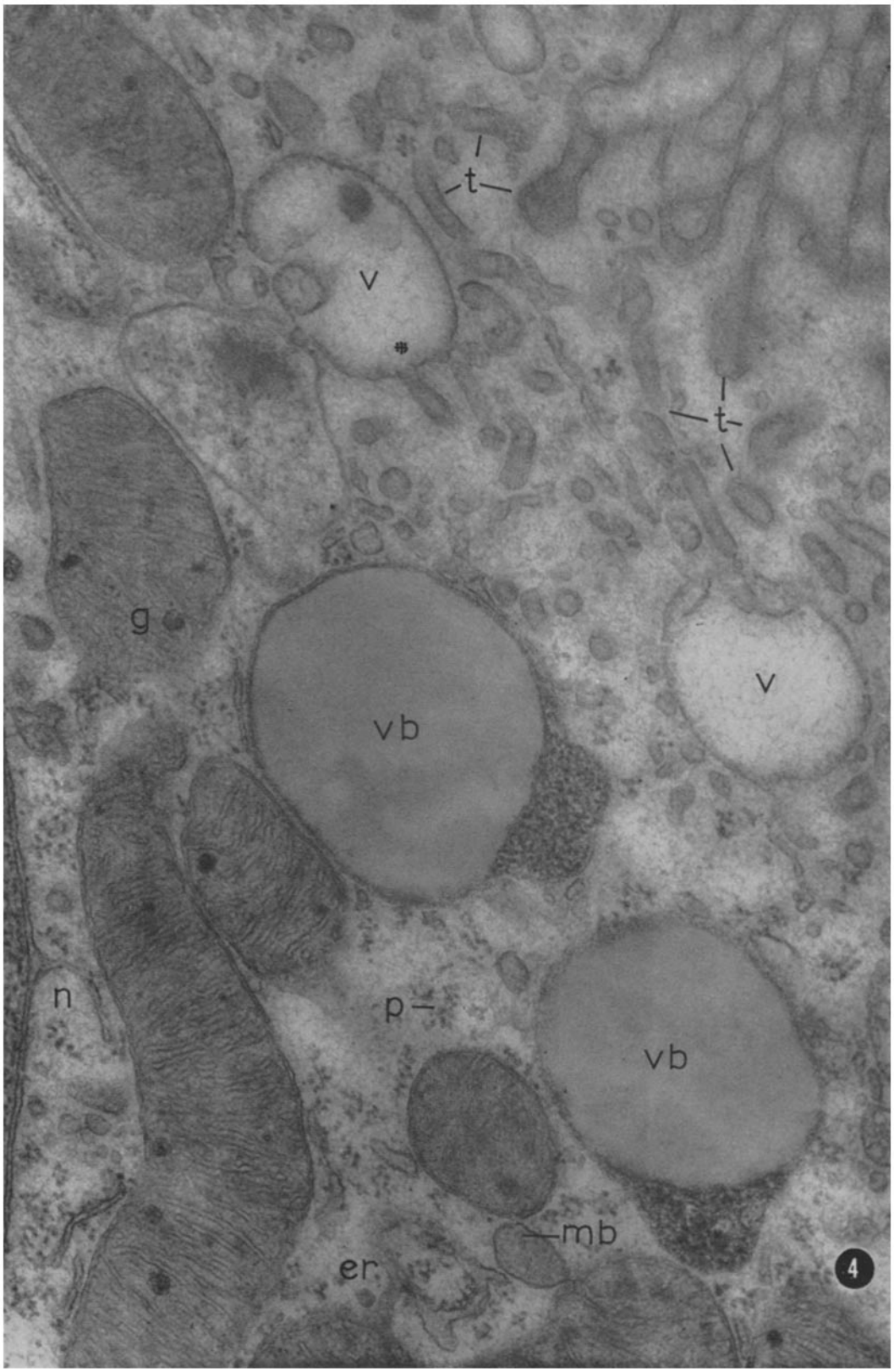
Apical and intermediate zone of a cell in the proximal convoluted tubule of a control mouse. At the basis of the brush border the cell membrane extends into the cytoplasm and surrounds profiles of tubular invaginations (arrows) the interior of which is continuous with the extracellular space in between the microvilli. Numerous other profiles of tubular invaginations and vacuoles with a light interior lie in the intermediate zone. At ⁜ a profile of a tubular invagination is in connection with a vacuole. Two large vacuolated bodies (*vb*) are in the lower part of the micrograph. × 43,000.

FIGURE 2

Cross-section through the brush border of a control mouse presenting a honeycomb pattern. A substance denser than the cytoplasmic interior of the microvilli surrounds them and holds them at a regular distance. × 61,000.

FIGURE 3

Tubular invaginations are seen to connect with two vacuoles (arrows) in the intermediate zone of a control mouse. Profiles of other tubular invaginations lie in the vicinity of the vacuoles. × 43,000.


t
v
t
g
vb
v
n
p
vb
mb
er
4

membranes were observed to be enclosed in tubular invaginations at the basis of the brush border, and this finding was discussed (16) in conjunction with secretion.

The intermediate zone contains fewer mitochondria than the basal zone, and they are smaller and not so elongated. Their dense inner granules are conspicuous (Fig. 4). Microbodies (16) are regularly present in the intermediate (Fig. 4) and basal zone. The ground cytoplasm appears clear and contains numerous profiles of the endoplasmic reticulum. Both rough and smooth surfaced elements are arranged without prevalent order; they represent cisternae of various sizes and degrees of dilatation with low density interiors. Continuity between smooth and rough surfaced endoplasmic reticulum was frequently observed (Fig. 4). Profiles of tubular invaginations can be distinguished from profiles of the smooth surfaced endoplasmic reticulum by their denser content and larger size. Connections between these two structures were not observed. Small clusters of unattached RNP-particles are found in all parts of the cell except the Golgi region and the brush border microvilli. The Golgi apparatus was found in the vicinity of the nucleus, as described by Rhodin (16).

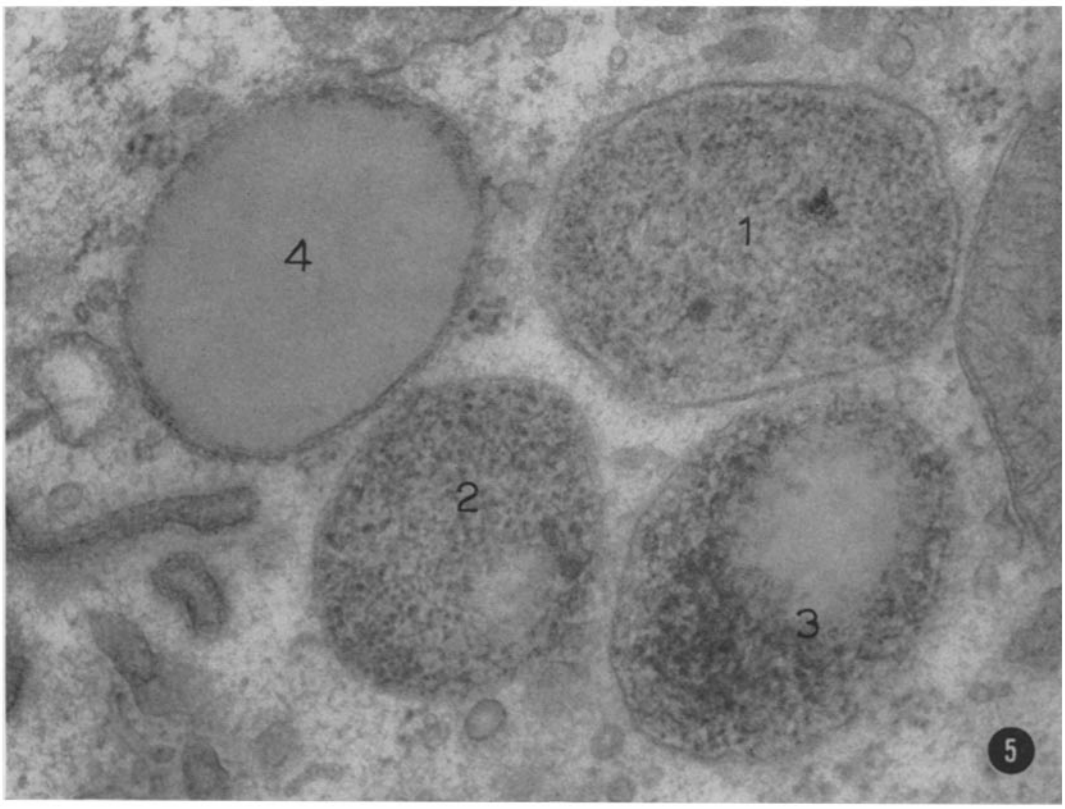
2. *Hemoglobin-Injected Animals*

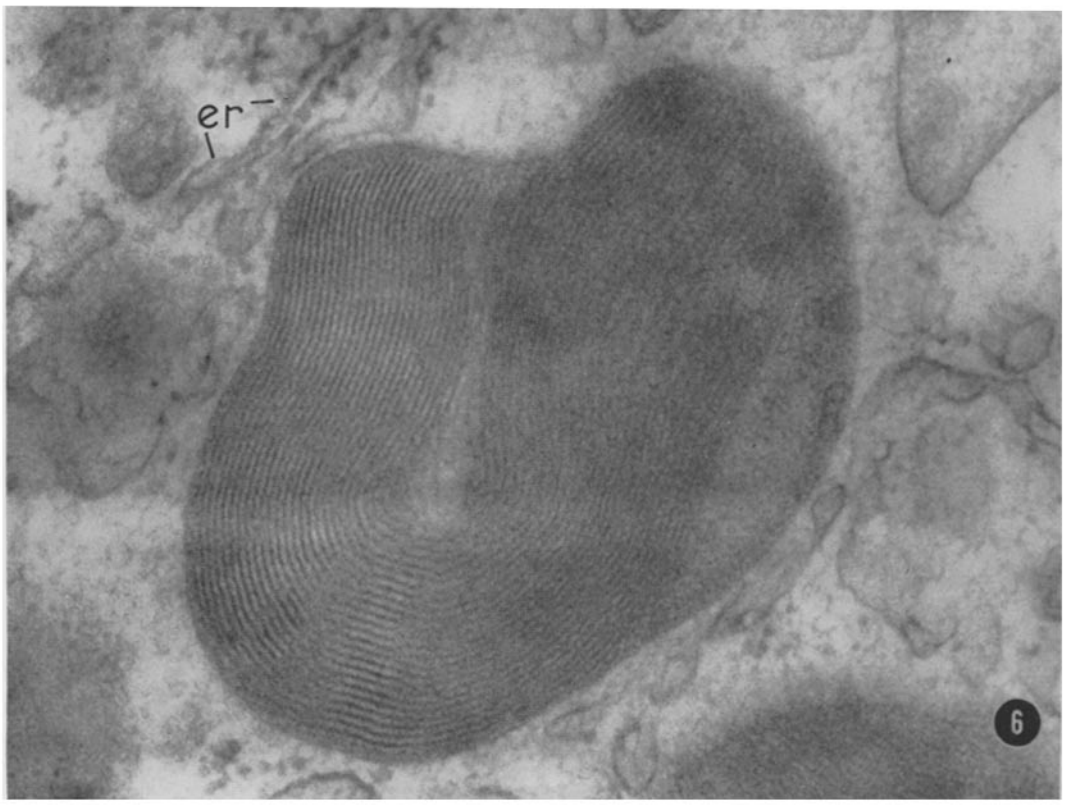
(*a*) *30 Minutes after Injection:* In the light and phase contrast microscope no change is visible. In the electron microscope, small patches of a dense substance are found in the narrow cleft of the tubular lumen. The spacing of the microvilli in the brush border is not increased, but the material among them is sometimes slightly denser than in controls. The content of the tubular invaginations is markedly denser than in controls (Fig. 7). Some vacuoles contain patches of a dense homogeneous substance spread out along the inside of their membrane and leaving the interior still clear (Fig. 8). Short finger-like projections of the vacuolar membrane of the size and shape of the tubular invaginations are assumed to indicate the connection between the two structures (Fig. 8). The vacuolated bodies (*vb*) appear unchanged.
(*b*) *1½ and 2½ Hours after Injection:* In the light microscope a few PAS-negative droplets are present in the intermediate zone below the brush border and these are larger than the tiny PAS-positive granules of the controls. They give as well a positive Lepehne reaction. In the phase contrast microscope they appear dark gray. Fine dark lines outline the luminal cleft of most proximal tubules. The open lumen of the distal convolutions, the loop of Henle, and the collecting ducts is also filled by a substance appearing gray under phase contrast optics and giving a positive Lepehne reaction.

In the electron microscope the density of the material among the microvilli of the brush border is slightly increased. The content of the tubular invaginations appears dense. The vacuoles contain patches of dense material along the inner surface of their wall or are almost filled with it (Fig. 9). A few very dense droplets are seen beside the vacuoles, and they are surrounded by the same single membrane that bounds the vacuoles (Fig. 9). The dense material among the microvilli, in the tubular invaginations, in the vacuoles, and in the droplets has the same fine, almost amorphous texture in all these locations, and practically fills the profiles of the tubular invaginations in the intermediate cell zone. We may deduce from this finding the functional continuity of the tubular invaginations with the lumen on one side and the vacuoles on the other.
(*c*) *4 and 6 Hours after Injection:* In the light microscope the number and size of the droplets

FIGURE 4

Two vacuolated bodies (*vb*) in the intermediate zone of a control mouse. The content of the vacuolar part is homogeneous while the excentrically located mass shows a coarsely granular appearance. Tubular invaginations (*t*) lie in the vicinity of vacuoles (*v*), and one of them is in connection with a vacuole at ⋕. The vacuole below shows a finely stranded content. Mitochondria have large internal granules (*g*). A microbody (*mb*) and unattached RNP-particles (*p*) are present. The endoplasmic reticulum is partly smooth surfaced and partly rough surfaced at *er*, and is continuous with the outer nuclear membrane at *n*. The brush border is in upper right corner. × 61,000.


4
1
2
3
5


er
6

giving a positive Lepehne reaction has increased. They are no longer confined to the intermediate regions, but are also found in the basal parts of the tubule cells (Fig. 15). Most of the cells of the proximal convolutions contain droplets, but some are still free of them.

In the electron microscope the picture is essentially the same as at the preceding time point. There are more droplets and fewer vacuoles only partially filled up with a dense substance, whereas at 2 hours the ratio between these two structures was reversed. The droplets are quite homogeneous and dense, and they tend to show compression waves even when cut with a diamond knife. Mitochondria lie frequently closely apposed to the membrane of either vacuoles or droplets, but are clearly separated from them and appear morphologically unaltered (Fig. 12). No change was observed in the vacuolated bodies or in microbodies.

The lumen of the distal parts of the nephron is filled with a homogeneous dense mass which corresponds to the hemoglobin cylinders seen in the light microscope (Figs. 10, 13, and 15). Hemoglobin penetrates between the apical parts of the cells in the distal convolution which sometimes bulge towards the lumen (Figs. 10, 15). The microvilli and the cell borders in the distal convolution (Fig. 10) and in the collecting ducts (Fig. 13) stand out clearly against the dark background which produces almost a negative staining effect. No reabsorption of hemoglobin was observed in the distal parts of the nephron. The penetration of hemoglobin between adjacent cell walls stops invariably at the height of the terminal bars (Figs. 11, 14). No hemoglobin is visible in the intercellular space beyond the terminal bar, demonstrating that this structure acts as an effective barrier against the penetration of large molecules.

(*d*) *15 and 18 Hours after Injection:* Droplet formation reaches its peak at this time point. All cells of the proximal convolutions with the exception of those in some distal portions are filled with droplets, some of them larger than the cell nucleus (Fig. 16). The droplets stain pale red with eosin at pH 7.8, give a positive Lepehne reaction, stain red with the Azan stain, and are PAS-negative. Some droplets stain blueish with Sudan black B. The Baker test is positive in a few droplets. The contents of the lumen give a positive Lepehne reaction and stain blue in parts with Sudan black B.

In the electron microscope the narrow lumen of the proximal convolution is filled with a dense substance which sometimes contains membranous material, organized in small whorls (Fig. 17). In one instance, triple-layered membranes of unit membrane (38) dimensions were seen in the lumen of a collecting duct (Fig. 18). They were spaced in pairs at a distance of *ca.* 55 A, and these pairs in turn separated by an interspace of *ca.* 135 A to form a repeat period of *ca.* 350 A. Unidentifiable debris was also found sometimes in the lumen of the distal parts of the nephron.

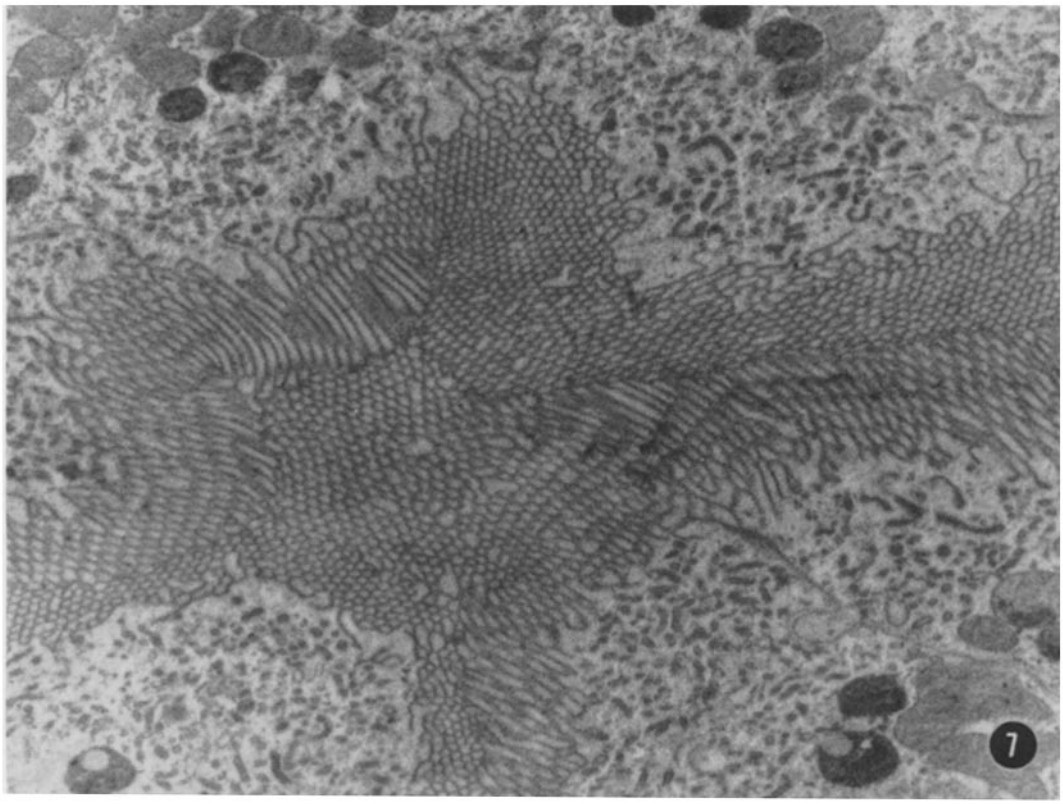
In cross- (Fig. 19) and longitudinal (Fig. 20)

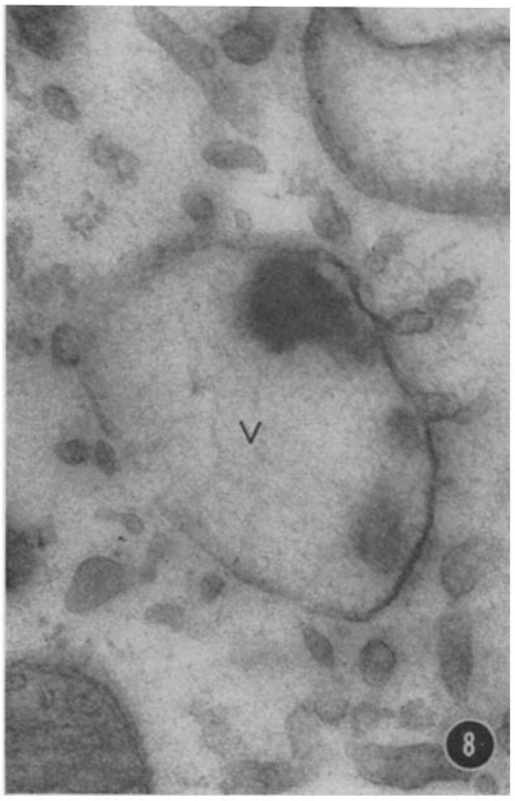
FIGURE 5

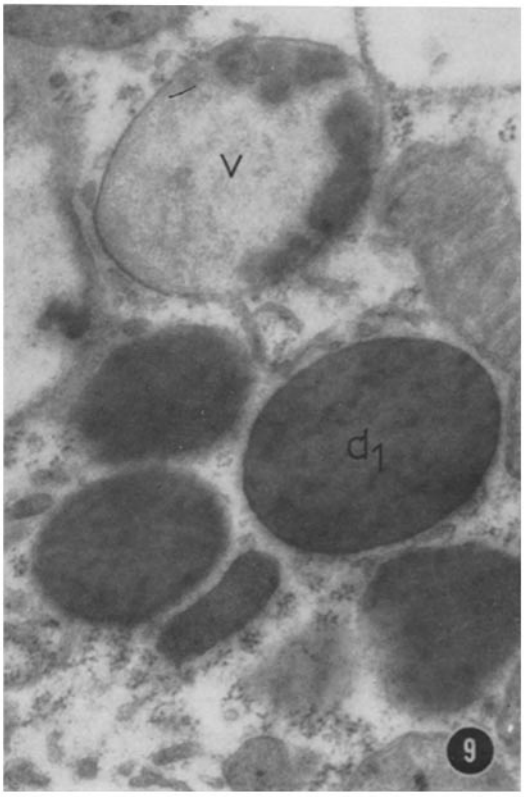
Four profiles of vacuolated bodies in the intermediate zone of a control mouse. Body No. *1* has a coarse granular content bounded by a membrane; bodies Nos. *2* and *3* show a lighter homogeneous area within the coarse granular interior, and body No. *4* appears as a vacuole with homogeneous interior and a narrow rim of coarse granular material. Such profiles could be obtained by cutting at different levels through the crescents of the vacuolated bodies shown in Fig. 4. Profiles of tubular invaginations are seen at lower left. × 78,000.

FIGURE 6

A dense body with layered dense and light bands in the intermediate zone of a control mouse. The dense bands in the upper left part of the body measure *ca.* 35 A and the repeating period is 78 A. The membrane around the body is undistinctly seen in the upper left part. The endoplasmic reticulum is partly smooth, and partly rough surfaced at *er*. We assume that this body corresponds to one of the tiny granules which give a positive Baker reaction in normal mice, and that the membranes are built of lipoproteins. × 131,000.


7


V
8


V
d_1
9

sections through the brush border the light microvilli stand out distinctly against a dark background. They are no longer closely packed to produce the honeycomb pattern of the normal brush border, but are separated by interspaces of varying width. The cell membrane around the microvilli remains clearly visible and no pinocytotic activity was observed here. No dense substance was ever observed in the intercellular space beyond the terminal bars which are found located at the base of the brush border between two adjacent microvilli (Fig. 20). Here, as in the distal parts of the nephron, the terminal bars seal the intercellular spaces against the passage of hemoglobin molecules.

Oblique sections near the base of the brush border show sometimes a striking pattern. The intervillous spaces form elongated interconnecting meandering channels 150 to 250 A wide (Fig. 21). On low power micrographs (Fig. 22) it is evident that this pattern evolves from the normal arrangement of the microvilli and becomes more complicated towards the base of the brush border. It is probably an indication of a severe distortion of the brush border during hemoglobin absorption.

The tubular invaginations are still filled with a dense substance and penetrate tortuously in some cells through the entire intermediate zone down to the level of the nucleus, taking up a much larger part of the volume of the intermediate cell zone than in controls (Fig. 23). These cells contain few droplets and still show vacuoles partly filled with dense material. Cells containing many droplets usually have fewer tubular invaginations filled with dense material in spite of the accumulation of dense material between the microvilli.

The dense droplets reach diameters of 2 to 3 μ and more and, as at earlier time points, are surrounded by a membrane which has the same thickness as the membrane around the vacuoles in the intermediate zone. Frequently, a single flattened cisterna of the smooth surfaced endoplasmic reticulum surrounds the droplets and separates them from adjacent mitochondria (Fig. 24). Some droplets are surrounded by 2 to 5 or more concentrically arranged cisternae of the endoplasmic reticulum, mostly of the smooth, but occasionally also of the rough surfaced variety. Small mitochondria are sometimes surrounded by several droplets. In thicker paraffin sections such complexes cannot be differentiated into their components because of overlap of structures.

While the majority of droplets is homogeneous at this time point, membranous material becomes visible in others (Fig. 25). The thickness of these membranes varies between 40 and 60 A. There are short stubs of membranes, circular forms resembling small vesicles or long meandering membranes disposed without order. Frequently, the membranes are arranged quite regularly, forming

---

FIGURE 7

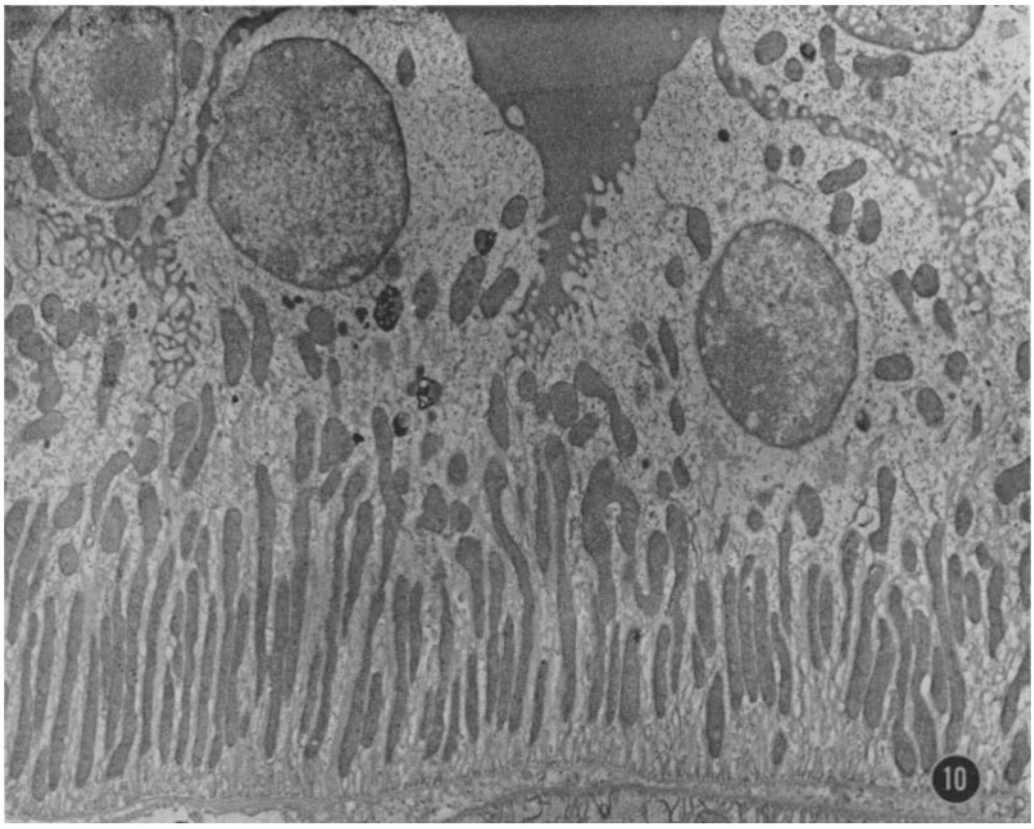
Low power micrograph of the apical and intermediate zone 30 minutes after hemoglobin injection. The space between the microvilli and the content of the tubular invaginations below the brush border appear denser than in controls. Two vacuolated bodies are near lower margin; the dense bodies near upper margin are membrane-containing bodies shown at higher magnification in Fig. 6. × 7000.

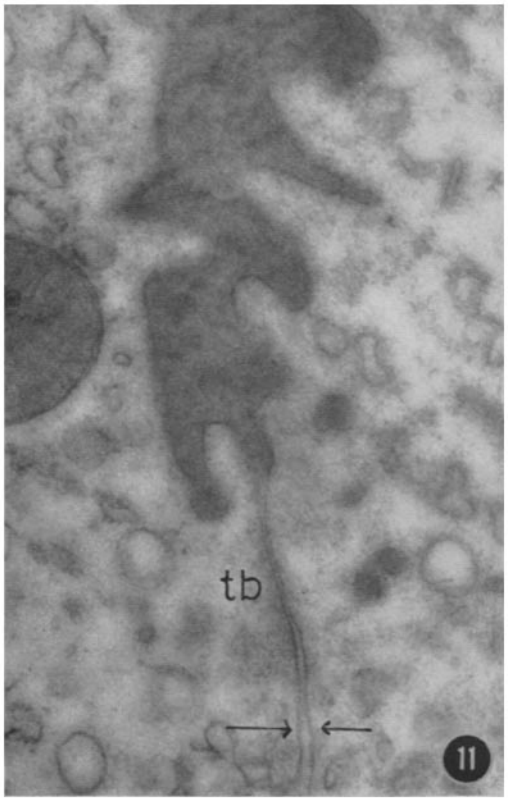
FIGURE 8

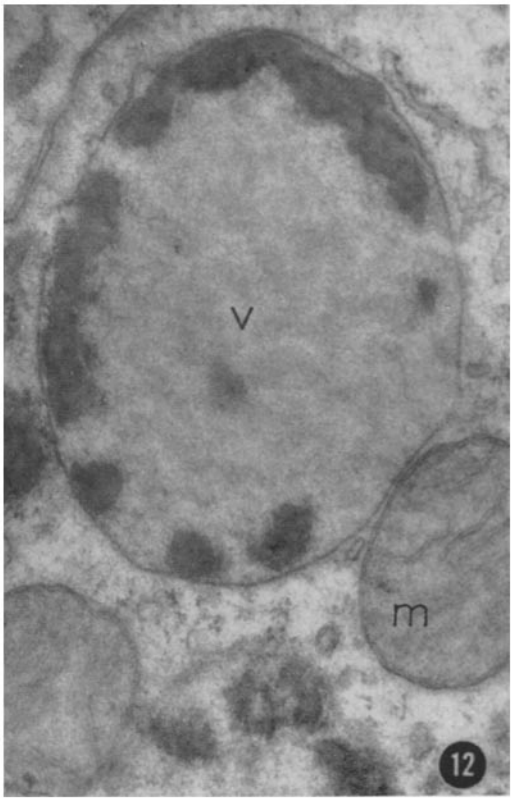
A vacuole (v) in the intermediate cell zone 30 minutes after hemoglobin injection with two small outpocketings at right thought to represent tubular invaginations in connection with the vacuole. Patches of a dense homogeneous substance lie inside the vacuole and profiles of tubular invaginations are in the vicinity. × 83,000.

FIGURE 9

A vacuole (v) in the intermediate cell zone 1½ hours after hemoglobin injection is partly filled with a homogeneous dense mass spread out along the inner surface. Droplets with dense homogeneous interior lie beside the vacuole, and in one (d_1) the surrounding membrane is visible. A mitochondrion is in the vicinity of droplet d_1. × 44,000


10


tb
11


v
m
12

layered semicircular, circular, or whorl-like systems, which comprise 10 to 25 individual membranes. The repeating period is between 80 and 100 A. The membranous material becomes more visible as the density of the hemoglobin absorption droplets decreases and is not seen in the droplets of the earlier stages. Some membrane-containing droplets display a few very dense particles measuring *ca.* 60 A (Fig. 26). No such particles were seen in the cytoplasm.

Only in two animals, 18 hours after injection, some droplets enclosed a structure suggestive of a mitochondrion trapped inside the droplet (Fig. 27). Rhodin (16) published a similar picture (Fig. 59 (16)) and concluded from this that mitochondria are coated by or coalesce with the reabsorbed protein to form the droplet. If a mitochondrion bulging into a droplet were cut transversely or obliquely through the inpocketing, a similar picture would result. In this case one should be able to see the outer membrane of the droplet surrounding the mitochondrion. There is, indeed, a membrane around such mitochondria in most of our pictures, but it is not visible all around the mitochondrion. This can be explained by low contrast of the membrane due to tangential sectioning. Although unequivocal evidence was not obtained because of the rarity of this finding, we believe that the picture of a mitochondrion inside a droplet is best explained by assuming that it represents a section through an inpocketing. That some mitochondria might get trapped between forming droplets and coalesce with them cannot be ruled out definitely, but if this happens, it is a very rare accident rather than a normal event in the life cycle of the droplet.

At all time points the content of the peritubular capillaries had the same appearance as in controls; no dense substances were found in the capillary lumina (Fig. 16).

(*e*) *24 and 48 Hours after Injection:* In the light microscope the number and size of the droplets begin to decrease. Tiny, iron-positive granules are seen in some tubule cells.

In the electron microscope the structure of the brush border returns to the normal state, and the content of the tubular invaginations is less dense. The droplets are still surrounded by a membrane, and their over-all density has further decreased. Most of them have a light or fuzzy interior and contain membranes which stand out clearly in rings, crescents, or whorls. Dense particles are visible in many droplets and are also found in the cytoplasm, either scattered or in small clusters, free of surrounding membranes. A few very dense bodies packed with layered, concentrically ar-

---

FIGURE 10

Distal convolution 2½ hours after hemoglobin injection. A dense homogeneous mass of hemoglobin fills the lumen (top) of the tubule and penetrates between the apical parts of the cells which appear slightly swollen. Slender and elongated mitochondria are typical for the distal convolution (41). No reabsorption of hemoglobin takes place in the distal convolution. Compare with Fig. 15. × 7000.

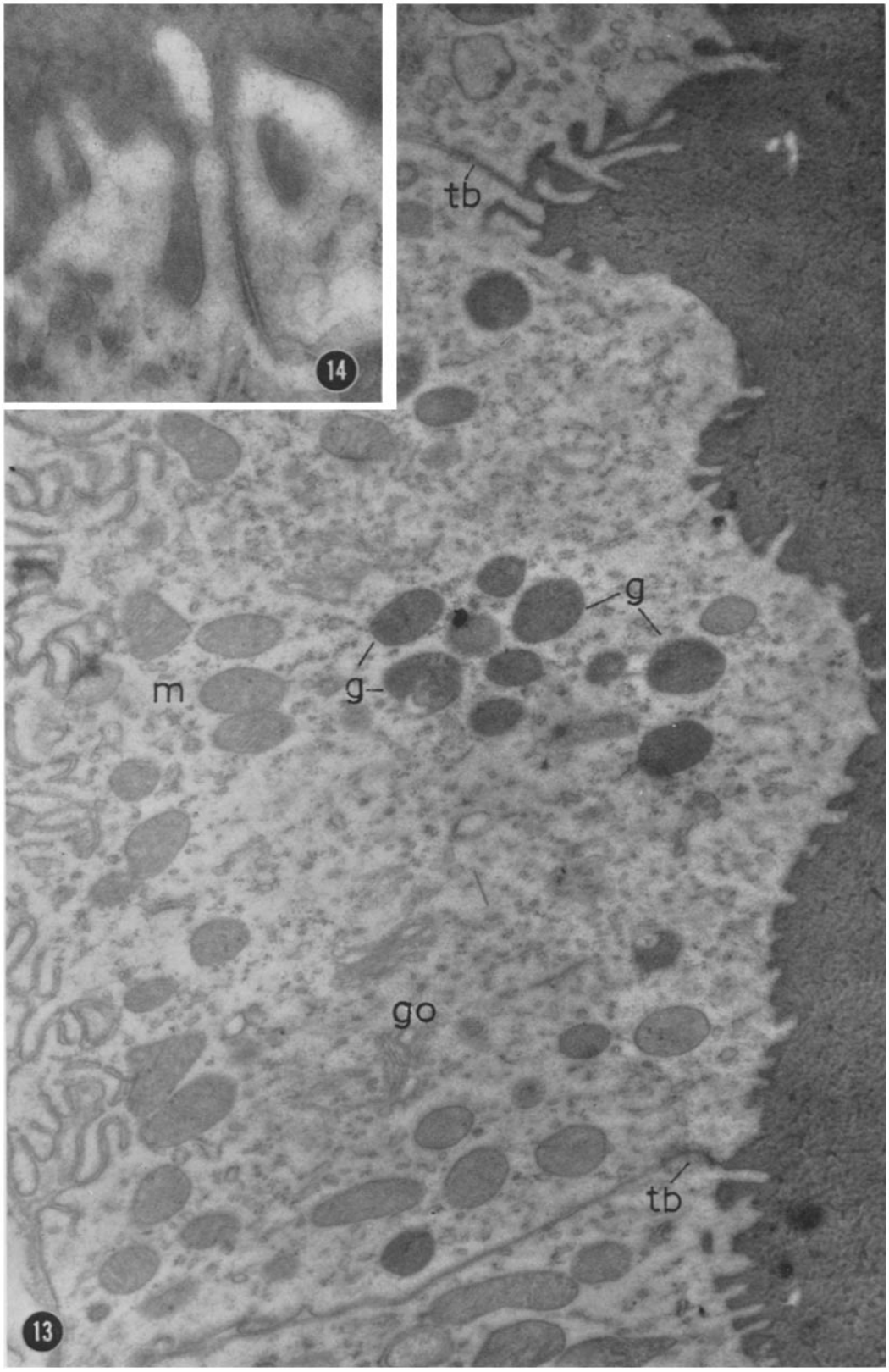
FIGURE 11

Same section as Fig. 10. The penetration of hemoglobin between the apical parts of two adjacent cells stops at the terminal bar (*tb*), and beyond this structure the intercellular space remains clear (arrows). × 61,000.

FIGURE 12

A large vacuole (*v*) in the intermediate cell zone 4 hours after hemoglobin injection is partly filled with a dense homogeneous mass spread out along the inner surface of the membrane. A mitochondrion (*m*) lies closely apposed to the vacuole, but an intervening space of *ca.* 70 A can be clearly resolved between the limiting membrane of the two structures which thus appear as separate units. × 44,000.

Figs. 8, 9, and 12 demonstrate that the dense hemoglobin absorption droplets arise from vacuoles when the latter are filled with hemoglobin.


14
tb
g
g
m
go
tb
13

ranged membranes were found surrounded by many elements of the smooth surfaced endoplasmic reticulum (Fig. 28).

(f) 3 and 4 Days after Injection: In the light microscope most of the droplets have disappeared, but the tubule cells contain tiny granules stained blue with the Berlin blue reaction.

In the electron microscope the brush border appears normal and the vacuoles in the intermediate cell zone are clear. Droplets surrounded by a membrane are still present but their size has decreased. They are filled by a matrix of moderate density and contain large aggregates of very dense particles without apparent order, measuring *ca.* 60 A. In favorable sections four subunits of about 25 to 30 A are visible (Fig. 29). Short stubs of membranes approximately 100 A thick lie sometimes in the vicinity of the dense particles. In some droplets containing dense particles an excentrically located vacuole with homogeneous content is present, and the structure then resembles the vacuolated bodies of the controls with exception of the presence of dense particles. Dense 60 A particles are also scattered freely in the cytoplasm and sometimes lie inside the microvilli of the brush border, but were not found within the tubular invaginations or in the intervillous space.

DISCUSSION

The investigation has clarified morphologically the mechanism of entrance of a protein, ox hemoglobin, into the cells of the proximal convoluted tubule, the mode of formation of protein absorption droplets, and their changes during intracellular digestion up to the 4th day after injection.

The high density of the hemoglobin solution passing through the nephron has permitted a clear visualization of the brush border. Under conditions of optimal preservation, either by osmium fixation (16, 25) or freeze-drying (39), the lumen of the proximal convolution is closed to a narrow slit. If we assume that this represents the state *in vivo* in the normal and protein reabsorbing animal, the brush border must be conceived of not only as a device that increases the surface area of the tubule cell, but also as a structure which distributes all the glomerular filtrate in thin layers and thus favors reabsorption. In the normal animal the space between the microvilli is filled by a substance with a distinct density which supposedly holds the microvilli at a regular distance. The chemical or physical changes of this substance during reabsorption have not been investigated, but its "cementing" capacity is obviously diminished as evidenced by a wider separation of the microvilli. No incorporation of hemoglobin into the brush border microvilli by a process of pinocytosis (40) has been detected.

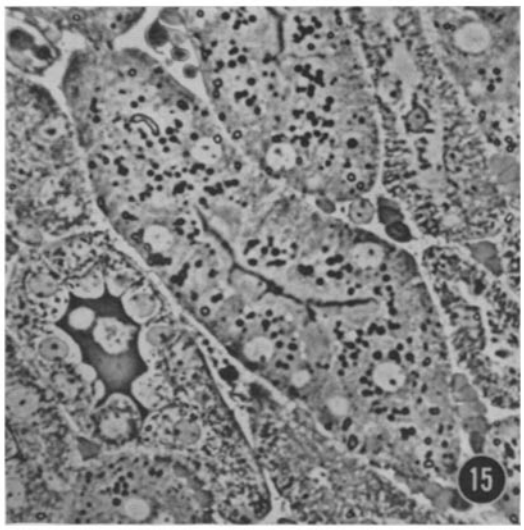
A direct connection from the base of the brush border via a tubular invagination to a vacuole has not been depicted clearly in the literature. Pease (27, 28), who first drew attention to this connection, showed only small dilatations below the base of the brush border in connection with the intervillous space, whereas the larger vacuoles deeper in the cell were not so connected. Clark

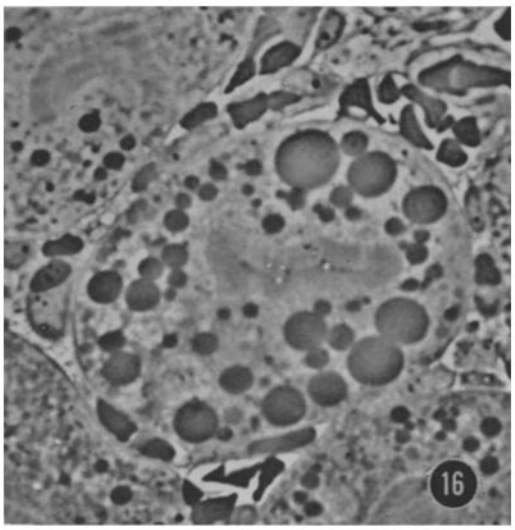
FIGURE 13

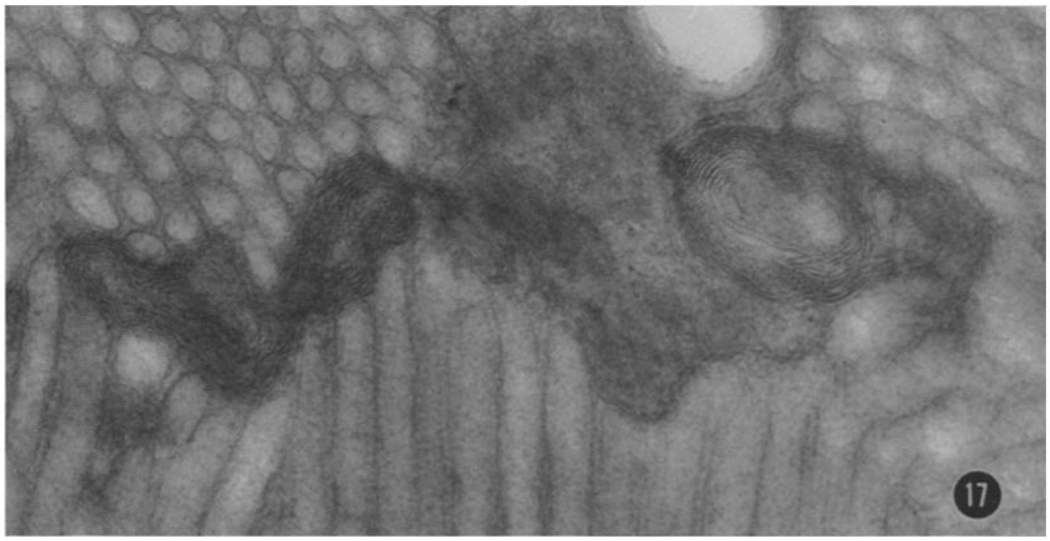
Light cell lining a collecting tubule, 4 hours after hemoglobin injection. The lumen at right is filled with a dense, almost homogeneous mass which corresponds to the hemoglobin cylinders seen in the light microscope. The luminal cell border with its microvilli stands out distinctly against the dark mass in the lumen. The Golgi apparatus is at *go*. The basal cell membrane at left is infolded. Dense granules (*g*) are distinct from mitochondria (*m*); they are present also in normal animals and are described elsewhere in this journal (78). Terminal bars (*tb*) are located close to the lumen. × 21,000.

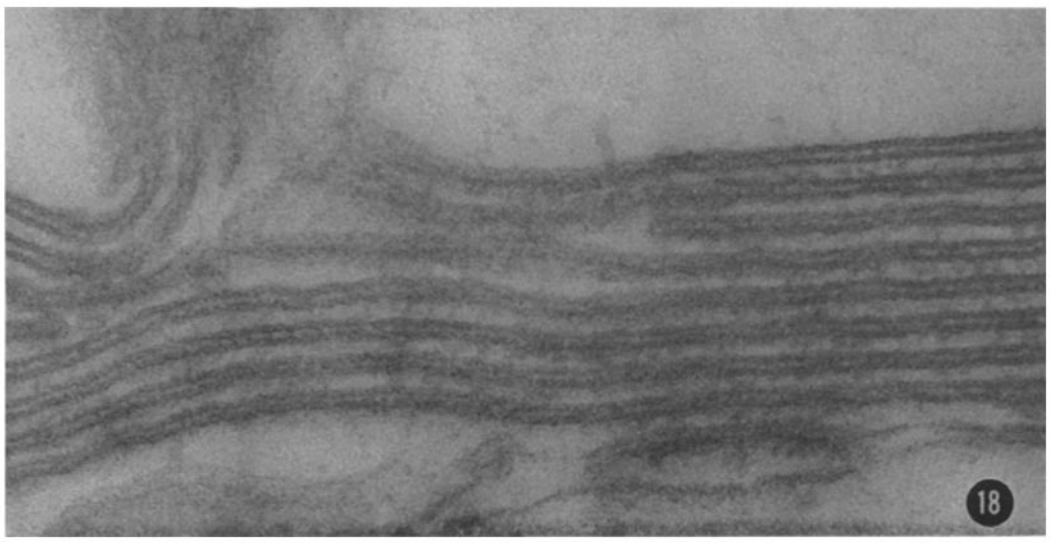
FIGURE 14

Terminal bar from same collecting tubule as in Fig. 13. Dense content in lumen (top) outlines microvilli. No hemoglobin penetrates into the intercellular space beyond the terminal bar. × 61,000.


15


16


17


18

(26) also indicated the existence of such connections, but did not furnish clearer evidence than Pease (27, 28). Sjöstrand and Rhodin (25), and Rhodin (16, 41) thought that the vacuoles were the equivalent of autofluorescent granules (39) after dissolution of their content during processing. Rhodin (16, 41) did not observe direct connections between vacuoles and intervillous space and stated (41) that the function of the vacuoles is unknown.

In the material derived from our experiments, the system of the tubular invaginations was filled with the same dense and almost amorphous mass that is also found in the lumen and in the vacuoles. Although a direct connection between the intervillous space and a vacuole was not included in any of our sections, our experiments demonstrate that the system of the tubular invaginations is functionally continuous with the lumen on one side and the vacuoles on the other. The pathway provided by these structures is used during hemoglobin reabsorption; hemoglobin enters the ducts of the tubular invaginations from the extracellular, intervillous space at the base of the brush border and is transported into vacuoles which are gradually filled up until the stage of a dense droplet, surrounded by a membrane, is reached. The continuity between intervillous space, tubular invaginations, and vacuoles may be permanent or intermittent. In the latter case, the picture observed in our sections would reflect the fact that the tubular invaginations become pinched off below the brush border and transport their content to the vacuoles in small portions. This would represent a mechanism of transport (across a part of the cytoplasm) related to pinocytosis and would resemble the "transport in quanta" (42) across the endothelial cell.

During reabsorption and droplet formation the system of the tubular invaginations becomes very elaborate and expanded. At the height of droplet formation the number of droplets observed in sections exceeds by far the number of vacuoles in the controls. A continuous new formation of cell membranes for the tubular invaginations and possibly also for the vacuoles must, therefore, be postulated. This would require energy and the transport of building blocks for lipids and proteins to these sites. No morphological evidence is available from our experiments to support this specu-

FIGURE 15

Kidney cortex 4 hours after hemoglobin injection. A dense mass fills the lumen of a distal convolution at left (compare with Fig. 10). Proximal convolution in center shows a dark line indicating the narrow lumen. Dark hemoglobin absorption droplets lie mainly in the intermediate cell zones above the nuclei, but some are seen also in the basal parts of the cells. × 700.

FIGURE 16

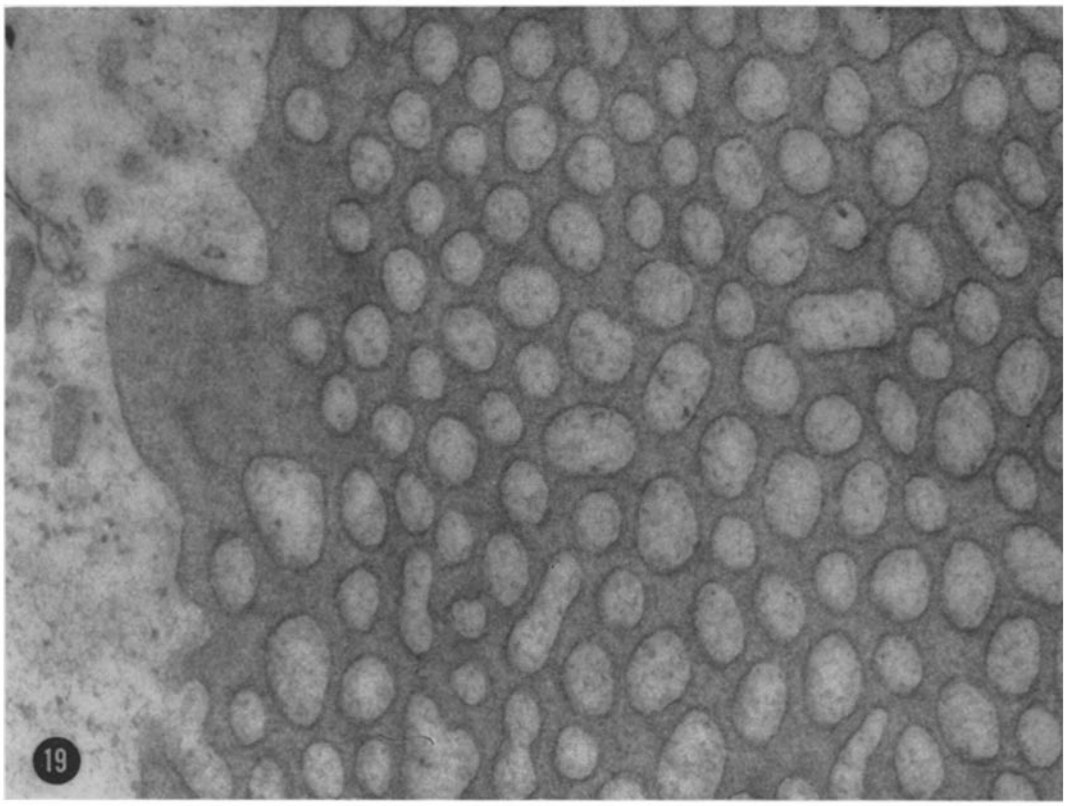
Cross-section through proximal convolution 15 hours after hemoglobin injection. Brush border in tubule in center appears as a gray fringe and the lumen is represented by a slightly denser line. Dark hemoglobin absorption droplets fill the cells, and some are larger than a nucleus. Peritubular capillaries (upper right and lower center) contain erythrocytes but no dense substance. × 1300.

FIGURE 17

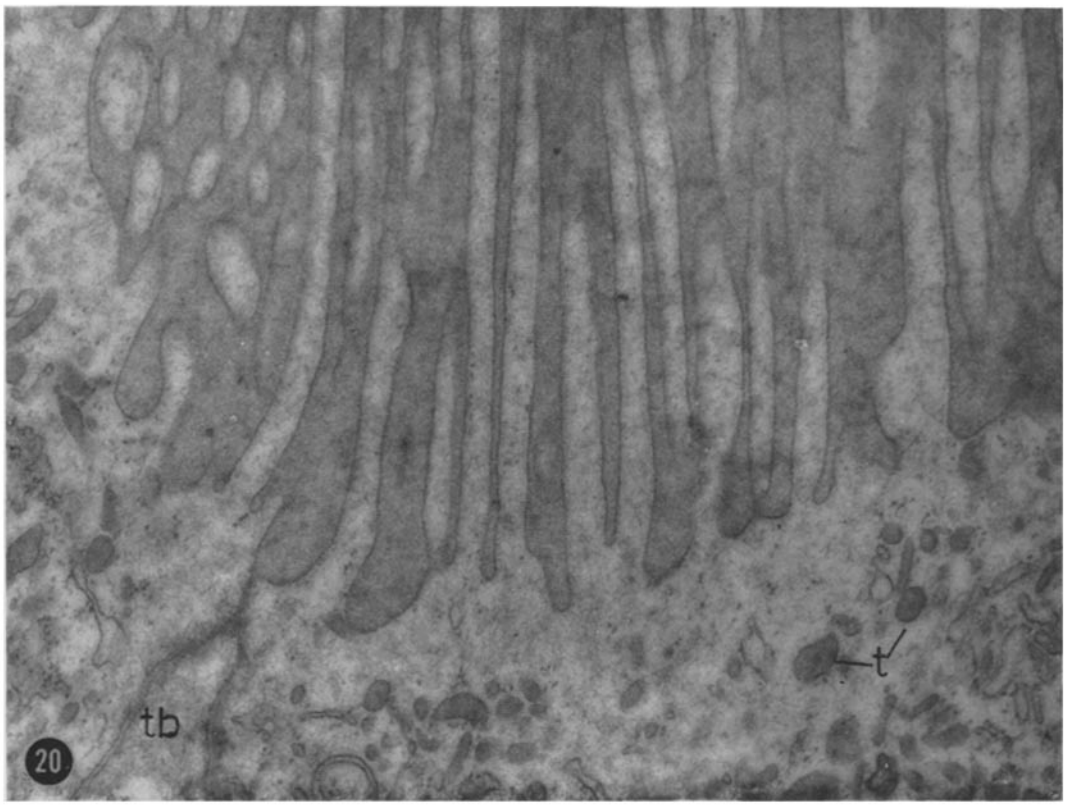
The lumen of a proximal convolution 15 hours after hemoglobin injection is filled with a dense mass which contains layered membranes. The brush border is seen in cross-section in the upper left and in longitudinal section in the lower part of the micrograph. × 61,000.

FIGURE 18

Parallel membrane systems found in the lumen of a collecting tubule 15 hours after hemoglobin injection. The spacings of the membranes are given in the text. × 130,000.



19



20

lation. It can only be pointed out that the machinery for such a process is present in the cells of the proximal convoluted tubule which have abundant mitochondria, a well developed smooth and rough surfaced endoplasmic reticulum, and a large Golgi apparatus. No significant changes were detected in the disposition of the Golgi apparatus at the beginning, the height or the end of reabsorption, although it was suggested (43) that the Golgi apparatus serves as an intracellular depot of membrane material.

It would also seem obvious that there is a limit to the reabsorptive capacity of each tubule cell. The appearance of a large number of protein absorption droplets is an indication that the cell has reabsorbed more than it can digest in a given time (4). The mechanism by which reabsorption is stopped could well be determined by the capacity of the cell to form new membranes for transport in tubular invaginations and storage in vacuoles. In support of this hypothesis we observed, 15 hours after injection, cells loaded with droplets and with a massive accumulation of hemoglobin in the intervillous space of the brush border which had little dense material in their tubular invaginations. This seems to indicate that no more hemoglobin is reabsorbed.

The growth of the droplets to a size many times larger than the vacuoles in which they form is conspicuous. Since they stay surrounded by a membrane, the latter must grow, too, and this is made possible by incorporation of the membrane around the tubular invaginations into the membrane bounding the vacuole. Thus, each time the vacuole receives a new quantum of hemoglobin, it also incorporates parts of the membrane necessary for its expansion from the transporting tubular invaginations. The droplets will cease to enlarge when the input of hemoglobin *via* tubular invaginations comes to a halt.

Probably some condensation of the hemoglobin solution takes place in the vacuoles by withdrawal of water. Fully matured droplets tend to develop compression waves during sectioning while vacuoles partly filled with hemoglobin do not, and this indicates that the consistency changes during droplet formation.

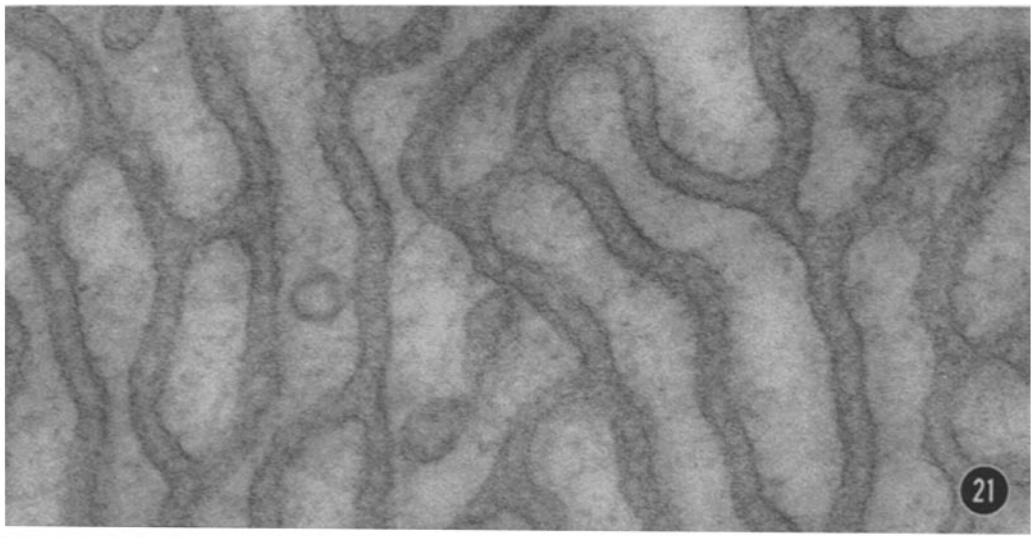
The hypothesis, advanced by Oliver and coworkers (3, 4, 6) and by other authors cited in the introduction, that mitochondria fuse or become coated with reabsorbed protein to form the droplet, could not be confirmed. Droplets are clearly separated from mitochondria in the great majority of the material examined. But the outer membranes of mitochondria and droplets are frequently separated by a narrow space of only 100 A or less, and groups of smaller mitochondria may be surrounded by several droplets. Such complexes, when stained supravitally with Janus green or in sections with the Baker procedure (3, 6, 7) could easily lead to the erroneous conclusion that the droplets contain substances derived from mitochondria because the aggregated structures cannot be resolved individually by such methods. Changes in mitochondrial fine structure, such as swelling during the earlier stages of protein reabsorption (17, 18), were not observed at any time point. Mitochondria trapped by, or protruding into, droplets were found so rarely that this event must be ruled out as a regular mechanism of

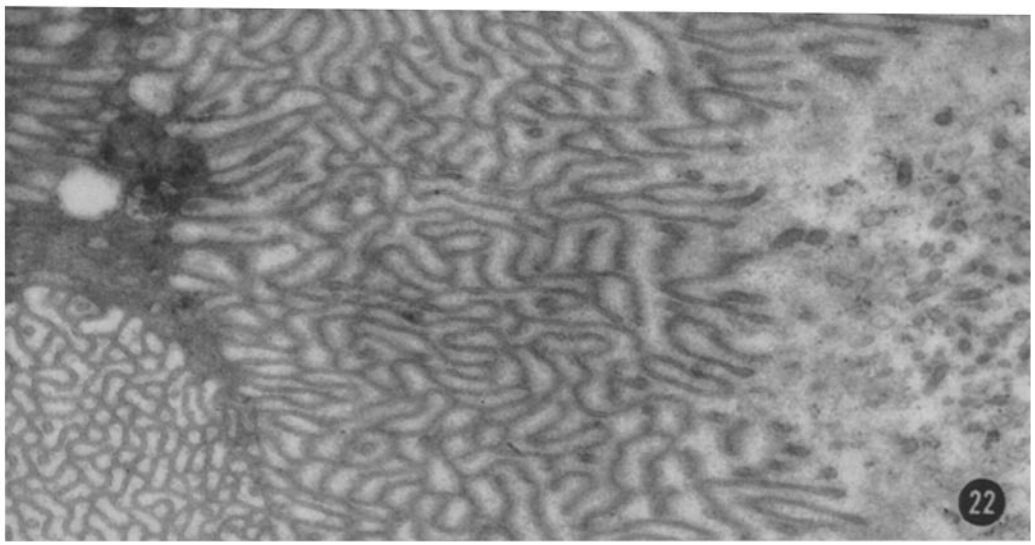
FIGURE 19

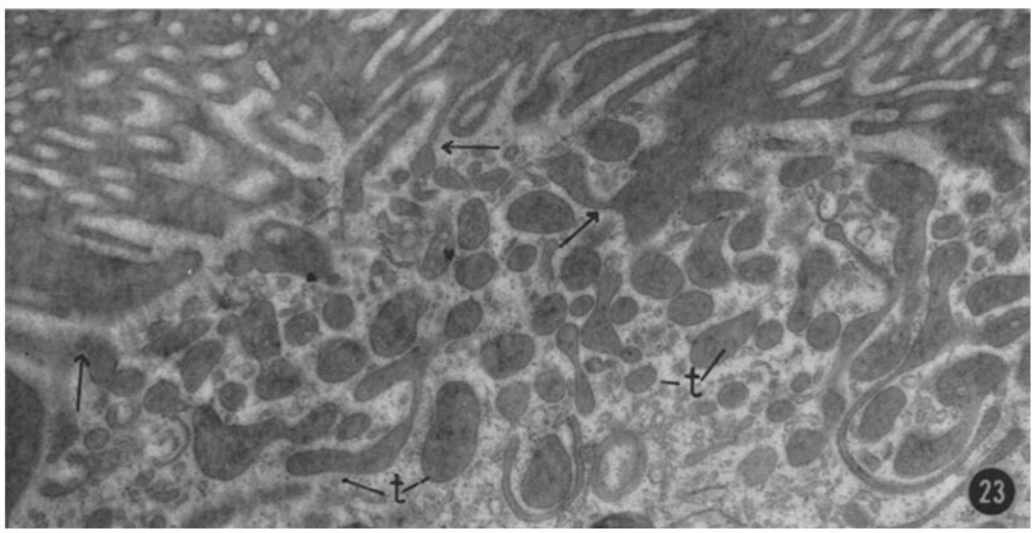
Cross-section through brush border 15 hours after hemoglobin injection. The microvilli appear light and are more widely separated than in controls because the intervillous spaces are filled by a dense homogeneous mass. Cell membrane around microvilli remains distinctly visible and shows no signs of pinocytotic activity. Cytoplasm of a tubule cell at left, cut below brush border, has the same density as the interior of the microvilli. × 87,000.

FIGURE 20

Longitudinal section through basal part of brush border 15 hours after hemoglobin injection. A dense homogeneous mass fills the widened intervillous spaces. Note same density in profiles of tubular invaginations (*t*). No dense substance is seen in intercellular space below terminal bar (*tb*). × 44,000.


21


22


t
t
23

droplet formation; it is an accident rather than a significant occurrence.

The appearance of membranous material in the droplets has misled investigators (16, 17, 26) into the belief that it was derived from, or represented altered mitochondria. With the better resolution now achieved, this possibility must be ruled out with exception of the rare instances in which mitochondria have been secondarily trapped in droplets.

Droplets with decreased density and layered internal membranes are morphologically identical with bodies that were described under a variety of names in different cells, such as alveolar macrophages (44–46), epithelial cells of the jejunum (47), tracheal epithelia (48), cells of the uterus (49, 50) and the thyroid (51), giant cells of rat placenta (52), visceral epithelial cells of the glomerulus after poisoning with uranyl nitrate (53), cultured cells of kitten cerebellum (54), and the adrenal cortex (55), to name a few. The term "cytosome," originally proposed by Lindner (56), was used by Schulz (44) for a variety of membrane-containing bodies assumed to have derived from microbodies and to develop either into mitochondria or into storage bodies. The schematic cycle of development proposed by Schulz (44) remains, however, hypothetical for the moment. The membrane systems in granules of mucus-secreting intermediary cells of the gill epithelium of axolotl were named delta-cytomembranes (57) and thought to be related to mucus secretion, although the membranes themselves were probably lipoproteins.

Interpretations of the membranous structures appearing in hemoglobin absorption droplets and in similar bodies in other cells must remain speculative as long as no chemical data on isolated droplets or bodies are available. The hypothesis that they might represent lipoprotein membranes is supported by the following arguments. Membrane systems of pure phospholipids have a repeating period of *ca.* 40 A and the width of the dark bands is around 18 to 20 A (58). The dark bands in the droplets measure 40 to 60 A and when ordered their repeating period is between 80 and 100 A. By addition of globin to phospholipids, Stoeckenius (58) observed an increase of the width of the dense lines on the outer surface of myelin figures up to 50 A. The dark lines in the droplets, 40 to 60 A in width, could be explained, then, as constructed of a film of protein plus the polar groups of a leaflet of phospholipids which appear dense after osmium fixation (59). The light bands could accommodate a bimolecular leaflet of fatty acid chains for which a longer chain length or a more unfolded state than in the phospholipids investigated by Stoeckenius (58) should be postulated. That phospholipids do occur in droplets is further confirmed by a positive Baker test in some droplets, a reaction already noticed by Oliver and coworkers (7). The basic protein,

FIGURE 21

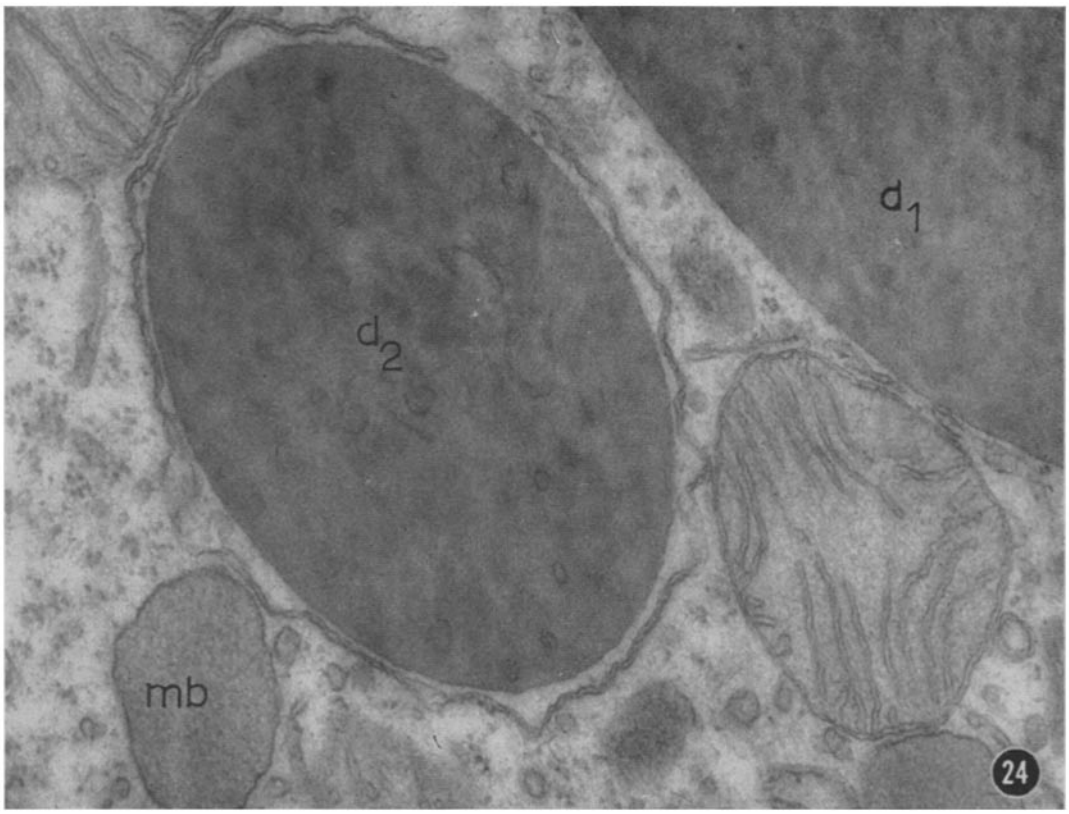
Oblique section near basis of brush border 15 hours after hemoglobin injection. The intervillous spaces form meandering interconnecting channels which are filled with a dense substance. Cytoplasmic interior of microvilli appears light. × 168,000.

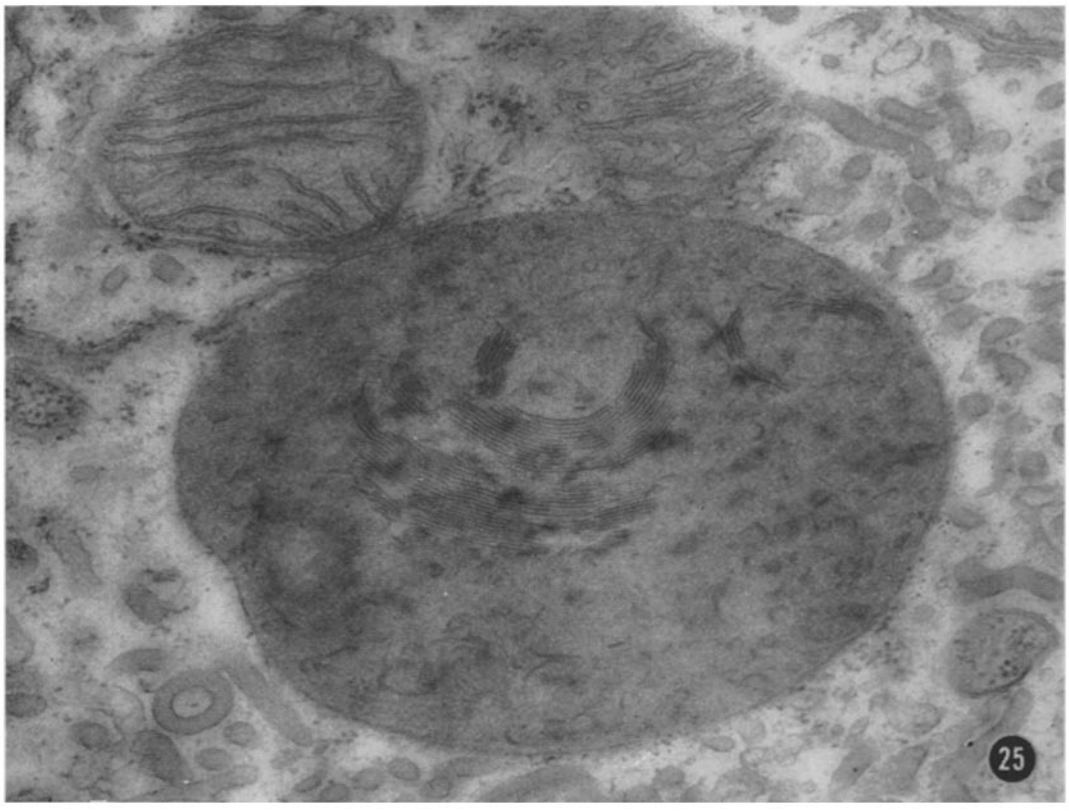
FIGURE 22

Low power micrograph of brush border 15 hours after hemoglobin injection. Dense mass in lumen at left penetrates in between the microvilli and fills the intervillous spaces which form meandering channels. Fig. 21 was taken from field at lower left. A few profiles of tubular invaginations are at right in the intermediate zone. × 30,000.

FIGURE 23

Brush border and intermediate cell zone 15 hours after hemoglobin injection. A homogeneous mass of the same density lies in the lumen (top), penetrates in between the microvilli, and fills the tortuous tubular invaginations (*t*) which reach deep into the cell. Connections between cell membrane around microvilli and tubular invaginations are marked by arrows. × 25,000.


d_1
d_2
mb
24


25

globin, reacts with certain phospholipids to produce salt-like components (60, 61), and was used by Stoeckenius (58) for the production of protein-coated myelin figures. The fact that membranes become visible in the droplets together with dense particles of ferritin 15 hours after injection indicates that within this time hemoglobin is broken up into smaller units and that globin could be available for the formation of lipoprotein membranes. The staining reaction of the droplets with eosin at pH 7.8 shows that they contain basic proteins.

It is not clear how phospholipids find their way into the droplets except in the case of accidentally trapped mitochondria which could furnish phospholipids upon disintegration of their membranes. Because this event was so rarely observed we assume that phospholipids enter the vacuoles at the time when hemoglobin is reabsorbed. We could not extract any lipids from the hemoglobin preparation used for injection. The reaction to Sudan black B in paraffin sections and the occurrence of membranes in the tubular lumen suggest the presence of lipids in the fluid passing through the tubules. Whether they derive from cells partly damaged during hemoglobin excretion as indicated by the observation of cellular debris in the lumen or whether they escape from the blood plasma through a damaged glomerular filter cannot be decided with the evidence at hand.

The dense particles mentioned in the droplets before, are undoubtedly ferritin. Ferritin molecules are composed of a dense core of a ferric hydroxide–phosphate complex surrounded by a shell of apoferritin (62). The ferritin molecule measures approximately 100 A across (62), but in sections only the ferric hydroxide–phosphate core measuring 55 to 60 A is visible (24). In favorable sections this core is seen to be composed of 4 subunits, each with a diameter of *ca.* 27 A (63, 64). Kerr and Muir (65) have recently postulated 6 subunits smaller than 20 A placed at the apices of a regular octahedron.

Ferritin cores become visible in small amounts in droplets 15 hours after injection and are amassed there as well as scattered in the cytoplasm at 3 and 4 days. Droplets with amassed ferritin correspond exactly to the "siderosomes" found by Richter (24, 66) in rat kidney after intraperitoneal injection of rat hemoglobin, by Wessel and Gedigk (67) in macrophages after subcutaneous injection of ox hemoglobin, and by Lindner (49) in the uterus of vitamin E–deficient rats. From what has been said before it seems evident that the ferritin-containing droplets or siderosomes, as they might be called at this stage, are not altered mitochondria as was initially claimed by Richter (24) but later corrected by him (68).

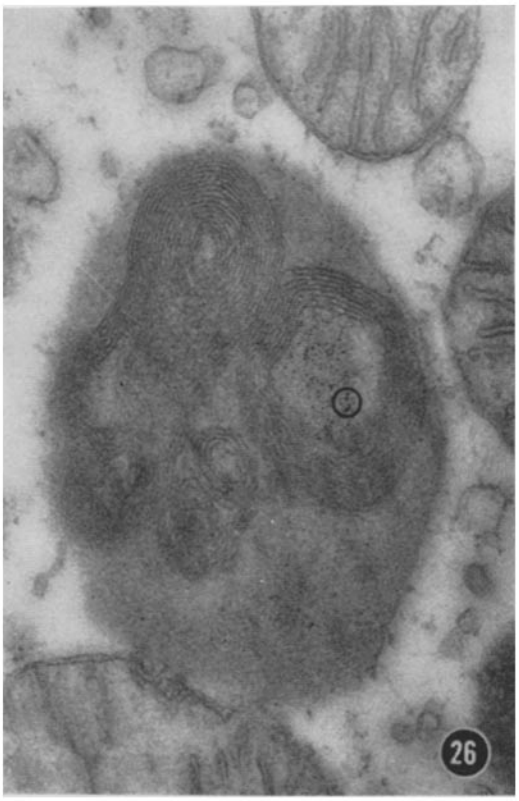
The appearance of dense ferritin cores implies that apoferritin has been synthesized by the tubule cell when iron becomes available from the breakdown of hemoglobin (69). Richter (68) discussing the formation of ferritin in the cell, has pointed out that apoferritin might be synthesized in the vicinity of the endoplasmic reticulum. It is of interest, then, that droplets are frequently surrounded by one or several elements of the endo-

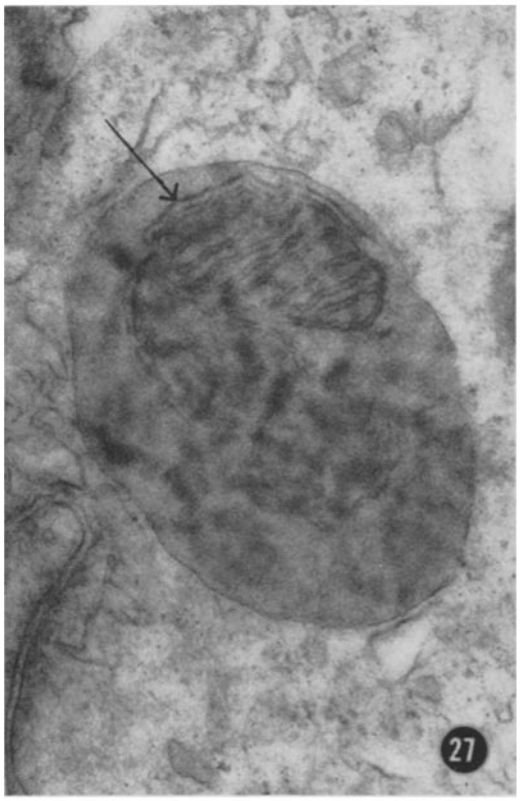
FIGURE 24

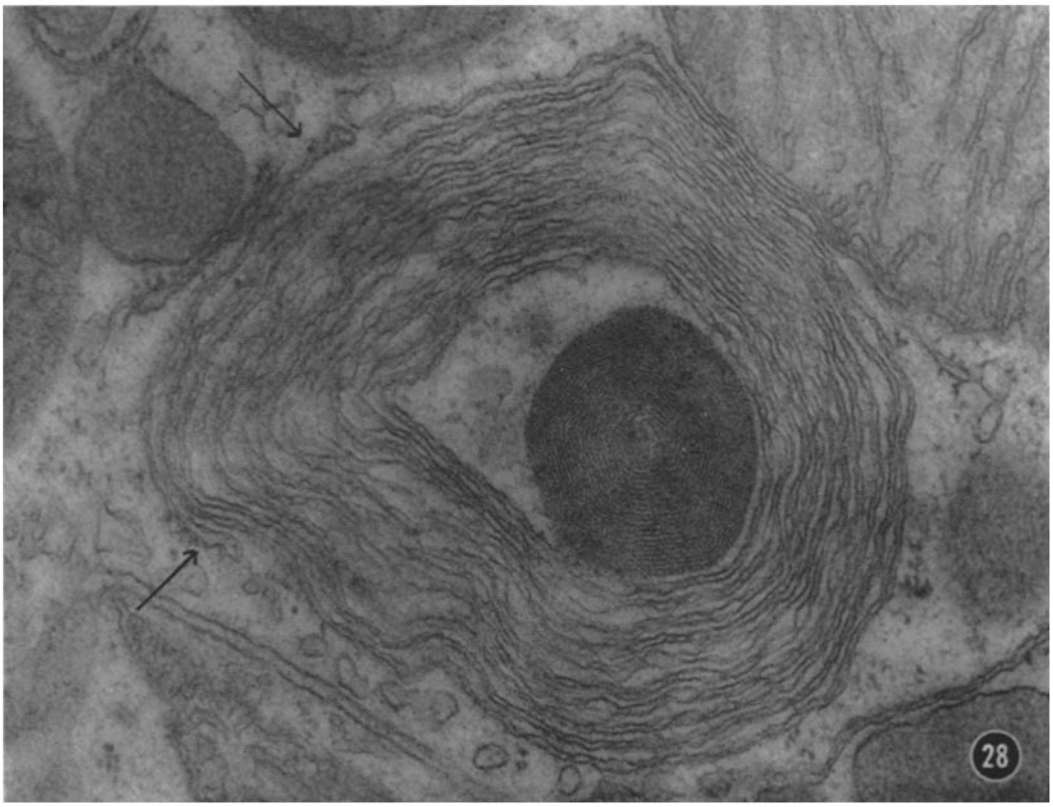
Hemoglobin absorption droplets 15 hours after injection. Droplet d_1 appears homogeneous, while droplet d_2 contains a few unordered membranes. The droplets are bounded by a distinct single membrane; droplet d_2 is almost completely surrounded by a single element of smooth surfaced endoplasmic reticulum. Note close apposition of mitochondria to droplets and elements of the endoplasmic reticulum between mitochondria and droplets. Microbody (*mb*) and mitochondria appear unaltered. × 61,000.

FIGURE 25

Hemoglobin absorption droplet 15 hours after injection. It contains layered membranes which appear as dense bands measuring *ca.* 50 A. The repeating period is 100 A. Two mitochondria lie closely apposed to the droplet and an element of the endoplasmic reticulum lies between the droplet and the mitochondrion at left. × 52,000.


26


27


28

plasmic reticulum when none or only a few ferritin particles are present, and that this relationship is not observed in later stages when the droplets are filled with ferritin. The reticulum around the droplets is, however, mostly composed of smooth surfaced elements not known to be engaged in protein synthesis, but is also continuous with rough surfaced elements. The latter were not found in close proximity to the droplets.

Iron liberated from the breakdown of hemoglobin is assumed to be in the ferrous state and must be oxidized to the ferric state for incorporation into ferritin, and oxidative enzymes of the mitochondria might be required for this process (68). The very close apposition of mitochondria to droplets could then indicate more than an accidental topical relationship.

The final disposal of ferritin has not been followed in our experiments, but it seems to remain in the tubule cell for a long time. Rather (23) found a positive iron reaction in rat tubule cells 13 days after hemoglobin injection, and Wessel and Gedigk (67) observed siderosomes loaded with ferritin in macrophages 3 months after hemoglobin injection.

The intracellular digestion of the protein moiety of hemoglobin cannot be followed morphologically. We assume that the decrease in density of the droplets indicates breakdown and removal of protein. The membranes thought to represent lipoprotein remain unaltered during the time period investigated. Whether the dense small bodies with tightly packed membranes represent a stage of further condensation, cannot be decided at present. The significance of the striking concentric arrangement of elements of the endoplasmic reticulum around these bodies remains also unknown; similar formations have been described in rat liver cells (70).

The dense content of the tubular lumen during reabsorption contrasts markedly with the content of the peritubular capillaries which appears, as in controls, to be composed of finely stranded or flocculated precipitates. This might indicate that a differential reabsorption of water takes place which precedes hemoglobin reabsorption and causes the concentration of hemoglobin in the tubular lumen. Whether concentration or size of molecules in the tubular lumen triggers the mechanism of reabsorption in bulk (43) through the tubular invaginations, and whether reabsorption of water and electrolytes occurs by a pinocytotic process into the brush border microvilli directly, is not known at present.

The newly developed concept of lysosomes (71) or phagosomes (13) as a group of cytoplasmic particles containing acid hydrolytic enzymes and involved in intracellular digestion has received additional support from this investigation. It could be shown that the reabsorption of hemo-

---

FIGURE 26

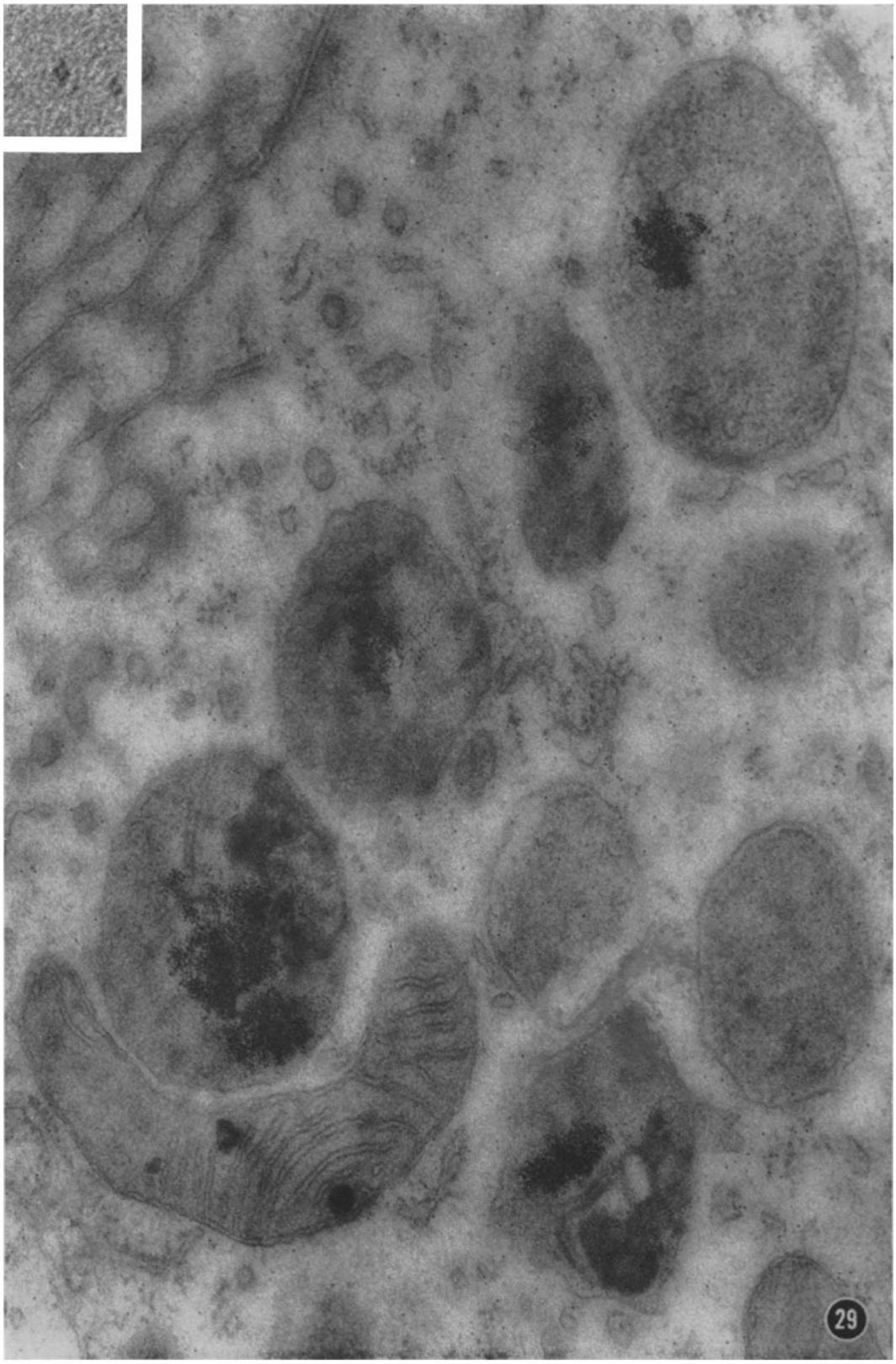
Hemoglobin absorption droplet (15 hours after injection) containing dense particles of ferritin (circle) and whorl-like membrane systems. × 61,000.

FIGURE 27

A hemoglobin absorption droplet (18 hours after injection) enclosing a structure suggestive of a mitochondrion. A membrane (arrow) surrounds part of the mitochondrion "inside" the droplet, and is thought to represent part of the outer droplet membrane which has been invaginated with the mitochondrion into the droplet. The mitochondrion is assumed to lie outside the droplet but protruding into it. Such pictures were very rarely obtained in only one animal and are further discussed in the text. × 75,000.

FIGURE 28

A dense body similar to the ones found in controls contains densely packed layered membranes and is surrounded by a closed ring of 10 elements of smooth surfaced endoplasmic reticulum. The outermost, eleventh element of the endoplasmic reticulum is both smooth and rough surfaced (arrows). Microbodies (at upper left and lower right) and mitochondria (at upper right) are not altered. × 66,000.


29

globin takes place into vacuoles bound by a membrane which transform into droplets. The decrease in density, the appearance of ferritin and of membranous structures suggest that intracellular digestion takes place in membrane bounded droplets. If apoferritin is synthesized outside the droplets and provides the template for the rearrangement of the ferric hydroxide into typical micelles (68), one could avoid the difficulty of postulating the occurrence of lytic and synthetic processes within the droplet.

Final confirmation of the identity of the vacuoles and droplets with lysosomes or phagosomes must await the development of topochemical methods for acid phosphatases or other hydrolytic enzymes at the electron microscopic level.

The fine structure of lysosomes has not been established, and it is not known whether all lysosomes have an identical structure at all. In the control animals several distinct structures have to be considered theoretically as possible bearers of hydrolytic enzymes, namely the microbodies, the vacuoles, and the vacuolated bodies. The latter would best fit a morphologist's concept of the structure of a lysosome: a digestive vacuole and a package of enzymes located excentrically and waiting to be called upon. The data at hand, however, have not shown any absorptive or digestive role either for microbodies or vacuolated bodies; in the early stages of hemoglobin reabsorption only the vacuoles and tubular invaginations are involved. Microbodies have not been found altered or increased during the entire period under investigation, and no clue was obtained whether they are precursors of mitochondria (18, 44, 72, 73). The vacuolated bodies must be further investigated. They were not seen to take part in the reabsorption of hemoglobin and no pictures were obtained that could indicate a role of these bodies in bringing enzymes to the vacuoles as suggested for the "microkinetospheres" in HeLa cells (74).

The tubular invaginations and the vacuoles, then, remain for the moment as the only structures found involved in hemoglobin reabsorption. Nothing definite is known about their content of hydrolytic enzymes. No attempt was made to demonstrate acid phosphatase in frozen sections of animals injected with hemoglobin because this does not permit a convincing correlation of ultrastructure with enzymatic activity in such restricted areas. Investigations by other authors seem to indicate a possible role of vacuoles as enzyme-containing structures. Novikoff (75) stated that the vacuoles of the cells in the proximal convolution of rats "have the same location, size, and frequency as the acid phosphatase droplets seen in light micrographs" but did not furnish unequivocal pictorial evidence for this assumption. Essner (76) investigated vacuoles in macrophages of the Novikoff ascites hepatoma phagocytizing erythrocytes and concluded on the basis of the acid phosphatase reaction in smears of these cells that the vacuoles contain acid phosphatase. He, therefore, equated the phagocytotic vacuoles plus their contents of fragmented erythrocytes with the phagosomes of Straus (13). Farquhar and Palade (77) followed the segregation of ferritin into vacuoles and droplets of the visceral glomerular epithelia and also discussed the possible role of these structures as bearers of acid hydrolytic enzymes.

I wish to express my gratitude to Dr. K. R. Porter for making available the facilities of his laboratory, to Dr. G. E. Palade for discussions, and to Miss Stephanie Walser for technical assistance.

BIBLIOGRAPHY

1. GÉRARD, P., and CORDIER, R., Esquisse d'une histophysiologie comparée du rein des vertébrés, *Biol. Rev.*, 1934, **9**, 110.
2. RANDERATH, E., Die Entwicklung der Lehre von den Nephrosen in der pathologischen Anatomie, *Ergebn. Path.*, 1937, **32**, 91.

FIGURE 29

Hemoglobin absorption droplets 4 days after injection. The droplets are surrounded by a membrane and contain dense aggregates of ferritin particles ("siderosomes" (24)). The four subunits of the ferritin molecule are visible in favorable sections (insert at upper left). Some droplets contain a few parallel membranes. Ferritin is also scattered in the cytoplasm. The brush border is at upper left. × 77,000. Insert: × 490,000.

3. OLIVER, J., The structure of the metabolic process in the nephron, *J. Mt. Sinai Hosp.*, 1948, **15**, 173.
4. OLIVER, J., and MACDOWELL, M., Cellular mechanisms of protein metabolism in the nephron. VII. The characteristics and significance of the protein absorption droplets (hyaline droplets) in epidemic hemorrhagic fever and other renal diseases, *J. Exp. Med.*, 1958, **107**, 731.
5. SMETANA, H., The permeability of the renal glomeruli of several mammalian species to labelled proteins, *Am. J. Path.*, 1947, **23**, 255.
6. OLIVER, J., MACDOWELL, M., and LEE, Y. C., Cellular mechanisms of protein metabolism in the nephron. I. The structural aspects of proteinuria; tubular absorption, droplet formation and the disposal of proteins, *J. Exp. Med.*, 1954, **99**, 589.
7. OLIVER, J., MOSES, M. J., MACDOWELL, M. C., and LEE, Y. C., Cellular mechanisms of protein metabolism in the nephron. II. The histochemical characteristics of protein absorption droplets, *J. Exp. Med.*, 1954, **99**, 605.
8. KRETCHMER, N., and DICKERMAN, H. W., Cellular mechanisms of protein metabolism in the nephron. IV. The partition of succinoxidase and cytochrome oxidase activities in the cells of the proximal convolution of the rat after intraperitoneal injection of egg white, *J. Exp. Med.*, 1954, **99**, 629.
9. KRETCHMER, N., and CHEROT, F. J., Cellular mechanisms of protein metabolism in the nephron. V. The intracellular partition and incorporation into protein of intravenously injected L-lysine, *J. Exp. Med.*, 1954, **99**, 637.
10. STRAUS, W., Isolation and biochemical properties of droplets from the cells of rat kidney, *J. Biol. Chem.*, 1954, **207**, 745.
11. STRAUS, W., and OLIVER, J., Cellular mechanisms of protein metabolism in the nephron. VI. The immunological demonstration of egg white in droplets and other cellular fractions of the rat kidney after intraperitoneal injection, *J. Exp. Med.*, 1955, **102**, 1.
12. STRAUSS, W., Segregation of an intravenously injected protein by "droplets" of the cells of rat kidneys, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 1037.
13. STRAUS, W., Rapid cytochemical identification of phagosomes in various tissues of the rat and their differentiation from mitochondria by the peroxidase method, *J. Biophysic. and Biochem. Cytol.*, 1958, **5**, 193.
14. MAYERSBACH, H., and PEARSE, A. G. E., The metabolism of fluorescein-labelled and unlabelled egg-white in the renal tubules of the mouse, *Brit. J. Exp. Path.*, 1956, **37**, 81.
15. NIEMI, M., and PEARSE, A. G. E., The relationship of the mitochondria to egg white absorption droplets in the rat kidney, *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 279.
16. RHODIN, J., Correlation of ultrastructural organization and function in normal and experimentally changed proximal convoluted tubule cells of the mouse kidney, Karolinska Institutet, Aktiebolaget Godvil, 1954.
17. MILLER, F., and SITTE, H., Elektronenmikroskopische Untersuchungen an Mäusenieren nach intraperitonealen Eiweissgaben, *Verhandl. deutsch. path. Ges.*, 1956, **39**, 183.
18. GANSLER, H., and ROUILLER, C., Modifications physiologiques et pathologiques du chondriome. Etude au microscope électronique, *Schweiz. Z. allg. Path. u. Bact.*, 1956, **19**, 217.
19. ZOLLINGER, H. U., Über hyalin-tropfige Veränderung der Nierenhauptstücke als Ausdruck von Eiweisspeicherung. Phasenmikroskopische Beobachtungen über Mitochondrienfunktionen. II, *Schweiz. Z. allg. Path. u. Bact.*, 1950, **13**, 146.
20. RÜTTIMANN, A., Über Aufbrauchserscheinungen und Neubildung der Mitochondrien in den Nierenhauptstücken nach Speicherung, *Schweiz. Z. allg. Path. u. Bact.*, 1951, **14**, 373.
21. FARQUHAR, M. G., VERNIER, R. L., and GOOD, R. A., The application of electron microscopy in pathology: Study of renal biopsy tissues, *Schweiz med. Woch.*, 1957, **87**, 501.
22. MILLER, F., Orthologie und Pathologie der Zelle im elektronenmikroskopischen Bild, *Verhandl. deutsch. path. Ges.*, 1959, **42**, 261.
23. RATHER, L. J., Renal athrocytosis and intracellular digestion of intraperitoneally injected hemoglobin in rats, *J. Exp. Med.*, 1948, **87**, 163.
24. RICHTER, G. W., A study of hemosiderosis with the aid of electron microscopy, *J. Exp. Med.*, 1957, **106**, 203.
25. SJÖSTRAND, F. S., and RHODIN, J., The ultrastructure of the proximal convoluted tubules of the mouse kidney as revealed by high resolution electron microscopy, *Exp. Cell Research*, 1953, **4**, 426.
26. CLARK, S. L., JR., Cellular differentiation in the kidneys of newborn mice studied with the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 349.
27. PEASE, D. C., Electron microscopy of the tubular cells of the kidney cortex, *Anat. Rec.*, 1955, **121**, 723.
28. PEASE, D. C., Fine structures of the kidney seen by electron microscopy, *J. Histochem. and Cytochem.*, 1955, **3**, 295.
29. PALADE, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95**, 285.

30. CAULFIELD, J. B., Effects of varying the vehicle for OsO_4 in tissue fixation, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 827.
31. SATIR, P. G., and PEACHEY, L. D., Thin sections. II. A simple method for reducing compression artifacts, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 345.
32. WATSON, M. L., Reduction of heating artifacts in thin sections examined in the electron microscope, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 1017.
33. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals. II. Applications of solutions containing lead and barium, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 727.
34. PEACHEY, L. D., A device for staining tissue sections for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 511.
35. WATSON, M. L., Staining of tissue sections for electron microscopy, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 475.
36. FAWCETT, D. W., Structural specialization of the cell surface, *in* Frontiers in Cytology, (S. L. Palay, editor), New Haven, Yale University Press, 1958.
37. PALADE, G. E., Studies on the endoplasmic reticulum. II. Simple dispositions in cells *in situ*, *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 567.
38. ROBERTSON, J. D., Structural alterations in nerve fibers produced by hypotonic and hypertonic solutions, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 349.
39. SJÖSTRAND, F., Über die Eigenfluoreszenz tierischer Gewebe mit besonderer Berücksichtigung der Säugetierniere, *Acta anat.*, 1944, suppl. No. 1.
40. SAMPAIO, M. M., BRUNNER, A., JR., and OLIVEIRA FILHO, B., Aspects of the ultrastructure of the brush border of the kidney of normal mouse, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 335.
41. RHODIN, J., Anatomy of kidney tubules, *Internat. Rev. Cytol.*, 1958, **7**, 485.
42. PALADE, G. E., Transport in quanta across the endothelium of blood capillaries (abstract), *Anat. Rec.*, 1960, **136**, 254.
43. PALADE, G. E., The endoplasmic reticulum, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 85.
44. SCHULZ, H., Zur submikroskopischen Pathologie der Cytosomen in den Alveolarmakrophagen der Lunge, *Beitr. path. Anat. u. allg. Path.*, 1958, **119**, 71.
45. KARRER, H. E., Electron microscopic study of the phagocytosis process in lung, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 357.
46. CAMPICHE, M., Les inclusions lamellaires des cellules alvéolaires dans le poumon du raton. Relations entre l'ultrastructure et la fixation, *J. Ultrastruct. Research*, 1960, **3**, 302.
47. ZETTERQVIST, H., The ultrastructural organization of the columnar absorbing cells of the mouse jejunum, Karolinska Institutet, Aktiebolaget Godvil, 1956.
48. RHODIN, J., and DALHAMN, T., Electron microscopy of the tracheal ciliated mucosa in the rat, *Z. Zellforsch.*, 1956, **44**, 345.
49. LINDNER, E., Der elektronenmikroskopische Nachweis von Eisen im Gewebe, *Ergebn. Path.*, 1958, **38**, 46.
50. NILSSON, O., Ultrastructure of mouse uterine surface epithelium under different estrogenic influences. 5. Continuous administration of estrogen, *J. Ultrastruct. Research*, 1959, **2**, 342.
51. HERMAN, L., An electron microscope study of the salamander thyroid during hormonal stimulation, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 143.
52. SCHIEBLER, T. H., and KNOOP, A., Histochemische und elektronenmikroskopische Untersuchungen an der Rattenplazenta, *Z. Zellforsch.*, 1959, **50**, 494.
53. BENCOSME, S. A., STONE, R. S., LATTA, H., and MADDEN, S. C., Acute reactions with collagen production in renal glomeruli of rats as studied electron microscopically, *J. Ultrastruct. Research*, 1959, **3**, 171.
54. DUNCAN, D., and HILD, W., Mitochondrial alterations in cultures of the central nervous system as observed with the electron microscope, *Z. Zellforsch.*, 1960, **51**, 123.
55. COTTE, G., Quelques problèmes posés par l'ultrastructure des lipides de la corticosurrénale, *J. Ultrastruct. Research*, 1959, **3**, 186.
56. LINDNER, E., Die submikroskopische Morphologie des Herzmuskels, *Z. Zellforsch.*, 1957, **45**, 702.
57. SCHULZ, H., and DE PAOLA, D., Delta-Cytomembranen und lamelläre Cytosomen. Ultrastruktur, Histochemie und ihre Beziehungen zur Schleimsekretion, *Z. Zellforsch.*, 1958, **49**, 125.
58. STOECKENIUS, W., An electron microscope study of myelin figures, *J. Biophysic. and Biochem. Cytol.*, 1959, **5**, 491.
59. STOECKENIUS, W., SCHULMAN, J. H., and PRINCE, L. M., The structure of myelin figures and microemulsions as observed with the electron microscope, *Kolloid Z.*, 1960, **169**, 170.
60. CHARGAFF, E., and ZIFF, M., The compounds between phosphatides and basic proteins, *J. Biol. Chem.*, 1939, **131**, 25.
61. CHARGAFF, E., ZIFF, M., and HOGG, B. M.,

The reaction between cephalin and hemoglobins, *J. Biol. Chem.*, 1939, **131**, 35.

62. FARRANT, J. L., An electron microscopic study of ferritin, *Biochim. et Biophysica Acta*, 1954, **13**, 569.
63. STOECKENIUS, W., Morphologische Beobachtungen beim intrazellularen Erythrozytenabbau und der Eisenspeicherung in der Milz des Kaninchens, *Klin. Woch.*, 1957, **35**, 760.
64. KUFF, E. L., and DALTON, A. J., Identification of molecular ferritin in homogenates and sections of rat liver, *J. Ultrastruct. Research*, 1957, **1**, 62.
65. KERR, D. N. S., and MUIR, A. R. A., A demonstration of the structure and disposition of ferritin in the human liver cell, *J. Ultrastruct. Research*, 1960, **3**, 313.
66. RICHTER, G. W., Electron microscopy of hemosiderin: Presence of ferritin and occurrence of crystalline lattices in hemosiderin deposits, *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 55.
67. WESSEL, W., and GEDIGK, P., Die Verarbeitung und Speicherung von phagocytiertem Eisen im elektronenmikroskopischen Bild, *Virchows Arch. path. Anat.*, 1959, **332**, 508.
68. RICHTER, G. W., The cellular transformation of injected colloidal iron complexes into ferritin and hemosiderin in experimental animals, *J. Exp. Med.*, 1959, **109**, 197.
69. FINEBERG, R. A., and GREENBERG, D. M., Ferritin biosynthesis. III. Apoferritin, the initial product, *J. Biol. Chem.*, 1955, **214**, 107.
70. EMMELOT, P., and BENEDETTI, E. L., Changes in the fine structure of rat liver cells brought about by dimethylnitrosamine, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 393.
71. DE DUVE, C., Lysosomes, a new group of cytoplasmic particles, *in* Subcellular Particles, (T. Hayashi, editor), New York, Ronald Press Co., 1959.
72. ROUILLER, C., and BERNHARD, W., "Microbodies" and the problem of mitochondrial regeneration in liver cells, *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, No. 4, suppl., 355.
73. WEISSENFELS, N., Über die Entstehung der Promitochondrien und ihre Entwicklung zu funktionstüchtigen Mitochondrien in den Zellen von Embryonal- und Tumorgewebe, *Z. Naturforsch.*, 1958, **13b**, 203.
74. ROSE, G. G., Microkinetospheres and VP satellites of pinocytic cells observed in tissue culture of Gey's strain HeLa with phase contrast cinematographic techniques, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 697.
75. NOVIKOFF, A. B., The proximal tubule cell in experimental hydronephrosis, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 136.
76. ESSNER, E., An electron microscopic study of erythrophagocytosis, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 329.
77. FARQUHAR, M. G., and PALADE, G. E., Segregation of ferritin in glomerular protein absorption droplets, *J. Biophysic. and Biochem. Cytol.*, 1960, **7**, 297.
78. MILLER, F., Lipoprotein granules in the cortical collecting tubules of mouse kidney, *J. Biophysic. and Biochem. Cytol.*, 1961, **9**, in press.